Selenium Tissue Thresholds:

Tissue Selection Criteria, Threshold Development Endpoints, and Potential to Predict Population or Community Effects in the Field

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Purpose of This Report

The U.S. Environmental Protection Agency (EPA) released a draft whole-body fish selenium (Se) criterion of 7.91 µg/g dry weight in 2004. When a criteria document for Se is finalized, the resulting approaches for acute and chronic criteria will not only have important implications in the U.S., but will also potentially set a precedent for regulatory decision making in Canada. Although there is general consensus that a fish tissue-based Se criterion is more appropriate than a water-based Se criterion for chronic toxicity, there are still important uncertainties associated with the EPA's draft chronic criterion. In addition, several new Se toxicity studies have been published since 2004.

The objective of the Tissue Threshold Workgroup was to conduct a vigorous evaluation of the state-of-the-science on Se toxicity in fish and implications for developing a broadly applicable criterion. This evaluation is not only intended to consolidate information that would be useful to members of the NAMC-SWG, but also to provide information to the EPA before the Se criterion is finalized. The following questions are addressed in this report:

- **What is the appropriate tissue for deriving a fish Se threshold?** This evaluation focuses on the relationships between Se in whole-body fish and individual tissues, and how these relationships vary among species and sites. Understanding these relationships has important implications for deriving a fish tissue-based Se criterion that is broadly applicable across sites and fish species assemblages.

- **What is the appropriate endpoint, life stage, and effect level for deriving a fish Se threshold?** This evaluation focuses on the relative sensitivity of different fish life stages and endpoints to Se and also considers which life stage and endpoints are most applicable for biological monitoring in the field. The evaluation also considers the appropriate statistical effect level for deriving a Se threshold. A tissue-based threshold is recommended based on these two sections.

- **Are fish Se thresholds predictive of population-level effects?** This evaluation focuses on whether various fish tissue thresholds can "field truth" population-level effects in the field. Considered in this evaluation is whether habitat-related impacts can be discerned from potential Se-related impacts.

Executive Summary

The Tissue Threshold Workgroup was charged with conducting a vigorous evaluation of the state-of-the-science on selenium (Se) toxicity in fish and implications for developing a broadly applicable tissue-based criterion. This evaluation was based on addressing three primary questions: (1) What is the appropriate tissue for deriving a fish Se threshold? (2) What is the appropriate endpoint, life stage, and effect level for deriving a fish Se threshold? (3) Are fish Se thresholds predictive of population-level effects? To further support this evaluation, evidence for winter stress syndrome, a term coined by Lemly (1993, 1996) to describe a condition of potential metabolic distress in warmwater fish, was critiqued and a standard operating procedure (SOP) for evaluating Se-related deformities in early life stages of fish was developed. Each of these components of the evaluation is summarized below, followed by the key conclusions and recommendations.

Tissue Selection Criteria: Selection of Tissue Types for the Development of a Meaningful Selenium Tissue Threshold in Fish (Adrian deBruyn, Al Hodaly, and Peter Chapman – Golder Associates)

In order to address the first question above, a review and quantitative meta-analysis of the literature on Se measurements in fish tissues, with an emphasis on studies that have compared different tissues in the same organisms and/or tissue measurements to biological effects, was conducted. While eggs and larvae could be considered "life stages" and not "tissues", per se, for the purposes of this analysis and the chapters herein, eggs are not considered a stage of development until they are fertilized and become embryos. Therefore, for convenience of reference, eggs and larvae are considered interchangeable with the term "tissues". The meta-analysis focused on assessing relationships among Se concentrations in various tissue types and between Se concentrations in various tissue types and toxic effects to early life stages. Overall, this analysis concluded that the egg Se concentration is the most useful basis for a Se tissue guideline or criterion, as this tissue concentration is most closely linked to exposure by developing early life stages. Relationships between Se concentrations in the eggs and adult tissues (e.g., whole-body, muscle) are species-specific, and therefore, use of generic relationships to estimate the Se concentration in one tissue from another is not recommended. However, if a species-specific tissue-to-tissue relationship has been developed, any candidate tissues (e.g., whole-body, muscle) should be a reliable surrogate for Se exposure by early life stages.

Threshold Development Endpoints: Review of Selenium Tissue Thresholds for Fish: Evaluation of the Appropriate Endpoint, Life Stage, and Effect Level and Recommendation for a Tissue-Based Criterion (David DeForest – Parametrix)

In order to address the second question above, the available Se toxicity studies with freshwater fish were reviewed. There are two basic types of Se toxicity studies with fish:

(1) assessment of larval abnormalities and mortality following Se exposures resulting from maternal transfer and (2) assessment of juvenile growth and mortality following direct dietary Se exposures. The existing Se toxicity data, albeit limited for comparisons within a species, suggest that the two types of studies are similarly sensitive. Overall, however, the maternal transfer Se toxicity studies appear to provide the most appropriate endpoint and life stage for developing a tissue-based Se threshold for the following reasons: (1) adult fish (including the eggs they produce) are a useful biomonitor for measuring tissue Se concentrations because this life stage is relatively insensitive to Se (a sensitive biomonitor would not be applicable across a broad range of Se exposure levels); (2) larval deformities represent a Se-specific endpoint that can be confirmed by site-specific evaluations; (3) exposure of adult fish to Se and maternal transfer to eggs and subsequent life stages represents an environmentally realistic exposure route in which fish are exposed to Se at the onset of development; and (4) data on Se concentrations in developing larvae and juveniles and associated effects are more limited than for exposure of adult fish, with at least one study suggesting that whole-body Se concentrations can be variable over time and the relationship between whole-body Se and effects varies with time. This review suggests that an egg Se EC10 of 17 μg/g dry weight, based on maternal transfer of Se, represents a broadly applicable tissue Se guideline (site-specific studies may still be used to develop an alternative egg Se guideline or criterion).

Field Application of Tissue Thresholds: Potential to Predict Fish Population or Community Effects in the Field (Steve Canton and Stephanie Baker – GEI Consultants)

In order to address the third question above, an analysis of studies that evaluated the impacts of Se on fish populations in streams was conducted. To date, most clearly documented cases of Se impacts on fish populations have been associated with coal fly ash discharges to lakes and reservoirs. The streams evaluated here include (1) the Arkansas River and tributaries in the vicinity of Pueblo, Colorado; (2) Dixon Creek and the Canadian River in the Texas panhandle; (3) the Sand Creek drainage in the vicinity of Denver, Colorado; (4) Stingy Run in southern Ohio; (5) the Thompson Creek watershed in central Idaho; and (6) the Snake River in Idaho. These streams have complete evaluations of fish populations at widely varying levels of Se and varying habitat conditions. These studies appear to indicate that Se may be a factor in structuring fish communities, especially with regard to specific fish families, such as Centrarchidae. However, based on these available studies, it is also apparent that habitat quality is a major factor to consider when trying to discern Se impacts to fish in streams, especially warmwater streams. Natural history factors, such as migration, immigration or emigration of fish, competition between fish species, and the balance between terrestrial (assumed low Se) versus aquatic (potentially high Se) food sources, are also important when determining if patterns observed in fish populations are Se related. Therefore, particularly in streams, a tissue-based Se guideline can be considered an indicator of possible Se impacts on fish populations, but field studies are necessary to fully attempt to elucidate the true impact of Se, if any, at a given site.

Standard Operating Procedure for Evaluating Selenium-Induced Deformities in Early Life Stages of Freshwater Fish (David Janz and Jorgelina Muscatello – University of Saskatchewan) – Appendix A

The previous evaluations recommended that the egg Se concentration associated with the threshold for developmental effects in larval fish is the most appropriate basis for determining a Se tissue guideline or criterion. Several studies have used a variety of techniques for evaluating larval deformities and edema in fish exposed to Se. Because different studies use varying techniques, it is not always possible to compare data between species and sites. Accordingly, a standard operating procedure (SOP) was developed that recommends the experimental design, statistical treatment of data, and detailed guidance for performing a Se effects evaluation in fish larvae and fry. The SOP is largely based on northern pike, white sucker, and lake trout embryo incubations and deformities evaluations conducted at the University of Saskatchewan's Toxicology Centre, but the SOP should also be applicable to other freshwater species.

A Critical Evaluation of Winter Stress Syndrome (David Janz – University of Saskatchewan) – Appendix B

The EPA's draft fish tissue Se criterion is based on "winter stress syndrome" in bluegill. In this study, bluegill exposed to an aqueous Se concentration of ~ 5 µg/L and a dietary Se concentration of ~ 5 µg/g had hematological changes and gill damage that reduced respiratory capacity, resulting in increased respiratory demand and oxygen consumption. When winter conditions were simulated simultaneously (reduction in temperature from 20°C to 4°C and reduced photoperiod), bluegills reduced activity and feeding. The increased respiratory demand combined with reduced feeding resulted in lipid depletion and subsequent mortality in a significant number of test fish. Bluegills exposed to the same Se levels, but warmer temperatures (i.e., 20°C), continued to feed and did not lose sufficient lipids for mortality to occur. Winter stress syndrome represents a scientifically sound hypothesis, although to date only laboratory studies exposing fish to Se have provided support for it. It is apparent that much more work is needed to investigate whether winter stress syndrome occurs in the field, whether other aquatic contaminants can cause it, and whether it is species-specific. As noted by Dr. Lemly, basic knowledge of life history characteristics and feeding ecology, particularly for young-of-the-year fish, would allow identification of potentially vulnerable fish species in temperate regions of the world. Unfortunately, there are few studies with direct observations and concrete conclusions regarding feeding ecology in juvenile fish species. It is possible that winter stress syndrome is most important in species at the northern limit of their ranges. Thus, future studies should focus on this aspect. Knowledge of local fish community ecology is essential when assessing the potential importance of overwinter mortality in aquatic ecotoxicological investigations of Se or other aquatic contaminants.

TISSUE THRESHOLD RECOMMENDATIONS AND SUGGESTED FUTURE RESEARCH

- The appropriate tissue and endpoint for developing a broadly applicable fish tissue Se guideline or criterion is the egg Se concentration, as associated with the effects threshold for larval deformities and mortality resulting from the maternal transfer of Se.

- In compiling the Se toxicity database for guideline or criteria development, Se concentrations in one tissue should not be estimated from another tissue using regression relationships based on other species and studies.

- Likewise, when implementing a Se criterion, tissue Se concentrations should not be estimated from another tissue for determining compliance. There is too much variability in these relationships between species and sites. However, use of species-specific and site-specific tissue-to-tissue relationships may be reasonable for evaluating Se compliance at a site.

- More field-based studies are necessary to understand the potential impacts of Se on stream fish populations at concentrations above recommended criteria and guidelines.

- Field studies are also necessary to evaluate the significance of winter stress syndrome, as to date this endpoint has been documented only in the laboratory.

- Additional field documentation of Se effects in standing waters (lakes and reservoirs) is needed to determine if effects from historic studies can be quantified at other sites.

- Future studies need to follow a consistent approach for analyzing Se and deformities in larval fish.

- Even seemingly minor information, such as the moisture content of fish tissues, needs to be reported since some studies report wet weight Se concentrations and others report dry weight concentrations.

References:

Lemly, A.D. 1993. Metabolic stress during winter increases the toxicity of selenium to fish. Aquatic Toxicology 27:133-158.

Lemly, A.D. 1996. Winter stress syndrome: An important consideration for hazard assessment of aquatic pollutants. Ecotoxicology and Environmental Safety 34:223-227.

Part I:
Tissue Selection Criteria

Selection of Tissue Types for the Development of a Meaningful Selenium Tissue Threshold in Fish

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1.0 Introduction

1.1 Conceptual Background

There is an emerging consensus in the scientific and regulatory community that protection of fish in Se-contaminated systems should be based on fish tissue concentrations rather than water, sediment, or dietary concentrations (Hamilton 2002; Sappington 2002; EPA 2004). However, there has been considerable debate regarding the development of a meaningful Se tissue residue guideline or criterion (hereafter, TRG) for fish. One element of this debate involves selection of the most appropriate tissue type to serve as the basis for the TRG.

There are conflicting requirements that must be considered in the selection of an appropriate tissue type for a TRG. A basic tenet of toxicology is that the most relevant measure of exposure to a toxic substance is the concentration at the site of toxic action. For Se in fish, the most ecologically relevant sites of toxic action are the developing tissues associated with early life stages (ELS). Basic toxicological principles suggest that Se concentrations in the eggs and newly-hatched larvae (i.e., prior to swim-up and the onset of exogenous feeding) of fish would be the most useful exposure measure for predicting or monitoring ecological effects. From a logistical perspective, however, it is far more practical to sample adult fish: most Se monitoring programs measure muscle tissue or whole-body concentrations in adults because ELS tissues are relatively difficult to sample and are available only at certain times of the year.

A common assumption, although not previously evaluated formally, is that Se concentrations in adult tissues will correlate to concentrations in ELS tissues because maternal transfer is the major source of Se to ELS prior to the onset of exogenous feeding. However, the relationship between adult and ELS tissue Se concentrations is not well understood and has the potential of becoming confounded or decoupled by intraspecific, interspecific, and temporal variation in factors such as toxicokinetics, feeding ecology, and physiology. Although several studies have demonstrated correlations between adult tissue concentrations and ELS tissue concentrations, using empirical regression equations developed to estimate concentrations in one tissue from concentrations in another (e.g., EPA 2004), the generality of these relationships has not been assessed. Furthermore, there is increased uncertainty in the derivation and application of a TRG when tissue-tissue regressions are required, due to the residual variance (which is often considerable but rarely explicitly considered) around these regression equations.

1.2 Objective

This report provides a review and quantitative meta-analysis of the literature on Se measurements in fish tissues, with emphasis on studies that have compared different

tissues in the same organisms and/or tissue measurements to biological effects. The meta-analysis focuses on assessing relationships among Se concentrations in various tissue types and between Se concentrations in various tissue types and toxic effects to ELS. The objective of this report is to make specific recommendations regarding the most appropriate tissue type for a Se TRG for fish.

2.0 Study Selection and Evaluation

2.1 Study Selection and Review Methods

Data were extracted from 20 studies that reported Se concentrations in at least two tissue types within the same adult fish or test group. Tissue types included in the present analysis were whole-body, muscle, muscle plug, liver, ovary, egg, and larva (Figure 1). Additional tissue types (e.g., kidney, gill) were not represented by sufficient data to provide a robust analysis and were therefore excluded from consideration. In all cases, original reports were reviewed and raw data were extracted from tables, appendices, or figures. Where available, corresponding ELS effects in the progeny of these fish were also extracted and reviewed. Tissue data from four of these studies (Bryson et al. 1985b; Coyle et al. 1993; Garcia-Hernandez et al. 2000; Hermanutz et al. 1996) were used by EPA (2004) as the basis for the generic regression relationships between whole-body and muscle, ovary, and liver tissue.

Studies were excluded from this review if the data presented were not relevant to assessing the potential utility of the candidate tissues as the basis for a Se TRG for fish. In particular, Se concentration and effects data for exogenously feeding larvae or juveniles were excluded from this review. This decision is appropriate because the focus of this review is on potential relationships between adult tissue Se and ELS tissue Se and/or ELS effects. Se concentrations in exogenously feeding larvae or juveniles are not expected to be directly related to adult or ELS Se concentrations in a consistent or predictable way because the influence of the post-swim-up dietary exposure of the larval or juvenile fish would rapidly outweigh the influence of maternal transfer. The effect of this influence is unpredictable. For example, consumption of Se-contaminated food may result in a net increase in larval fish Se concentration and thus bias the tissue-effect relationship in one direction. In contrast, consumption of clean food may result in a net decrease in larval fish Se concentrations (via the influence of growth dilution) which would bias the tissue-effect relationship in the opposite direction.

This decision results in exclusion of several studies cited by existing toxicity reference value (TRV) derivation documents. For example, Hunn et al. (1987) related growth effects to concentrations in post-swim-up fry feeding on a Se-contaminated diet, and therefore, these data cannot be directly related to maternal Se concentrations. Thus, this study was excluded from the current review, despite the fact that Hunn et al. (1987) strongly influenced the whole-body Se concentration of 4 µg/g dry weight cited as a toxicity threshold by Lemly (1993, 1996).

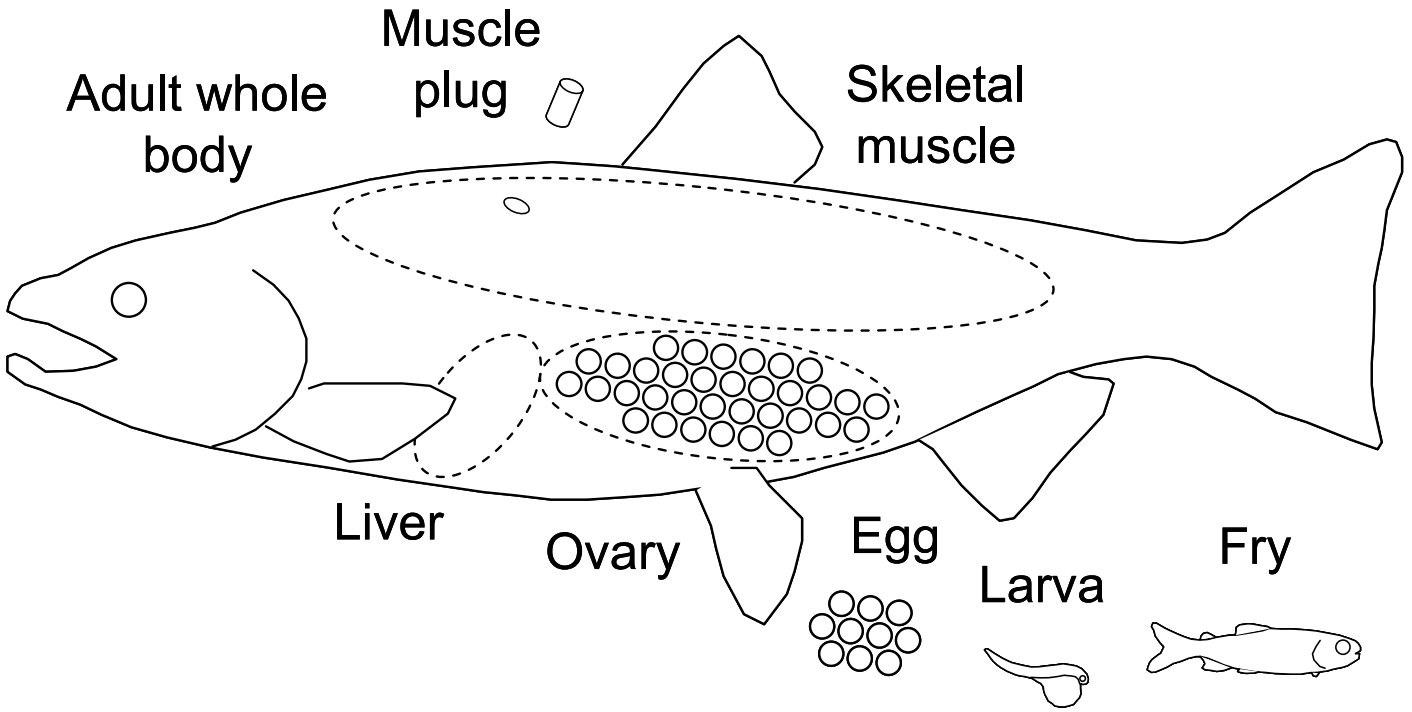

Figure 1: Candidate fish tissues for a Se guideline or criterion.

2.2 Summary of Reviewed Studies

Brief summaries of the 20 reviewed studies are provided below. Additional details are provided in Appendix I.

- **Bryson et al. 1984.** A 28-day study reported survival and weight of embryos and larvae obtained from multiple crosses between bluegill sunfish (*Lepomis macrochirus*) from the Hyco Reservoir (hereafter "Hyco", a cooling lake for Carolina Power & Light that receives discharge from an ash storage pond) and a nearby control lake (Roxboro City Lake). Mean Se concentrations were reported for adult female muscle, liver, and ovary. Fertilized eggs were exposed to 0, 20, and 50 percent ash pond effluent and percent hatch and swim-up success were measured. Due to ongoing exposure to high levels of Se during incubation (i.e., Se concentrations in these embryos may not be reflective of maternal transfer), egg and larval data were excluded from this review. This study was followed up by an ingestion study in which larvae surviving past the swim-up stage were released to exposure tanks containing control water and fed zooplankton from either Hyco Reservoir or the control lake. Data for exogenously feeding larvae were excluded from the present analysis.

- **Bryson et al. 1985a.** In a repeat of the Bryson et al. (1984) 28-day embryo-larval study, embryos were obtained from three crosses: Hyco female and Hyco male; control female and Hyco male, and control female with control male (n = 1 for each combination). Mean Se tissue concentrations in muscle, liver, and ovary were reported for adult males and females. Embryos were exposed to 0, 20, and 50 percent ash pond effluent for 28 days. Percent hatch, percent swim-up success, and survival were measured to 28 days post-hatch. Two replicate treatments were fed zooplankton from either Hyco or the control lake. Larvae were observed for 28 days at which time survival and weight were measured. Data for exogenously feeding larvae were excluded from the present analysis. In an additional embryo-larval study up to the swim-up stage, embryos were obtained from crosses of fish from "affected" and "unaffected" areas of Hyco. Percent hatch, percent swim-up, and survival until swim-up were measured.

- **Bryson et al. 1985b.** Adult fish were collected from either Hyco Reservoir or the control lake and exposed to either Hyco or control water in flow-through spawning tanks for 4 to 7 months. The results of the study concluded that fish from the control lake did not spawn. Embryos of Hyco adults from each treatment were maintained to swim-up. Data for exogenously feeding larvae were excluded from the present analysis. This study also included a 60-day ingestion study using juvenile, hatchery-raised bluegill exposed to Se in five different diets. Each test group consisted of 40 fish, with a test duration of 60 days, after which total Se was measured in liver and

whole-body. Liver and whole-body data from this study were used in the EPA's derivation of a draft chronic Se criterion.

- **Casey and Siwik 2000.** The objective of this survey was to obtain seasonal data for Se in surface water and sediments of headwater streams, mainstem sites, and lakes of the Gregg, McLeod, Pembina, and Smoky River Basins, Alberta. Fish collection and analysis (primarily rainbow trout, *Oncorhynchus mykiss*) was an additional objective. Samples collected include surface water, rainbow trout muscle and egg, sediment from two lakes, and additional sediment and biota from flowing-water sites. Se concentrations ranged from 0.13 to 9.34 µg/g wet weight in muscle and from 0.02 to 28.9 µg/g wet weight in eggs. With few exceptions, the lowest Se concentrations in muscle and eggs were found at the reference sites, compared to sites downstream of mines. Wet weight concentrations were converted to dry weight using a moisture content of 80 percent (EPA 2004).

- **Coyle et al. 1993.** In a chronic toxicity study evaluating the effects of dietary and waterborne Se on the reproductive success of adult bluegills, Se concentrations were determined in adult fish, eggs, and 30-day old fry. Two-year-old bluegill were exposed for a total of 140 days to six combinations of dietary and waterborne Se, including a control. Measurements taken include effects on adult growth, mortality, gonad weight, whole-body Se tissue concentration, spawning frequency, number of eggs per spawn, hatch success of eggs, fry 30-day survival, growth, and whole-body fry Se tissue concentration.

- **Garcia-Hernandez et al. 2000.** Three fish species (n = 8 fish total) were collected in a Se survey of a brackish wetland. This study reported differential accumulation of Se observed among different organs and tissues of carp (*Cyprinus carpio*), largemouth bass (*Micropterus salmoides*), and tilapia (*Tilapia* sp.). Se concentrations were higher in kidneys than in other tissues and lower in gonad/egg tissue than other organs. An important limitation of this study is that the data reported as "whole-body" concentrations – subsequently used in the EPA's derivation of a draft chronic Se criterion – are simply the medians of all the various tissues for each species. These estimated whole-body values are considered to be unreliable and have been omitted from the present analysis.

- **Golder 2005.** Samples of water, fish, invertebrates, algae, and plant tissues were collected from various locations in the Elk River Valley, British Columbia, between 2001 and 2003 to evaluate Se concentrations. Cutthroat trout (*Oncorhynchus clarkii*) were sampled in April 2001 and 2002 prior to spawning. Se tissue concentrations ranged between 3 and 42 µg/g dry weight and were highest in the liver, followed by the ovary and muscle. Mountain whitefish (*Prosopium williamsoni*) were sampled in October 2001 prior to spawning. Se tissue concentrations ranged between 4.6 and 45.7 µg/g dry weight and varied among tissues, but did not appear to be related to

water concentrations or location. In general, mountain whitefish liver and ovary tissue Se concentrations were slightly higher than those measured for cutthroat trout. Conversely, concentrations in mountain whitefish muscle were lower than those found in the trout. Tissue concentrations did not appear to be related to age or size of cutthroat trout or mountain whitefish.

- **Hamilton et al. 2005a.** Adult razorback suckers (*Xyrauchen texanus*) were exposed to various Se concentrations in ponds and isolated river channels near Grand Junction, CO to determine effects on growth and Se accumulation over an 11-month period. During a depuration period, adults lost 1 to 2 percent of their Se burden in 32 days and 14 to 19 percent in 66 days. Se accumulated in razorback sucker above toxic thresholds reported in other studies, yet those residues were less than those reported in muscle plugs of 40 percent of wild razorback sucker caught in the Green River, UT. Mean Se concentrations (µg/g dry weight) in adult razorback muscle, muscle plug, liver, and ovary were reported.

- **Hamilton et al. 2005b.** This study reported effects on hatching and development of fertilized eggs in adult razorback sucker exposed to Se in flooded bottom land sites near Grand Junction, CO. After 9 months of exposure, fish were collected and induced to spawn. The results of the study found that egg Se concentrations were as high as 46 µg/g dry weight. The study also reported significant correlations between Se concentrations in adult muscle plugs and percent hatch, egg diameter, and deformities in embryos. No differences in viability, survival, hatch, hatchability, or mortality of deformed embryos or larvae were observed.

- **Hamilton et al. 2005c.** Razorback sucker larvae from adults exposed to Se for 9 months were used in a 30-day waterborne and dietary Se study. Waterborne Se concentrations ranged from < 1.6 to 10.7 µg/L, and dietary Se concentrations ranged from 2.7 µg/g wet weight in brine shrimp to 39 µg/g wet weight in zooplankton. Se concentrations of ≥ 4.6 µg/g wet weight in food resulted in rapid mortality of larvae from the exposed adults.

- **Hardy 2005.** This study was designed to determine the effects of various dietary concentrations of Se, added as selenomethionine, on feed intake, growth, and reproductive performance of cutthroat trout from Blackfoot River and Henry's Lake, ID. The study was divided into two portions: an acute exposure phase and a chronic exposure phase, extending to maturation and spawning between 2 and 3 years. Gamete samples were collected from wild mature fish in the Blackfoot River over 2 years to assess evidence of reproductive impacts, e.g., elevated levels of deformed fry. Adult whole-body Se concentrations ranged from 0.7 to 11.4 µg/g dry weight and egg Se concentrations ranged from 1.0 to 18.0 µg/g dry weight.

- **Hermanutz et al. 1992.** In this study, replicated outdoor experimental streams were exposed to control water, 10 µg/L, and 30 µg/L Se as sodium selenite to evaluate whether standard laboratory test results accurately predict the effects of Se on fish in freshwater ecosystems. Dosing of the streams began ~ 25 weeks before the fish were introduced. Adult fish (3 to 4 years old) were exposed for a total of 356 days. Prespawning exposure lasted 40 weeks. The authors concluded that standard laboratory tests, which expose fish only through the water pathway, have underestimated the toxicity of Se and that 10 µg/L of Se in the water of a natural ecosystem may adversely affect bluegills. Wet weight Se concentrations were converted to dry weight using a moisture content of 80 percent (EPA 2004).

- **Hermanutz et al. 1996.** Bluegill sunfish were continuously exposed to sodium selenite in duplicate outdoor experimental streams at nominal concentrations of 2.5 and 10 µg/L in water. Effects on survival, growth, and reproduction of adults and effects on the early life stage of their progeny were studied. No statistically significant effects on adults were found at either 2.5 or 10 µg/L; however, there were indications that progeny were affected by both concentrations. The authors suggest that the chronic EPA criterion (5 µg/L) might not provide adequate protection for aquatic life. Se residues in a stream that previously contained 30 µg/L appeared to affect both adults and progeny one year after the addition of Se ended. The authors concluded this was likely due to uptake of Se by bluegills from food-chain organisms. Adult fish in streams were exposed to Se for about 35 weeks before spawning in Study II and 31 weeks before spawning in Study III. This study was used in the EPA (2004) criterion derivation. Values reported in the original study were given in wet weight and have been converted to dry weight assuming 80 percent moisture content.

- **Hilton et al. 1982.** This paper describes a study in which the influence of different levels of dietary Se on the metabolism of Se in rainbow trout was investigated using ^{75}Se as a tracer. Gastric absorption of Se by the trout appeared to be efficient, and the highest tissue concentrations of Se were noted in the liver and kidney. Liver and kidney tissue also appeared to be involved in Se excretion based on high tissue concentrations and variation in half-life with Se loading. Blood did not concentrate Se and plasma was the major transport medium through the body. The biological half-life of Se in tissues decreased with increased Se loading, except in the liver, where at toxic dietary concentrations the half-life became longer, suggesting a rate-limiting metabolic transformation of Se for excretion in this organ. Whole-body concentrations were not calculated; however, with additional data (i.e., tissue masses), weighted average whole-body concentrations could be estimated.

- **Holm et al. 2005.** Spawning rainbow trout and brook trout (*Salvelinus fontinalis*) were collected and gametes were stripped prior to muscle sampling. Se concentrations in eggs were positively correlated to Se in muscle tissue for both rainbow and brook

trout based on a subsample of fish in which both tissues were analyzed. The slope of the brook trout correlation was steeper than that for rainbow trout, indicating that rainbow trout accumulated higher Se concentrations in eggs at lower muscle body burdens. Regression analysis showed no significant relationship between the weight of females and the concentration of Se in their eggs. All Se data were reported in wet weight concentrations and no moisture data were reported. The authors used an assumed moisture content of 61 percent in eggs to estimate dry weight concentrations. Dry weight egg concentrations estimated in this way have relatively high uncertainty due to the potentially large variation in the water content of fish eggs during maturation (Craik and Harvey 1984).

- **Kennedy et al. 2000.** This study was designed to determine if waterborne Se released by coal mining in the Elk River in southeastern British Columbia could be causing reproductive or teratogenic effects in wild cutthroat trout. Se concentrations were measured in adult cutthroat trout muscle, liver, ovary, and eggs. Despite elevated Se concentrations in eggs (range 8.7 to 81.3 µg/g dry weight), there was no apparent effect on fertilization, time to hatch, percent hatch, or egg, larvae, and fry deformities or mortalities. Reproductive failure and embryonic terata have been reported at much lower egg concentrations in other fish species. The authors suggest that the lack of any toxic response may be due to an evolved tolerance to higher tissue Se concentrations in a population of fish living in a seleniferous river system.

- **Minnow 2006.** This study involved prespawning monitoring of fish in selected lentic areas of the Elk River watershed, British Columbia, collection of gametes from longnose suckers (*Catostomus catostomus*) possessing high- and low-tissue Se concentrations, on-site fertilization and incubation of fish embryos to hatch, and a detailed analysis of larval deformities. Se concentrations in eggs from Elk River Upper Oxbow females ranged from 6.0 to 12.2 µg/g dry weight, whereas those from Goddard Marsh females ranged from 15.5 to 65.4 µg/g dry weight. Mortality was variable among batches and in some cases complete mortality was observed (particularly in Goddard Marsh batches that began incubation after June 2). Most larvae surviving until collection had one or more deformities regardless of the maternal collection area or egg Se content. No significant correlations were found between egg Se concentrations and embryo-larval mortalities or deformities. The authors concluded that if longnose suckers are sensitive to Se concentrations in the observed range of 15.5 to 65.4 µg/g dry weight in eggs, the effects were masked in this study by the influence of other unknown factors. A strong correlation between Se concentrations measured in expelled eggs and in the undeveloped eggs retained in ovaries of each female was presented.

- **Muscatello et al. 2006.** Eggs were obtained from northern pike (*Esox lucius*) collected from a reference site and three sites downstream of a uranium milling operation. Embryos were incubated following a two-way analysis of variance design

that allowed discrimination between effects due to maternal transfer to eggs and effects due to site water exposure in the developing embryos. The authors reported a significant increase in the frequency of individual deformities and edema in fry originating from high and medium exposure site females (mean Se concentrations of 48 and 31 µg/g egg dry weight, and 38 and 16 µg/g muscle dry weight, respectively) compared to reference site females.

- **Rudolph et al. 2008.** Eggs from 12 cutthroat trout from an exposed site (Clode Pond) and 16 from a reference site (O'Rourke Lake) in southeastern British Columbia were collected and reared in the laboratory. Egg Se concentrations ranged from 12.3 to 16.7 and 11.8 to 140 µg/g dry weight from fish collected at the reference and exposed sites, respectively. Eggs with Se concentrations > 86.3 µg/g dry weight were not successfully fertilized or were nonviable at fertilization, whereas eggs with concentrations of > 46.8 and < 75.4 µg/g dry weight were fertilized (96 percent reached the eyed stage) but did not produce viable fry. A significant positive relationship between egg Se concentration and alevin mortality was observed. Deformities were analyzed in surviving fry that developed from eggs with Se concentrations between 11.8 and 20.6 µg/g dry weight. No relationship between Se concentration in eggs and deformities or edema was found in this range, leading the authors to suggest that the no-effect threshold for deformities or edema is > 20.6 µg/g dry weight.

- **Waddell and May 1995.** A single muscle plug was collected from each of 25 live razorback suckers and analyzed for Se. Eleven fish exhibited Se concentrations exceeding 8 µg/g dry weight. Se concentrations in eggs and milt were significantly correlated with Se in muscle plugs. Muscle plugs (< 50 mg) and muscle tissue (20 g) were collected from dorsal, anterior, and posterior areas of common carp, flannelmouth sucker (*Catostomus latipinnis*), and an archived razorback sucker and analyzed for Se. The authors report that Se concentrations in muscle plugs were significantly correlated with Se concentrations in muscle tissue from the same location and fish (r = 0.97). Coefficients of variation for Se concentrations were < 6.5 percent for muscle tissue, but ranged from 1.5 to 32.4 percent for muscle plugs. Increased variation in muscle plugs was attributed to lower Se concentrations found in the anterior muscle plugs of flannelmouth suckers. The authors concluded that mean Se concentrations in muscle plugs and tissue from dorsal and posterior areas of the razorback sucker and other fish species may provide an accurate assessment of Se concentrations in adjacent muscle tissue.

2.3 Summary of Reviewed Data

The data obtained for this review are outlined in Table 1 (by study) and in Table 2 (by tissue-tissue relationship). In total, the 20 different studies contained the following data:

- **Whole-body.** Six studies provided measured whole-body concentrations for fish to correlate to other tissue types;

- **Muscle.** Thirteen studies reported muscle concentrations, of which three reported concentrations in wet weight, one reported only one mean muscle concentration for all females, and one reported only median muscle concentrations;

- **Muscle Plug.** Two studies provided muscle plug data;

- **Liver.** Eleven studies reported liver concentrations; however, four of these reported data as wet weights, median values, or provided only a single mean value;

- **Ovary.** Ten studies reported ovary concentrations; of these, two studies presented ovary data in wet weight, one presented ovary concentrations as the medians of gonads/eggs (n = 2), and one presented undeveloped eggs retained in the ovary after ripe eggs were expelled;

- **Egg.** Eleven studies reported egg concentrations; as with ovary data above, two studies presented egg data in wet weight and one presented concentrations as the medians of gonads/eggs (n = 2), or just eggs (n = 1); and

- **Larvae.** Only one study provided useful larval concentration data. The majority of larval Se data reported in the literature are for larvae feeding exogenously on a Se-contaminated diet, and are not expected to show a consistent or generalizable relationship with adult or egg Se concentrations.

Table 1: Summary of evaluated studies and available tissue-effects data.

Study	Tissue Se Concentrations							Biological Effects		
	Whole-body	Muscle	Muscle Plug	Liver	Ovary	Egg	Larvae	Egg Mortality	Larval Mortality	Larval Terata
Bryson et al. 1984		○[d]		○	○				●	
Bryson et al. 1985a		●		●	●			●	●	
Bryson et al. 1985b	○[a]			●				●	●	
Casey and Siwik 2000		○[e]				○[j]				
Coyle et al. 1993	●				●	●		●	●	
Garcia-Hernandez et al. 2000	×[b]	○[f]		○[f]	○[h]	○[h]				
Golder 2005		●		●	●					
Hamilton et al. 2005 a,b,c		●	●	●	○[i]	●	●			
Hardy 2005	●					●		●		●
Hermanutz et al. 1992	○[c]	○[e]		○[e]	○[e]			●	●	●
Hermanutz et al. 1996	●	●		●	●			●	●	●
Hilton et al. 1982				×[g]						
Holm et al. 2005		○[e]				○[k]		●		●
Kennedy et al. 2000		●		●	●	●		●		
Minnow 2006					●	●		●	●	●
Muscatello et al. 2006		●		●		●		●	●	●
Rudolph et al. 2008		●				●		●	○[l]	○[l]
Waddell and May 1995		●	●			●				

× Data from this study not analyzed.
○ Data available with limitations and/or uncertainty (e.g., small sample size, wet weight, not tissue-specific), however still included (often with conversion/uncertainty) in analysis.
● Data readily available and utilized in analysis.

a Hatchery-raised juveniles fed different Se diets.
b Whole-body concentrations (n=2) extrapolated from the medians of the different tissues for each species (essentially, median liver value presented as whole-body).
c Se residues (n=2) presented as µg/g wet weight and converted to dry weight assuming 80 percent moisture content (EPA 2004).
d Only one mean muscle value reported.
e Reported as wet weight and converted using 80 percent moisture content (EPA 2004).
f Only median values reported.
g For the purpose of this review, tissues measured in this study were not analyzed herein (*i.e.,* brain, gill, carcass, etc.). This study may be analyzed further if weights of various tissues were available to determine weighted averages and thereby calculate whole-body concentrations (from [carcass] + [all other tissues]). Note: liver concentrations reported.
h n=3 reported median values, 2 of which are reported as gonad/egg concentrations, and only one reported as median egg concentration.
i Mean values reported for gonads, therefore this may include concentrations of Se in testes.
j Egg concentrations reported in wet weight and as no moisture content reported, converted to dry weight using 80 percent as per EPA (2004).
k Reported as wet weight, however assumed species specific moisture content of 61 percent thereby increasing accuracy of conversion to dry weight.
l Data available via extraction from study figures.

Table 2: Total n (paired) and number of species for tissue-tissue correlations.

Total n (Paired)

Adult whole-body[a]	1	-	1	1	2	-
20	Skeletal muscle	3	8	6	8	1
-	30	Muscle plug	1	1	1	1
26	176[b]	8	Liver	6	5	1
25	110[c]	9	109[c]	Ovary	4	1
16	142	7	29	48	Egg	1
-	3[d]	4[d]	2[d]	3[d]	4[d]	Larvae

Number of Species

a Note that for whole-body concentrations, data from Garcia-Hernandez et al. (2000 – median liver concentrations presented as whole-body) and Hilton et al. (1982 – carcass concentrations not representative of whole-body) have been excluded from analysis.

b Includes Golder (2005), n=114 (grey literature, raw data available in Appendix 1).

c Includes Golder (2005), n=58 (grey literature, raw data available in Appendix 1).

d Whole-body reference larval concentrations were measured at both 7d and 30d. (Hamilton et al. 2005c).

3.0 Tissue-Tissue Relationships

3.1 Tissue-Tissue Se Data Analysis

Tissue Se data were extracted from the studies summarized above. Study-specific moisture content was used to convert reported wet weight Se concentration data into dry weight where available. In studies where moisture content was not reported, a default moisture content of 80 percent (EPA 2004) was applied. Most data were reported as Se concentration values in various tissues within individual fish; however, data reported as means or medians of multiple fish for a particular tissue were also retained for analysis. The data reported by Garcia-Hernandez et al. (2000) as "whole-body concentrations" were determined to be medians of all sampled tissues for a species; these data were excluded from the analysis.

All Se concentration data were $\log_{10}$-transformed to linearize tissue-tissue relationships and stabilize variance for regression analysis. Regression analyses were performed for each tissue-tissue pairwise combination, both for individual species and for all species combined. Analysis of covariance (ANCOVA) was used to compare tissue-tissue log Se relationships among species where data were sufficient. Regression equations, summarized below, are presented in Table 3.

3.2 Summary of Tissue-Tissue Results

- **Whole-body vs. Muscle, Liver, Ovary, and Egg.** Six of the 20 studies reviewed present whole-body concentrations, but only one of these studies presents data for a species other than bluegill sunfish. In general, whole-body concentrations in bluegill sunfish were strongly correlated with other tissues (Table 3). Bluegill sunfish whole-body concentrations correlated with muscle (Hermanutz et al. 1992, 1996), liver (Hermanutz et al. 1992, 1996; Bryson et al. 1985b), ovary (Hermanutz et al. 1992, 1996; Coyle et al. 1993) and egg (Coyle et al. 1993). Cutthroat trout whole-body concentrations also correlated with egg (Hardy 2005) but followed a significantly different relationship to that exhibited by the bluegill sunfish data (Figure 2; ANCOVA main effect of species $F_{1,13} = 5.2$, $p = 0.04$; interaction $F_{1,12} = 0.4$, $p = 0.26$). The whole-body-ovary relationship was similar in form to the whole-body-egg relationship (Table 3), although this may be a function of the timing of collection of the ovary samples in these studies (i.e., ovaries were generally collected around the time of egg maturity); it is not known whether this relationship is representative of the non-spawning portion of the year.

Table 3: Log-log regression equations for all pairwise comparisons of tissue Se concentrations.

Log [Y-Value]	Log [X-Value]	Slope	Y-intercept	r^2	n	Species
Whole-body	Muscle	1.06	-0.03	0.96	20	bluegill sunfish
Muscle Plug	Muscle	0.81	0.10	0.94	12	razorback sucker
		0.93	0.07	0.97	9	common carp
		1.65	-0.53	0.36	9	flannelmouth sucker
		0.88	0.06	0.85	30	all species
Liver	Muscle Plug	1.18	0.07	0.89	8	razorback sucker
Ovary	Muscle Plug	1.38	-0.14	0.80	9	razorback sucker
Egg	Muscle Plug	0.78	0.28	0.33	7	razorback sucker
Larvae	Muscle Plug	1.55	-0.18	0.98	4	razorback sucker
Whole-body	Liver	1.07	-0.65	0.80	26	bluegill sunfish
Whole-body	Ovary	0.73	0.06	0.76	25	bluegill sunfish
Whole-body	Egg	0.90	-0.31	0.99	6	bluegill sunfish
		0.75	0.04	0.59	10	cutthroat trout
		0.77	-0.05	0.71	16	all species
Muscle	Liver	1.05	-0.49	0.70	27	bluegill sunfish
		0.50	0.19	0.32	89	cutthroat trout
		0.18	0.47	0.10	40	mountain whitefish
		0.78	0.13	0.94	8	razorback sucker
		0.57	0.61	0.88	9	northern pike
		-	-	-	1	carp
		-	-	-	1	largemouth bass
		-	-	-	1	bull trout
		0.51	0.19	0.25	176	all data
Muscle	Ovary	0.79	0.12	0.72	25	bluegill sunfish
		0.56	0.24	0.28	47	cutthroat trout
		0.80	-0.46	0.42	27	mountain whitefish
		0.57	0.40	0.76	9	razorback sucker
		-	-	-	1	carp
		-	-	-	1	largemouth bass
		-0.0023	1.04	0.11	110	all species
Muscle	Egg	0.65	-0.07	0.68	72	rainbow trout
		0.77	0.09	0.85	38	cutthroat trout
		1.085	-0.07	0.87	17	brook trout
		0.44	0.78	0.88	9	northern pike
		0.62	0.31	0.76	3	razorback sucker
		-	-	-	1	carp
		-	-	-	1	largemouth bass
		-	-	-	1	tilapia
		0.59	0.15	0.54	142	all species
Muscle	Larvae	0.80	0.10	0.91	3	razorback sucker

Log [Y-Value]	Log [X-Value]	Slope	Y-intercept	r^2	n	Species
Ovary	Liver	1.20	-0.62	0.84	25	bluegill sunfish
		0.43	0.65	0.33	47	cutthroat trout
		0.34	1.01	0.26	27	mountain whitefish
		1.10	-0.16	0.85	8	razorback sucker
		-	-	-	1	carp
		-	-	-	1	largemouth bass
		0.89	0.05	0.51	109	all species
Liver	Egg	0.73	0.58	0.49	16	cutthroat trout
		0.74	0.34	0.88	9	northern pike
		1.10	-0.02	1.00	2	razorback sucker
		-	-	-	1	carp
		-	-	-	1	largemouth bass
		0.80	0.42	0.74	29	all species
Liver	Larvae	1.14	-0.11	1.00	2	razorback sucker
Ovary	Larvae	1.07	-0.08	0.99	3	razorback sucker
Egg	Larvae	1.09	0.09	1.00	4	razorback sucker
Ovary	Egg	0.97	0.06	1.00	6	bluegill sunfish
		0.57	0.61	0.75	16	cutthroat trout
		0.87	0.15	0.91	3	razorback sucker
		0.93	0.08	0.98	23	longnose sucker
		0.87	0.18	0.92	48	all species

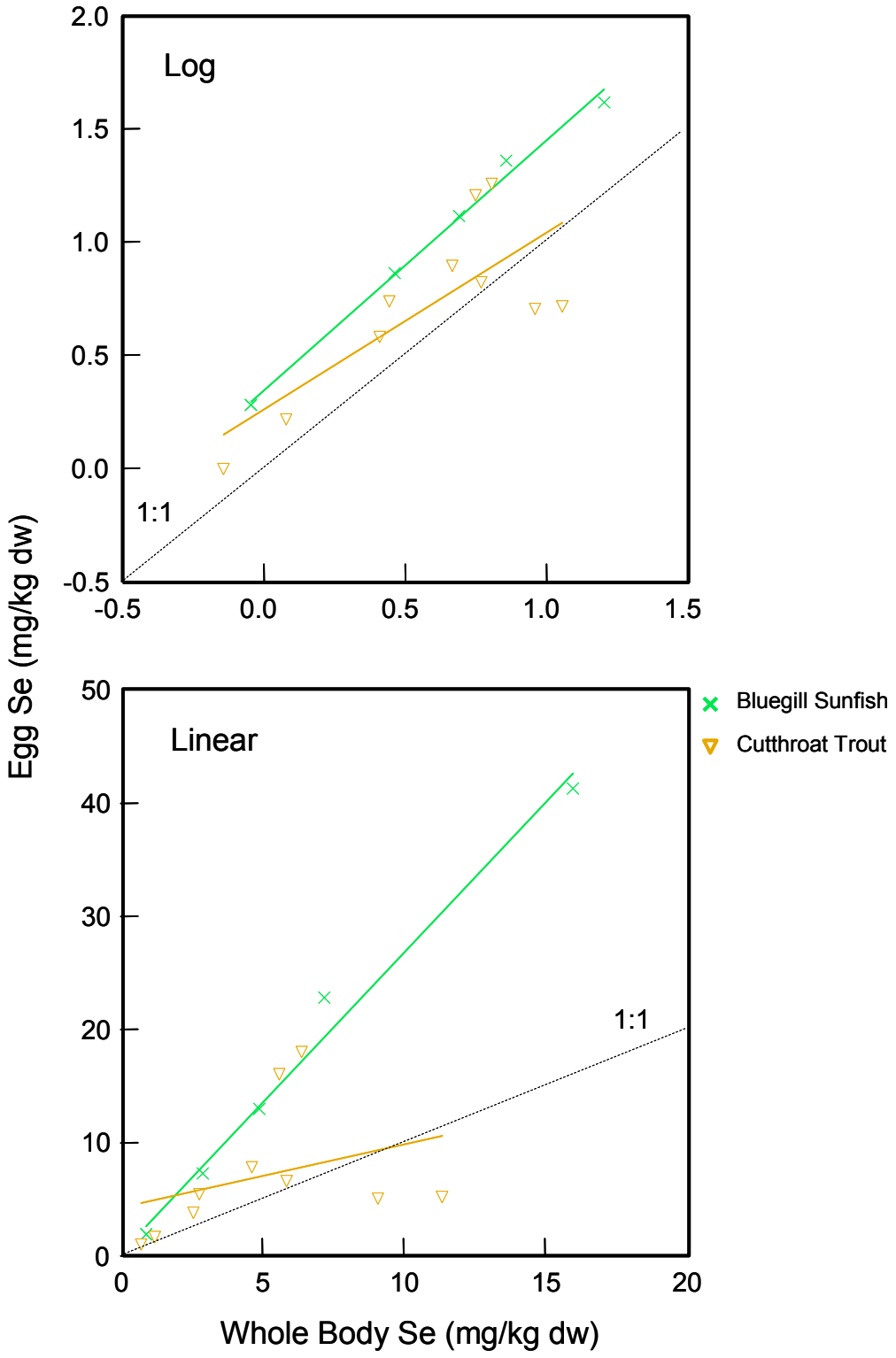

Figure 2: Scatterplot of Se concentrations in whole-body and egg.

- **Muscle Plug vs. Muscle.** Two studies reported a correlation of muscle-to-muscle plug Se for a total of three species (razorback sucker, flannelmouth sucker, and common carp). Waddell and May (1995) reported a significant correlation between Se concentrations in muscle plugs and muscle tissue from the same location and fish for three species, excluding anterior muscle plug sampling sites. Hamilton et al. (2005a) observed similar results with razorback suckers. Table 3 reports a significant relationship for combined data from all three species ($r^2 = 0.85$); all species exhibited a similar relationship (ANCOVA main effect of species $F_{2, 26} = 3.0$, $p = 0.68$; interaction $F_{2, 24} = 1.3$, $p = 0.28$).

- **Muscle Plug vs. Egg.** Data combined from two razorback sucker studies (total n = 7) resulted in an r^2 of 0.33, a value much lower than reported for each individual study (Hamilton et al. 2005a,b, $r^2 = 0.89$; Waddell and May 1995, $r^2 = 0.99$); this indicates an apparent inter-study difference in tissue-tissue relationships for razorback sucker.

- **Muscle Plug vs. Liver, Ovary, and Larva.** Hamilton et al. (2005a) reported a correlation between muscle plug and liver, ovary, and larval Se concentrations. These correlations were generally strong (Table 3), but are based on relatively small sample sizes.

- **Muscle vs. Liver.** Data from nine studies (paired n = 178) were used to assess the potential relationship between muscle and liver data. The data set included eight fish species, three of which were represented by n = 1 data. Muscle-liver correlations were significant but varied in strength (r^2 values ranged from 0.10 to 0.94) and were significantly different among species (ANCOVA main effect of species $F_{6,165} = 6.3$, $p < 0.001$; interaction $F_{5,165} = 4.5$, $p = 0.001$).

- **Muscle vs. Egg.** Data from eight studies (paired n = 142) were used to assess potential correlation between muscle and egg data. The data set included eight fish species, three of which were represented by n = 1 data. Muscle-egg correlations were significant and generally strong, with $r^2 > 0.7$ for the five species with $n > 1$. Muscle-egg correlations varied significantly among species (Figure 3; ANCOVA main effect of species $F_{5, 129} = 5.0$, $p < 0.001$; interaction $F_{6,129} = 3.4$, $p = 0.004$). Muscle-egg correlations also showed significant variation within a species for the rainbow trout data of Casey and Siwik (2000); when unripe eggs were removed from the data set, the r^2 increased from 0.66 to 0.79.

- **Muscle vs. Ovary.** Data from eight studies (paired n = 110) were used to assess potential correlation of muscle-to-ovary data. The data set included six fish species, two of which were represented by n = 1 data. Muscle-ovary correlations varied in strength among species and varied significantly among species (Figure 4; ANCOVA main effect of species $F_{3,102} = 9.6$, $p < 0.001$; interaction $F_{5,102} = 4.2$, $p = 0.002$).

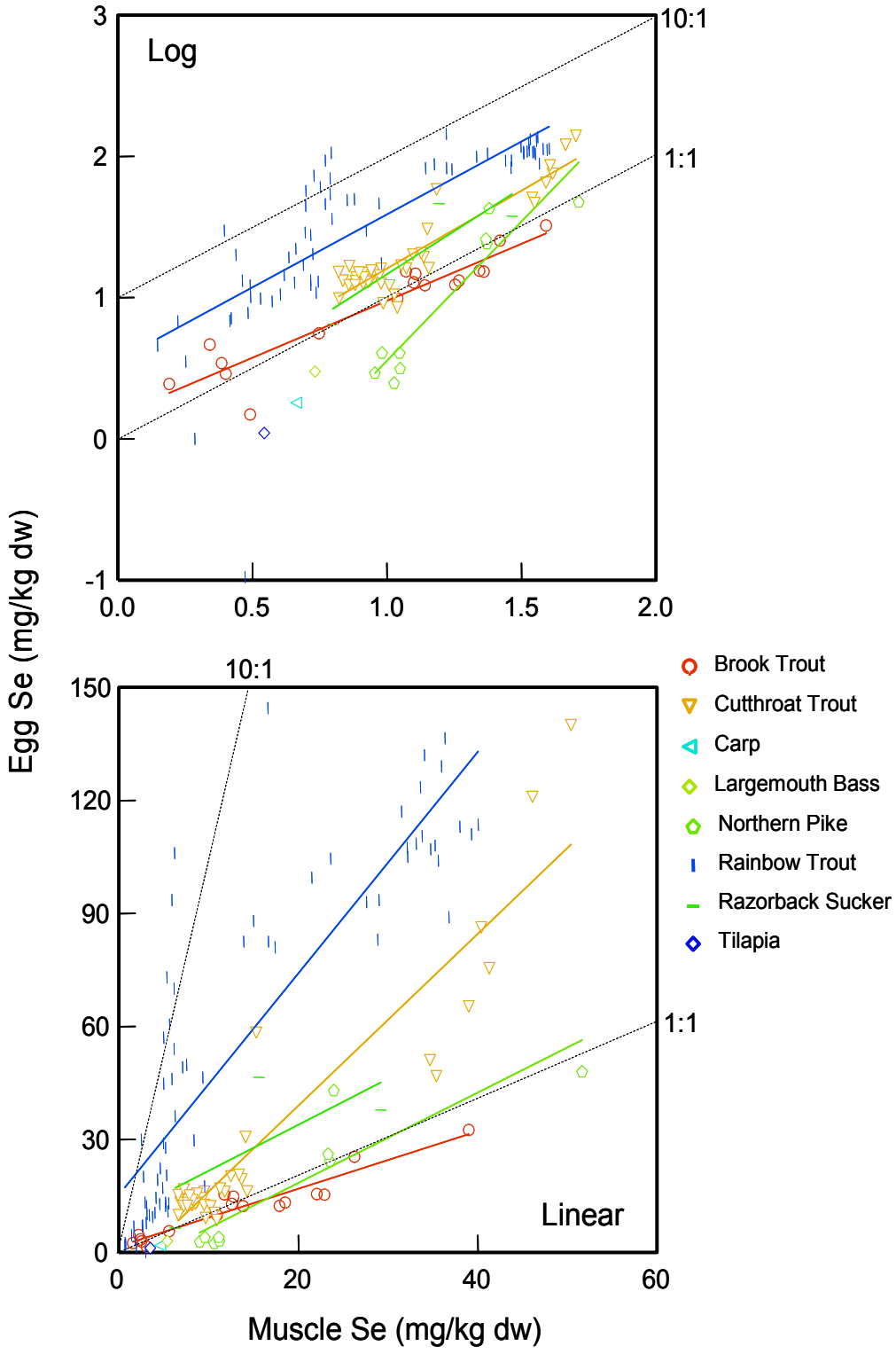

Figure 3: Scatterplot of Se concentrations in muscle and egg.

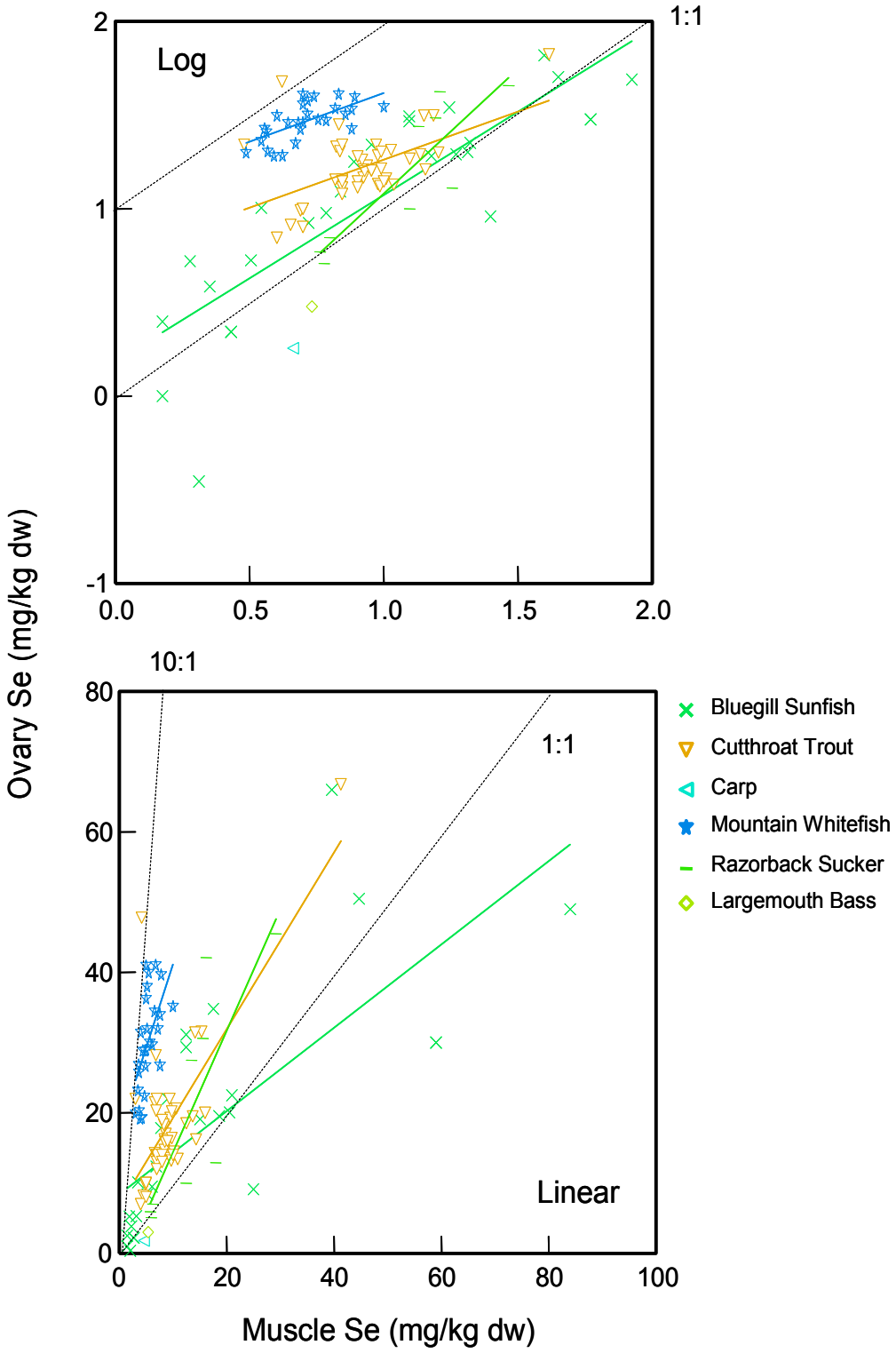

Figure 4: Scatterplot of Se concentrations in muscle and ovary.

- **Liver vs. Egg.** Only two studies provided n > 2 for correlation of liver to egg Se concentrations. Cutthroat trout data presented by Kennedy et al. (2000) revealed a significant but weak relationship ($r^2 = 0.49$), whereas northern pike data from Muscatello et al. (2006) revealed a much stronger relationship ($r^2 = 0.88$). Garcia-Hernandez et al. (2000) and Hamilton et al. (2005b) provided limited data ($n \leq 2$) for common carp, largemouth bass, and razorback sucker. Liver-egg correlations varied significantly among species (Figure 4; ANCOVA interaction $F_{4, 21} = 3.0$, $p = 0.041$).

- **Ovary vs. Egg.** Paired ovary and egg Se data were available for four species (bluegill sunfish n = 6; cutthroat trout n = 16; razorback sucker n = 3; longnose sucker n = 23). The ovary-egg relationship was strong for all species ($r^2 > 0.75$). Ovary-egg correlations did not vary significantly among species (Figure 6; ANCOVA main effect of species $F_{5, 43} = 2.3$, $p = 0.059$; interaction $F_{4, 40} = 0.9$, $p = 0.50$).

3.3 Summary of Tissue-Tissue Relationships

Tissue-tissue Se correlations were generally strong for a single species within a single study, although the rainbow trout data of Casey and Siwik (2000) indicate that even intra-study variability can be large. Variation among studies for a single species can also be large, as demonstrated by the reduction in r^2 observed when the razorback sucker muscle plug-egg data of Hamilton et al. (2005a,b) are combined with those of Waddell and May (1995).

The greatest variation in tissue-tissue relationships in our data set was among species. Analysis of covariance revealed statistically significant differences among species for most tissue-tissue combinations. Inspection of the data (e.g., Figure 3) shows these differences among species can be large. In general, tissue-tissue combinations for which relatively more data were available (e.g., muscle-egg, muscle-ovary) showed the greatest intraspecific and interspecific variability. Variability is likely just as large for the less data-rich tissue-tissue combinations (e.g., whole-body-egg).

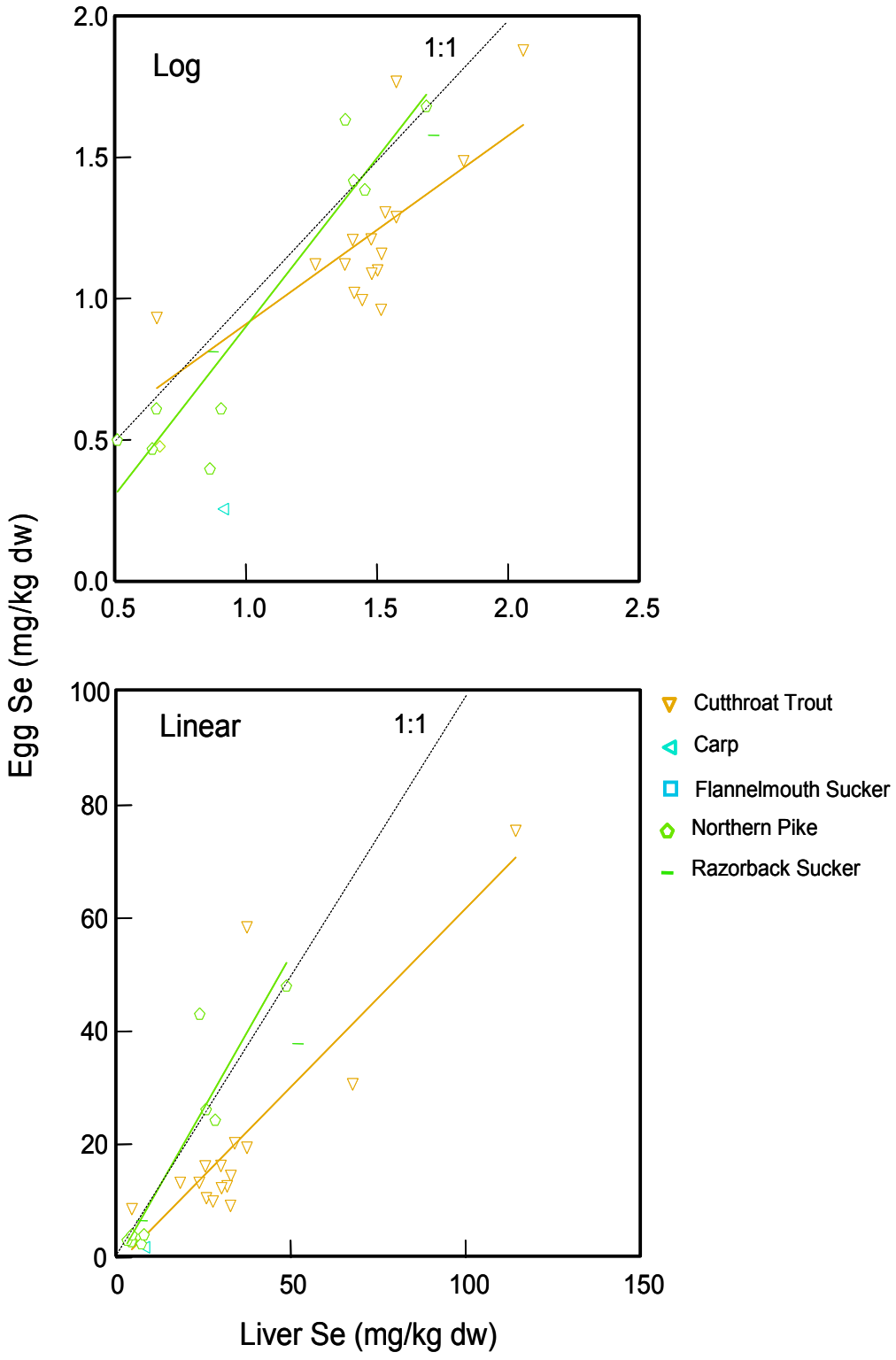

Figure 5: Scatterplot of Se concentrations in liver and egg.

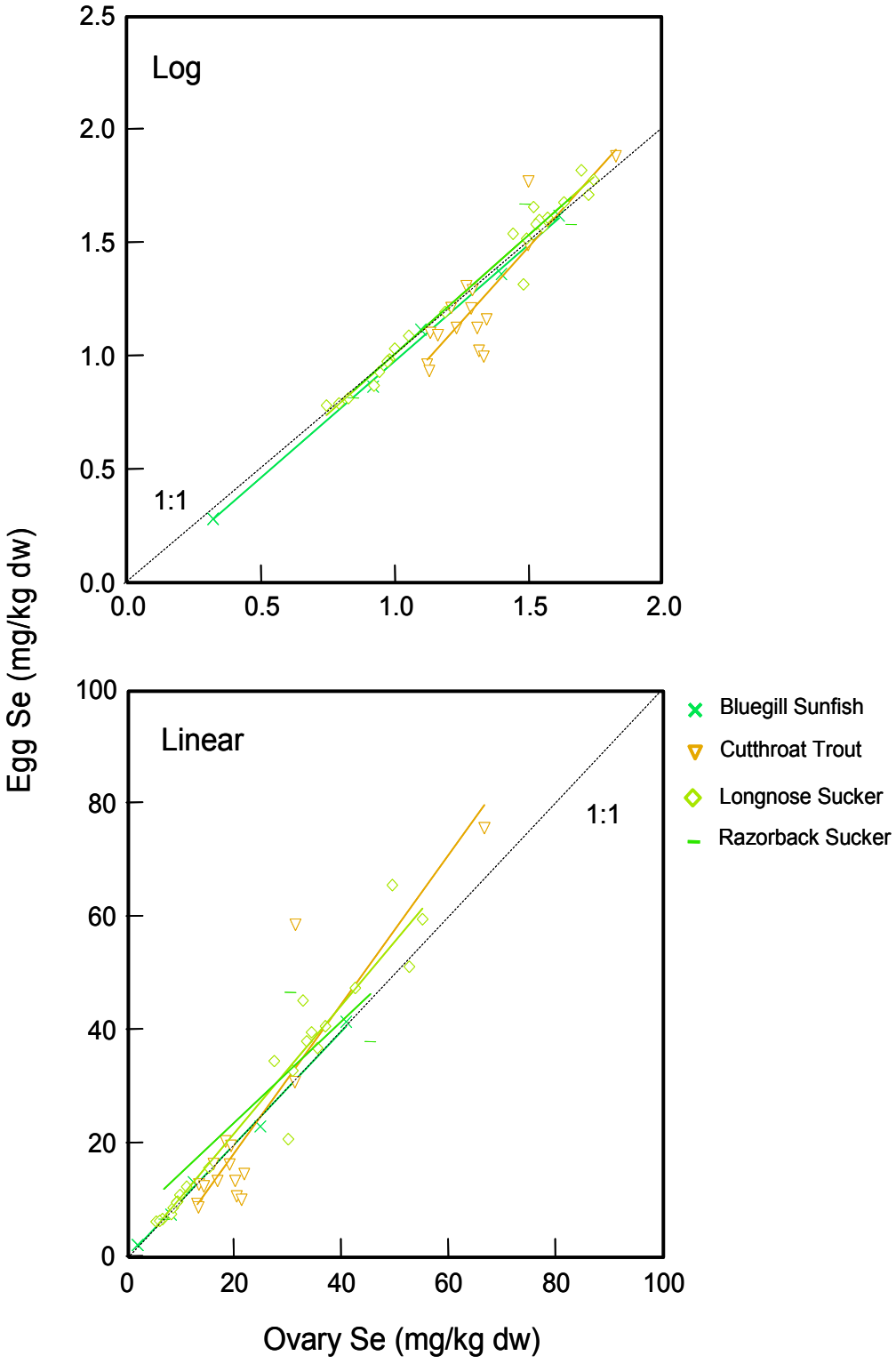

Figure 6: Scatterplot of Se concentrations in ovary and egg.

4.0 Biological Effects

4.1 Biological Effects Results

Studies providing Se concentration data for eggs, at least one adult tissue, and/or at least one biological effect (percent hatch, percent mortality/survival, percent swim-up, or percent terata) are listed in Table 4 and briefly summarized below.

- **Bryson et al. 1984.** A 28-day embryo/larval study reported 100 percent mortality of swim-up larvae obtained from females from Hyco. An ingestion study reported 97.3 percent mortality in larvae fed zooplankton containing 45 µg/g dry weight Se (from Hyco) compared to 23.7 percent mortality in larvae fed zooplankton containing 1.9 µg/g dry weight Se (from the control lake). No differences in survival were observed during the 30 days prior to this study, when the larvae were being raised in different concentrations of ash pond effluent. These data were not included in the EPA criterion derivation.

- **Bryson et al. 1985a.** A 28-day embryo/larval study reported 100 percent mortality of swim-up larvae obtained from females from Hyco; these females had 30, 33, and 59 µg/g dry weight Se in ovaries, liver, and muscle, respectively. Percent swim-up of larvae of parents collected from the "non-affected" Hyco site averaged 93 percent, compared to 12 percent for larvae of parents collected from the "affected" Hyco site. Some groups showed decreasing hatch or swim-up survival with increasing effluent concentration. EPA (2004) used muscle concentrations in Hyco females to calculate a chronic value.

- **Bryson et al. 1985b.** An ingestion study reported no effect on juvenile growth in any treatment. A source and exposure embryo-larval study using Hyco fish reported percent hatch and percent swim-up values of > 83 and > 83, respectively, suggesting no effect on these endpoints. EPA (2004) calculated a chronic value from liver concentrations. This study was used in the EPA criterion derivation.

- **Coyle et al. 1993.** Adults with whole-body tissue concentrations of 16 µg/g dry weight Se produced eggs with normal hatchability rates. However, survival of fry (at 5 days post-hatch) from these exposed adults was significantly affected; these data were used to calculate an EC20 for fry survival based on egg and whole-body Se tissue concentrations in adults at 60 days. A marked decrease in fry survival was associated with the highest measured egg and whole-body Se concentrations in exposed adults. A residue-response relationship was not evident between tissue Se and ELS effects below the highest tissue/egg concentration.

Table 4: Review of studies providing ELS biological effects and Se measured in egg or adult tissue.

Study	Species and Sample Size	Whole-body	Muscle	Muscle Plug	Liver	Ovary	Egg	Effect Measured
Bryson et al. 1984	bluegill sunfish (n = 1)		n = 1		n = 1	n = 1		M/S
Bryson et al. 1985a	bluegill sunfish (n = 5)		n = 5		n = 5	n = 5		H; SU; M/S
Bryson et al. 1985b	bluegill sunfish (n = 2)				n = 2			H; SU; M/S; T
Coyle et al. 1993	bluegill sunfish (n = 6)	n = 6				n = 6	n = 6	H; M/S
Hamilton et al. 2005b	razorback sucker (n = 2–4)		n = 3	n = 4	n = 2	n = 3	n = 4	H; M/S; T
Hardy 2005	cutthroat trout (n = 6)	n = 6					n = 6	H; M/S; T
Hermanutz et al. 1992	bluegill sunfish (n = 2-3)		n = 3		n = 3	n = 2		H; M/S; T
Hermanutz et al. 1996	bluegill sunfish (n = 8)	n = 8	n = 8		n = 8	n = 8		H; M/S; T
Holm et al. 2005	brook trout (n = 17)		n = 17				n = 17	M/S; T
	rainbow trout (n = 7)		n = 7				n = 7	M/S; T
Kennedy et al. 2000	cutthroat trout (n = 16)		n = 16		n = 16	n = 16	n = 16	H; M/S; T
Minnow 2006	longnose sucker						n = 23	H; M/S; T
Muscatello et al. 2006	northern pike (n = 9)		n = 9		n = 9		n = 9	H; M/S; T
Rudolph et al. 2008	cutthroat trout (n = 22)		n = 22				n = 22	H; M/S; T
Total (13 studies)	6 species (n = 106)	n = 20	n = 91	n = 4	n = 46	n = 41	n = 87	H; M/S; T

H % hatch.
SU % swim-up.
M/S % mortality and/or % survival.
T % terata (includes at least one of: edema, skeletal deformities, craniofacial deformities, and/or fin deformities).

- **Hamilton et al. 2005b.** A 9-day study of eggs from four different sources reported no differences in viability, survival, hatching success, or mortality of deformed embryos or larvae. The authors reported an incongruity in Se concentrations in eggs from Adobe Creek (46.5 µg/g) and North Pond (37.8 µg/g) because North Pond had higher Se concentrations in water, sediment, and fish muscle plugs than Adobe Creek. This study reports 12 to 26 percent deformities in embryo larvae from Adobe Creek adults and 20 to 27 percent in larvae from North Pond adults. The authors suggest this may be the result of substantially higher Se concentrations in zooplankton during vitellogenesis in the fall prior to spawning. Furthermore, the authors reported 10 to 18 percent deformities in embryo larvae from Horsethief Pond, indicating razorback sucker are sensitive to Se exposure; adults had Se concentrations of 5.8 to 6.3 µg/g in muscle. Inbreeding effects may also explain the high number of deformities in this study. Overall, Se concentrations were substantially elevated in eggs from adults from Adobe Creek and North Pond. Mean egg concentrations were reported for adults from each of four sites; a qualitative analysis of these data indicates that a relationship between egg Se concentrations and biological effects could not be established based on the n = 3 paired data available.

- **Hardy 2005.** Percent survival to the eyed stage varied among dietary groups. The single clutch produced by a female fed the second-highest dietary Se concentration (8 µg/g dry weight diet) had the highest percent hatch value (n = 1). Clutches from fish fed the control diet had 85 percent hatch, similar to the egg lots from adults fed the highest-Se diet. Percent hatch of eggs from fish fed lower-Se diets had the lowest percent hatch values. No Se dose-related pattern in percent egg hatch was evident. Fish fed intermediate levels of Se had the highest deformity rate. A qualitative review of egg Se concentrations (Table 12 in Hardy [2005]) and biological effects (Table 10 in Hardy [2005]) suggests no consistent relationship between egg concentrations and biological effects.

- **Hermanutz et al. 1992.** Reduced growth of adults was observed in the first 258 days. Significant mortality was observed in the 30 µg/L Se treatment. No significant effect on spawning activity was observed. Significant increases in deformities in both treatments included edema, lordosis, and internal hemorrhaging. Mortality rates in the first 4 days post-hatch were higher than control in the 30 µg/L Se treatment. Most larvae with lordosis and hemorrhaging failed to survive more than 1 day. Larvae with edema survived the first 5 days but failed to develop. The authors concluded that exposures of adults for 40 weeks prior to spawning resulted in reduced embryo and larval survival and produced larvae with higher incidences of edema, lordosis and internal hemorrhaging.

- **Hermanutz et al. 1996.** The authors reported a whole-body NOAEC (5.55 µg/g dry weight) and LOAEC (26.46 µg/g dry weight) based on percent larval survival and percent larvae exhibiting edema.

- **Holm et al. 2005.** A significant relationship between egg Se concentrations and craniofacial deformities, skeletal deformities, and edema was observed in rainbow trout. An association between the occurrence of deformities and egg Se concentrations was not evident for brook trout.

- **Kennedy et al. 2000.** Neither the percent fertilization nor percent hatch of eggs differed significantly between females with from reference and Se-exposed areas. Characteristic deformities associated with excessive Se in eggs were not observed. Despite egg concentrations up to 81 µg/g dry weight Se, the overall frequency of deformities and mortalities in the exposed population was < 1 percent (0.04 percent pre-swim-up deformity) and 3.3 percent, respectively. Some clutches of eggs suffered 100 percent mortality; however this was not significantly correlated with egg Se content. More than 80 percent of eggs from females from the exposed site had Se concentrations greater than the apparent critical level of 10 µg/g; the authors suggest this lack of response in this population of cutthroat trout indicates a tolerance at the cellular level.

- **Minnow 2006.** The majority of longnose sucker larvae surviving until collection had one or more deformities regardless of the maternal collection area or egg Se content. Egg Se concentrations ranged from 15.5 to 65.4 µg/g dry weight. No significant correlations were found between egg Se concentrations and embryo-larval mortalities or deformities.

- **Muscatello et al. 2006.** Mean egg diameter, fertilization success, cumulative embryo mortality, condition factor of fry, degree-days to 50 percent eyed embryos, 50 percent hatch, and 50 percent swim-up did not differ between reference or exposed sites. However, a significant increase in incidence of edema, skeletal deformities, craniofacial deformities, and fin deformities did occur in fry of parents collected at the medium exposure site. Deformities were also greater in fry of parents collected from the high exposure site (n = 1). Whole-body EC20s for total deformities were derived based on EC20s for eggs and muscle. The authors reported significant linear relationships between Se concentrations in northern pike eggs and percentage of fry exhibiting edema, skeletal, craniofacial and fin deformities.

- **Rudolph et al. 2008.** The authors presented larval effects data, including percent skeletal, craniofacial, and finfold deformities, percent edema, and alevin mortality. Cutthroat trout eggs with Se concentrations > 86.3 µg/g dry weight were not successfully fertilized or were nonviable at fertilization, whereas eggs with concentrations between 46.8 and 75.4 µg/g dry weight were fertilized but did not

produce viable fry. A significant positive relationship between egg Se concentration and alevin mortality was observed. Deformities were analyzed in surviving fry which developed from eggs with Se concentrations between 11.8 and 20.6 µg/g dry weight. No relationship between Se concentration in eggs and deformities or edema was found in this range, leading the authors to conclude that the no-effect threshold for fry deformity is > 20.6 µg/g dry weight.

4.2 Summary of Residue-Response Relationships

There is a growing body of evidence that egg Se concentrations are predictive of ELS effects. Relationships were observed between egg Se and ELS effects in studies with cutthroat trout (Rudolph et al. 2008), bluegill sunfish (Coyle et al. 1993), rainbow trout (Holm et al. 2005) and northern pike (Muscatello et al. 2006). However, similar relationships could not be developed for brook trout (Holm et al. 2005), longnose sucker (Minnow 2006) or razorback sucker (Hamilton et al. 2005b). Relationships were rarely observed between adult tissue Se and ELS effects. At this time, there is no compelling evidence that adult tissue Se is consistently predictive of ELS effects.

5.0 Conclusions and Recommendations

Based on the analyses presented above, the following recommendations are made:

1. It is not recommended to use generic tissue-tissue relationships. Regression equations for estimating Se concentrations in one tissue from measurements in another (as in EPA 2004) must be species-specific. Interspecific variability in tissue-tissue relationships, both within and among studies, further suggests that site-specific relationships should be developed whenever possible.

2. After a species-specific tissue-tissue relationship has been developed, any of the candidate tissues should be a reliable surrogate for ELS Se exposure. Within a species and/or study, tissue-tissue relationships are generally strong. With appropriate recognition of the predictive power of the derived relationship (i.e., recognizing the uncertainty in an estimated value that arises from residual scatter around the regression line), it should be possible to obtain reliable estimates of ELS Se exposure from concentrations measured in adult tissues.

3. If no species-specific tissue-tissue relationship is available, it is not possible to use adult tissue Se to estimate potential ELS exposure.

4. Egg Se concentration is recommended as the most useful basis for a Se tissue guideline or criterion. This conclusion is supported by basic toxicological principles (i.e., the best measure of exposure for ELS effects is ELS Se), the observed residue-response relationships (i.e., residue-response relationships are more often apparent based on egg Se than on any adult tissue), and the observed variability in tissue-tissue relationships. As noted above, several adult tissues can also be useful for monitoring, provided a species-specific tissue-egg relationship is developed.

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APPENDIX I

Raw Data

STUDY	SPECIES	ALL DATA (ug/g dw)							
		Sample ID	[Whole Body]	[Muscle]	[Muscle Plug]	[Liver]	[Ovary]	[Egg]	[Larvae]
Bryson et al. 1984	bluegill sunfish	1		84		130	49		
Bryson et al. 1985a (part 1)	bluegill sunfish	1		59		33	30		
		2		2.7		4.4	2.2		
Bryson et al. 1985a (part 2)	bluegill sunfish	1		25		26	9.1		
Bryson et al. 1985b	bluegill sunfish	0	0.45			3.9			
		1	2.16			9.23			
		2	3.74			16.33			
		3	2.46			10.85			
		4	1.21			11			
		5	2.35			9.1			
Casey and Siwik 2000	rainbow trout	1		21.5				99.5	
		2		17.4				81	
		3		13.9				82.5	
		4		23.6				104.5	
		5		5.2				27.75	
		6		5.3				20.4	
		7		15				88	
		8		5				57	
		9		5.35				73	
		10		6.2				106	
		11		16.65				82.5	
		12		16.6				144.5	
		13		2.485				29.85	
		14		4.965				28.95	
		15		7.1				49.15	
		16		6.25				36.15	
		17		9.35				46.4	
		18		5.45				10.85	
		19		5.65				60.5	
		20		6.15				54	
		21		4.985				44.75	
		22		7.55				49.8	
		23		5.9				46.05	
		24		5.9				93.5	
		25		6.15				70	
		26		0.75				2.175	
		27		0.665				1.81	
		28		0.685				2.495	
		29		32.15				107.5	
		30		34.75				107	
		31		36.35				136.5	
		32		31.5				117	
		33		35.6				104	
		34		33.15				108.5	
		35		33.8				110.5	
		36		33.6				123.5	
		37		36.8				89	
		38		35.25				108	
		39		35.95				129	
		40		40.05				113.5	
		41		27.6				93	
		42		34.05				132	
		43		28.85				83	
		44		39.3				111	
		45		38				113	
		46		32.2				105	
		47		29				93.5	
		48		4.585				22.25	
		49		4.32				19.3	
		50		2.965				0.105	
		51		3.745				9.4	
		52		2.605				6.85	
		53		3.115				10.15	
		54		2.635				7.15	
		55		3.1				12.25	
		56		3.385				9.85	
		57		5.55				13	
		58		1.67				6.8	
		59		2.9				13.2	
		60		5.2				13.25	
		61		5.2				12.3	
		62		4.02				10.5	
		63		2.75				20.05	
		64		4.145				14.45	
		65		3.04				7.8	

STUDY	SPECIES	ALL DATA (ug/g dw)							
		Sample ID	[Whole Body]	[Muscle]	[Muscle Plug]	[Liver]	[Ovary]	[Egg]	[Larvae]
Coyle et al. 1993	bluegill sunfish	1	0.9				2.1	1.9	
		2	0.9				2.1	1.9	
		3	2.9				8.3	7.3	
		4	4.9				12.5	13.0	
		5	7.2				25	22.8	
		6	16				41	41.3	
Garcia-Hernandez 2000	tilapia	1		3.5				1.1	
	carp	1		4.6		8.2	1.8		
	largemouth bass	1		5.4		4.7	3		
Golder 2005.	cutthroat trout (1996)	1		6.81		33.5	28.2		
		2		4.18		16.2	47.8		
		3		3.02		16.3	22		
		4		4.04		23.3			
		5		4.64		16.6			
		6		3.98		11.7			
		7		4.15		23.6			
		8		3.64		14.9			
		9		4.53		25.7			
		10		3.82		16.3			
		11		14.7		51.5			
		12		6.62		34.9			
		13		4.75		14.5			
		14		5.16		24.3			
		15		4.81		23.5			
	mountain whitefish (1996)	1		3.6		35	26.9		
		2		3.66		26	25.8		
		3		3.07		21.3	20		
	bull trout (1996)	1		3.53		18.4			
	cutthroat trout (2001-2002)	1		4.9		10.0	9.8		
		2		4.5		10.0	8.2		
		3		5.0		17.0			
		4		4.4		18.2			
		5		5.1		16.0			
		6		5.4		13.0			
		7		5.8		18.0			
		8		4.4		11.0			
		9		4.7		15.0			
		10		5.7		16.0			
		11		4.0		11.0	7.0		
		12		5.0		21.0	10.0		
		13		5.0		15.0	10.0		
		14		5.0		15.0	8.0		
		15		5.0		23.0			
		16		5.0		19.0			
		17		5.0		17.0			
		18		5.0		23.0			
		19		5.0		18.0			
		20		5.0		18.0			
		21		8.4		18.0	16.2		
		22		8.3		15.0	18.3		
		23		7.0		19.0	14.3		
		24		8.4		16.0			
		25		6.6		22.0	14.3		
		26		8.3		27.1			
		27		7.8		24.9			
		28		6.9		41.0			
		29		8.1		28.0			
		30		8.5		29.3			
		31		8.4		14	14.7		
		32		9.8		17	16.4		
		33		8.5		19	15.9		
		34		9.2		27.2			
		35		8.1		25.5			
		36		8.5		24.0			
		37		16.0		25.0	20.0		
		38		7.0		15.0	14.0		
		39		8.0		18.0	19.0		
		40		7.0		16.0	14.0		
		41		7.0		16.0	14.0		
		42		9.0		39.0	16.0		
		43		7.0		16.0	13.0		
		44		7.0		16.0	14.0		
		45		8.0		15.0	14.0		
		46		8.0		27.0			
		47		9.8		32.0	20.2		

STUDY	SPECIES	ALL DATA (ug/g dw)							
		Sample ID	[Whole Body]	[Muscle]	[Muscle Plug]	[Liver]	[Ovary]	[Egg]	[Larvae]
Golder 2005.	cutthroat trout (2001 - 2002)	48		10.4		42.0			
		49		7.0		6.0	22.0		
		50		9.0		14.0	16.0		
		51		7.0		17.0	12.0		
		52		8.0		17.0	13.0		
		53		10.0		20.0	14.0		
		54		13.0		37.0			
		55		6.0		41.0			
		56		8.0		24.0			
		57		6.0		35.0			
		58		10.0		38.0			
	mountain whitefish (2001)	1		4.2		14	19.3		
		2		3.9		15	19.2		
		3		3.5		19	23.2		
		4		5		30			
		5		7.2		58			
		6		4.3		28			
		7		4.1		29			
		8		4.3		40			
		9		4.9		43			
		10		4.8		46			
		11		5.2		40	38		
		12		5		20	41		
		13		5.2		37	32		
		14		7.6		17	34		
		15		7.2		29	32		
		16		5.5		51	40		
		17		8.2		61			
		18		6		50			
		19		6.7		108			
		20		6		44			
		21		7.8		18	39.7		
		22		3.7		12	20.3		
		23		4.7		15	22.4		
		24		4.4		15	28.9		
		25		5.7		28	30.1		
		26		4		22	31.5		
		27		10		20	35.2		
		28		4.9		22	26.7		
		29		7.6		21	26.8		
		30		6.1		22	29.7		
		31		6.8		34	41.1		
		32		5		25	29		
		33		6.6		21	34.5		
		34		5		17	36.3		
		35		4.8		16	28.9		
		36		7		42			
		37		6.6		39			
Hamilton et al. 2005a,b,c	razorback sucker	1		6.3	4.5	7.5	7	6.5	7.3
		2		15.6	11.7		30.6	46.5	32.1
		3		29.2	16.6	52.1	45.5	37.8	39.7
		4			5.1			6	7.2
		5		5.8	4.5	7.9	5.9		
		6		13.5	11.5	17.6	27.5		
		7		16.2	16.4	29.6	42.1		
		8							
		9		6	4.5	6.8	5.1		
		10		12.5	9.5	12.2	10		
		11		18	14.2	23.7	12.9		
		12							
Hardy 2005	cutthroat trout	1	0.72					0.99	
		2	2.57					3.8	
		3	2.78					5.45	
		4	6.4					18.0	
		5	1.2					1.64	
		6	4.64					7.82	
		7	5.87					6.61	
		8	9.1					5.05	
		9	11.37					5.18	
		10	5.61					16.04	

STUDY	SPECIES	ALL DATA (ug/g dw)							
		Sample ID	[Whole Body]	[Muscle]	[Muscle Plug]	[Liver]	[Ovary]	[Egg]	[Larvae]
Hermanutz et al. 1992	bluegill sunfish	1		1.5		7.5	2.5		
		2		9		46.5	22		
		3		14		79			
		4	2.0	1.5		5.5	1		
		5	23.0	21		36.5	22.5		
Hermanutz et al. 1996	bluegill sunfish	1	1.95	2.05		5.4	0.35		
		2	22.85	20.55		36.05	20.05		
		3	2.45	1.9		13.2	5.25		
		4	1.95	2.25		7.2	3.85		
		5	3.5	3.5		29.2	10.1		
		6	6.15	6.9		26.45	12.35		
		7	15.45	17.55		119	34.8		
		8	26.45	44.7		68.5	50.5		
		9	11.85	12.45		64	29.35		
		10	30.6	39.6		100.5	66		
		11	3.35	3.35		9.95			
		12	2.3	3.2		9.4	5.3		
		13	6.3	5.25		13.85	8.4		
		14	5.3	6.1		16.3	9.5		
		15	12	12.45		33.25	31.15		
		16	13	18.6		37.15	19.55		
		17	8.35	7.75		21	17.85		
		18	17.35	15.05		31.9	19.1		
Holm et al 2005	brook trout	1		3.1				1.5	
		2		1.6				2.5	
		3		2.4				3.4	
		4		2.2				4.7	
		5		2.5				2.9	
		6		5.6				5.6	
		7		10.9				9.9	
		8		11.8				15.4	
		9		12.6				12.8	
		10		12.8				14.8	
		11		13.8				12.2	
		12		17.9				12.4	
		13		18.5				13.2	
		14		22.1				15.5	
		15		22.9				15.3	
		16		26.3				25.4	
		17		39.0				32.5	
	rainbow trout	1		1.9				1.0	
		2		1.8				3.5	
		3		1.4				4.6	
		4		4.5				12.8	
		5		4.9				17.1	
		6		9.5				17.5	
		7		8.4				29.7	
Kennedy et al 2000	cutthroat trout	1		41.3		114.40	66.8	75.4	
		2		15.3		37.50	31.6	58.4	
		3		14.1		67.75	31.4	30.6	
		4		12.5		34.04	18.5	20.2	
		5		13.7		37.50	19.5	19.4	
		6		14.3		30.08	16.2	16.2	
		7		9.5		25.56	19.3	16.1	
		8		9.4		32.92	22.0	14.4	
		9		8.7		18.42	17.0	13.2	
		10		9.5		31.81	13.6	12.6	
		11		10.2		30.25	14.5	12.3	
		12		10.7		25.89	20.6	10.5	
		13		6.6		27.79	21.5	9.9	
		14		9.7		32.81	13.2	9.1	
		15		10.9		4.58	13.4	8.5	
		16		6.9		23.88	20.3	13.2	

STUDY	SPECIES	ALL DATA (ug/g dw)							
		Sample ID	[Whole Body]	[Muscle]	[Muscle Plug]	[Liver]	[Ovary]	[Egg]	[Larvae]
Minnow 2006	longnose sucker	1					10	10.71	
		2					8.35	7.36	
		3					9.55	9.6	
		4					11.26	12.18	
		5					5.56	6.01	
		6					9.43	9.45	
		7					8.74	8.46	
		8					6.72	6.47	
		9					6.19	6.14	
		10					9.38	9.39	
		11					52.81	51.01	
		12					30.21	20.57	
		13					42.72	47.24	
		14					26.89		
		15					15.39	15.51	
		16					49.61	65.4	
		17					31.05	32.65	
		18					27.6	34.35	
		19					35.66	36.65	
		20					32.94	45.03	
		21					34.56	39.44	
		22					33.65	37.84	
		23					55.27	59.37	
		24					37.13	40.5	
Muscatello et al. 2006	northern pike	1		51.6		48.8		48.1	
		2		24.0		24		43.1	
		3		23.3		25.8		26.2	
		4		23.5		28.4		24.4	
		5		9.6		4.6		4.1	
		6		11.2		3.2		3.2	
		7		9.0		4.4		3.0	
		8		10.6		7.3		2.5	
		9		11.1		8		4.1	
Rudolph et al. 2007	cutthroat trout	1		7.7				13.9	
		2		8.2				12.5	
		3		8.0				15.0	
		4		8.1				14.9	
		5		6.6				15.2	
		6		8.5				12.9	
		7		7.2				12.3	
		8		7.3				16.7	
		9		7.6				13.1	
		10		8.7				15.6	
		11		8.2				13.9	
		12		7.9				15.1	
		13		7.6				12.3	
		14		11.8				16.1	
		15		40.4				86.3	
		16		46.1				121.0	
		17		50.4				140.0	
		18		34.7				51.0	
		19		39.0				65.3	
		20		35.4				46.8	
		21		11.3				16.9	
		22		13.4				20.6	

STUDY	SPECIES	ALL DATA (ug/g dw)							
		Sample ID	[Whole Body]	[Muscle]	[Muscle Plug]	[Liver]	[Ovary]	[Egg]	[Larvae]
Waddell and May 1995	razorback sucker	1			11.5				
		2			3.55				
		3			4.38			3.7	
		4			7.14			4.7	
		5			32			10.6	
		6			4.51				
		7			7.37				
		8			26				
		9		3.2	3.4				
		10		2.7	3.2				
		11		3.2	3.6				
	common carp	1		5.8	5.9				
		2		5.9	6.8				
		3		6	5.6				
		4		8.5	8.7				
		5		8.7	9.2				
		6		8.9	8.8				
		7		13.2	13				
		8		14	13				
		9		13.8	14				
	flannelmouth sucker	1		4.6	4.8				
		2		4.5	3.9				
		3		4.7	2.4				
		4		5.8	6.5				
		5		6.6	7.2				
		6		6.1	5.6				
		7		5	5.2				
		8		5.1	5.4				
		9		5.4	2.9				

Part II:
Threshold Development Endpoints

Review of Selenium Tissue Thresholds for Fish: Evaluation of the Appropriate Endpoint, Life Stage, and Effect Level and Recommendation for a Tissue-Based Criterion

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1.0 Introduction

It is generally agreed that a selenium (Se) criterion or guideline for aquatic biota should be based on the Se concentration in fish tissue. The EPA's (2004) current draft Se criterion is based on whole-body Se in fish tissue and, although implementation guidance has not yet been released, presumably any size of fish (larvae through adult) could be sampled at a site to determine compliance with this whole-body-based criterion. The previous chapter of this report, prepared by Dr. Adrian deBruyn and Alan Hodaly of Golder Associates, Ltd. (Golder), evaluated the different tissues that could be considered in developing a tissue-based Se criterion and concluded that the egg was the most appropriate tissue for setting a criterion. The objective of this chapter is to evaluate the available Se toxicity data for fish and recommend the appropriate endpoint, life stage, and statistic for developing a broadly applicable criterion. As such, the objective of this evaluation is complementary with the previous chapter in determining the appropriate tissue for developing a broadly applicable criterion. It should be emphasized that the term "broadly applicable criterion" is being used because site-specific studies could support development of an alternative Se criterion to that recommended in these chapters.

There are two general types of biomonitors for evaluating chemical concentrations in organism tissues: (1) biomonitors of exposure; and (2) biomonitors of effects. The biomonitor of exposure and biomonitor of effects may be the same or different organisms. The biomonitor of exposure should have increasing chemical concentrations in its tissue as the external chemical concentration increases. Organisms that are able to regulate or metabolize the chemical(s) of interest are not useful biomonitors of exposure because tissue concentrations will not reflect different concentration gradients. A biomonitor of exposure should be relatively tolerant to the chemical of interest if it is to be applied over a wide range of concentrations, while a biomonitor of effects should be sensitive to ensure that it is protective of other effects-based endpoints. If the biomonitor of exposure is based on a sensitive organism, it would be one of the first organisms impacted or eliminated from the aquatic community at a sufficiently elevated chemical concentration. These same concepts apply when determining the appropriate life stage for developing a tissue-based threshold for Se.

The classic pathway of documented Se poisoning in fish is exposure of adult female fish to Se, maternal transfer of the Se to the ovaries and then eggs, and then, if sufficiently high egg Se concentrations are reached, larval deformities and mortality. For example, in Belews Lake (North Carolina, USA), the site of one of the most clearly documented cases of Se poisoning in fish, the presence of adult fish hid the fact that early life stages were being adversely affected by Se. In addition to the maternal transfer pathway for Se poisoning in fish, juvenile fish directly exposed to dietary Se in the laboratory have been shown to be relatively sensitive. In fact, the EPA's draft criterion for Se is based on the sensitivity of juvenile bluegill simultaneously exposed to Se and winter conditions (i.e., cold temperature

and short photoperiod). In terms of developing a tissue-based Se criterion, however, there are several reasons why the maternal transfer exposure scenario is desirable. First, adult fish are relatively insensitive to Se (Coyle et al. 1993), which makes this life stage an ideal biomonitor across a range of Se exposure gradients. Second, the larval deformity endpoint is amenable to confirmatory field studies (i.e., the endpoint is Se-specific). Third, the larval deformity endpoint is directly linked to the egg Se concentration, the tissue type recommended as the basis for a Se tissue guideline in the previous chapter. In other words, there is a direct linkage between the collection of adults (a generally insensitive life stage) for Se analysis in the eggs to toxicity in developing embryos and larvae. In addition, although not a critical requirement, development of a fish Se criterion based on maternal transfer is consistent with the Se criterion model for birds, which is based on the maternal transfer of Se to bird eggs and associated effects on hatchability.

The other objective of this evaluation was to recommend an appropriate toxicity test statistic for developing a broadly applicable Se criterion for fish tissue. A typical toxicity study conducted in the laboratory may include a control (with no test chemical added) and a series of five increasing concentrations. There are typically 2 to 4 replicates for the control and each treatment. Ideally, although not always the case, the level of effect (e.g., mortality, reduced growth, etc.) increases with increasing chemical concentration. Toxicity results are then typically evaluated using one of two basic approaches: (1) hypothesis testing or (2) point estimation. Hypothesis testing entails use of a statistical test, such as analysis of variance (ANOVA), to determine whether there are significant differences between treatments at some defined probability level (e.g., $p \leq 0.05$). If there are differences, a multiple comparisons test is conducted to determine which treatments resulted in a level of effect that was significantly different from the control. The highest chemical concentration that did not result in statistically significant effects is typically called the no observed effect concentration (NOEC) and the lowest chemical concentration that did result in statistically significant effects is typically called the lowest observed effect concentration (LOEC). Using the point estimation approach, a statistical model (e.g., probit) is fit to the concentration-response data to identify a chemical concentration associated with a given level of effect. For example, the results of acute toxicity tests are often reported as the LC50, or the concentration lethal to 50 percent of the test organisms. In developing a toxicity threshold to be used as a guideline or criterion, a lower effect level is typically desired. For example, the EPA has considered EC20 values in developing ambient water quality criteria (AWQC) for ammonia (EPA 1999) and the draft tissue-based criterion for Se (EPA 2004). The EC10 is another effect level often identified as a threshold for toxicity.

The Se toxicity studies available for identifying tissue-based thresholds are highly variable in terms of experimental design, making it difficult to develop a consistent effect level across studies. This is particularly true for many of the studies based on field exposures, which include a reference site and often only one to two Se exposure sites. The EPA's (2004) draft Se criteria document also struggled with this issue, which resulted in a combination of

EC20s, NOECs, and LOECs being considered in developing the draft criterion. Ultimately, the EPA's draft criterion was based on the Lemly (1993a) winter stress syndrome study that considered one Se treatment; therefore, the draft criterion is not truly based on an EC20, NOEC, or LOEC.

This chapter evaluates whether existing Se toxicity data for fish support the concept that larval deformities and mortality resulting from maternal transfer of Se to the eggs represents the appropriate endpoint and life stage for developing a broadly applicable criterion. A key component of this evaluation is to assess whether such a maternal transfer-based criterion is protective of other life stages and endpoints (namely juvenile survival and growth). Based on the results of this evaluation, an appropriate toxicity test statistic for quantifying the recommended criterion is presented.

1.1 Methods

In nature, adult fish are simultaneously exposed to inorganic and organic Se forms in both the water and the diet; however, dietary exposure to organic Se tends to be the dominant exposure pathway at environmentally relevant levels (Bertram and Brooks 1986; Woock et al. 1987; Besser et al. 1993; Coyle et al. 1993). Accordingly, laboratory toxicity studies were only considered in this evaluation if fish were provided organic Se in the diet (in some studies adult Se exposures occurred in the field and implicitly included dietary organic Se exposures). If fish were exposed only to aqueous Se, or an inorganic Se-spiked (e.g., selenite) diet, the study was not considered in this evaluation. Aqueous and dietary inorganic Se have different bioaccumulation properties and toxicodynamics than environmentally relevant organic Se forms. In addition, this evaluation only considered measured Se concentrations in fish; that is, no attempt was made to estimate Se concentrations in one tissue from concentrations measured in another tissue due to species-specific and study-specific variability in these relationships.

There are two basic types of fish Se toxicity studies. In the most common type of study, adults are exposed to dietary Se in the laboratory or field, and effects on offspring are evaluated. In the second type of study, previously unexposed (via maternal transfer) juveniles are provided dietary Se and effects on survival and growth are evaluated. Relative sensitivity of the two study types is not necessarily based on the level of effects associated with whole-body Se, but rather the external (i.e., dietary) Se concentration to which they are exposed. For example, suppose a water body contains Species A and Species B. The whole-body Se EC10 values for Species A and Species B are 10 and 12 µg/g, respectively. However, Species A is not necessarily more sensitive than Species B because the Se trophic transfer factors (i.e., bioaccumulation potential) for the two species are not necessarily the same. If the trophic transfer factors are 1 and 1.5 for Species A and B, respectively, Species B thus requires a lower dietary Se concentration to achieve the whole-body EC10 than Species A (Table 1). Thus, assuming a similar diet, Species B would be more sensitive than Species A despite having a higher whole-body Se EC10.

Table1: Generic example for determining the relative sensitivity of organisms to Se as a function of dietary Se and whole-body Se.

	Trophic Transfer Factor	X	Dietary Se (µg/g)	=	Whole-body Se EC10 (µg/g)
Species A:	1		10		10
Species B:	1.5		8		12

The same concept applies when evaluating which fish life stages are more sensitive to Se. Accordingly, when assessing whether the larval development and survival endpoints (resulting from maternal transfer) are more sensitive than the juvenile survival and growth endpoints, one must consider the exposure concentration. For Se, as noted above, the most relevant exposure concentration to consider is dietary organic Se. Relevant toxicity studies for this comparison are limited to laboratory studies with known dietary Se concentrations. The fish species with the most data available for making this comparison is the bluegill (*Lepomis macrochirus*).

To evaluate and recommend an appropriate test statistic for defining a tissue-based Se criterion, NOECs, LOECs, EC10s, and EC20s were calculated from each study, to the extent possible, and compared. Consideration was given to how the EC10 and EC20 relate to hypothesis testing results. For example, if the EC10 is consistently at a level below the NOEC for Se, then perhaps it is not a useful level for evaluating Se effects in the field.

1.2 Results and Discussion

1.2.1 Endpoint and Life Stage Evaluation

1.2.1.1 Availability of Toxicity Data for Different Life Stages and Endpoints

Overall, Se toxicity studies based on maternal transfer have been conducted for seven freshwater species based on 17 different studies (Table 2). The studies include laboratory exposures (bluegill, cutthroat trout [*Oncorhynchus clarkii*], fathead minnow [*Pimephales promelas*]), mesocosm exposures (bluegill, fathead minnow), and field-based exposures for (bluegill, fathead minnow, brook trout [*Salvelinus fontinalis*], cutthroat trout, northern pike [*Esox lucius*], white sucker [*Catostomus commersoni*]). Various tissues were analyzed for Se, but either egg or ovary Se was analyzed in each study. Six studies evaluated the effects of long-term (≥ 60 days) dietary Se exposure on juvenile fish and measured Se in whole-body tissue (Table 2). Se toxicity studies which measured fish tissue Se concentrations but were excluded from this evaluation are summarized in Table 3 with the rationale for their exclusion.

Table 2: Summary of available tissue-based Se toxicity data for fish based on maternal transfer and direct juvenile exposures.

Species	Studies Based on Maternal Transfer						Studies Based on Direct Juvenile Exposures[1]	
	Exposure Type	Tissues Analyzed				Reference	Whole-body Tissues Analyzed	
		Whole-body	Muscle	Ovary	Egg		Reference	
Bluegill	Field		X	X		Bryson et al. 1984		
	Field		X	X		Bryson et al. 1985a		
	Field			X		Bryson et al. 1985b	Cleveland et al. 1993	
	Lab	X		X	X	Coyle et al. 1993	Lemly 1993a	
	Lab		X	X	X	Doroshov et al. 1992	McIntyre et al. 2008	
	Field			X		Gillespie and Baumann 1986		
	Mesocosm	X	X	X		Hermanutz et al. 1996		
Brook trout	Field		X		X	Holm et al. 2005		
Chinook salmon	Lab						Hamilton et al. 1990	
Cutthroat trout	Lab				X	Hardy 2005		
	Field	X	X	X	X	Kennedy et al. 2000		
	Field		X		X	Rudolph et al. 2008		
Fathead minnow	Lab	X		X		Ogle and Knight 1989		
	Mesocosm		X	X		Schultz and Hermanutz 1990	Bertram and Brooks 1986	
	Field/Lab	X		X		GEI Consultants 2008		
Northern pike	Field		X		X	Muscatello et al. 2006		
Rainbow trout	Field		X		X	Holm et al. 2005	Vidal et al. 2005	
White sucker	Field				X	de Rosemond et al. 2005		
						Studies Based on Maternal Transfer	**Studies Based on Direct Juvenile Exposures**	
					Total # of Species:	**7**	**4**	
					Total # of Studies:	**17**	**6**	

[1] Studies where juvenile fish were exposed to dietary organic Se for ≥60 days.

The toxicity data set for maternal transfer studies is more extensive than that for direct juvenile exposure studies, both in the number of species tested and because multiple studies have been conducted for some species (i.e., bluegill, fathead minnow, cutthroat trout). As discussed below, data from maternal transfer studies, both within species and between species, is quite consistent. This increases the confidence in the basic study design, study results, and appropriateness of using the maternal transfer endpoint to develop a broadly applicable Se criterion for fish tissue. As discussed below, Se toxicity studies using juvenile fish exhibit greater variability in interspecies response. In addition, the design of juvenile toxicity studies may limit the applicability of results to field settings.

1.2.1.2 Relative Sensitivity of Maternal Transfer and Juvenile Toxicity Studies

Because Se toxicokinetics and toxicodynamics may differ between adults and juveniles, the relative sensitivity of different fish life stages to Se may be more closely related to dietary Se concentrations than internal tissue concentrations. Three laboratory studies exposed adult bluegill to dietary organic Se and evaluated effects in offspring: (1) Woock et al. (1987) (note that internal Se concentrations in fish tissue were not measured in this study); (2) Doroshov et al. (1992); and (3) Coyle et al. (1993). The most sensitive endpoints in these three studies were larval mortality in the Woock and Coyle studies and larval edema in the Doroshov study. The concentration-response curves relating dietary Se concentration to the percentage of effect are shown in Figure 1. The concentration-response relationships are similar between the studies, with effects being low up to a dietary Se concentration of approximately 12 µg/g dry weight Se, and then a rapid increase to a 90 to 100 percent effect level at dietary Se concentrations above approximately 21 µg/g dry weight Se. For comparison to embryo/larval studies, there are three studies which exposed juvenile bluegills to dietary organic Se.

Table 3. Summary of tissue-based toxicity studies (or specific tests within a study) excluded from this evaluation.

Reference	Species	Basis for exclusion
Goettl and Davies 1978	Rainbow trout	Exposed to diet spiked with selenite (limited relevance to a natural diet)
Hilton et al. 1980	Rainbow trout	Exposed to aqueous selenite (limited relevance to a natural Se exposure)
Hodson et al. 1980	Rainbow trout	Exposed to diet spiked with selenite (limited relevance to a natural diet)
Hicks et al. 1984	Rainbow trout	Exposed to diet spiked with selenite (limited relevance to a natural diet)
Hunn et al. 1987	Rainbow trout	Exposed to aqueous selenite (limited relevance to a natural Se exposure)
Bennett et al. 1986	Fathead minnow	Test durations from three experiments ranged from 7-9 days (not evaluated because exposure duration was not sufficiently long)
Hamilton et al. 1986	Chinook salmon	Fish were fed mosquitofish collected from areas with potential contamination from other chemicals
Hamilton et al. 1990	Chinook salmon	In one test from this study fish were fed mosquitofish collected from areas with potential contamination from other chemicals (this study included another test in which fish were fed an organic Se-spiked diet. This test was included in the evaluation [Table 1])
Cleveland et al. 1993	Bluegill sunfish	Exposed to aqueous 6:1 selenate:selenite (limited relevance to natural Se exposure; this study included another test in which fish were fed an organic Se-spiked diet This test was included in the evaluation [Table 1])
Dobbs et al. 1996	Fathead minnow	Test duration was 25 days (not evaluated because exposure duration was not sufficiently long)

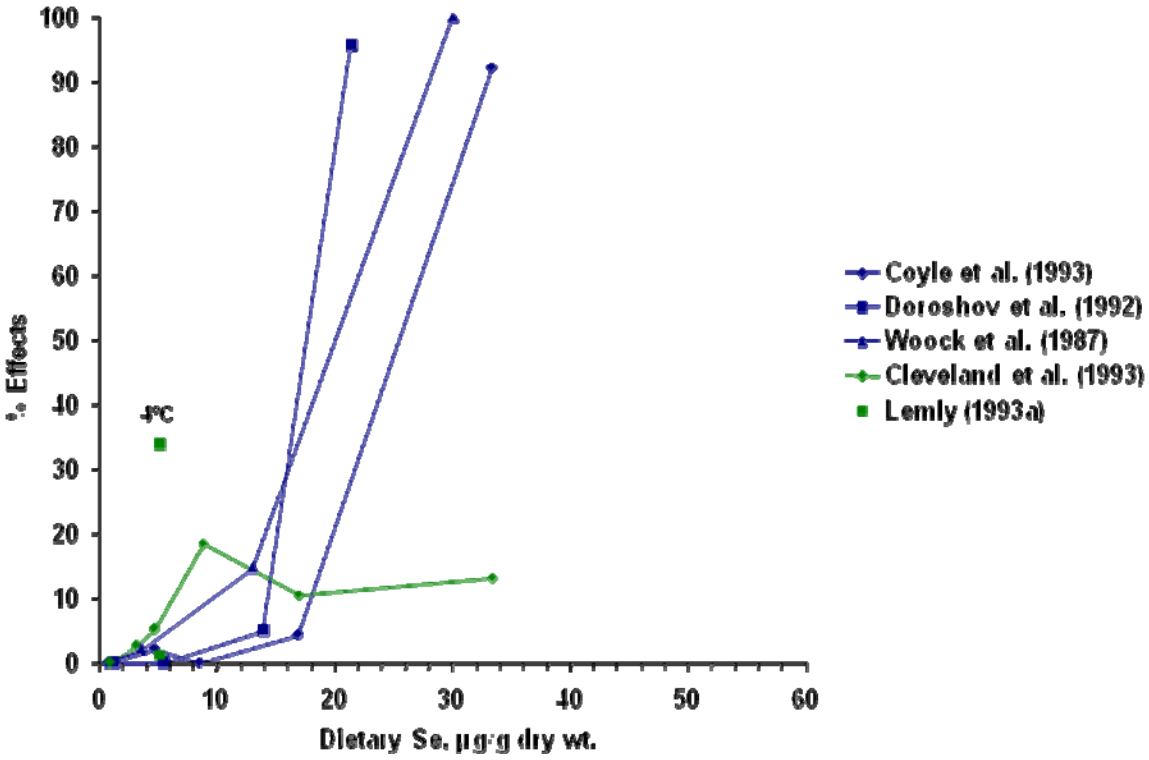

Figure 1: Se effects on bluegill larvae (from maternal transfer) and juveniles (from direct dietary exposures) as a function of dietary Se.

- **Cleveland et al. 1993.** This study exposed 3-month-old bluegill to dietary Se for 90 days. Endpoints evaluated included mortality, body weight, and condition factor, with mortality the most sensitive endpoint compared to the control. As shown in Figure 1, the concentration-response curve from this study is similar to the maternal transfer studies at low dietary Se concentrations. However, the same rapid increase in adverse effects is not observed at dietary Se concentrations greater than approximately 12 µg/g dry weight. Rather, the mortality response generally reaches a plateau between dietary Se concentrations of 9 to 33 µg/g dry weight.

- **Lemly 1993a.** Juvenile bluegills were exposed to a dietary Se concentration of 5.1 µg/g and waterborne 1:1 selenate:selenite concentration of 4.8 µg/L for 180 days. Some of the fish were simultaneously exposed to a water temperature gradually reduced to 4°C, while others were maintained at a water temperature of 20°C. The fish under simulated winter stress had significantly higher mortality (33.8 percent)[1] than fish continuously exposed to Se at 20°C (5.8 percent) (Figure 1). This study suggested that juvenile bluegill could be more sensitive than embryos/larvae exposed via maternal transfer, but due to limitations in the study design (one Se concentration and one coldwater regime were tested) this conclusion is uncertain. A follow-up study by McIntyre et al. (2008) was conducted to address these uncertainties.

- **McIntyre et al. 2008.** The authors conducted a series of tests with juvenile bluegills. One test essentially repeated the Lemly (1993a) study, while other tests were conducted using a range of aqueous and dietary Se concentrations and two coldwater temperature regimes (4 and 9°C). Although the final study results have not been published, it appears that significant juvenile mortality does not begin to occur until dietary and whole-body Se concentrations reach approximately 25 µg/g and 11 µg/g, respectively. Consequently, the juvenile bluegill in this study appear to be no more sensitive than offspring from adults exposed to similar dietary Se concentrations.

Overall, the weight-of-evidence evaluation suggests that juvenile bluegill are not more sensitive than bluegill embryos/larvae exposed to Se via maternal transfer and, in fact, the relative sensitivities of the two life stages appear to be relatively similar based on dietary Se exposures. Although the data available for this comparison are not extensive, the results for bluegill, if translatable across species, suggest the maternal transfer endpoint is more sensitive relative to dietary exposures to juveniles. At this time, similar comparisons cannot be made for other species because data from laboratory adult exposure studies are not available. The following section evaluates juvenile Se toxicity data for species other than bluegill.

[1] Although this is the level of mortality reported in Lemly (1993a), the actual level is probably closer to 50 percent if removal of fish during the test is properly accounted for. Fish were sub-sampled during the test for various analyses, but the mortality level of 33.8 percent assumed all sub-sampled fish would have survived. If the sub-sampled fish are removed from the total sample size, the mortality level is closer to 50 percent.

1.2.1.3 Life Stage Considerations for a Biomonitor of Selenium Exposure

The basic life stage options for developing a fish tissue-based Se threshold are adults, juveniles, larvae, and eggs. In general, field and laboratory data support the concept that Se concentrations in fish tissue tend to increase with increasing exposure concentration. A possible exception is lotic (flowing) systems, where whole-body Se concentrations may be relatively constant up to a water Se concentration of 20 µg/L or greater. However, these relatively constant whole-body Se concentrations at lower exposure concentrations are below those that may result in effects. Most of the Se bioaccumulation data, whether from field or laboratory studies, are based on adult tissue (e.g., whole-body or ovaries) or, to a much lesser extent, eggs.

It is less clear how Se concentration-response relationships for larval and juvenile tissues vary in response to the Se exposure concentrations. Furthermore, concentrations may vary as a function of age. Vidal et al. (2005), for example, exposed juvenile rainbow trout (24 days old at test initiation) to dietary seleno-L-methionine concentrations of 4.6, 12, and 18 µg/g dry weight for 90 days. Growth, measured as weight, of fish was reduced by 33 percent, 33 percent, and 26 percent, respectively, relative to control fish. The 33 percent reductions were significantly different from the control ($p \leq 0.05$), but the 26 percent reduction was not ($p > 0.05$). Whole-body Se concentrations and burdens were measured at 30, 60, and 90 days; whole-body Se concentrations peaked at day 60 and then decreased by day 90, even in the control fish (Figure 2a). In addition, whole-body Se concentrations were lower at day 90 than day 30. The authors suggested that the decreases in whole-body Se from day 60 to day 90 likely occurred because of the relative increases in total body mass, which would have decreased overall concentrations. However, the observed reduction in Se concentrations does not appear to be entirely a growth dilution effect because Se burdens (µg Se per fish), calculated based on the whole-body Se concentration and the weight of the fish, also declined from day 60 to 90 (Figure 2b). The changes in whole-body Se during the course of the test were substantial enough that by day 60, whole-body Se in control fish was 1.24 µg/g wet weight, or more than two times the effects-based whole-body LOEC of 0.58 µg/g wet weight. An observation from this study, which was also noted by the authors, is that the results may demonstrate the difficulty in using whole-body tissue residues as toxicity thresholds for Se in individuals that are developmentally immature. However, studies with juvenile Chinook salmon (*Oncorhynchus tshawytscha*) (Hamilton et al. 1990) and bluegill (Lemly 1993a) did not observe the same fluctuations in tissue Se over time.

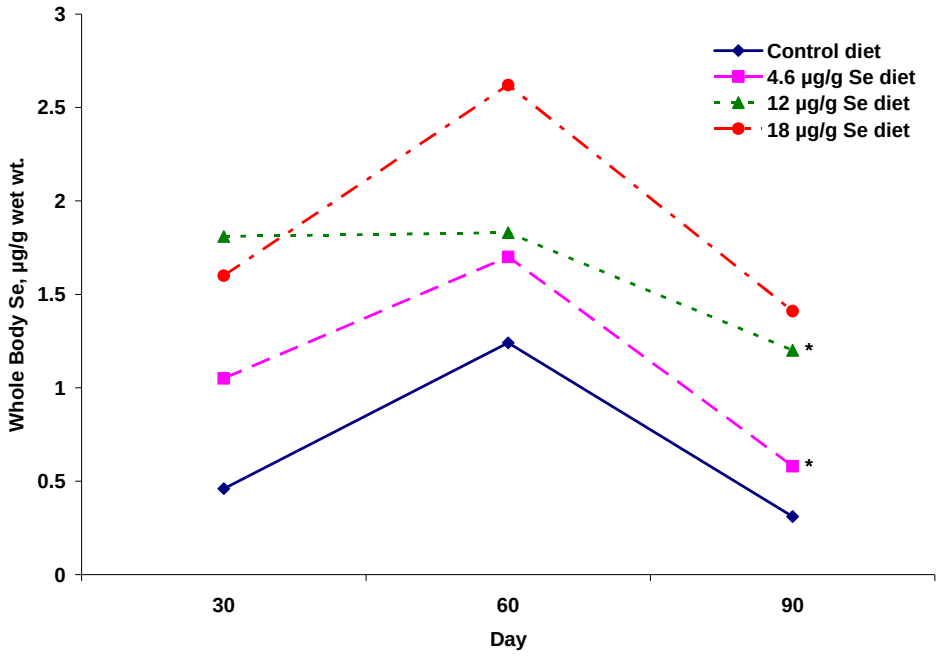

Figure 2a: Whole-body Se concentrations in juvenile rainbow trout following 30, 60, and 90 days. Data from Vidal et al. (2005). Asterisks (*) denote where growth was significantly reduced relative to the control.

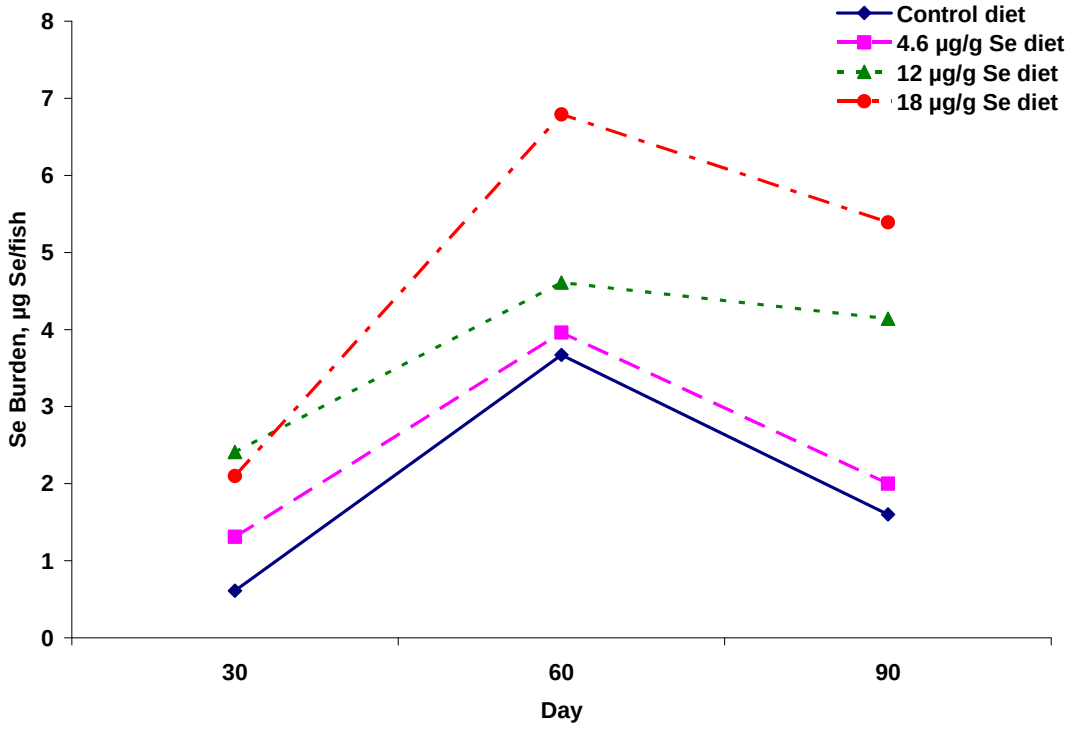

Figure 2b: Whole-body Se burdens in juvenile rainbow trout following 30, 60, and 90 days. Data from Vidal et al. (2005).

The Chinook salmon study conducted by Hamilton et al. (1990) also resulted in a very different relationship between juvenile whole-body Se and growth effects relative to the Vidal et al. (2005) rainbow trout study, despite the two studies having very similar test designs. Hamilton et al. (1990) exposed juvenile Chinook salmon to dietary organic Se for 90 days. However, due to high control mortality (survival decreased from 99 percent to 66.7 percent) between days 60 and 90, only 60-day data are presented. The most sensitive endpoint was growth, which is compared to the Vidal et al. (2005) rainbow trout toxicity data in Figure 3. An increasing relationship between whole-body Se and reduced growth was observed for Chinook salmon, but not rainbow trout. This variability in basic concentration-response relationships between two closely related species and similar study designs suggests that the sensitivity of young fish directly exposed to dietary Se is variable, or at least not well understood based on the limited data available.

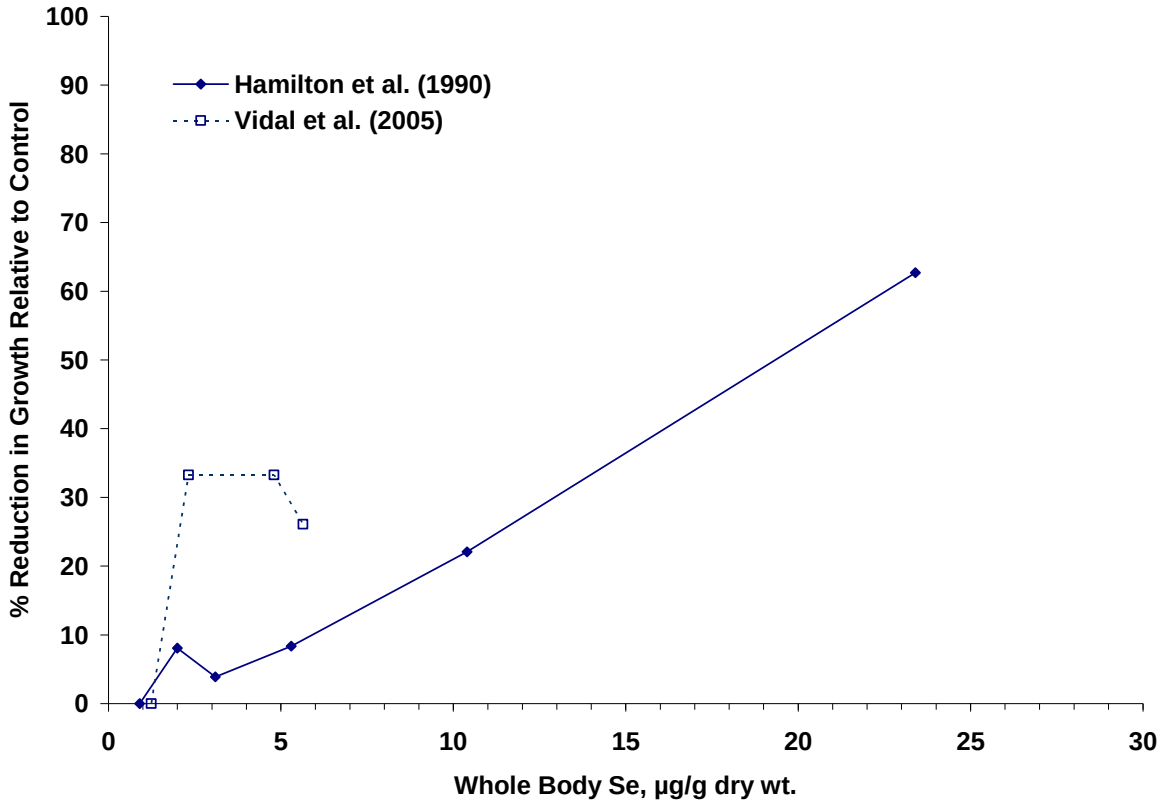

Figure 3: Comparison of Se effects on juvenile Chinook salmon and rainbow trout growth as a function of whole-body Se.

Teh et al. (2004) provided another example of variability in concentration-response relationships for juvenile fish. The authors exposed Sacramento splittail (*Pogonichthys macrolepidotus*) to dietary organic Se for 9 months. An anomalous response was observed between dietary Se and larval mortalities; this is the only study that has observed deformities in fish not exposed to egg Se as a developing embryo (Figure 4). Greater than 50 percent deformities were observed at a dietary Se concentration of 6.6 µg/g and then the level of

deformities decreased with increasing dietary Se concentration. Growth and survival was not significantly reduced at dietary Se concentrations up to 12.6 µg/g. The growth data from this study are more consistent with that observed in Hamilton et al. (1990) for Chinook salmon – weight of fish fed a dietary Se concentration of 12.6 µg/g was reduced by 6 percent relative to the control and by 41 percent in fish fed 26 µg/g dietary Se. The basis for the anomalous concentration-response data for deformities is unclear. The study is unique due to its longer duration than typical studies with juveniles so there are not results available for direct comparison.

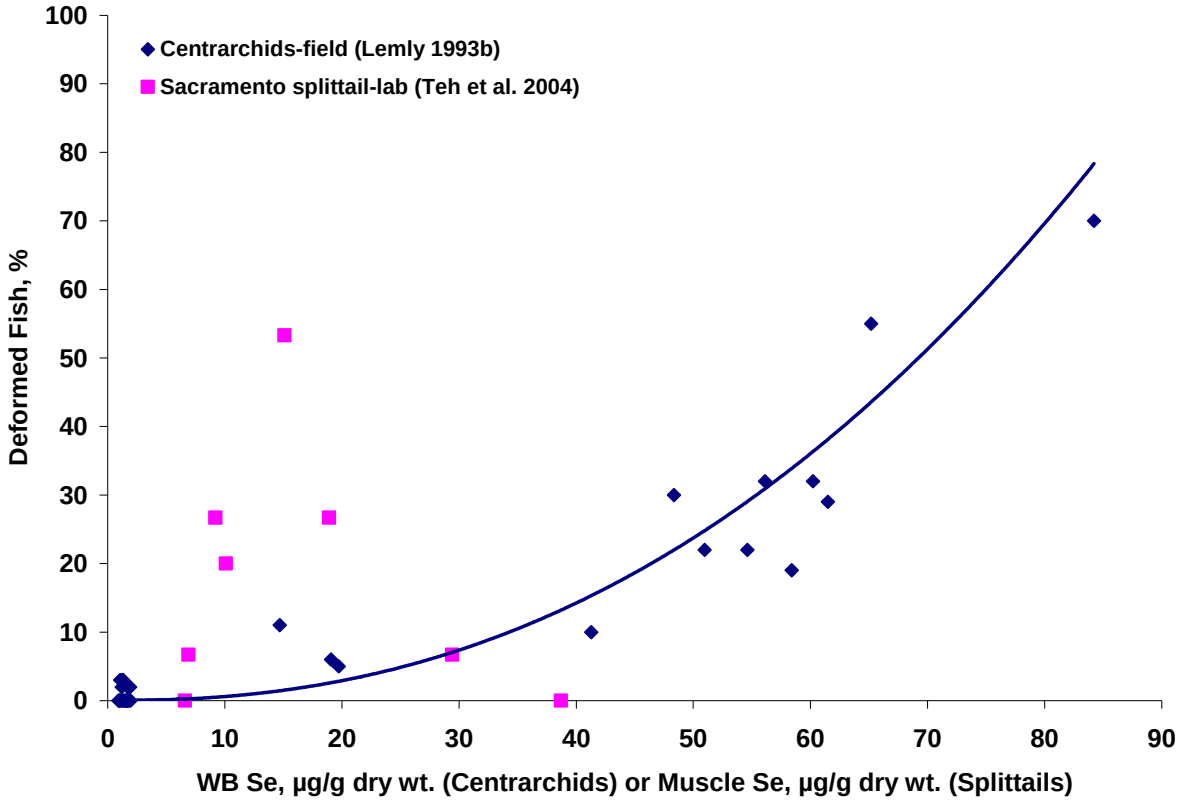

Figure 4: Relationship between fish deformities and whole-body Se in Belews Lake centrarchids (Lemly 1993b) and muscle Se in Sacramento splittails (Teh et al. 2004).

Although Teh et al. (2004) did not measure whole-body Se concentrations during the study, muscle Se concentrations did increase with increasing dietary Se concentrations, suggesting whole-body Se would have also increased with increasing dietary Se concentration. As a point of comparison, Lemly (1993b) assessed abnormalities and whole-body Se concentrations in 22 species of fish representing eight families from Belews Lake and two reference reservoirs. Observed abnormalities included spinal deformities (e.g., lordosis), accumulation of body fluid (e.g., edema), missing or abnormal fins, abnormally shaped head or mouth, and cloudy eye lens or cornea. Lemly (1993b) observed a significant positive relationship between whole-body Se and the prevalence of abnormalities (Figure 4). The relationship between deformities and whole-body or muscle Se is different between the field data for centrarchids and the laboratory data for the splittail (Figure 4).

Whereas relatively consistent concentration-response curves are typically observed for maternal transfer studies, data from juvenile studies are fewer, highly variable, and poorly understood. Overall, Se concentrations in fish tissue tend to increase with increasing exposure concentration. However, the Vidal et al. (2005) study observed substantial variability in whole-body Se concentration in juvenile rainbow trout over time, which may have been due to developmental changes in the fish. Teh et al. (2004) observed an inverted U-shaped relationship between muscle Se and larval deformities in splittail, which is not consistent with the field data of Lemly (1993b) for centrarchids and other fish in Belews Lake. Accordingly, the juvenile toxicity data suggest there may be greater uncertainty in using early life stage fish as biomonitors of exposure than using adult fish or reproductive tissues (e.g., eggs).

1.2.1.4 Environmental Relevance of Toxicity Study Designs

As discussed above, results from Se toxicity tests with juveniles often have different concentration-response relationships between species and, in some cases, unexpected results when compared to field data. It is possible that this variability in responses is an effect of the typical study design for juvenile Se toxicity tests. A key distinction between the toxicity studies based on maternal transfer and those based on exposure of juveniles to dietary Se is that fish in the latter study were not previously exposed to Se via maternal transfer. This exposure scenario is unlikely to be broadly relevant to field applications because, if juveniles are exposed to elevated dietary Se concentrations, it is likely that the parent fish were also exposed to elevated dietary Se concentrations. Unless Se concentrations increased at a site from a recent event, these fish would also be from a population with a prior Se exposure history. It is unclear how the sensitivity of juveniles with no elevated Se exposure history would compare to those that were initially exposed to Se as developing embryos due to maternal transfer. For highly mobile fish species or fish with unique life history characteristics, such as anadromous salmon, it is possible that typical adult Se exposure concentrations would be lower than concentrations at rearing grounds. Nevertheless, it can be assumed that, under most scenarios, juvenile fish exposed to elevated dietary Se will be offspring of parent fish that were also exposed to an elevated Se diet.

1.2.1.4.1 What is the Appropriate Test Statistic

As discussed in the introduction, common statistics for defining toxicity "thresholds" include the NOEC, LOEC, EC10, and EC20. The test results from each of the maternal transfer toxicity studies are summarized in Table 3. As shown, depending on the Se toxicity test designs and results, it is not always possible to calculate each test statistic (NOEC, LOEC, EC10, EC20) for each test. In ecological toxicology, it is generally accepted that the point estimate approach is more desirable than the hypothesis testing approach for several reasons. Determination of the NOEC and LOEC, for example, directly depends on the test concentrations tested and is highly influenced by the number of replicates and variability in the toxicity response. Accordingly, NOEC and LOEC values from different tests can be associated with greatly different effects levels. In the Se toxicity literature, reported LOECs range from a 5 percent effect level for edema (Doroshov et al. 1992) to a 93 percent effect level for larval mortality (Coyle et al. 1993), although both LOECs fit well on the concentration-curve based on the data from both studies (Figure 5). A primary benefit of the point estimation approach is that a consistent effect level may be defined, which allows for more appropriate comparisons of sensitivities between species and studies. The effect level may also be adjusted based on management decisions for a site.

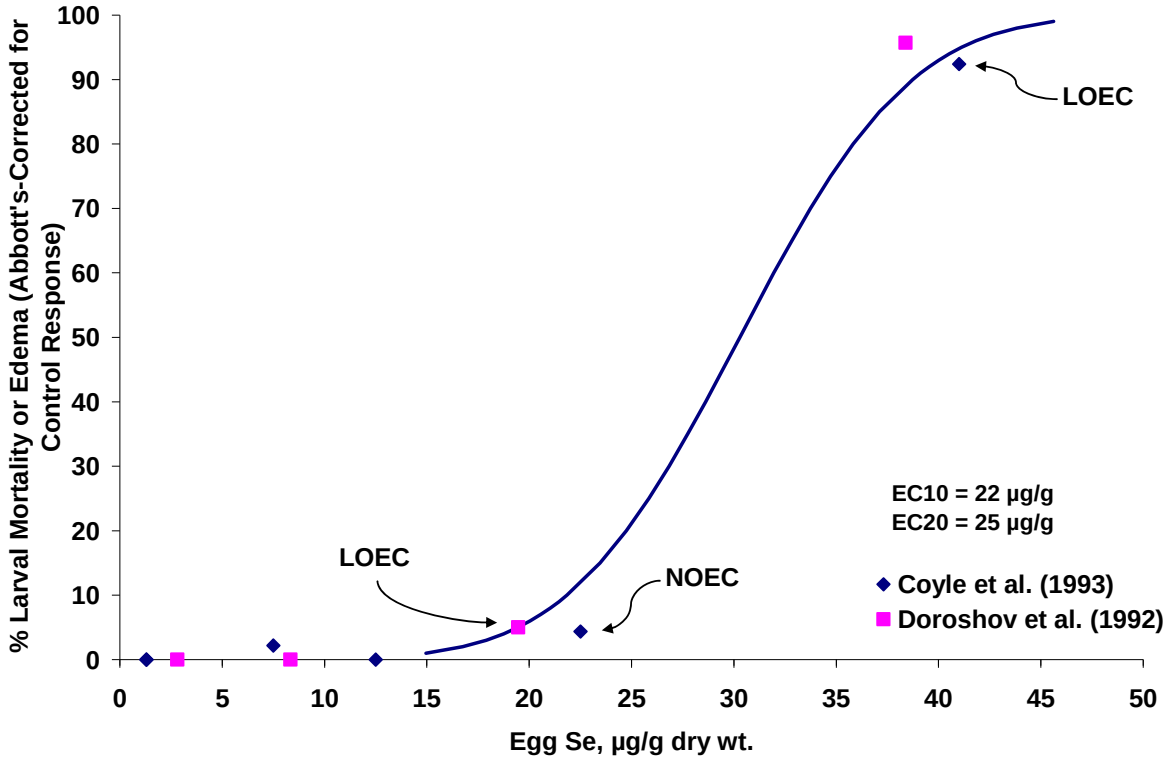

Figure 5: Comparison of egg-based lowest observed effect concentrations (LOECs) for Se in larval bluegill. Data from Doroshov et al. (1992) and Coyle et al. (1993).

Two common effect levels used in criteria or guideline development include the EC10 and EC20. The EPA has used the EC20 in its latest AWQC for ammonia (EPA 1999) and in the draft AWQC for Se (EPA 2004), while the EC10 is most recently being considered in developing a site-specific Se criterion for the Great Salt Lake. Ultimately there are numerous factors to consider in selecting an appropriate effect level, including whether the toxicity data available are likely to be conservative for a given site. For example, an EC20 may be conservative for one site, but not for another, depending on site-specific differences. As a point of comparison, egg-based EC10 values were plotted versus egg-based NOEC and LOEC values (Figure 6). As shown in Figure 6, the range in fish EC10 values generally falls between the available NOEC and LOEC values (for Doroshov et al. [1992], the hatched NOEC and LOEC values reflect alternative values that are more biologically meaningful since 5 percent edema is unlikely to be biologically significant). Overall, the EC10 appears to be an appropriate effect level for developing a broadly applicable tissue-based Se criterion that should provide an adequate level of protection. Selection of a lower effect level would obviously add conservatism to the criterion, but this may result in a criterion that is overly stringent for areas with naturally elevated Se levels, as well as an effect level that is statistically indistinguishable from control or reference site fish.

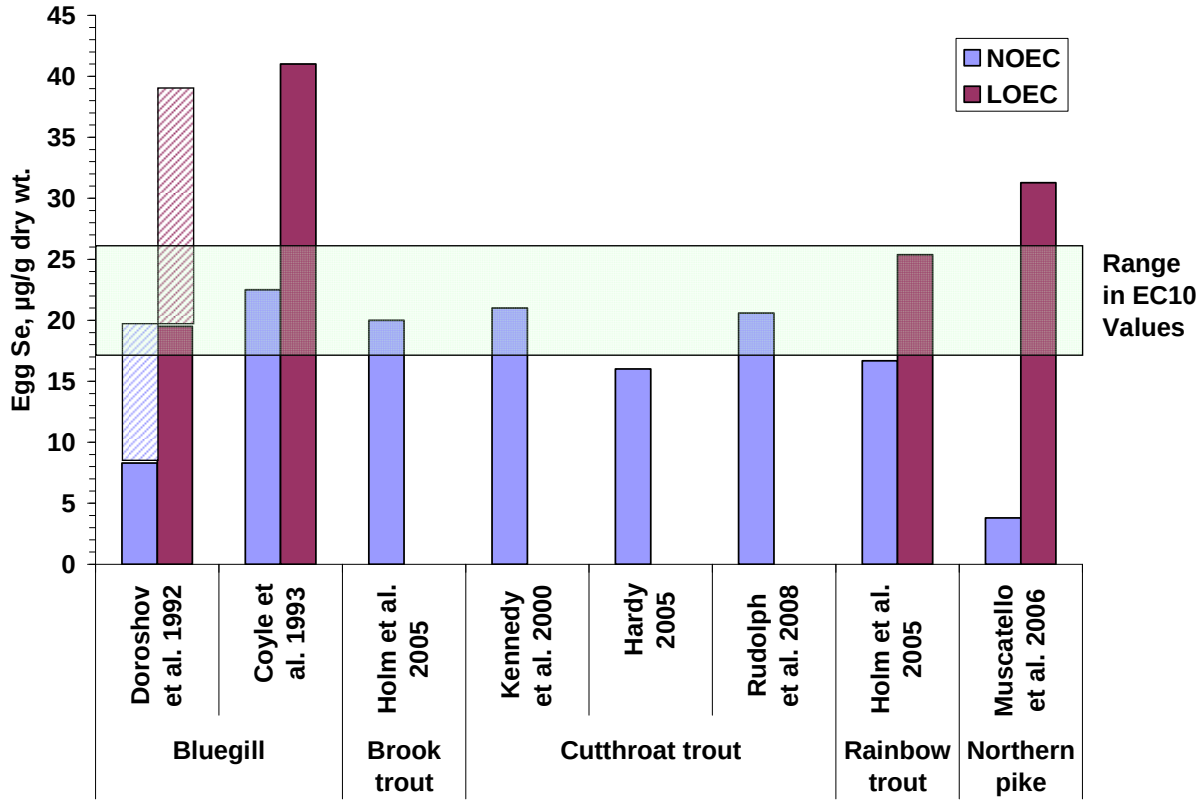

Figure 6: Comparison of egg-based Se EC10 values to egg-based NOECs and LOECs.

2.0 Summary and Conclusions

The weight-of-evidence supports that a broadly applicable Se criterion should be based on embryo-larval effects resulting from the maternal transfer of Se from the parent fish to the eggs for the following reasons. First, more toxicity data are available (more studies and more fish species) than for studies in which juveniles were fed dietary Se. The toxicity data from these Se maternal transfer studies are consistent within and between species, while the toxicity results from juvenile toxicity studies are variable. Second, the maternal transfer exposure scenario is expected to be the predominant exposure model for fish in the wild. Dietary exposure of juvenile fish to dietary organic Se, without a prior maternal transfer exposure as a developing embryo, may result in a Se toxicity response by these fish that is not translatable to a natural exposure. Third, Se toxicokinetics and toxicodynamics in young, developing fish is not well understood. For example, whole-body Se bioaccumulation was highly variable in the Vidal et al. (2005) study with young rainbow trout, but similar patterns have not always been observed with juveniles of other fish species. Fourth, sampling adult fish to collect eggs is broadly applicable to sites with a wide range of Se exposure levels because adults are relatively insensitive to Se. Fifth, the maternal transfer endpoint is a sensitive endpoint that is directly linked to the insensitive biomonitor (i.e., eggs collected from adults). Sixth, the larval deformity endpoint is Se-specific and thus amenable to confirmatory studies in the field.

As a companion to this paper (see Appendix A), Dr. David Janz and his colleagues at the University of Saskatchewan in Saskatoon, Canada developed standardized methods for evaluating Se-induced deformities in early life stages of freshwater fish. The method describes universal procedures, conditions and recommendations for gamete collection, embryo incubations, and evaluation of Se-induced deformities in freshwater fish. The methods are based largely on northern pike (*Esox lucius*), white sucker (*Catostomus commersoni*) and lake trout (*Salvelinus namaycush*) embryo incubations and deformity evaluations developed at the Toxicology Centre at the University of Saskatchewan. These methods are broadly applicable to other freshwater fish species, but some modifications from the procedures described here could be justified under special circumstances, based on spawning characteristics, fish size, and field conditions.

An egg-based species sensitivity distribution (SSD) for larval deformities and mortality can be derived from the Se EC10 values summarized in Table 4. As shown in Figure 7, the egg Se EC10s between species are not highly variable, with the maximum EC10 being only a factor of 1.5 greater than the minimum EC10. The minimum egg Se EC10 of 17 µg/g dry weight for cutthroat trout is based on the relationship between alevin mortality and egg Se reported in Rudolph et al. (2008). This EC10 is based on a simple linear regression between a reference site and a Se exposed site, and is less than the egg Se NOEC of 20.6 µg/g dry weight for larval deformities from this same study (Table 4). Nevertheless, 17 µg/g dry

weight is assumed to represent a conservative egg Se criterion that should be protective of larval effects. Conveniently, and perhaps not surprisingly, given that Se is transferred from the ovaries to eggs and that inter-species sensitivity is not highly variable as a function of egg Se, an ovary-based EC10 of 17 µg/g dry weight is estimated by fitting a probit curve to the ovary-based concentration-response data for Se in bluegill (Figure 8). This ovary EC10 appears to also be more than adequately protective of fathead minnows, the other fish species in which ovary Se was measured in maternal transfer-based Se toxicity studies (Table 4).

In addition to augmenting the Se toxicity database with maternal transfer studies for a more diverse range of species (representing additional families), another important data gap to address is the sensitivity of exogenously feeding juvenile fish that were previously exposed to Se via maternal transfer versus those that were not. It is possible, and perhaps likely, that exposure to Se as a developing embryo may influence the toxicokinetics and toxicodynamics of Se in young developing fish relative to those not previously exposed to Se via maternal transfer. Nevertheless, the available toxicity data support that an egg or ovary Se EC10 of 17 µg/g dry weight, based on larval deformities resulting from maternal transfer of Se, is an appropriately protective tissue Se criterion that should be broadly applicable to a range of sites, although site-specific values could be higher and still protective.

Table 4: Summary of toxicity studies that evaluated Se toxicity to embryos/larvae resulting from maternal transfer. WB = whole-body.

Species	Reference	Adult Exposure	Endpoint	Tissue	Se Concentration (µg/g dry weight)			
					NOEC	LOEC	EC10	EC20
Bluegill	Bryson et al. 1984	Field	Larval mortality	Ovary	-	<49	-	-
	Bryson et al. 1985a	Field	Hatchability/swim-up	Ovary	>9.1	-	-	-
		Field	Hatchability/swim-up	Ovary	-	<30	-	-
	Bryson et al. 1985b	Field	Hatchability/swim-up	Ovary	>14.8	-	-	-
		Field	Hatchability/swim-up	Ovary	>9.2	-	-	-
	Gillespie and Baumann 1986	Field	Larval edema	Ovary	-	<38.6	-	-
	Doroshov et al. 1992	Lab	Larval edema	Ovary	3.94	21.10	15	17
		Lab	Larval edema	Egg	8.55	25.81	21	23
	Coyle et al. 1993	Lab	Larval mortality	WB	7	16	8	8.5
		Lab	Larval mortality	Ovary	20	35	24	27
		Lab	Larval mortality	Egg	22.5	41.3	22	26
	Hermanutz et al. 1996	Mesocosm	Larval edema	WB	4.4	21.8	-	-
		Mesocosm	Larval edema	Ovary	17.3	69	-	-
Fathead minnow	Ogle and Knight 1989	Lab	Reproduction	WB	>7.5	-	-	-
		Lab	Reproduction	Ovary	>10.92	-	-	-
	Schultz and Hermanutz 1990	Mesocosm	Larval edema/lordosis	Ovary	-	<39.3	-	-
	GEI Consultants	Field	Larval deformities/edema	WB	-	-	33	-
		Field	Larval deformities/edema	Ovary	-	-	45 [a]	-
Brook trout	Holm et al. 2005	Field	Larval deformities	Egg	>20	-	20 (EC06)	-
Cutthroat trout	Kennedy et al. 2000	Field	Larval deformities/ mortality	Egg	>21	-	-	-
	Hardy 2005	Lab	Larval deformities/ mortality	WB	>11.37	-	-	-
		Lab	Larval deformities/ mortality	Egg	>16.04	-	-	-
	Rudolph et al., 2008	Field	Larval deformities	Egg	20.6	46.8	-	-
		Field	Alevin mortality	Egg	-	-	17	23
Rainbow trout	Holm et al. 2005	Field	Larval deformities	Egg	17	25	26	29
Northern pike	Muscatello et al. 2006	Field	Larval deformities	Egg	3.80	31.28	20.4	33.55
White sucker	de Rosemond et al. 2005	Field	Larval deformities	Egg	-	-	26 (EC13)	-

[a] An ovary-based EC10 of 44.6502 µg/g was estimated from a whole-body EC10 of 33.07 µg/g based on the whole-body-ovary Se relationship for fathead minnows (FHM) presented in GEI Consultants (2008): FHM [Se] dw WB = 0.75826*(FHM ovary [Se] dw) - 0.78645.

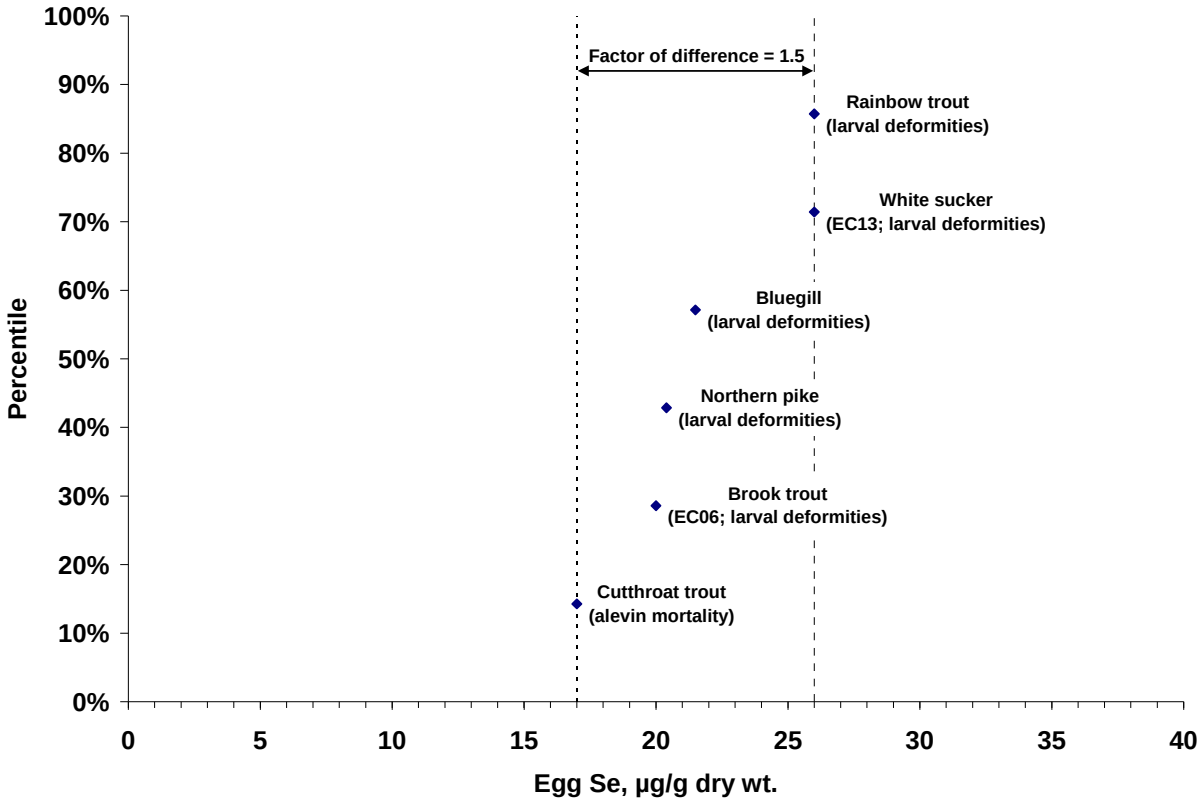

Figure 7: Species sensitivity distribution (SSD) for Se in eggs.

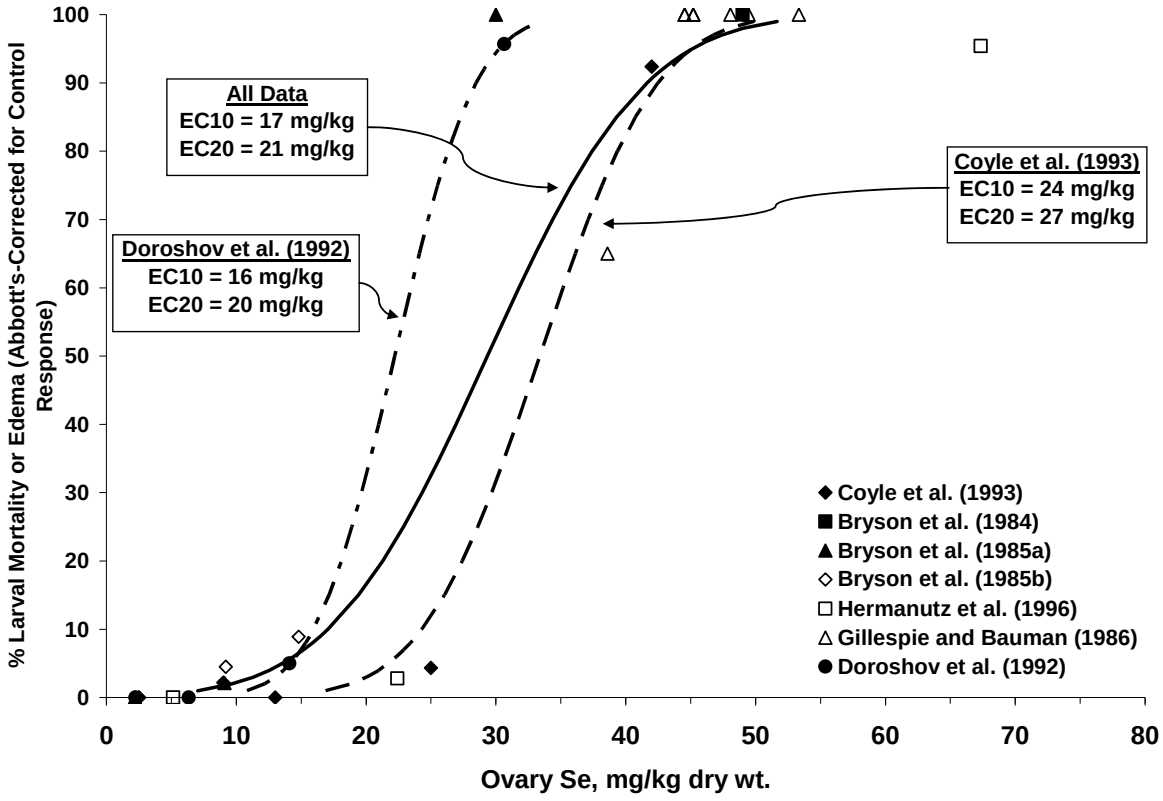

Figure 8: Concentration-response relationship for larval effects as a function of Se in ovaries.

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Part III:
Field Application of Tissue Thresholds

Potential to Predict Fish Population or Community Effects in the Field

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1.0 Introduction

The previous chapters of this report evaluated tissue thresholds based on tissue type and predictive values. The first chapter, prepared by Dr. Adrian deBruyn and Alan Hodaly at Golder, evaluated the different tissues that could be considered in developing a tissue-based Se criterion and concluded that the egg was the most appropriate tissue for setting a criterion. The second chapter, prepared by David DeForest at Parametrix, evaluated the available Se toxicity data for fish and recommended the appropriate endpoint, life stage, and statistic for developing a broadly applicable criterion. The resulting recommendation was an EC10 of 17 µg/g dry weight egg tissue, based on an analysis of larval deformities and mortality.

Selenium (Se) is an essential micronutrient required by most aquatic and terrestrial species in order to maintain metabolic function (EPA 2004). Se occurs in virtually all environmental media at trace concentrations, including rocks, soils, water, and living organisms. Anthropogenic activities such as irrigating seleniferous soils, coal and phosphorus mining, coal-fired power plants, and oil refining have increased Se beyond background concentrations in many aquatic ecosystems (Lemly 1997a).

Given that Se is an essential micronutrient, aquatic organisms readily assimilate organic forms of Se (e.g., selenomethionine), yet frequently are not able to excrete Se at the same rate of consumption at elevated concentrations. This imbalance of intake and excretion can lead to elevated tissue concentrations that can be toxic to the organism. As noted in the previous chapters, direct toxic effects have been measured in adult organisms via decreased survival or growth and in young by decreased survival (reproductive success), growth, or increased occurrences of larval deformities. Additionally, the margin between required concentrations and those that may become toxic is narrow, perhaps as low as one order of magnitude for some vertebrate species, and highly variable within and between species making toxic thresholds difficult to define.

While a tissue-based standard is likely more relevant than a water-column based value, this approach raises a potential issue: are chronic Se threshold tissue concentrations applicable in the field? "Ground-truthing" of Se-effects research is not a common analysis; however, it is important for something relatively new like a tissue-based national criterion for the protection of aquatic life. It is also important to note that "aquatic life" in the context of this approach and that used by EPA (2004) is limited to fish species. As such, protection of fish using a tissue threshold is assumed to also protect other aquatic organisms.

Many factors that are not issues in a laboratory setting come into play when dealing with field application of a tissue-based chronic criterion: effects of prior exposure, determining whether mortality is additive or natural, migration/immigration, increased sensitivity of

juveniles versus adults, effects of other contaminants, density-related effects, seasonal effects, habitat quality, water flow regime, and so on.

Little research on Se-related population effects has been performed. The most well-known case study involving population-level effects of Se is Belews Lake, where, at a mean water column Se concentration of 10 µg/L, reproductive failure resulted in the disappearance of 19 out of 20 fish species (Lemly 2002a). Only Se-tolerant mosquitofish (*Gambusia affinis*) survived.

Several studies which address the issue regarding applicability of tissue-based standards to natural populations are described in this chapter. The majority of research to-date has documented population-level Se impacts (elimination of fish populations) in lakes and reservoirs receiving coal fly ash discharges (Bryson et al. 1984; Garrett and Inman 1984; Lemly 1985). However, there are few population-level evaluations of fish from other lakes with naturally elevated Se inputs. Given this general lack of lake data, the following analysis necessarily concentrates on studies that evaluate the impacts of Se to fish populations in streams.

In addition, while the previous chapters concluded that egg tissue is the best predictive tissue for Se toxicity, with a recommended threshold of 17 µg/g dry weight egg tissue Se, few data exist on egg or ovary concentrations from field studies. As such, the analyses that follow necessarily rely on comparisons of fish population metrics to whole-body Se concentrations.

2.0 Field-Based Selenium Tissue Threshold Studies

2.1 Warmwater Stream Studies

A number of studies on warmwater stream fish communities from locations with natural geologic Se sources (Colorado) and industrial sources (refinery discharge in Texas and power plant discharge in Ohio) were evaluated. Although data on other warmwater streams were found, they could not be used in this analysis due to a lack of a key component, such as no fish Se tissue data, no water column Se concentration data, or no habitat data for comparison to population effects. It is important to have information on all potential stressors, not just Se concentrations, to best evaluate trends in fish populations with elevated tissue levels. This requirement limited the number of available studies, but did allow a thorough analysis of stressors in those studies.

2.1.1 Arkansas River (Pueblo) CO (2004-2007)

2.1.1.1 Study Background and Methods

The objectives of this study were to collect aquatic biological data, determine background chemical data, and define physical habitat characteristics of a portion of the Arkansas River and nearby tributaries in the vicinity of the City of Pueblo, Colorado, to evaluate relationships between Se concentrations and fish populations (GEI 2007a).

Data collected to accomplish the study goals included 1) seasonal sampling over a period of 2 to 3 years of fish and macroinvertebrate populations to determine the "health" of the aquatic biota, in terms of the species composition and relative abundance of aquatic organisms; 2) collection of whole-body fish tissue, composite macroinvertebrate tissue, and sediment samples for the analysis of Se bioaccumulation pathways; and 3) physical habitat measurements, including sediment particle size, to determine relationships between the biota and their environment (GEI 2007a). Data were collected from fall 2004 to fall 2006 from the Arkansas River, Fountain and Wildhorse Creeks, and the St. Charles River (Figure 1). Together, these data were used to evaluate potential population-level effects due to elevated Se.

Water column samples were collected by City of Pueblo personnel as part of the larger Se load allocation study conducted by ARCADIS G&M, Inc. (2006). All samples were analyzed for total Se and dissolved sulfate by the City of Pueblo.

Not all sites could be sampled for all data during all sampling episodes due to flow, access, changes in site location, changes in scope or changes resulting from review of past data. Figures below note those sites or dates where data are not available by an asterisk.

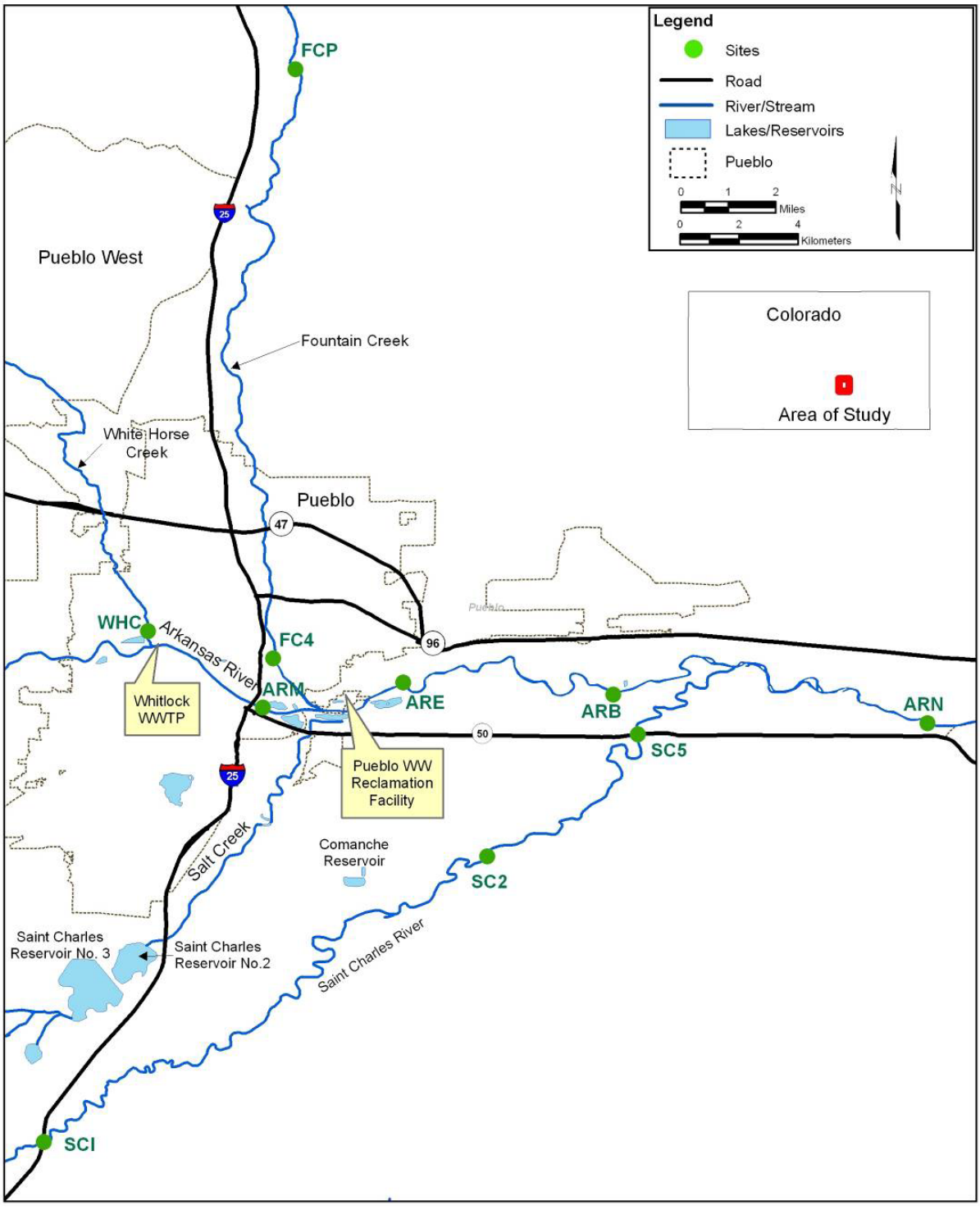


Figure 1: Location of study sites on the Arkansas River, Wildhorse Creek, Fountain Creek, and the St. Charles River, 2004 - 2006.

Total Se concentrations for whole-body fish tissue, macroinvertebrate tissue, and sediment were obtained. All whole-body fish tissue Se concentrations were then converted to dry weight concentrations using fish sample-specific percent solids data to facilitate comparisons between media (e.g., fish vs. sediment) and sites, as well as to be consistent with the draft Se criteria document (EPA 2004).

Potential relationships between fish population metrics, Se concentrations (species-specific, macroinvertebrate, and sediment Se concentrations) and habitat characteristics were evaluated with all possible regressions and linear regression using NCSS statistical software (Hintze 2000). Paired data were evaluated at the site level (e.g., site mean fish tissue vs. total number of species) and at the fish family level (e.g., mean replicate tissue concentrations for species within the family Cyprinidae vs. species-specific density). Given paired data are necessary for such analysis, only population data collected in 2005 and 2006 were included in these analyses.

An accompanying analysis had also been conducted to evaluate the sources of Se within the reach of the Arkansas River from upstream of the city of Pueblo to the confluence with the Huerfano River (ARCADIS G&M, Inc. 2006). The purpose of that study was to determine if irrigation of the Se-rich lands along the Arkansas River was contributing significantly to the elevated Se levels noted within this reach. Measurements taken during this study showed marked increases in Se concentrations in the Arkansas River in a downstream direction. Similar increases were observed in Fountain Creek, with the Se concentrations near the confluence of Fountain Creek and the Arkansas River about four times higher than concentrations in Fountain Creek near the city of Fountain, about 30 miles north of Pueblo. Sulfate concentrations in both these streams also increased in a downstream direction (ARCADIS G&M, Inc., 2006).

Analysis of surface and groundwater sources of Se clearly indicated that the amount of Se discharged into the Arkansas River from nonpoint sources, including groundwater, as a result of irrigation return flows was small relative to other natural sources (ARCADIS G&M, Inc., 2006). Wildhorse Creek and Fountain Creek were the largest contributors of Se to the Arkansas River from the measured surface water sources to the river within the reach studied, adding approximately 22 percent (2.8 kg/day) and 21 percent (2.6 kg/day) of the total Se load, respectively. The Pueblo Water Reclamation Facility, a permitted source, was the seventh largest contributor of Se, with an average of 0.8 kg/day. However, Se from this source is not originating from any of the processes occurring at the facility. Instead, the Se is entering the facility through the infiltration of groundwater into the sanitary sewer system, and can therefore also be considered a natural source of Se (ARCADIS G&M, Inc., 2006).

Groundwater Se concentrations were influenced more by geology than irrigation practices, with average Se concentrations in shale-influenced zones estimated as being 100 times higher than concentrations in the alluvial zones, regardless of irrigation practices in those areas. Irrigated alluvial zones did not have notably higher Se concentrations than non-irrigated

areas, indicating that irrigation practices did not significantly affect Se levels in these materials (ARCADIS G&M, Inc., 2006). The results of this study indicated that Se in this reach of the Arkansas River and tributaries occurs from natural sources, with insignificant human-induced additions to total loading. This was an important consideration in the evaluation of effects of elevated Se to fish populations. Since elevated concentrations of Se are naturally occurring at the study sites, this study provided a unique evaluation of Se impacts to fish populations that have been exposed naturally to elevated Se for potentially thousands of years.

2.1.1.2 Summary of Fish Populations

2.1.1.2.1 Arkansas River Mainstem Sites

Twenty-one species of fish and one hybrid fish were collected in the Arkansas River during the 2005 and 2006 sampling efforts (Table 1). The number of fish species collected at each site ranged from nine to 14 species. Only white suckers were collected from every site during both years. Other species commonly collected from these sites included central stonerollers, fathead minnows, flathead chubs, green sunfish, largemouth bass, longnose dace, red shiners, sand shiners, and smallmouth bass. Black bullheads, channel catfish, green sunfish/bluegill hybrids, orangespotted sunfish, plains killifish, saugeye, white crappie, and yellow bullheads were only rarely collected from one or two sites over the two years of sampling.

Density estimates ranged from 238 fish/acre to 1,300 fish/acre (Figure 2). Density was higher at most sites in 2005, although Site ARB had a slightly higher density of fish collected in 2006. No single fish species dominated in terms of density across all the Arkansas River sites, but central stonerollers, flathead chubs, red shiners, sand shiners, and smallmouth bass were numerically dominant at one or more sites and sampling times and were generally common in relatively large numbers throughout this reach of the Arkansas River (GEI 2007a).

Table 1: Density estimates for fish species collected from the Arkansas River mainstem sites in fall 2005 and 2006.

Fish Species			Density (number/acre)							
			2005 Sampling					2006 Sampling		
Family	Common Name	Scientific Name	ARM	ARE	ARB	ARN	ARM	ARE	ARB	ARN
Catostomidae										
	Longnose Sucker	*Catostomus catostomus*	110	--	12	7	--	--	--	5
	White Sucker	*Catostomus commersoni**	186	208	186	64	30	50	49	30
Centrarchidae										
	Bluegill	*Lepomis macrochirus*	135	--	--	--	50	--	20	--
	Green Sunfish	*Lepomis cyanellus**	219	6	--	6	30	--	10	--
	Green Sunfish x Bluegill Hybrid	*NA*	--	--	--	--	30	--	--	--
	Largemouth Bass	*Micropterus salmoides*	203	19	--	39	--	50	59	43
	Orangespotted Sunfish	*Lepomis humilus**	203	--	--	--	10	--	--	--
	Smallmouth Bass	*Micropterus dolomieu*	228	47	35	32	110	--	--	--
	White Crappie	*Pomoxis annularis*	8	--	--	13	--	--	--	--
Cyprinidae										
	Central Stoneroller	*Campostoma anomalum**	--	13	244	6	380	--	118	--
	Common Carp	*Cyprinus carpio*	--	--	47	13	--	--	--	15
	Fathead Minnow	*Pimephales promelas**	--	32	221	26	20	60	--	--
	Flathead Chub	*Platygobio gracilis**	--	231	23	26		460	196	5
	Longnose Dace	*Rhinichthys cataractae**	8	--	35	--	60	10	20	--
	Red Shiner	*Cyprinella lutrensis**	--	33	81	286	--	420	118	101
	Sand Shiner	*Notropis stramineus**	--	334	93	102	--	--	69	10
Fundulidae										
	Plains Killifish	*Fundulus zebrinus**	--	--	35	--	--	10	--	--
Ictaluridae										
	Black Bullhead	*Ameiurus melas*	--	--	--	--	30	10	--	--
	Channel Catfish	*Ictalurus punctatus**	--	--	12	--	--	10	--	--
	Yellow Bullhead	*Ameiurus natalis**	--	--	--	6	--	--	--	--
Percidae										
	Saugeye	*Stizostedion canadense*	--	--	--	--	--	10	--	10
Poeciliidae										
	Mosquitofish	*Gambusia affinis*		--	--	13	--	--	10	19
		Total Density	1,300	923	1,024	638	750	1,090	669	238
		# of Native Species	4	7	9	8	6	7	7	4
		Total # of Species	9	9	12	14	10	10	10	9

* = Native to the Arkansas River basin.

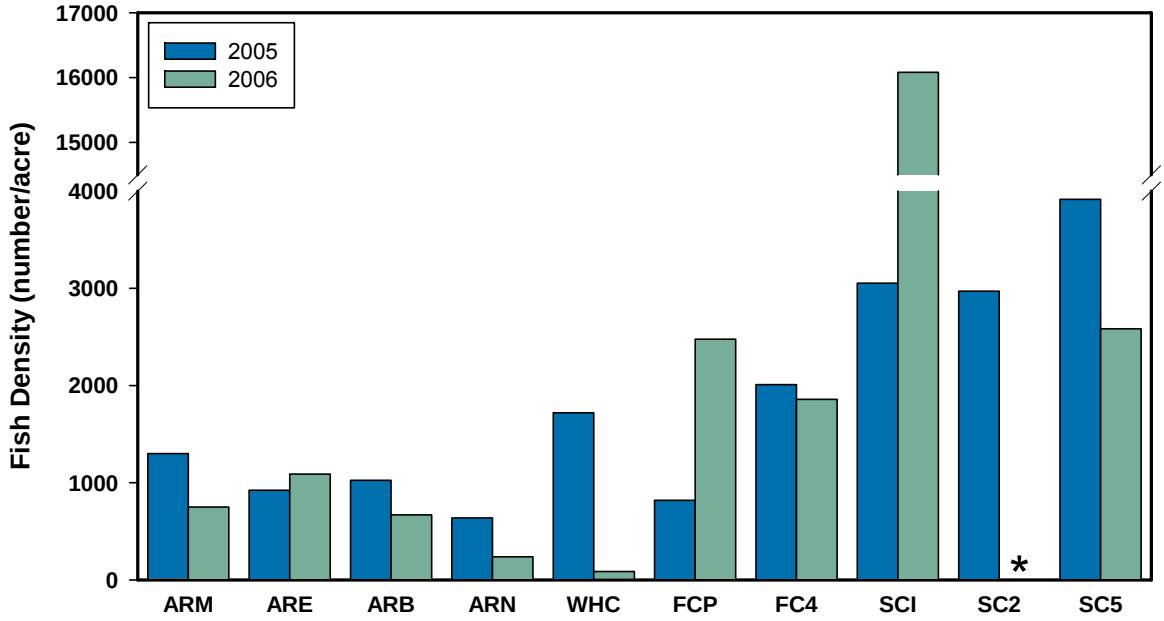


Figure 2: Fish density estimates (number/acre) at sites on the Arkansas River, Wildhorse Creek, Fountain Creek, and St. Charles River in fall 2005 and 2006.

The length-frequency data collected for the fish species at these sites indicates multiple age groups present for most of the species at the sites. Weight-length "condition" parameters, such as the Fulton condition factor (K) and relative weight (Wr), also showed that the fish species collected from the Arkansas River sites are generally healthy in regards to being an appropriate weight for their length (GEI 2007a).

Fish at each site were observed for any visual abnormalities (i.e., deformities) or injuries. Few abnormalities were noted for fish from the Arkansas River sites, with the observed abnormalities (caudal lesions and black spotted fins) probably due to a parasitic infection. A single central stoneroller from Site ARM collected in 2006 had a deformed tail, and a fathead minnow collected from Site ARE in 2005 was missing one eye. These abnormalities and injuries were noted on a very low percentage of the total fish collected at each site (< 6 percent), and are likely related to factors other than Se, according to the Lemly (1997b) deformity index.

Based on these results, the fish populations within this reach of the Arkansas River are similar in species composition, but are highly variable in terms of density, biomass, and population characteristics between sites and between years. While total densities were generally lower in 2006 than in 2005, no single fish species was responsible for this trend throughout these study sites.

Higher water was noted at all the Arkansas River sites in 2006 in comparison to 2005, with accompanying physical habitat changes that likely contributed to the variation between years

at these sites. Monthly mean discharge data from U.S. Geological Survey gages within the area (Gages 07099970, 07106300, 07109500, and 07106500) support these observations, with discharges near or more than twice as high during the month of sampling in 2006 in comparison to 2005. At Site ARE in particular, the higher water resulted in the elimination of some of the riffle and bank habitat that was present the previous year. The hydrologic differences between years may have made the habitat more homogenous and less hospitable to certain fish species or displaced organisms. Higher water levels may also have affected sampling efficiency.

Several fish species collected in this reach of the Arkansas River appear to have established stable, reproducing populations, with juveniles and adults present at one or more of the Arkansas River sites during the study. However, others were much more variable over the two years of sampling, with some species present in one year in high numbers, and either absent or in low numbers in the other year. These data indicate that some species possibly moved out of these sites or were temporarily eliminated from them over the two year span of the study, while other species became established. The fish condition factors indicate that the fish populations are generally in fair to good condition, with a low rate of abnormalities.

2.1.1.2.2 *Wildhorse Creek Sites*

Two species of fish, central stonerollers and white suckers, were collected in 2005 and 2006 from the site on Wildhorse Creek. Both of these species were common in the Arkansas River sites. Central stonerollers were numerically dominant during both years of sampling (Table 2). Density estimates at Site WHC were 20 times higher in 2005 than they were in 2006 (Figure 2). Both species contributed to this decrease, with central stonerollers decreasing by 91 percent and white suckers by 99 percent between 2005 and 2006.

Despite the large differences between years, the age class distribution of central stonerollers was similar between years, indicating a reproducing population that includes both juvenile and adult fish in both years. White suckers did not follow that pattern, as the fish collected in 2005 were YOY and age class 1+ fish, while the fish collected in 2006 did not include any YOY fish (GEI 2007a).

Table 2: Fish density estimates for sites on Wildhorse Creek, Fountain Creek, and the St. Charles River, fall 2005 and 2006.

Fish Species			Density (number/acre)										
			2005 Sampling						2006 Sampling				
Family	Common Name	Scientific Name	WHC	FCP	FC4	SCI	SC2	SC5	WHC	FCP	FC4	SCI	SC5
Catostomidae													
	White Sucker	*Catostomus commersoni**	794	74	15	51	--	780	5	124	11	833	--
Centrarchidae													
	Bluegill	*Lepomis macrochirus*	--	--	--	--	--	--	--	--	--	83	
	Green Sunfish	*Lepomis cyanellus**	--	--	--	--	--	8	--	--	--	83	--
	Largemouth Bass	*Micropterus salmoides*	--	--	--	--	--	16	--	--	--	--	--
Cyprinidae													
	Central Stoneroller	*Campostoma anomalum**	926	107	15	1,061	20	764	81	94	198	7,250	95
	Fathead Minnow	*Pimephales promelas**	--	8	--	667	2,495	228	--	--	55	1,333	27
	Flathead Chub	*Platygobio gracilis**	--	598	1,330	61	202	1,433	--	2,093	1,385	--	14
	Longnose Dace	*Rhinichthys cataractae**	--	8	--	--	--	220	--	51	11	167	--
	Red Shiner	*Cyprinella lutrensis**	--	25	15	242	--	323	--	--	99	3,500	770
	Sand Shiner	*Notropis stramineus**	--		618	889	40	134	--	114	77	2,333	1,662
Fundulidae													
	Plains Killifish	*Fundulus zebrinus**	--	--	--	81	212	8	--	--	22	500	14
Ictaluridae													
	Black Bullhead	*Ameiurus melas**	--	--	15	--	--	--	--	--	--	--	--
		Total Density	1,720	819	2,010	3,052	2,969	3,914	86	2,476	1,858	16,082	2,582
		# of Species	2	6	6	7	5	10	2	5	8	9	6

* = Native to the Arkansas River basin.

The two fish species at Site WHC are in fair condition based on their length-weight ratios (GEI 2007a). No abnormalities were observed for fish collected in 2005, but an eroded caudal fin and spotted fins were observed on one central stoneroller each in 2006. Condition values are within the ranges observed at the Arkansas River sites.

As with the Arkansas River sites, the differences between the two years of study are likely correlated to the increased water levels and accompanying habitat changes that occurred at this site. These changes appear to have had a more pronounced effect at Site WHC, possibly due to its smaller size. Observations in the field indicated that higher water levels had decreased habitat complexity at this site in 2006, and resulted in muddy water throughout the site.

2.1.1.2.3 Fountain Creek Sites

Nine species were collected from the two Fountain Creek sites in 2005 and 2006, all of which were species that had also been collected from the Arkansas River sites (Table 2). Central stonerollers, flathead chubs, and white suckers were collected from both sites and years, and longnose dace, red shiners, and sand shiners were also collected during most sampling events. Black bullheads and plains killifish were rare, being collected only from Site FC4 at low abundances. The number of species collected from each site ranged from five to eight.

Densities at these two sites were generally higher than densities at the Arkansas River sites (Figure 2). Flathead chubs dominated the fish populations numerically at both sites, composing 66 percent to 85 percent of the total density. Central stonerollers, sand shiners, and white suckers were also abundant at one or both sites. Length-frequency analysis of the flathead chubs indicated that the populations are reproducing, with juvenile and older adult fish present in relatively high numbers at both sites and years (GEI 2007a). Fish condition index values indicated that most species of fish present in Fountain Creek are in only fair condition, possibly indicating that food resources are limited for some species. The only abnormality or injury observed at these sites was a tail lesion noted on a single flathead chub, potentially indicating a parasitic infection.

2.1.1.2.4 St. Charles River Sites

Eleven species of fish were collected from the St. Charles River sites between 2005 and 2006 (Table 2). All of the species present in the St. Charles River sites were also present in one or more of the Arkansas River sites. The number of species collected at each site ranged from five to ten. No trend towards higher or lower species richness was observed between years. Four species were collected from each site each year: central stonerollers, fathead minnows, plains killifish, and sand shiners. Flathead chubs and red shiners were also commonly collected, but bluegills and largemouth bass were rare.

Densities were higher than those seen at the Arkansas River sites and other tributary sites (Figure 2). No consistent trend of increasing or decreasing densities was observed, with total density estimates that were similar between sites and years, with the exception of the much higher density estimate observed at Site SCI in 2006. The high density at Site SCI resulted largely from substantial increases in central stonerollers, red shiners, and sand shiners which, when combined, made up 81 percent of the total density that year. No single species numerically dominated all of the St. Charles River sites, but central stonerollers, fathead minnows, flathead chubs, and sand shiners were dominant at sites for one or both years.

Length-frequency analysis of the fish populations at the St. Charles River sites indicated that sites had reproducing populations of central stonerollers, fathead minnows, and sand shiners, with juvenile and adult fish collected during both years (GEI 2007a). Fish populations at

Site SC5 varied extensively between years, with several species that were present in relatively high numbers during 2005 not being collected at all or only in small numbers in 2006. These differences between years are probably related to the changes in habitat that occurred at this site. In 2005, this site was dominated by riffle and run habitat, but by the following year, beaver activity had resulted in the entire site being inundated and consisting of standing water only. Despite these changes, both years of sampling indicated that central stoneroller, red shiner, and sand shiner populations were present and reproducing at this site.

Compared to sites on the other streams, a higher number of abnormalities and injuries were observed in the St. Charles River. Black-spotted fins were observed on several central stonerollers, fathead minnows, flathead chubs, red shiners, and sand shiners. While such abnormalities are of concern, spotted fins are likely the result of parasitic infections rather than Se (Lagler 1956; Herman 1990).

No abnormalities were observed in fish from Site SC2 in 2005, but at Site SC5, collected flathead chubs, red shiners, and white suckers had lesions on their fins. However, the fish with these lesions made up less than two percent of the fish collected, and no lesions were observed during the 2006 sampling event. Again, lesions are more of a sign of a parasitic infection rather than a result of possible high Se concentrations. Additionally, a shortened opercule, a deformed jaw, and a growth on the underside of the mouth (potential Se-related deformities) were noted in one flathead chub each in 2005. These three fish composed less than 1 percent of the fish collected at this site.

2.1.1.2.5 *Fish Population Summary*

Composition of fish populations in the Arkansas River, Wildhorse Creek, Fountain Creek, and the St. Charles River within the study area are similar, with the same species present in the tributary sites as were found in the Arkansas River mainstem sites. Population parameters such as density and biomass varied substantially between sites and years, and were often higher at the Fountain Creek and St. Charles River sites than Arkansas River sites. No other consistent trends were noted in biomass or density among sites or streams. Several sites, most notably those on Wildhorse Creek and the St. Charles River, had substantial changes in the populations of certain fish species from 2005 to 2006, with fish present one year in high numbers and a variety of age classes, and either absent the other year or present only in low numbers.

Such changes are likely linked to the higher stream flows present in 2006 and significant habitat changes due to beaver activity at some sites. Variable population parameters are not uncommon in plains streams with highly variable flow regimes and habitat conditions (Schlosser 1987). No or few fish were observed as having abnormalities or injuries at most sites, and the abnormalities noted at the remaining sites were more consistent with signs of parasitic infections than increased Se levels. There is also the potential for "survivor bias" with regard to deformities that is inherent in analyses of field populations – i.e., Se-related

deformity effects could be occurring, but masked by mortality (Lemly 2002b). In part, this can be accounted for in analysis of potential Se effects through "weighting" the presence of Se-related deformity occurrence with age of the fish (Lemly 2002b).

2.1.1.3 Habitat Evaluation Summary

Habitat at sites on the Arkansas River, Wildhorse Creek, and Fountain Creek consisted primarily of fast water habitats such as runs and riffles, and the habitat generally changed little between the two years of the study at most sites. The St. Charles River sites were more variable between years, with substantial habitat and flow alterations at Sites SCI and SC5 resulting from beaver activity.

In general, the sites on the Arkansas River and the Fountain Creek sites were more similar to each other than the sites on Wildhorse Creek and the St. Charles River. The segments of the Arkansas River and Fountain Creek within the study area are wider and have higher velocities than sites on the other two streams. They also had higher amounts of bank cover, and some sites had backwater areas which provide increased habitat diversity for fish. The sites on Wildhorse Creek and the St. Charles River had more limited cover and less habitat complexity than most sites on the other streams.

Substrate composition has many significant impacts on the aquatic biota in streams. Heterogeneous substrate provides a variety of microhabitats for organisms to colonize, resulting in a diverse macroinvertebrate community. Fish feeding, spawning, and habitat requirements are likewise affected by the types of substrate available in streams. Substrate in the Arkansas River, Wildhorse Creek, Fountain Creek, and St. Charles River was relatively homogenous, and tended to be dominated by sand at most sites (Figure 3). Streambeds composed largely of fine substrates such as sand and silt are typical for plains streams, and are generally less stable than streambeds with higher percentages of larger substrate. Sites on the St. Charles River were the only two sites to show substantial changes in substrate composition over the two study years. As previously discussed, beaver activity altered the habitat noticeably at both sites between 2005 and 2006. As a result, the percent of the substrate composed of silt was over twice as high at those two sites in 2006 in comparison to 2005.

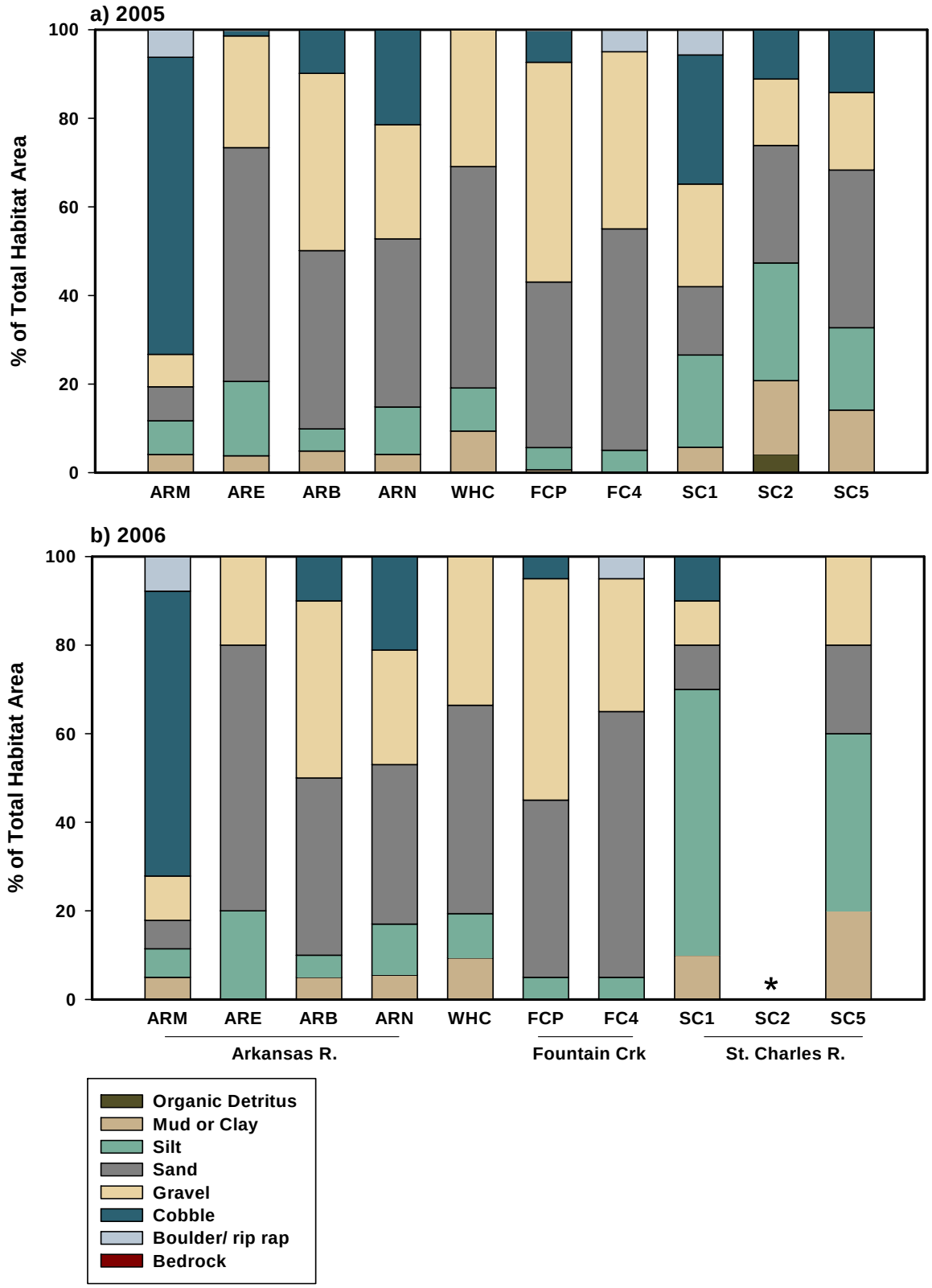


Figure 3: Percent substrate for sites on the Arkansas River, Wildhorse Creek, St. Fountain Creek, and the St. Charles River in fall of a) 2005 and b) 2006 (* = no data collected).

2.1.1.4 Summary of Selenium Effects Analyses

Fine sediment mean Se concentration was greater than coarse sediment Se concentration at all sites (Figure 4). A positive relationship between total mean sediment Se concentration (mean includes coarse and fine sediment concentrations), mean dissolved Se water column concentration, and mean sediment TOC (mean includes coarse and fine sediment percentages) was observed (Figure 5) when all sites are included in a regression analysis, similar to that reported by Van Derveer and Canton (1997).

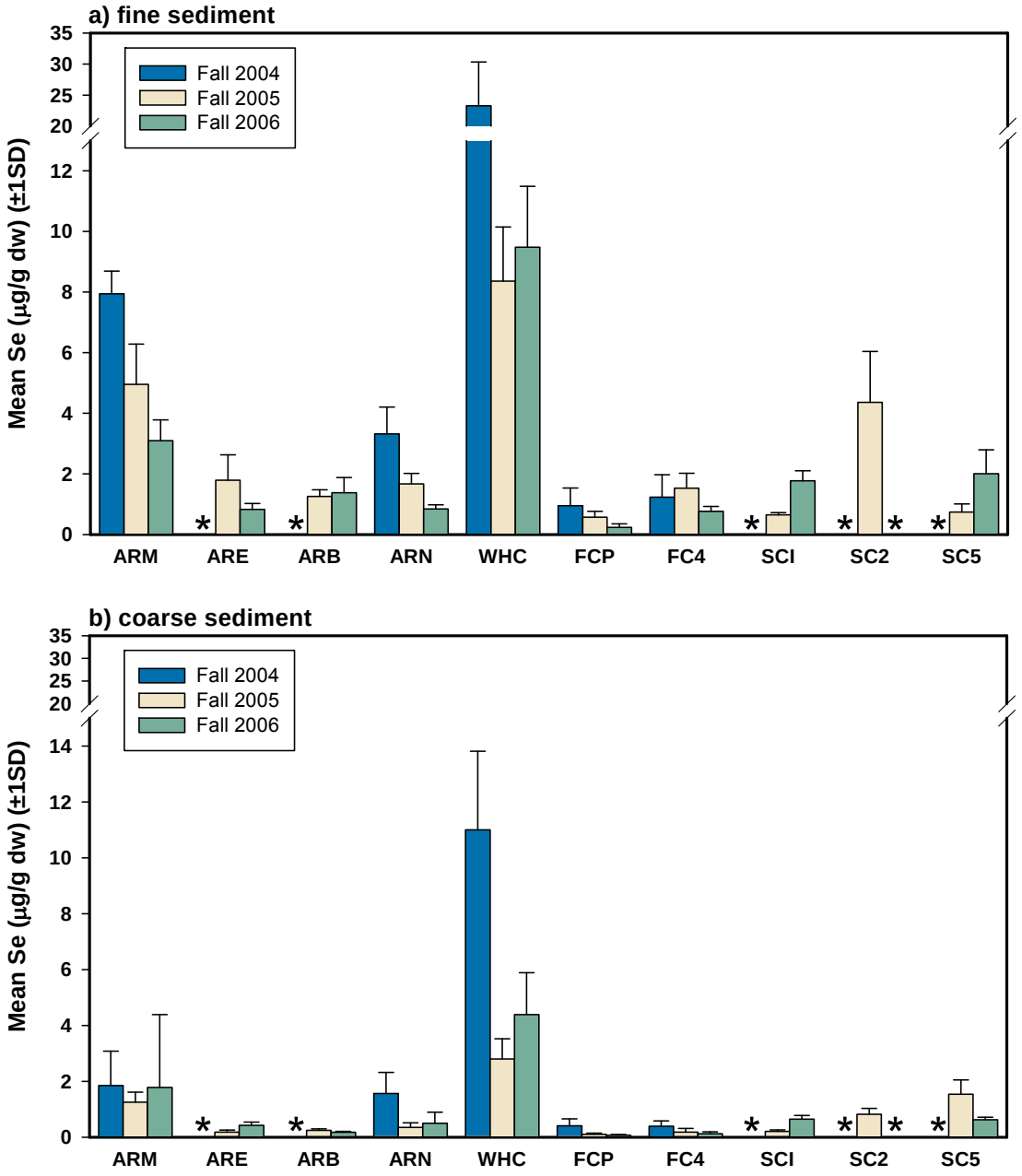


Figure 4: Mean a) fine sediment and b) coarse sediment Se concentrations (n = 5) collected during fall sampling in 2004 - 2006 (* = no data collected).

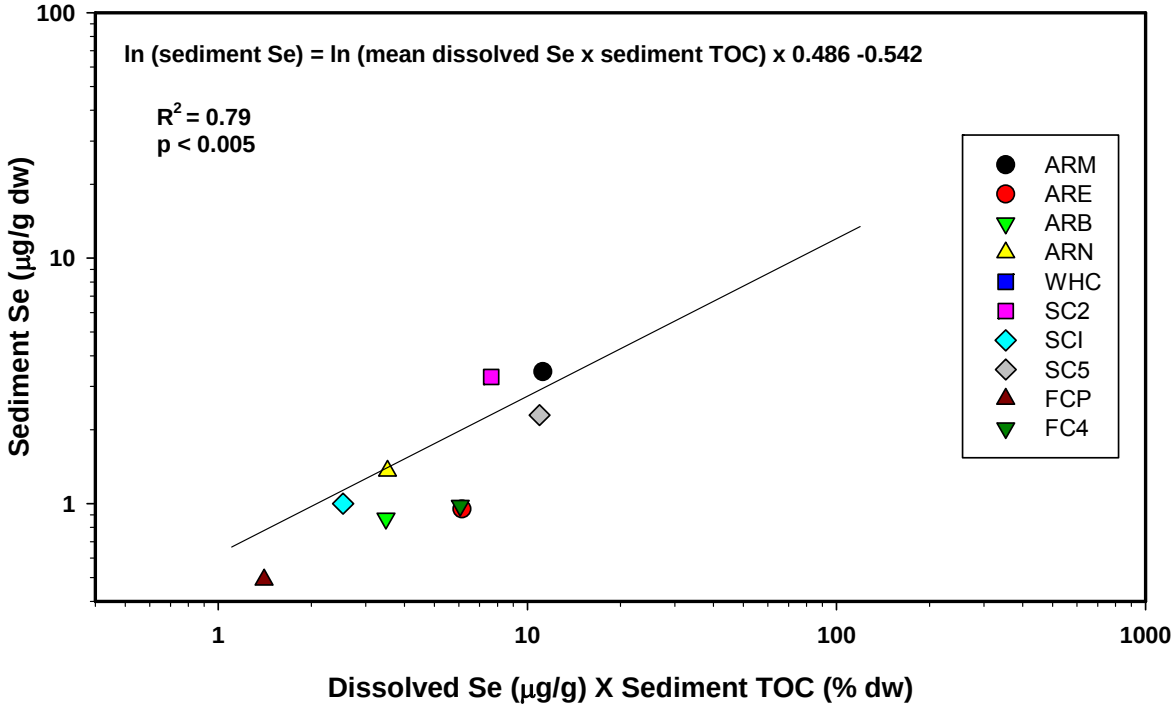


Figure 5: Relationship between mean total sediment Se concentration and the mean dissolved Se-sediment total organic carbon (TOC) interaction.

Total Se water column concentrations were generally elevated throughout the study area, with only the upper reaches on the St. Charles River and Fountain Creek having mean Se concentrations below the EPA chronic standard of 5 µg/L Se (Table 3). Se concentrations measured during the biological study at the Wildhorse Creek site were more than 20 times greater than all of the other biological sampling locations, with a mean concentration of 418 ± 115 µg/L. Even the minimum concentration measured at WHC (315 µg/L) were 7 times greater than the maximum Se concentration measured at other study sites (43.6 µg/L as SC5).

Table 3: Total Se concentration summary for paired surface water and biological (GEI) sampling locations.

Water Quality Sampling Location	GEI Sampling Location	Start	End	Total Se (µg/L)					
				n	Min	Max	Mean	±	SD
Arkansas River									
AR-17 Arkansas at Moffat	ARM	1/4/05	8/24/06	15	2.33	14.1	7.05	±	3.69
AR-19 Arkansas at CS-10	ARE	1/6/05	2/25/06	9	4.48	15.5	10.6	±	4.06
AR-18 Offtake at Excelsior Ditch	ARB	1/13/05	10/15/05	7	3.02	13.4	8.72	±	4.00
AR-27 Arkansas at Nyberg	ARN	1/6/05	10/15/05	8	4.64	11.7	8.81	±	2.85
Tributaries									
AR-09 WP-118 Wildhorse near mouth	WHC	1/4/05	6/8/06	17	315	715	418	±	115
FC-05 Fountain Creek above Pinon	FCP	1/5/05	2/25/06	9	1.72	4.93	3.43	±	1.05
FC-09 Fountain Creek above confluence	FC4	1/5/05	6/8/06	12	7.49	21.1	12.1	±	4.34
AR-36 St. Charles R. at I-25	SCI	1/22/05	6/8/06	6	1.43	4.75	3.09	±	1.37
AR-22 St. Charles below Bessemer	SC2	1/14/05	6/8/06	11	2.64	19.8	11.7	±	6.22
AR-23 St. Charles at Hwy 50	SC5	1/6/05	6/8/06	13	3.74	43.6	20.3	±	13.0

The mean Se concentration increased in the Arkansas River downstream of the confluence with Fountain Creek. In the St. Charles River, mean Se concentrations were generally greater than the Arkansas River (Table 3). The most downstream site (SC5) had the second highest mean Se concentration of 20.26 ± 13.0 µg/L. Although mean Se concentrations were high, variability in concentrations was also high, which suggests high Se exposure to aquatic life via the water column may not be constant.

Mean invertebrate Se tissue concentrations varied by season, year, and site and ranged from 6.0 µg/g dry weight to 45.50 µg/g dry weight (Table 4). Individual tissue concentrations ranged from less than the minimum detection limit to 90.4 µg/g dry weight (Figure 6). Although mean macroinvertebrate Se concentrations varied by site and watershed, only the mean macroinvertebrate Se concentration for WHC was significantly different (higher) from other sites.

Table 4: Mean benthic macroinvertebrate Se tissue concentration by site for all years and seasons.

Site	Mean [Se] (µg/g dw)	Standard Deviation	n
Arkansas River			
ARM	8.74	1.65	5
ARE	16.80	4.78	4
ARB	12.05	5.64	4
ARN	9.40	2.86	5
Tributaries			
WHC	45.50	28.58	5
FCP	7.90	2.92	5
FC4	14.78	4.19	5
SCI	6.00	2.91	3
SC2	19.66	7.58	3
SC5	16.69	14.68	4

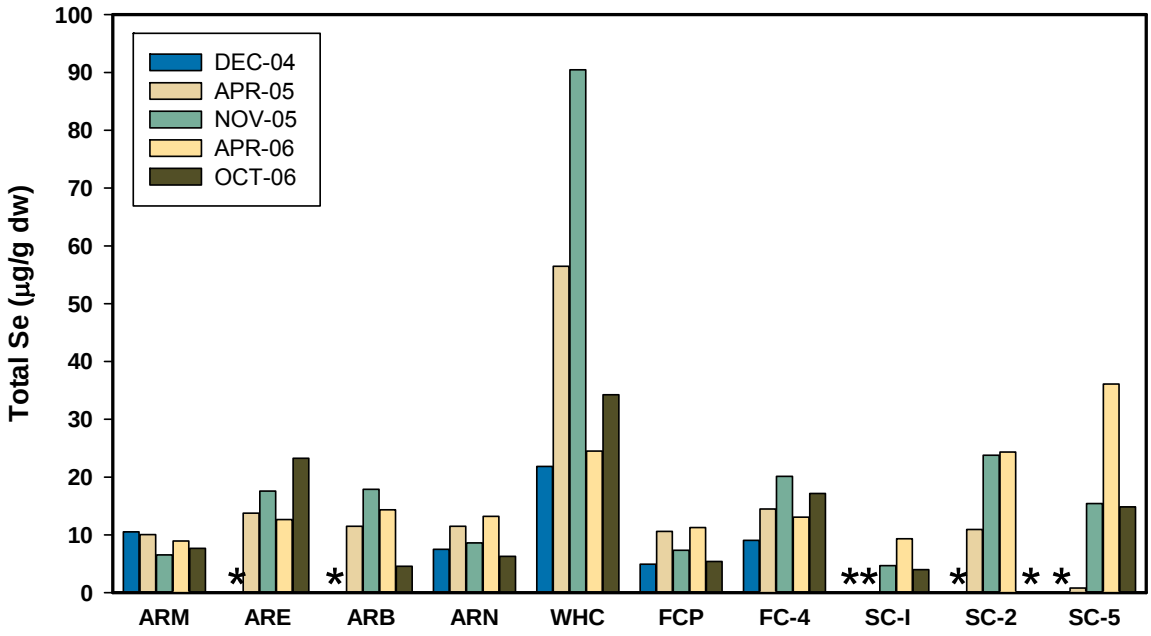


Figure 6: Total Se concentration of composite benthic macroinvertebrate tissues (n = 1) for each sampling date and location (* = no data collected).

Mean Se concentrations in fish tissue generally increased from the most upstream sampling location to the most downstream location within a watershed. Statistical differences between sites and years were observed (one-way ANOVA $P < 0.001$), with lower fish Se concentrations during the 2004 sampling than both 2005 and 2006. No statistical differences were observed between the most upstream sites in the Arkansas River, Fountain Creek, and St. Charles River (Figure 7). Furthermore, the Arkansas River sites that are downstream of the confluence with Fountain Creek were not significantly different from the Fountain Creek site located immediately upstream from the confluence (FC4).

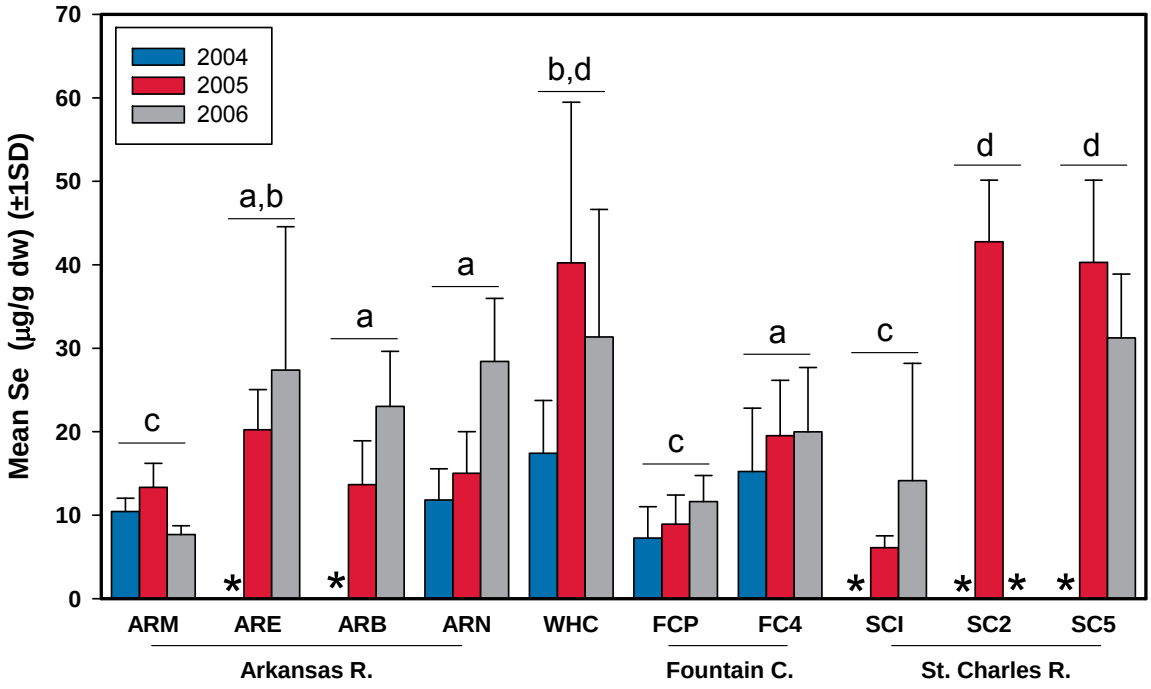


Figure 7: Mean fish tissue Se concentration by site and year. Means equal the arithmetic mean of all species and replicates sampled that year. WHC = Wildhorse Creek. Different letters indicate significant differences between site means (one-way ANOVA on site means followed by Bonferroni, P < 0.005) (* = no data collected).

The greatest fish tissue concentrations were collected from the two downstream St. Charles River sites (Table 5). Equally high Se concentrations were measured in fish collected from Wildhorse Creek (Figure 7). The most upstream sites on the Arkansas River, Fountain Creek, and the St. Charles River were all statistically similar ($p > 0.05$; Figure 7) and were generally among the lowest fish tissue concentrations observed.

Table 5: Site mean Se whole-body fish tissue concentration. Means include all species and sampling dates.

Site	Mean Fish Selenium (µg/g dw)	Standard Deviation	n
Arkansas River			
ARM	10.30	3.03	32
ARE	23.81	12.91	30
ARB	18.68	7.58	28
ARN	18.31	8.94	35
Tributaries			
WHC	31.56	17.50	25
FCP	9.25	3.82	30
FC4	18.60	7.30	19
SCI	10.75	11.27	19
SC2	42.75	7.38	6
SC5	37.64	9.39	17

Se tissue concentrations varied noticeably by fish family. Se tissue concentrations were measured for three cyprinids (central stoneroller, sand shiner, red shiner), one catostomid (white sucker), and three centrarchids (green sunfish, smallmouth bass, and largemouth bass) (Figures 8 and 9). Mean concentrations in all cyprinids were greater (21.06 µg/g dry weight; SE = 1.38) than either centrarchids (19.73 µg/g dry weight; SE = 1.32) or catostomids (17.52 µg/g dry weight; SE = 1.52). However, there was no statistical difference between the overall mean for the three families (p = 0.19). Most mean whole-body Se concentrations were well above the EPA (2004) chronic tissue criterion of 7.91 µg/g dry weight.

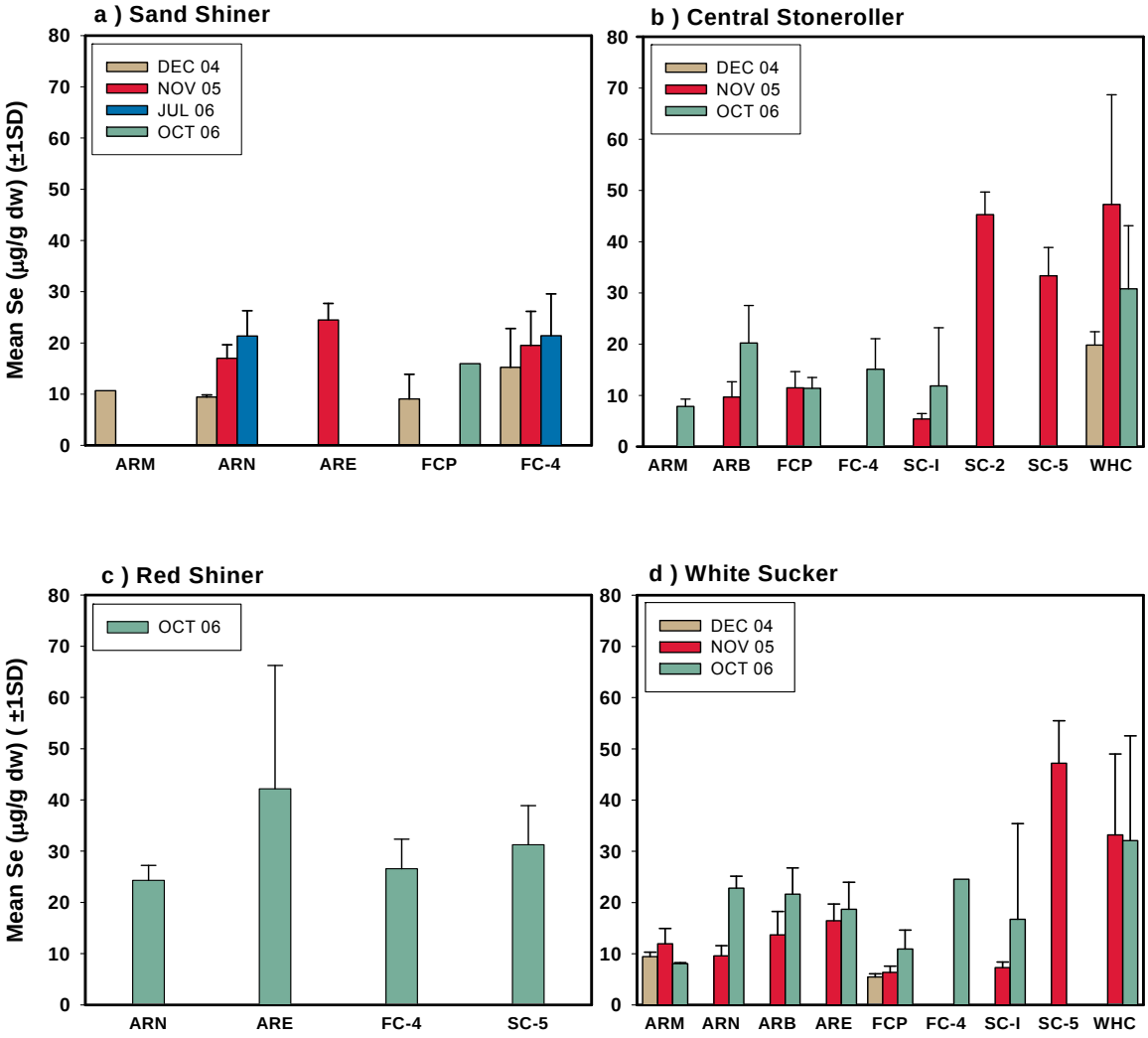


Figure 8: Mean whole-body Se concentration of cyprinid species sampled for tissue analyses including a) sand shiner, b) central stoneroller, and c) red shiner and one catostomid represented by the d) white sucker. Lack of a bar indicates samples were not collected during that sampling event.

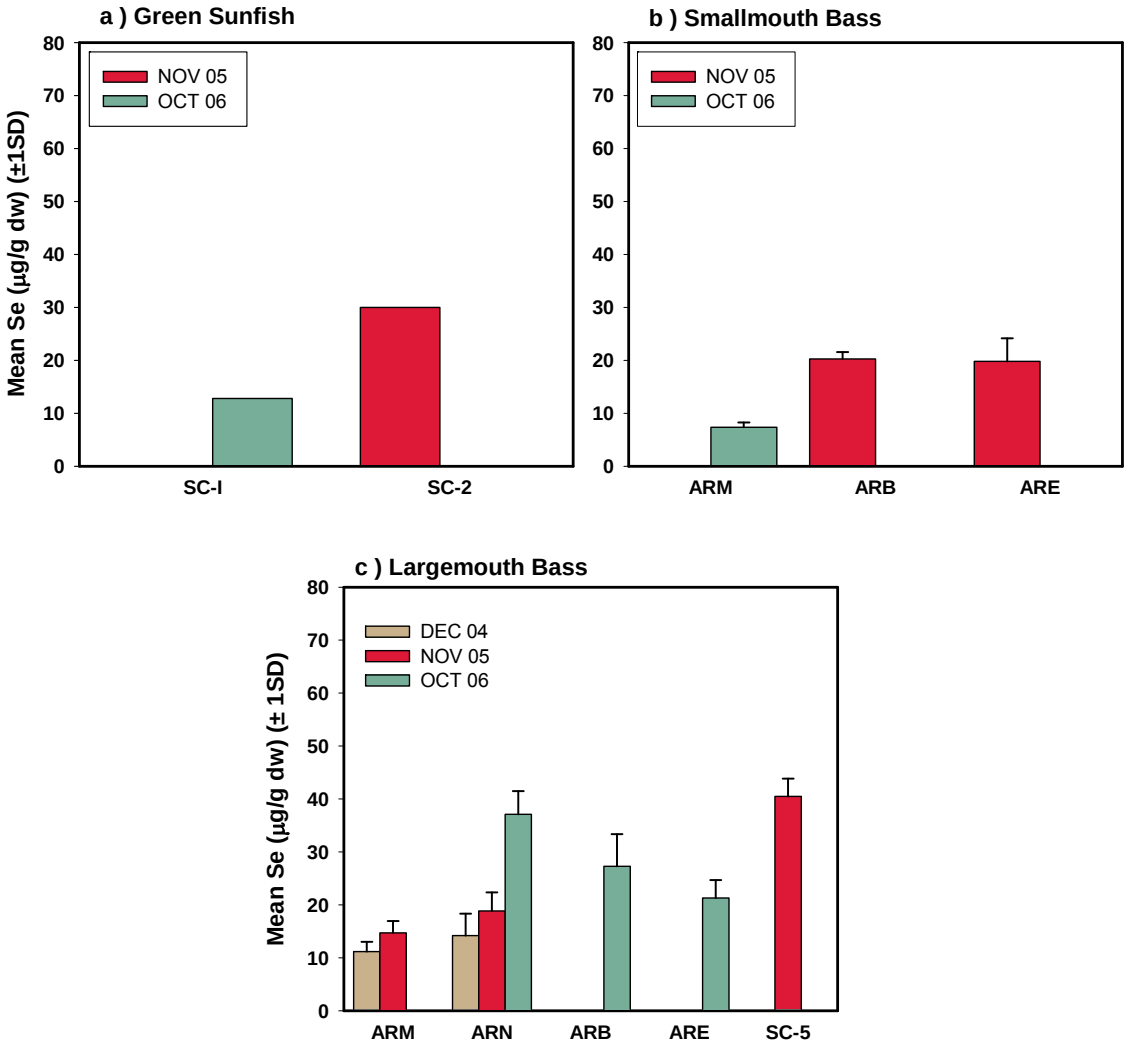


Figure 9: Mean whole-body Se concentration of centrarchid species sampled for tissue analyses including a) green sunfish, b) smallmouth bass, and c) largemouth bass. Lack of a bar indicates samples were not collected during that sampling event.

Media with measured Se concentrations and sediment TOC collected simultaneously with fall fish population sampling were analyzed in an all possible regression analysis (Hintze 2000) to determine the most likely parameters affecting bioaccumulation pathways for Se throughout the study area. For the fall data, site-mean fish tissue Se concentrations (all species combined) were most influenced by macroinvertebrate tissue concentrations, followed by coarse sediment Se concentration and sediment TOC. Out of these three variables, only macroinvertebrate Se had a significant positive relationship with whole-body fish tissue concentration ($R^2 = 0.38$; $p = 0.001$) for the fall data (Figure 10). Although these three variables were most influential, together they explained only 45 percent of the variability in fish tissue Se concentrations.

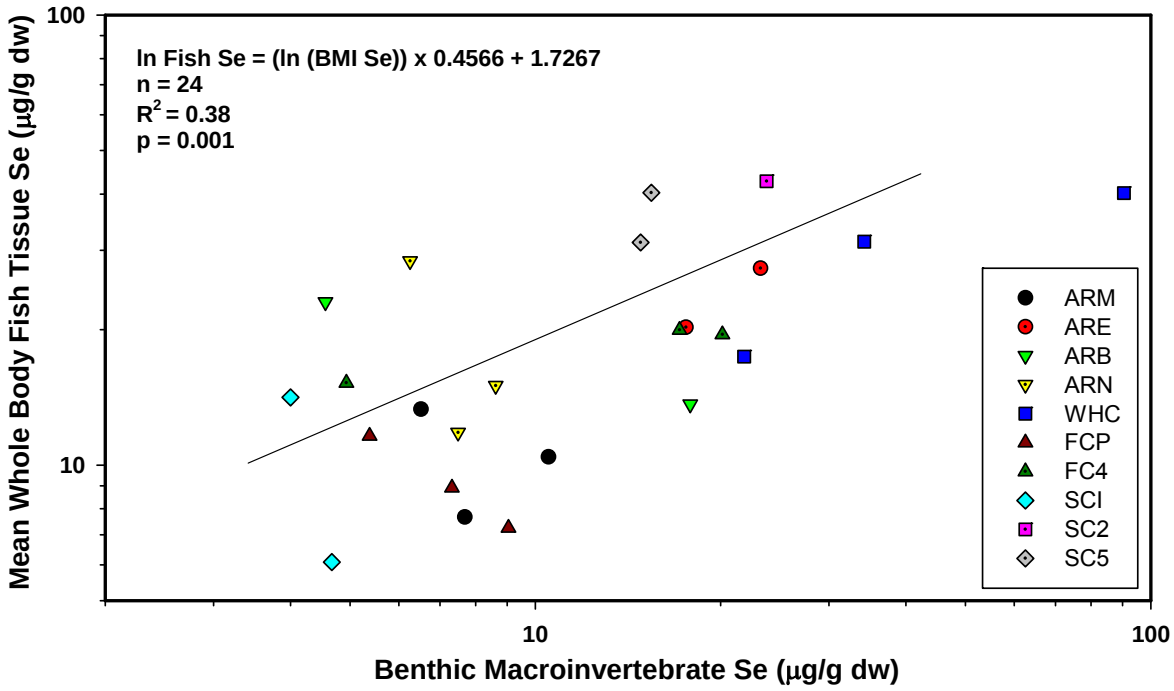


Figure 10: Relationship between benthic macroinvertebrate (BMI) Se tissue concentration and mean whole-body fish tissue Se concentration.

Fish density, when weighted by habitat availability, was not significantly related to tissue Se. Rather, substrate conditions, represented by percent silt and boulder/rip rap, explained most of the variability in total fish density weighted by habitat ($R^2 = 0.44$, two-parameter model). Although silt by itself likely does not directly contribute to greater fish densities, high silt may be an indicator of high primary production that could influence food availability, or diverse habitat that contains slow backwater or eddy refugia.

While a slight negative relationship was observed between densities of cyprinid species and their whole-body Se concentrations (i.e., as Se concentrations increased, density decreased), this relationship was not statistically significant ($R^2 = 0.0239$; $p = 0.5120$). However, when habitat parameters, substrate characteristics, and Se data are included in an all possible regressions analysis, the strongest two-parameter model suggests percent silt and whole-body Se have the greatest influence on cyprinid densities. Together, percent silt and Se explain 49 percent of the variability in the data, with percent silt explaining most of the variability. A three-parameter model of percent silt, whole-body Se, and percent sand explains 57 percent of the variability in the densities of cyprinid species, indicating a potential habitat-related confounding factor in the evaluation of potential Se effects.

Grouping species-specific data by the family Catostomidae results in trends that are similar to trends observed with cyprinids. Catostomid densities (equivalent to white sucker density) were not significantly correlated with whole-body Se concentrations ($R^2 = 0.02$; $p = 0.65$).

Rather, a significant positive relationship was once again observed with percent silt ($R^2 = 0.25$; $p = 0.05$).

A significant negative relationship was observed between whole-body Se and species-specific densities for the family Centrarchidae ($R^2 = 0.5257$; $p = 0.02$). However, densities of centrarchid species were also significantly correlated with primary habitat average depth ($R^2 = 0.74$; $p < 0.001$); greater population densities were associated with greater habitat depths. A three-parameter model identified primary habitat depth, percent sand, and percent cobble as having the greatest influence on centrarchid density. Together, these three parameters explain 90 percent of the variability in centrarchid density. These results and the significant negative relationship between mean primary habitat depth and centrarchid Se suggest population differences between sites are more related to habitat parameters than whole-body Se concentrations.

Despite a wide range of Se concentrations in the water, sediments, invertebrates and fish, no significant relationships were observed between total fish densities or fish species richness and site mean fish tissue Se concentrations. When individual fish families are considered, it does appear there is a significant relationship between species-specific whole-body Se concentrations and densities within the family Centrarchidae (bass and sunfish). No significant relationships were observed in the other two dominant families in the study sites, Cyprinidae or Catostomidae.

However, it is important to note that attempts to determine Se effects were highly confounded by significant correlations between fish population metrics and primary habitat average depth. In fact, habitat parameters, in general, consistently explained more of the variability in fish parameters than whole-body fish tissue Se, suggesting that tissue concentrations were not driving populations despite the wide range of tissue levels.

2.1.2 Dixon Creek/Canadian River (Conoco) TX (2007)

2.1.2.1 Study Background and Methods

This study determined relationships between aquatic life and instream Se concentrations in Dixon Creek, an effluent-dominated stream in the panhandle of Texas (Figure 11). This project utilized biological, chemical, and physical data from Dixon Creek and the Canadian River, as well as sites on two reference streams (White Deer Creek and Rock Creek), to examine potential Se effects in reference, upstream, and downstream aquatic sampling sites, analysis of fish population metrics versus Se in water and tissues, and overall fisheries health (GEI 2007b).

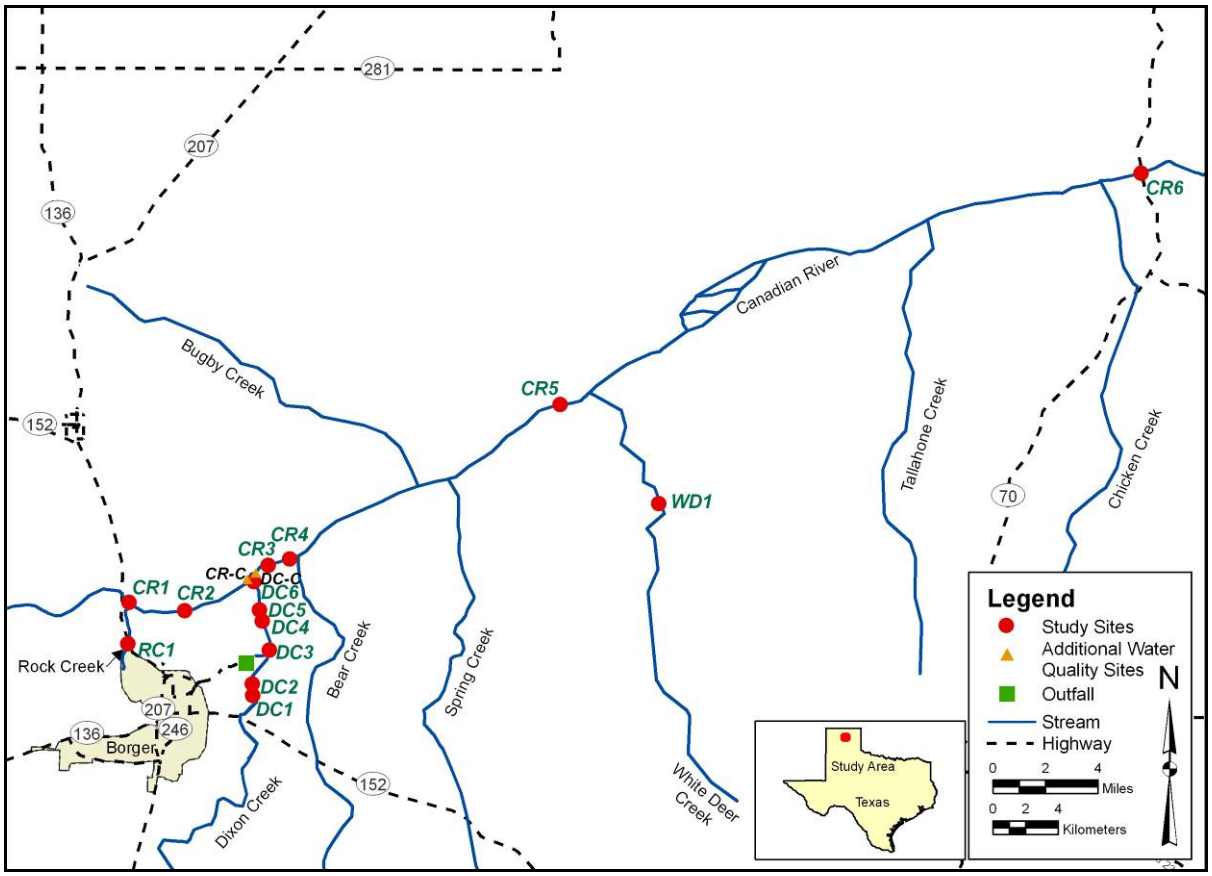


Figure 11: Location of sampling sites in northern Texas.

Dixon Creek receives effluent from the Borger Refinery approximately 5 miles from its confluence with the Canadian River. Historically, this segment of Dixon Creek has not met the state of Texas chronic Se water quality standard of 5 µg/L.

Field data were collected during early summer and late fall of 2006 for habitat quality, fish population, and fish tissue data following Texas Commission on Environmental Quality (TCEQ) guidance. Fish tissues were analyzed for total Se and percent solids. In addition to population metrics, the collected fish were examined for abnormal and teratogenic phenotypes. This information was used to estimate potential population mortality from Se exposure according to the Lemly (1997b) deformity index. Long-term water quality data were also used to determine ambient Se exposure in fish.

In addition to data collected during the current study, results from previous studies were also examined (RMT 1999; Weston Solutions 2004). The purpose of the 1999 RMT study was to determine if site-specific Se water quality criteria were appropriate for Dixon Creek. The second study by Weston Solutions, Inc. (2004) was conducted in response to the EPA's request to perform additional modeling for confirmation that the water quality standard of 5 µg/L would be met in the Canadian River during low-flow, steady state conditions. As part

of this study, fate and transport of Se in Dixon Creek were evaluated and provided to the TCEQ for Se fate modeling.

Physical habitat and substrate characteristics were measured during the first sampling event at each site; conditions were evaluated using the TCEQ (2005) Texas Habitat Quality Index (THQI) scoring system. The THQI protocol scores habitat condition using nine instream habitat, substrate stability, channel characteristics, and riparian condition categories. Habitat was also surveyed using a method based on the R1/R4 fish habitat inventory procedures developed by the U.S. Forest Service (Overton et al. 1997). This method includes measurements of a variety of physical parameters related to habitat types, channel configuration, and substrate composition. In each habitat unit, substrate composition was characterized by visual observation as organic detritus, fine sediment (silt and clay), coarse sediment (sand), gravel, rubble (cobble), boulder, or bedrock. The relative distribution of substrate types was used to characterize fish habitat and Se bioaccumulation potential for each site (Canton and Van Derveer 1997).

Fish collection methods followed guidelines outlined by the state of Texas for collection of freshwater fish (TCEQ 2005). All collected fish were observed for deformities in the field to determine species and site teratogenic deformity index (TDI) ratings as described by Lemly (1997b). Deformities examined for in the field included abnormal spots, fin erosion, tail erosion, lesions, red fins, and spinal, mouth, eye, opercula, and gill abnormalities.

Whole-body fish tissue samples were collected for Se tissue analyses from each site during fish population sampling. The goal was to collect five replicate whole-body samples from one species from each fish family at each site. All Se concentrations were converted to dry weight concentrations to facilitate comparisons between species and sites, as well as to be consistent with the EPA draft Se criteria document (EPA 2004).

Water samples were collected from the established sampling sites on a weekly or monthly basis by Borger Refinery personnel. Water quality analyses included total Se and sulfate, which is a selenate acute toxicity mitigating factor and used for final acute criteria derivation (EPA 2004). Borger Refinery water quality data were available from January 2005 through December 2006. Water column Se data at many of the study sites were also collected during previous studies conducted by RMT (1999) and Weston Solutions, Inc. (2004).

To elucidate potential influences of Se exposure on Dixon Creek and Canadian River fish populations, relationships between fish population metrics, tissue Se concentrations, water quality parameters, and habitat variables were analyzed. To allow for the most biologically relevant comparisons between variables, some analyses required condensation of results into site, species, and family (Centrarchidae, Cyprinidae, and Catostomidae) level effects.

2.1.2.2 Results

Evaluation of the stream habitat within the study area using the THQI scoring system indicated that the upstream sites on Dixon Creek and the Canadian River offered less quality habitat than downstream sites on these streams or reference stream sites due to limited flows. Instream habitat at all Canadian River and Dixon Creek sites was dominated by slow water habitat types such as pools and glides, with higher complexity and more fast water habitat types observed at the reference sites on White Deer Creek and Rock Creek. Generally, higher proportions of fine substrates and organic matter were observed at the downstream Dixon Creek sites than at the other study sites, indicating a higher potential to accumulate and cycle Se at these sites (Canton and Van Derveer 1997).

A total of 16 fish species and one hybrid were collected from the study sites when data from summer and fall were combined. A total of 2,733 fish in summer and 4,633 fish in fall were inspected for deformities in 2006. The overall occurrence of Se-related deformities was low for all species and sampling sites. Of all deformities observed, spinal deformities were most common (n = 37 out of 7,366 fish), followed by cloudy eyes (n = 7 out of 7,366 fish). When evaluating all sites, a slightly greater frequency of deformities was observed in the summer (2 percent) compared to the fall (less than 1 percent) with no more than five percent of the total number of fish collected at each site having some type of deformity. Deformity rates were used to determine TDI ratings as described by Lemly (1997b, 2002b). The percent deformities of all species that exhibited terata deformities for both seasons were less than 20 percent for all sites analyzed, and thus would receive a TDI rating of 1. Therefore, the expected Se-induced mortality would be less than five percent. The TDI index scores and the evaluation of the deformity frequency in the 7,366 observed fish indicates that the anticipated impact from Se is negligible (Lemly 2002b).

Whole-body fish tissues for Se analysis were collected from a total of 13 different species representing six families during the summer and fall of 2006. In total, 296 fish tissue samples were analyzed for Se and percent solids. The families and species sampled for Se analyses included the family Centrarchidae represented by bluegill, green sunfish, green sunfish x bluegill hybrid, smallmouth bass, and longear sunfish; the family Cyprinidae represented by bullhead minnows, carp, red shiners, suckermouth minnows, and sand shiners; the family Fundulidae represented by plains killifish; the family Cyprinodontidae represented by Red River pupfish; the family Poeciliidae represented by the western mosquitofish; and the family Ictaluridae represented by the yellow bullhead.

Analysis of the Se concentration data from the reference and upstream sites suggests that the "background" site mean Se concentrations would be expected to be 5 μg/g or less within the study area. The fish tissue results clearly show greater Se concentrations in fish collected in the downstream Dixon Creek and Canadian River sites than those at upstream sites (Figure 12). Western mosquitofish were the only species found at every downstream site and Poeciliidae had the greatest tissue Se concentration of any fish family. Centrarchid and

cyprinid tissue concentrations were also relatively high when compared to other family mean concentrations. Tissue data collected from several fish species during a previous study in 1999 (RMT 1999) were not statistically different from the 2006 concentrations.

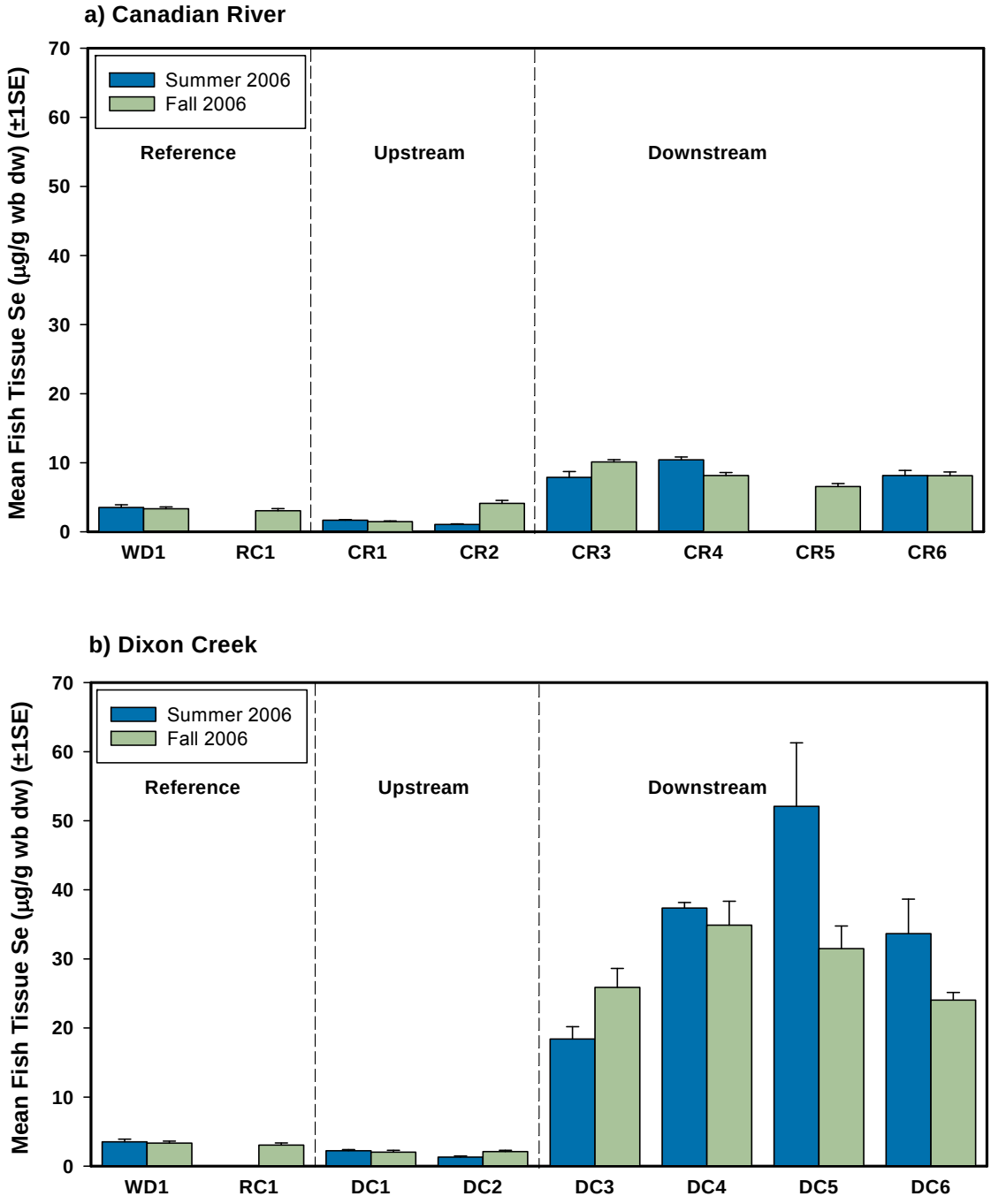


Figure 12: Mean Se fish tissue concentrations (µg/g whole-body dry weight) of all fish species collected in a) the Canadian River and b) Dixon Creek sites near the Borger Refinery. Sites CR5 and RC1 were not sampled in summer 2006.

Total Se concentrations in the water column and effluent were provided by the Borger Refinery from 2005 through 2006. An increase in Se concentrations within the water column in Dixon Creek was observed downstream of the Borger Refinery. Trends in the Dixon Creek Se data suggest that Se is removed from the water column or diluted before reaching the Canadian River (Figure 13).

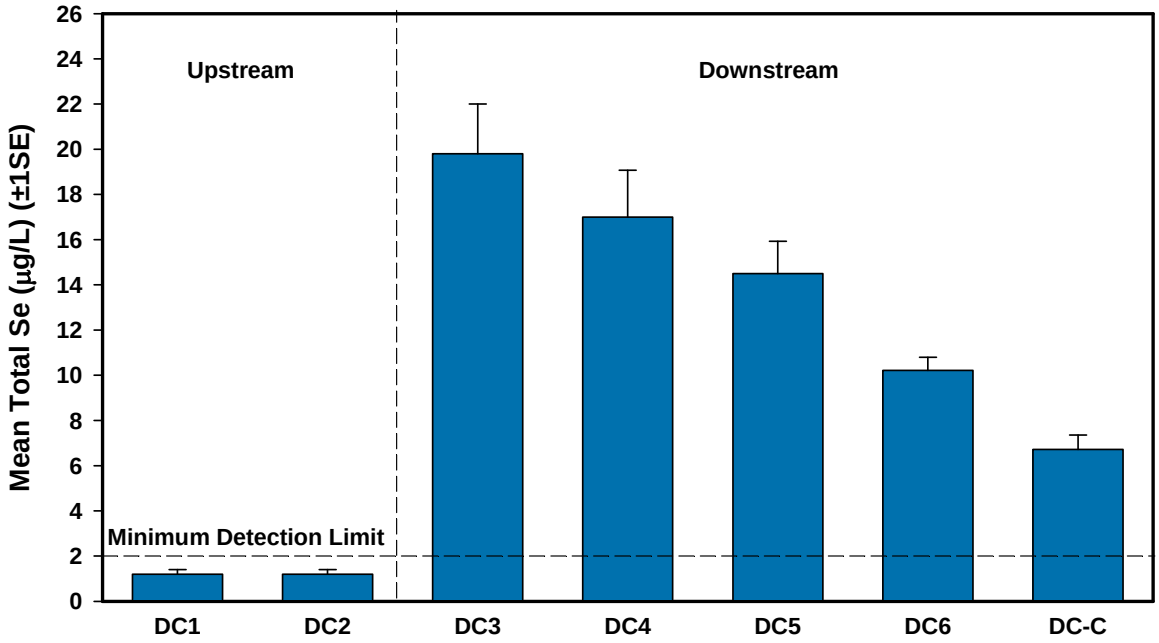


Figure 13: Mean water column total Se concentrations for the Dixon Creek sites from 2005 through 2006. DC-C = water quality sampling location on Dixon Creek downstream of DC6, immediately upstream of the Canadian River-Dixon Creek confluence.

A statistically significant negative relationship was observed between ln-transformed mean Se tissue concentrations and centrarchid species density ($p = 0.003$; Figure 14). In contrast, a weak positive relationship was found between cyprinid Se tissue concentrations and density, but it was not statistically significant ($p = 0.849$; Figure 15). These trends for centrarchids and cyprinids are consistent with trends observed in the Arkansas River Basin (GEI 2007a). Regression analyses with species in the families Cyprinodontidae, Fundulidae, and Poeciliidae resulted in similar weak positive relationships between Se tissue concentrations and density, as seen with the cyprinids. None of these relationships were statistically significant.

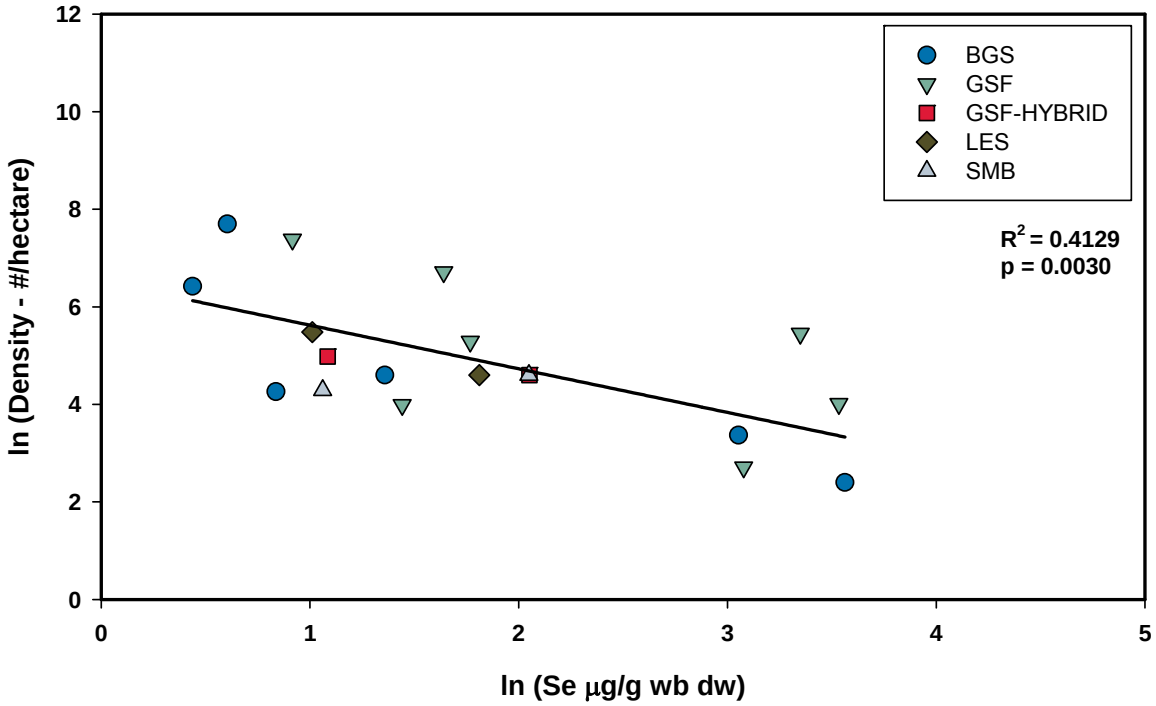


Figure 14: **Relationship between ln-transformed mean Se concentrations in centrarchid species and corresponding species density estimates for fish collected during 2006 summer and fall sampling events.**

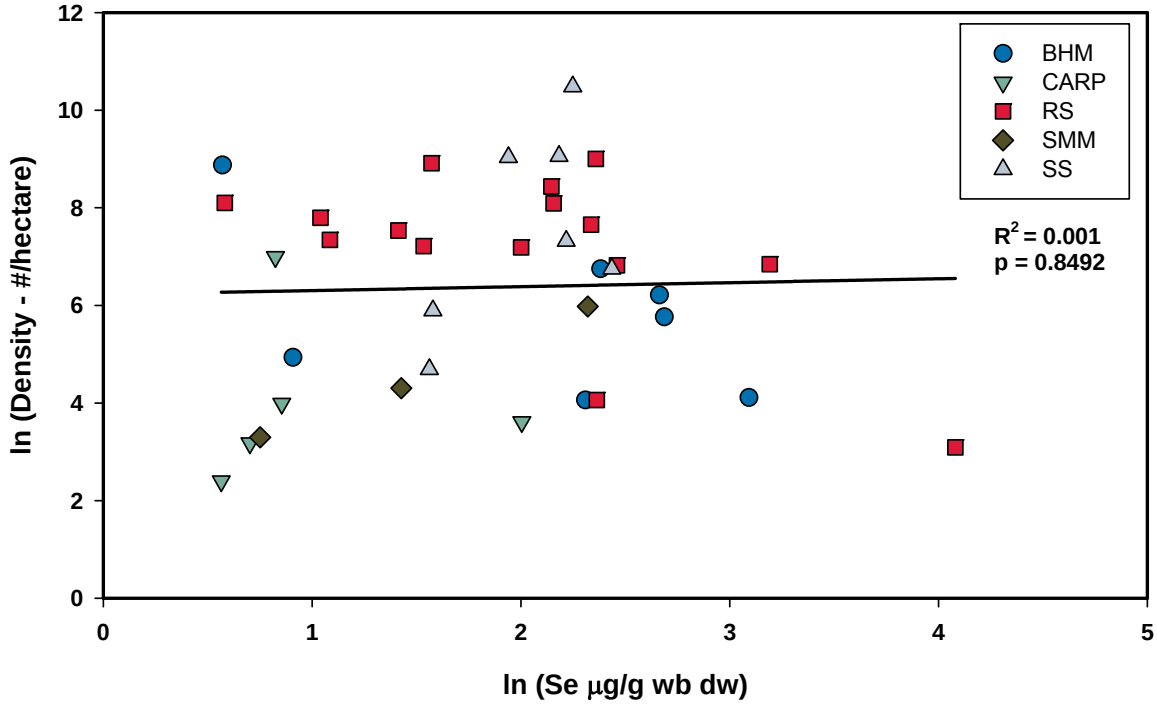


Figure 15: **Relationship between ln-transformed cyprinid species mean Se concentration and corresponding species density estimates for fish collected during 2006 summer and fall sampling events.**

To investigate the influences of habitat quality on fish population metrics, the relationship between fish IBI scores and THQI scores was examined. A statistically significant positive relationship was observed between fish IBI and THQI scores for the study sites ($R^2 = 0.245$; $p = 0.012$; Figure 16). The increase in fish community health associated with increasing habitat quality would be expected, but the relative contribution of habitat to fish health compared to Se impacts is unknown.

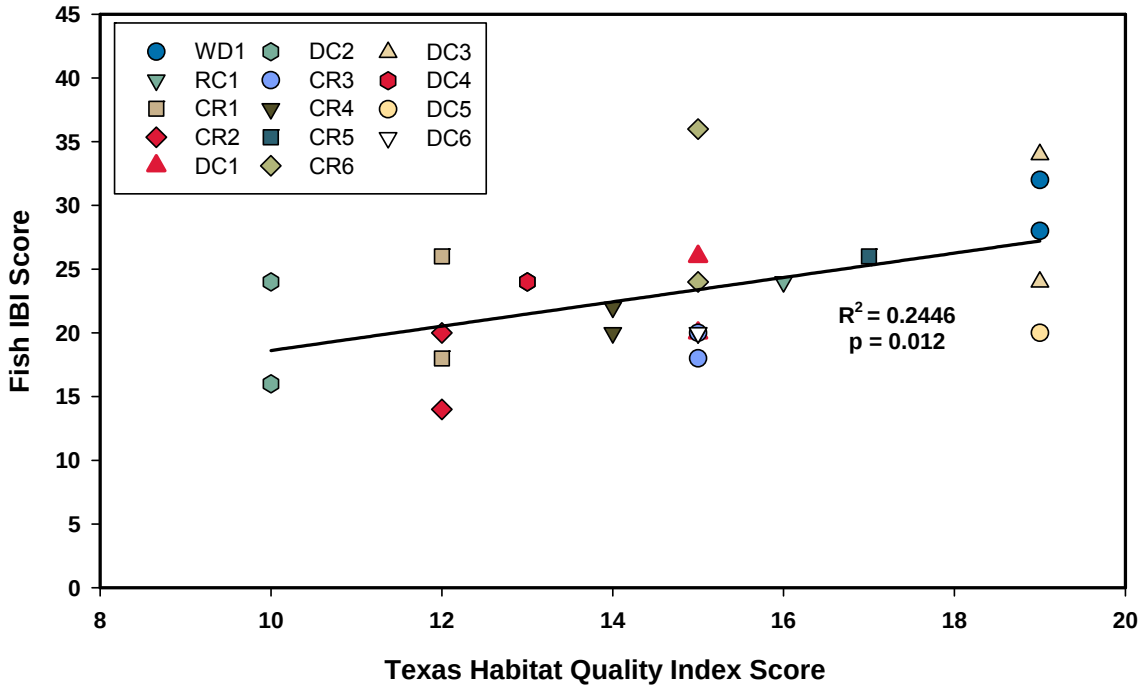


Figure 16: Relationship between Texas Habitat Quality Index scores and corresponding fish Index of Biological Integrity (IBI) scores.

Comparison of fish population metrics to Se concentrations in fish tissues indicated that centrarchids may be more sensitive to elevated Se tissue concentrations than the other fish species sampled. This was the only family of fish that showed a significant negative correlation between density and concentration of Se in respective fish tissues. However, it is important to note that fish community health also was significantly correlated with habitat quality. The relative contribution of Se or habitat on community health for these streams is unknown, but these correlations suggest that Se is not the only potential impact or factor limiting resident fish populations. Furthermore, the differences in species composition in summer and fall at many of the sites suggest that parameters that vary seasonally, including temperature and flow, may also strongly influence fish communities throughout the study area.

2.1.3 Sand Creek Drainage (CWWUC Se database, 2004) CO

2.1.3.1 Study Background and Methods

In order to determine whether site-specific Se criteria would be protective of the aquatic communities in Sand Creek and Segment 15 of the South Platte River, GEI Consultants, Inc. (formerly Chadwick Ecological Consultants, Inc.) and Brown and Caldwell investigated the current status of fish populations in these stream segments (Brown and Caldwell and Chadwick Ecological Consultants, Inc. 2003; CWWUC 2004). Density and biomass data were collected to indicate whether current fish populations were abundant or reduced in relation to measured Se levels in the water column and sediment. In addition, macroinvertebrate and tissue Se concentrations were quantified to determine actual Se uptake and its potential population-level effects.

Water column samples were collected monthly through South Platte Coalition for Urban River Evaluation (SPCURE) sampling. Samples were collected from June 2001 to December 2002. Sediment sampling took place on a quarterly basis from June 2001 to November 2002. Beginning March 2002, sediment and water column samples were also collected on a quarterly basis from seven reference stream sites.

The instream habitat at each site sampled for fish population data was evaluated during 2002 sampling efforts. At each site the habitat was broken up into principal types: riffle, run, glide, or pool. A Rapid Bioassessment Protocol (RBP) habitat assessment (EPA 1999) was conducted at all sites from which population data were collected. This procedure evaluates 13 different habitat parameters, giving a score for each one, based on visual observations of habitat conditions at the site. These separate parameter scores were added together to get a single RBP score for each site.

Composite benthic macroinvertebrate samples were collected from four sites in Sand Creek, as well as single sites in Tollgate Creek, West Tollgate Creek, Cherry Creek (CH-1), Big Dry Creek, and Lakewood Gulch. Samples were collected from all representative habitat types at each site. These samples were analyzed for total Se and percent solids by ACZ Laboratories (Steamboat Springs, CO).

Fish populations were sampled in the Sand Creek Drainage to determine species composition, abundance, and size structure of fish communities. Fish sampling occurred at four sites in both Sand Creek and the South Platte River, two sites on Cherry Creek, as well as single sites in Tollgate Creek, East Tollgate Creek, West Tollgate Creek, Big Dry Creek, Box Elder Creek, West Bijou Creek, Kiowa Creek, Plum Creek, and Lakewood Gulch. Samples were collected from all representative habitat types at each site.

Wherever possible, five replicate samples of fathead minnow ovary/eggs and five replicate whole-body fathead minnow samples were collected from each site in 2002. The fathead minnow was the only fish species found at virtually all sites. These samples were analyzed

for both Se and percent solids by University of Missouri, Research Reactor Center in Columbia, Missouri (ovary/eggs) and by ACZ Laboratories in Steamboat Springs, Colorado (whole-body). The data that follow are presented on a dry weight basis.

2.1.3.2 Results

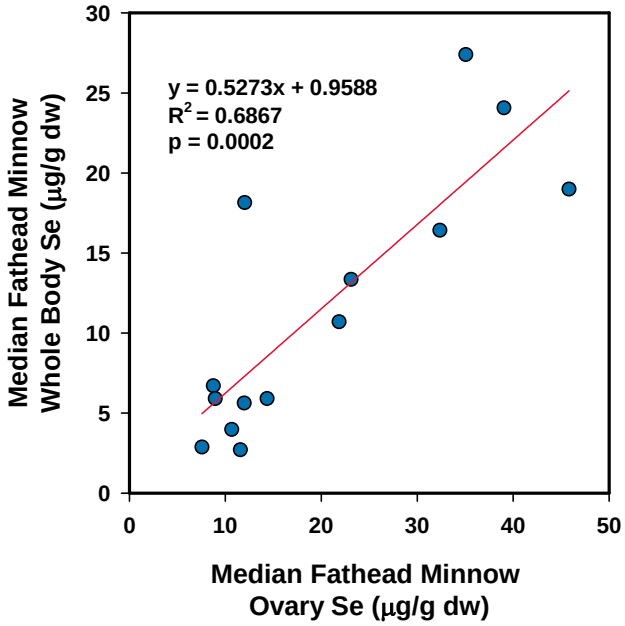
RBP scores ranged from a low of 104 to a high of 166. These data indicate that the available habitat was of lower quality at the lower two Sand Creek sampling sites relative to the upper Sand Creek and other reference sites.

Dry-weight concentrations of Se in macroinvertebrate tissue samples followed an increasing downstream trend in Sand Creek from a low of 3.52 µg/g to 10.47 µg/g. In general, Se toxicity thresholds applied to benthic macroinvertebrates view these organisms as a dietary item and source of Se for fish. This is how benthic macroinvertebrates are viewed in the toxicity thresholds suggested by both DeForest et al. (1999) and NIWQP (1998). The DeForest et al. study (1999) suggests a dietary toxicity threshold of 10 µg/g dry weight for warmwater fish, while the NIWQP (1998) study suggests a dietary threshold of 3 to 8 µg/g dry weight. The dry weight concentration of Se in benthic macroinvertebrates at all but two sample sites exceeded the lower limit suggested by the NIWQP study. Se concentrations measured in the benthic macroinvertebrates collected from two sample sites exceed both the high end of the range proposed in the NIWQP study (8 µg/g dry weight) and the threshold of 10 µg/g dry weight proposed by DeForest et al. (1999).

The mean concentration of Se in whole-body fathead minnow samples ranged from a low of 3.2 µg/g dry weight to a high of 19.4 µg/g dry weight. The mean Se concentration measured in ovary/egg samples ranged from a low of 7.8 µg/g dry weight to 45.3 µg/g dry weight. Overall mean Se concentrations measured in ovary/egg samples were higher than those measured in the whole-body samples from the corresponding sites. However, the ranking of the sites by Se concentration remains roughly the same, indicating that the concentrations of Se in these tissues are related and likely bioaccumulated in the same manner. Values observed at the three lower Sand Creek Sites and the Tollgate Creek sites also exceeded the ovary/egg Se toxicity threshold of 17 µg/g dry weight proposed above by DeForest et al. (1999).

The Se concentration measured in the fathead ovary/egg and whole-body fathead minnow tissues collected in 2002 were positively and significantly ($P = 0.0002$) related, with this relationship accounting for 68 percent of the observed variance ($R^2 = 0.687$; Figure 17). This relationship suggests that, while final concentrations in these tissues may be different, fathead minnows take up and sequester Se in both the ovary/egg and whole-body tissues in similar manners.

Figure 17:
Relationship between the median concentration of Se in fathead minnow whole-body versus the median concentration of Se in fathead minnow ovary/egg samples.



The observed Se concentrations in both the ovary/egg and whole-body tissue samples were significantly ($P < 0.05$) and positively correlated, with up to 72 percent of the variance accounted for ($R^2 = 0.72$ and 0.51, respectively; Figures 18 and 19, respectively) by the relationship between tissue levels and the concentration of dissolved Se in collected water samples. These relationships appear to indicate that the concentration of Se in the water column of these systems is related to the amount of Se present in fish tissues. The relationship between tissue Se concentrations and those concentrations in the sediment were not significant for either ovary/egg or whole-body tissues. This appears to indicate that the concentration of Se in the sediments of these systems is not directly related to the Se concentrations in fish tissues in these systems.

Figure 18:
Relationship between the median concentration of Se in fathead minnow ovary samples versus the median dissolved Se concentrations at sample sites.

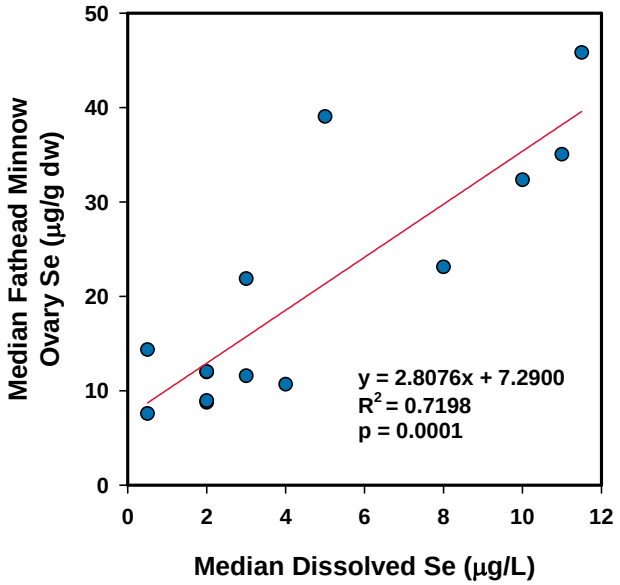
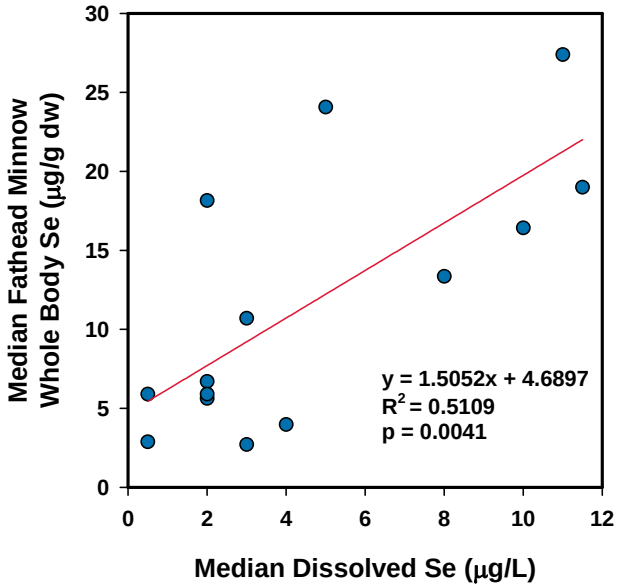
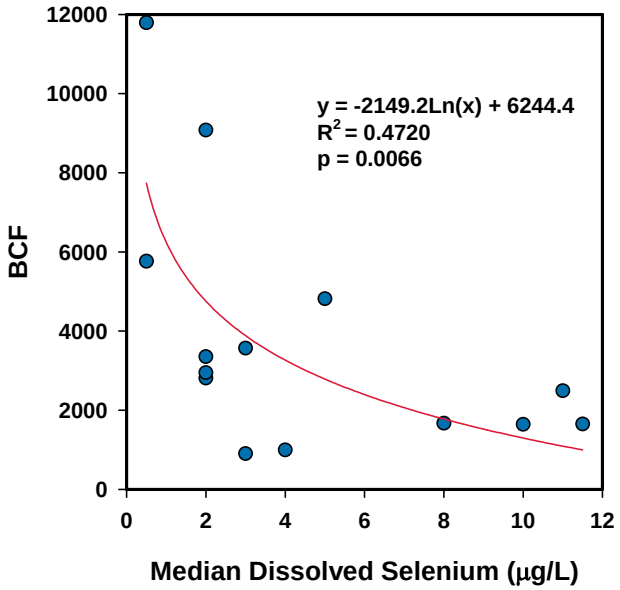


Figure 19:
Relationship between the median concentration of Se in fathead minnow whole-body samples versus the median dissolved Se concentrations at sample sites.



Using the whole-body fathead minnow Se concentration and the dissolved concentration of Se in the water column, bioconcentration factors (BCFs) were determined for each site. A BCF, defined as the ratio of the chemical concentration in the organism to that in its environment, incorporates the dietary pathway specific to the site. These BCFs ranged from 993 to 11,790. There was a significant inverse relationship ($p = 0.007$) between BCFs and dissolved Se concentrations; i.e., as the concentration of Se in the water column decreased, the BCF decreased (Figure 20). This inverse relationship is not surprising, and has been documented in the literature. Because Se is an essential nutrient, fish actively accumulate Se at low environmental concentrations to satisfy metabolic requirements. However, once the concentration of Se reaches a certain point, fish appear to actively manage Se through depuration, sequestration in tissues, and passing Se to the eggs.

Figure 20:
Relationship between the bioconcentration factor (BCF) versus the median dissolved Se concentrations at sample sites.



Positive relationships were observed between water column and sediment Se concentrations and between fish density and fish taxa richness. While not indicating a causal relationship (i.e., it cannot be said that "more Se means more fish"), these relationships do tend to indicate that Se is not a significant toxicant in these systems at observed levels. Significant relationships were also found for fish population parameters and a number of habitat variables, such as stream width (Figure 21) and channel flow status (Figure 22). A positive relationship, although not statistically significant ($p = 0.082$), was found between total fish density and sediment deposition scores (Figure 23). Slightly negative relationships, although not statistically significant ($p = 0.3389$ and 0.7395, respectively), were found between the number of fish taxa and total fish density versus the number of habitat units per kilometer.

Figure 21:
Relationship between the number of fish taxa versus the average stream width at sample sites.

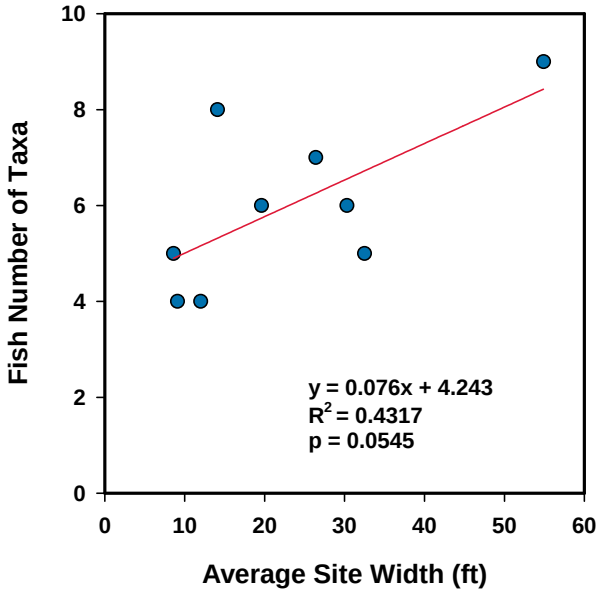


Figure 22:
Relationship between the number of fish taxa versus the channel flow status at sample sites.

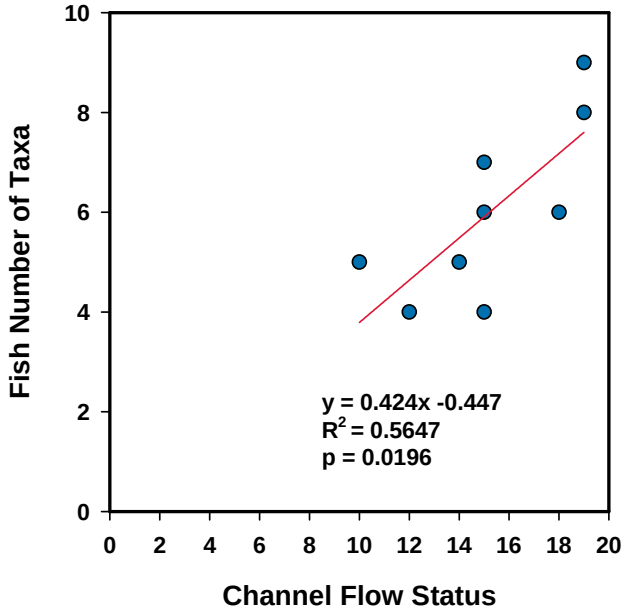
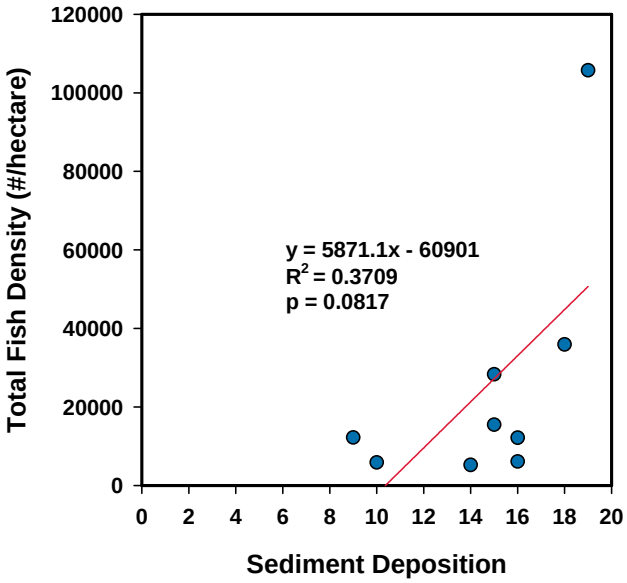


Figure 23:
Relationship between the total fish density (#/ha) versus the sediment deposition scores at sample sites.



The basic fish population parameters of total fish abundance and species richness exhibited positive relationships with Se water column and sediment concentrations (Figure 24a-d). However, these relationships were not statistically significant ($p > 0.05$) and are probably artifacts rather than actual causal relationships. Weak relationships, although not statistically significant, were found between mean tissue Se concentrations and ln-transformed population density (Figure 25); while fathead minnows demonstrated increasing population densities with increasing Se tissue concentrations, density of catostomids and other cyprinids decreased with increasing Se concentrations. When two years of data were examined, positive relationships, although not statistically significant, were observed for fathead

minnow whole-body and ovary Se concentrations (p = 0.2617 and 0.0651, respectively) versus population density (Figure 26). This could be an artifact of few mean whole-body Se concentrations above the chronic criterion.

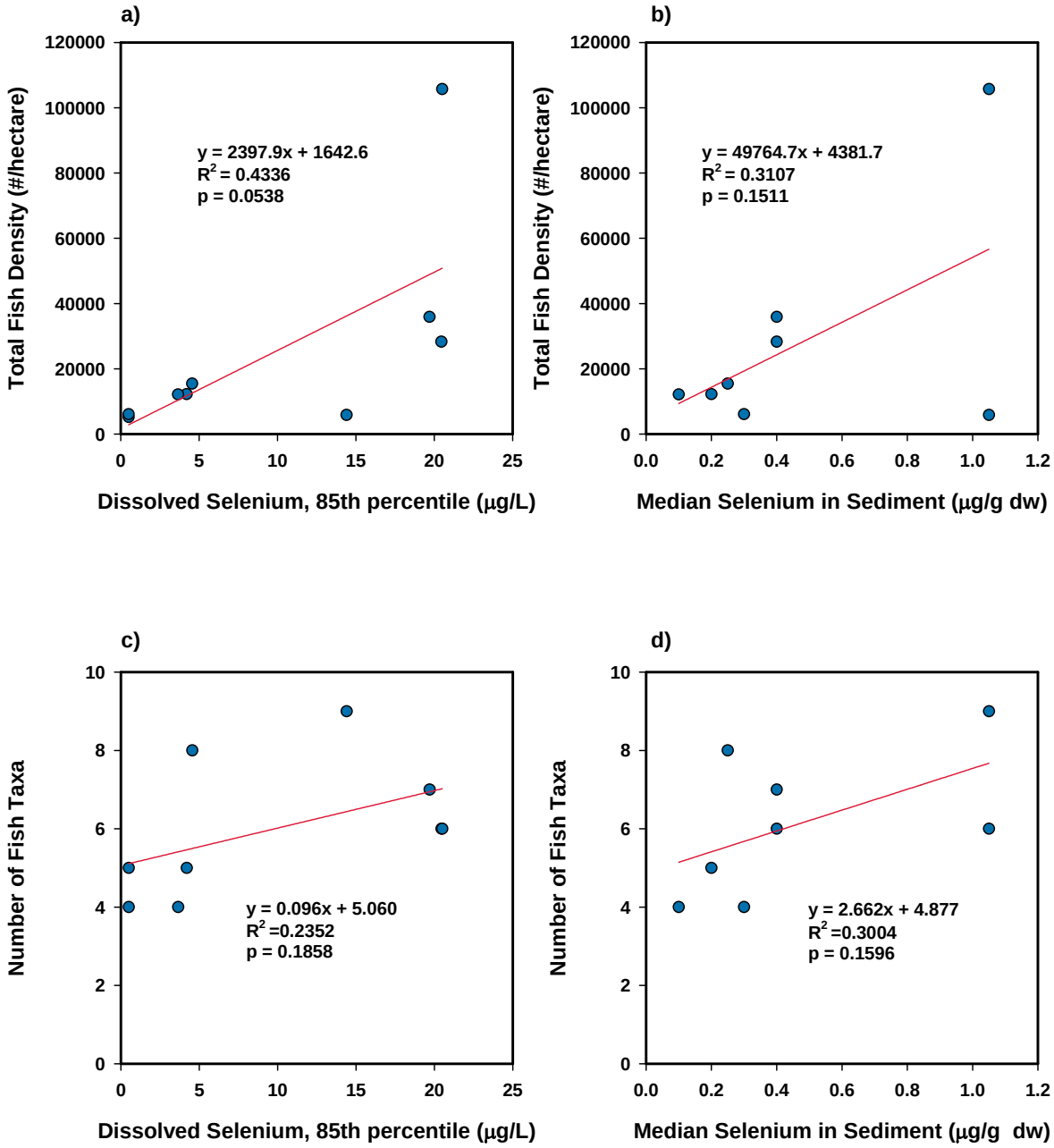


Figure 24: Relationships between total fish density and fish number of taxa and the 85th percentile of dissolved Se concentrations in the water column and mean Se in the sediment at the study sites – 2002 data.

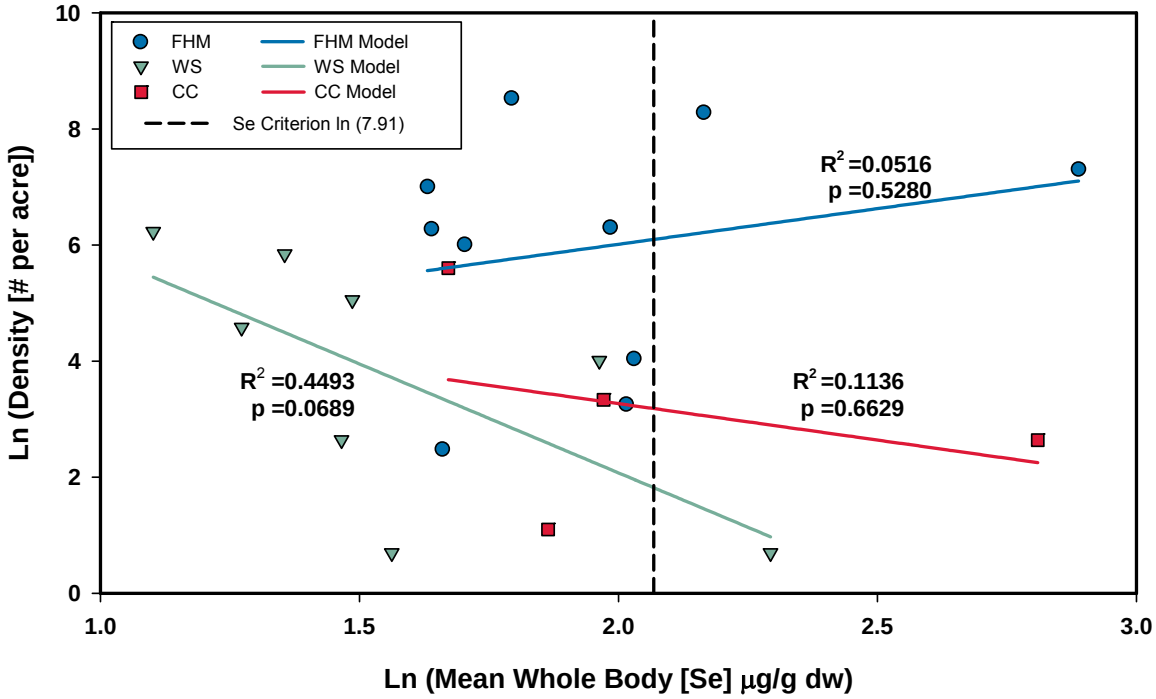


Figure 25: **Relationship between fish population density and mean whole-body Se concentrations for Sand Creek Drainage study sites (South Platte, Sand Creek, Box Elder Creek, Big Dry Creek, and Cherry Creek), 2001.**

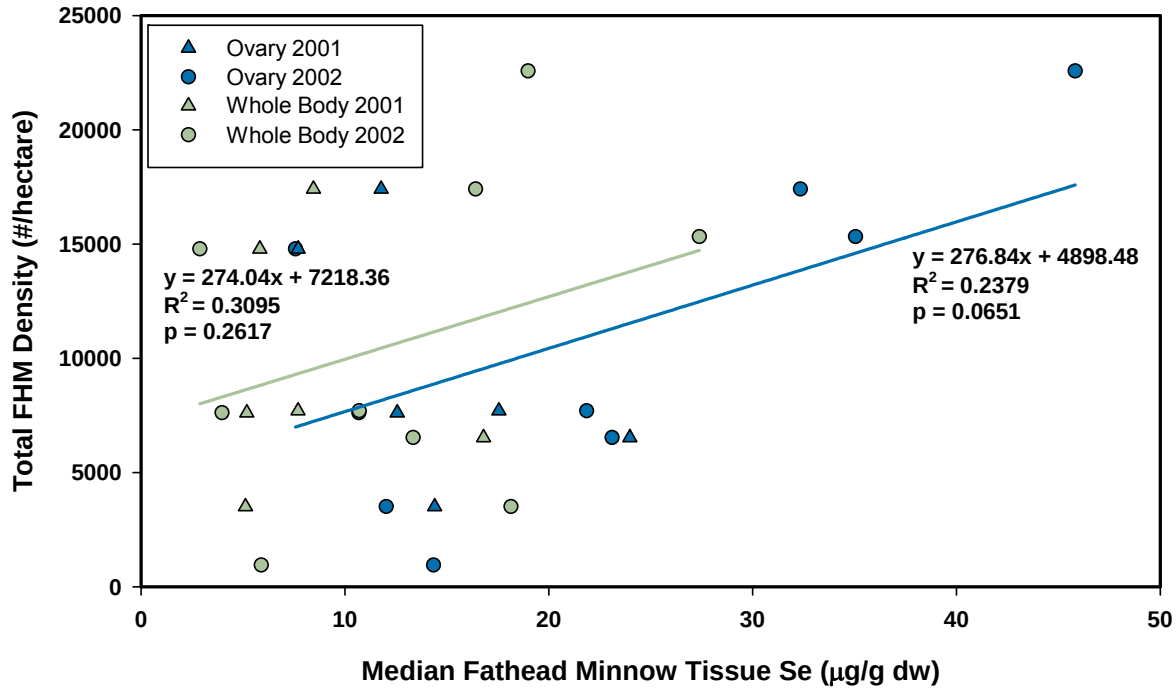


Figure 26: **Relationship between fathead minnow population density and mean whole-body and ovary Se concentrations for Sand Creek Drainage study sites (South Platte, Sand Creek, Box Elder Creek, Big Dry Creek, and Cherry Creek), 2001 – 2002.**

Although Se thresholds were exceeded at many sites, widespread reproductive impairment for fathead minnows was not observed. The calculated chronic level for whole-body Se (40 µg/g) to protect fathead minnows (GEI 2008) was not exceeded at any of the sites (based on mean whole-body concentrations), perhaps explaining the lack of observable population effects – i.e., tissue levels are below effects levels for Se and fathead minnows.

2.1.4 Stingy Run (Reash et al. *1988) OH*

2.1.4.1 Study Background and Methods

In 1974, Stingy Run, a tributary of Kyger Creek in southern Ohio, was impounded to form a fly ash receiving stream for the Ohio Power Company's coal-fired power plant. Prior to impoundment, Stingy Run drained strip mine refuse areas and was dominated by a limited acid-tolerant macroinvertebrate community and supported no fish. Changes in water chemistry following impoundment resulted in a significantly different aquatic life community. In their study, Reash et al. (1988) investigated these changes, including the increase in Se concentrations resulting from the accumulation of fly ash.

In September and December 1973, prior to impoundment of Stingy Run, the researchers conducted water quality, habitat, benthic macroinvertebrate, phytoplankton, and fish assessments. Following impoundment, water chemistry evaluations were performed two to four times per year from 1975 – 1986. Benthic macroinvertebrate samples were collected during multiple sampling sessions in 1975, 1978, and 1980 – 1983. Fish were sampled on eleven occasions between February 1980 and October 1986. Diversity indices, community composition, species richness, relative abundance, and feeding guilds were established for all biological samples. Stomachs were collected from Stingy Run fish in May 1983 and October 1986 to determine food sources and monitor any temporal dietary changes.

2.1.4.2 Results

Post-impoundment water quality analyses indicated a significant increase in pH and decrease in aluminum and manganese. Mean Se concentrations from 1974 – 1982 and 1982 – 1986 were 33 µg/L and 19 µg/L, respectively. These values are significantly higher than the current chronic standard of 5 µg/L. However, despite these elevated Se concentrations, fish and invertebrate species richness and diversity significantly increased from1983 – 1986.

An increase in habitat quality may be partially responsible for the increase in aquatic life richness and diversity. Cobble substrate was added to a stretch of Stingy Run to stabilize the channel; this new substrate provided more suitable habitat for a variety of invertebrates. The observed increase in fish density and diversity may be a result of the concurrent increase in density and diversity of invertebrates, a source of food for many fish.

Overall, post-impoundment macroinvertebrate diversity and richness were relatively low, likely due to water quality issues; the authors demonstrated that 99 percent of the variation in

number of taxa could be explained by a linear relationship with ammonia, zinc, and copper concentrations.

In general, fish that initially colonized Stingy Run are tolerant, omnivorous species. Following the increase in invertebrate density, invertebrate-feeding fish became more prevalent. Species found at the site included common carp, channel catfish, bluegill, green sunfish, gizzard shad, bluntnose minnow, black bullhead, brown bullhead, golden shiner, and fathead minnow.

While most Stingy Run fish are known to be fairly tolerant species, it is still important to note how they appeared to thrive in a high-Se environment. Their extreme tolerance to Se raises potential questions, such as whether the colonizers had developed tolerance through prior Se exposure, or whether other contaminants in the water had antagonistic effects.

2.2 Coldwater Stream Studies

Few studies that combine quantitative fish population sampling and fish tissue Se concentrations in cold water streams could be found. While a number of studies using coldwater fish have been conducted to determine threshold effects (see earlier chapters), these studies did not relate tissue concentrations to population "health" in the environment. Two studies were located, both from areas with mine influence in Idaho, and are summarized below.

2.2.1 Thompson Creek (TCMC) ID

2.2.1.1 Study Background and Methods

The Thompson Creek Mining Company (TCMC) operates an open-pit molybdenum mine located in the Thompson Creek watershed in central Idaho (Figure 27). The mine and associated waste rock deposition areas are near the upstream reaches of Buckskin Creek and Pat Hughes Creek. Both Buckskin Creek and Pat Hughes Creek are intermittent, first-order tributaries of Thompson Creek, and each receives runoff from the mine area through permitted Outfalls 001 and 002, respectively. Neither Buckskin Creek nor Pat Hughes Creek receives active discharge or process waters from the mine. However, they do receive runoff associated with stormwater conveyance and drainage from waste rock piles. Sediment control ponds are also located on both streams.

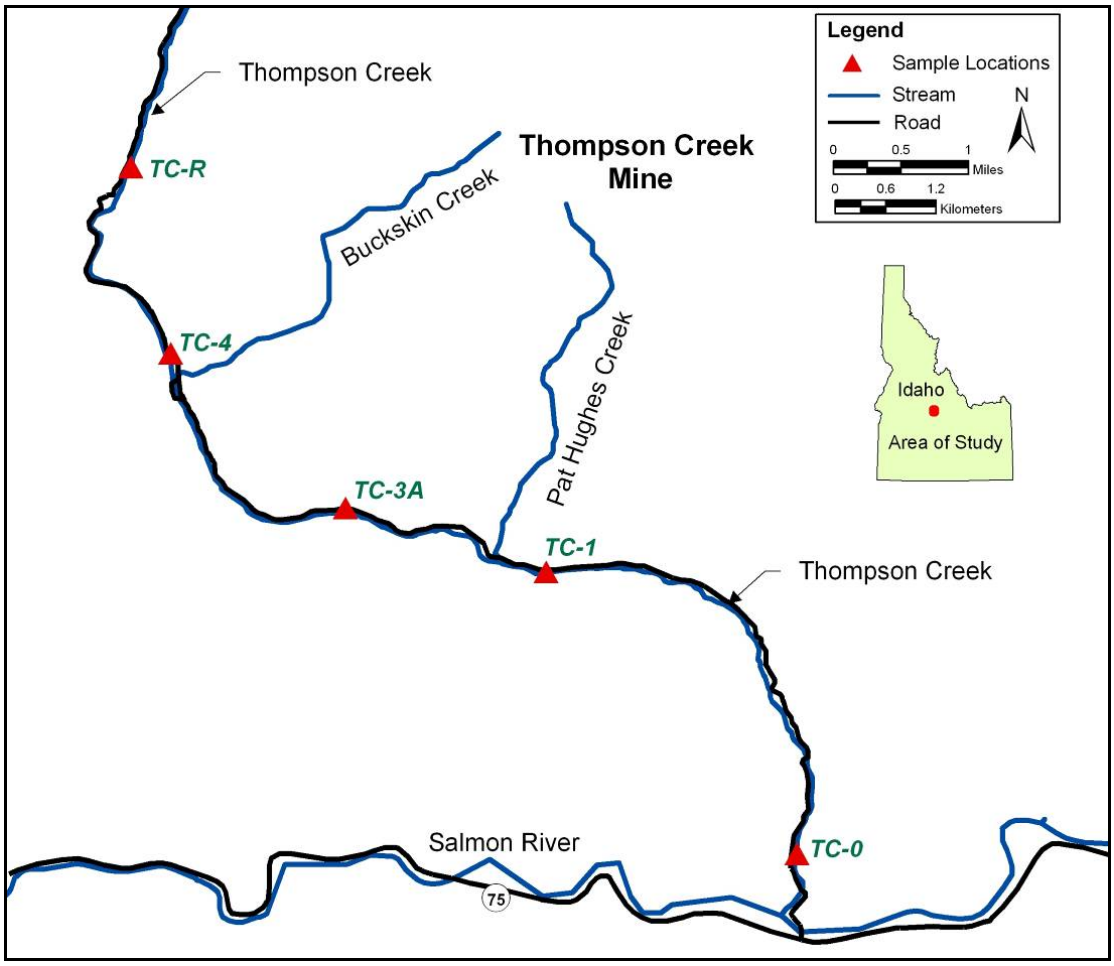


Figure 27: Locations of Se bioaccumulation monitoring sites on Thompson Creek, Idaho.

An annual monitoring program was initiated in 1980 to evaluate any potential effects of continued operations of the Thompson Creek Molybdenum Mine on the aquatic communities of Thompson Creek. Sediments, benthic macroinvertebrates, and fish from Thompson Creek were tested for bioaccumulation of mercury and Se for the first time in April 2000. Se levels in sediment, macroinvertebrates, and fish collected in April 2000 indicated a low bioaccumulation risk for Se (CEC 2001a).

A Se bioaccumulation monitoring plan (CEC 2001b) was developed to satisfy requirements set forth in TCMC's Draft National Pollutant Discharge Elimination System (NPDES) Permit (No. ID-002540-2) and by the Idaho Department of Environmental Quality ([IDEQ], 2000). Sampling was conducted in October 2001 and monitoring has continued since 2002. The primary goal of monitoring is to evaluate the concentration of total recoverable Se in the aquatic biota and sediments in Thompson Creek both upstream and downstream of the discharge points (001 and 002). The following components of the aquatic environment were sampled from 2002 through 2007 (GEI 2007c): sediment, detritus (represented by fine particulate organic matter [FPOM]), macroinvertebrates, sculpin, and trout.

Field personnel collected five replicate sediment samples at each site. Sediment samples were collected from slow water-depositional areas (pools and stream margins), and represent the potential worst case for waterborne Se to accumulate in stream habitats (Van Derveer and Canton, 1997). Five replicate samples of FPOM, or particulates smaller than 4 mm (Wallace et al. 1995), were also collected at each site and analyzed for Se content.

Five replicate composite macroinvertebrate samples were collected at each site. Macroinvertebrate Se analysis results were provided in wet weight and converted to dry weight by dividing the wet weight value by percent solids (geometric mean percent solids = 18 percent).

Five replicate samples of whole-body adult shorthead sculpin (*Cottus confusus*, one individual per replicate) and whole-body cutthroat/rainbow trout hybrids (*Oncorhynchus clarkii lewisi x O. mykiss*, one individual per replicate) were collected from each site and examined for Se bioaccumulation. Fish tissue Se analysis results were provided in wet weight and converted to dry weight by dividing the each individual wet weight value by the individual percent solids value.

2.2.1.2 Results

Se concentrations in sediment increased steadily with downstream distance (Figure 28). Sediment Se concentrations in individual replicate samples since 2002 ranged from 0.05 µg/g dry weight to 4.26 µg/g dry weight; only one value (4.26 µg/g dry weight from Site TC-3A in 2005) exceeded the 4 µg/g dry weight that has been suggested as a possible effects threshold (VanDerveer and Canton 1997) for sediment.

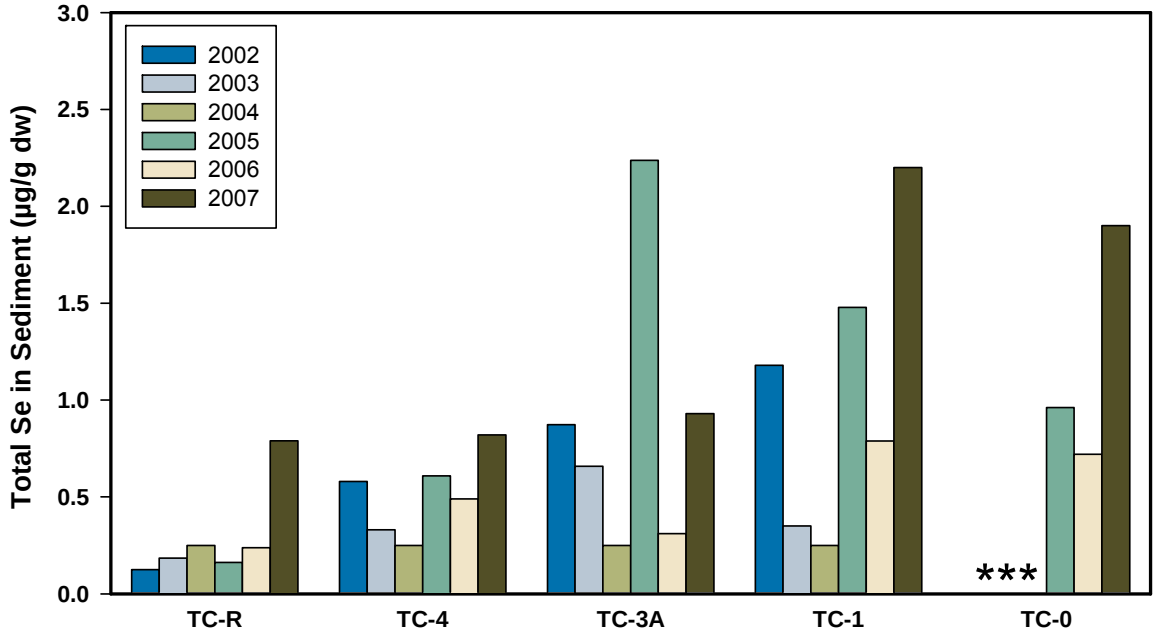


Figure 28: Summary of geometric mean Se concentrations (µg/g dry weight) in sediment samples collected from Thompson Creek, 2002-2007 (* = no data collected).

Se concentrations in FPOM were far more variable than those in sediment or biota (Figure 29). The spatial pattern for FPOM Se suggests that there are multiple sources for Se within the study reach, but one of the largest inputs may be between sites TC-1 and TC-0, which bracket a historic mill site.

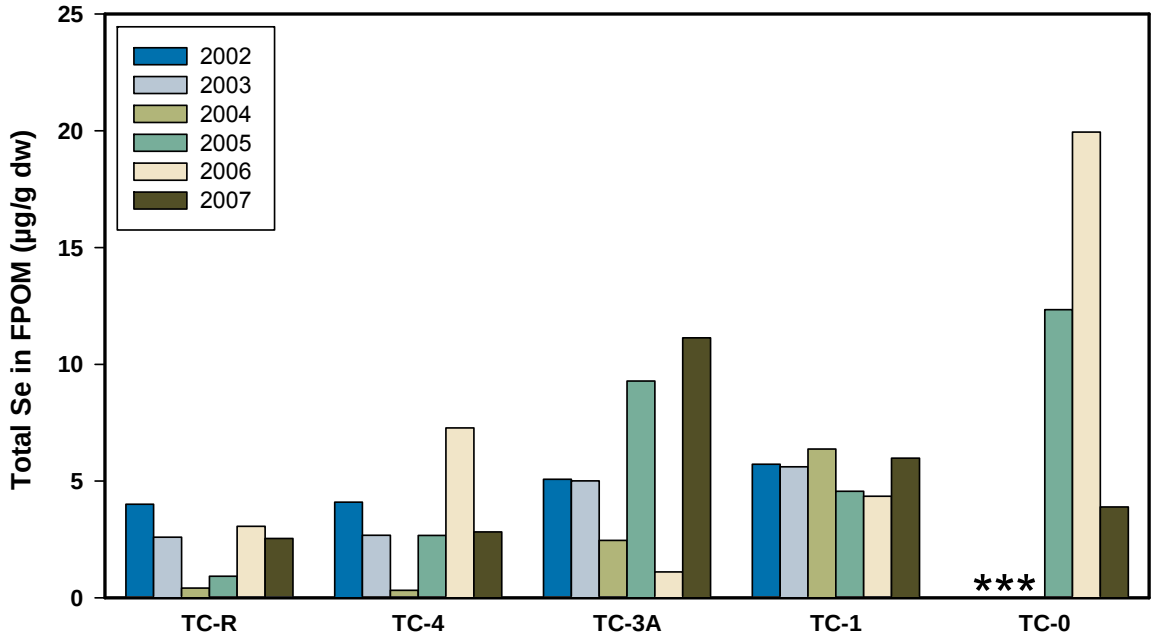


Figure 29: Summary of historic geometric mean Se concentrations (µg/g dry weight) in FPOM samples collected from Thompson Creek, 2002 - 2007. The literature-derived effects threshold of 11µg/g is included for comparison (* = no data collected).

Temporal changes in macroinvertebrate-based Se were also observed within sites (Figure 30). Se appears to be increasing in a consistent manner over time at all sites except one. Exceedances of 11 µg/g dry weight, which has been suggested as a potential dietary threshold for coldwater fish (DeForest et al. 1999), occurred in individual replicate samples at all sites since 2002.

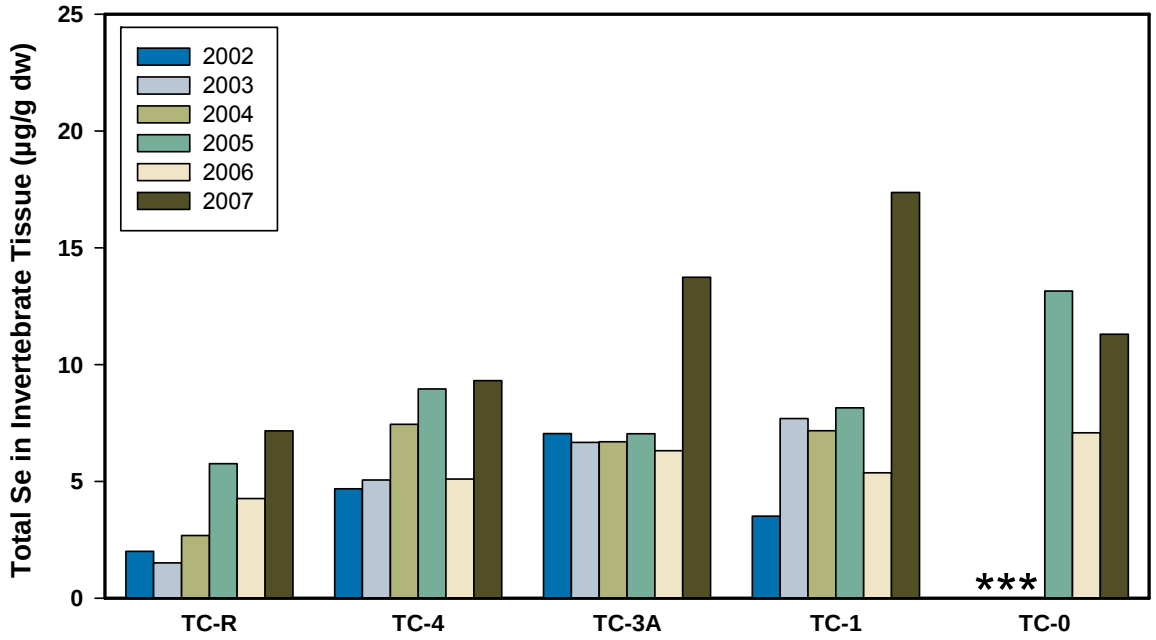


Figure 30: Summary of historic geometric mean Se concentrations (µg/g dry weight) in macroinvertebrate tissue samples collected from Thompson Creek, 2002-2007. The literature-derived effects threshold of 11 µg/g is included for comparison (* = no data collected).

Se in sculpin tissue has increased over time at all of the studied sites, and linear regression demonstrated that all of these increases are statistically significant ($p \leq 0.018$ for all five sites; Figure 31). This result suggests that increasing Se sculpin body burdens are occurring throughout the study area on Thompson Creek. Increases in mean tissue concentrations between macroinvertebrates and sculpin tissue ranged from 0 to 47 percent. The spatial pattern in Se in macroinvertebrate tissue is similar to that of sculpin tissue, suggesting that macroinvertebrate and sculpin tissue levels of Se may be linked. However, the variability between sites in the increase in Se concentrations between macroinvertebrates and sculpin also suggests that the fate of Se is complex.

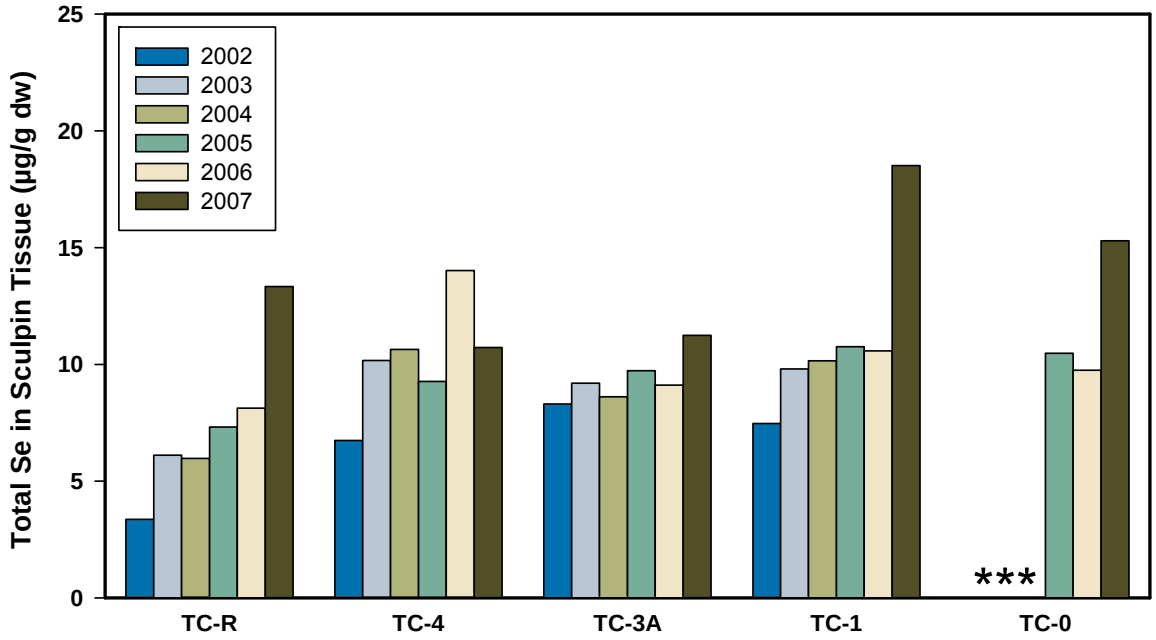


Figure 31: Summary of historic geometric mean Se concentrations (µg/g dry weight) in whole-body sculpin tissue samples collected from Thompson Creek, 2002 - 2007 (* = no data collected).

Trout tissue concentrations of Se also increased over time at all sites (Figure 32). Linear regression showed that the increase was statistically significant at all sites except one ($p \leq 0.0104$ at all sites except Site TC-0). While bioaccumulation of Se is suggested in fish collected from Thompson Creek, it is variable. The increase in tissue levels of Se between macroinvertebrates and trout is not as great as that between macroinvertebrates and sculpin. Increases between macroinvertebrates and trout ranged from 0 to 20 percent; therefore, trout may not accumulate as much Se as sculpin because of their dietary habits. Trout in small streams often consume large amounts of terrestrial insects (Nakano et al. 1999; Webster and Hartman 2005; Saunders and Fausch 2007), which may contain lower levels of Se than drifting macroinvertebrates. Because of their close association with the benthos, sculpin primarily consume drifting macroinvertebrates (e.g., Johnson 1985). Therefore, sculpin may be accumulating more Se than trout by feeding upon prey with higher body burdens of Se.

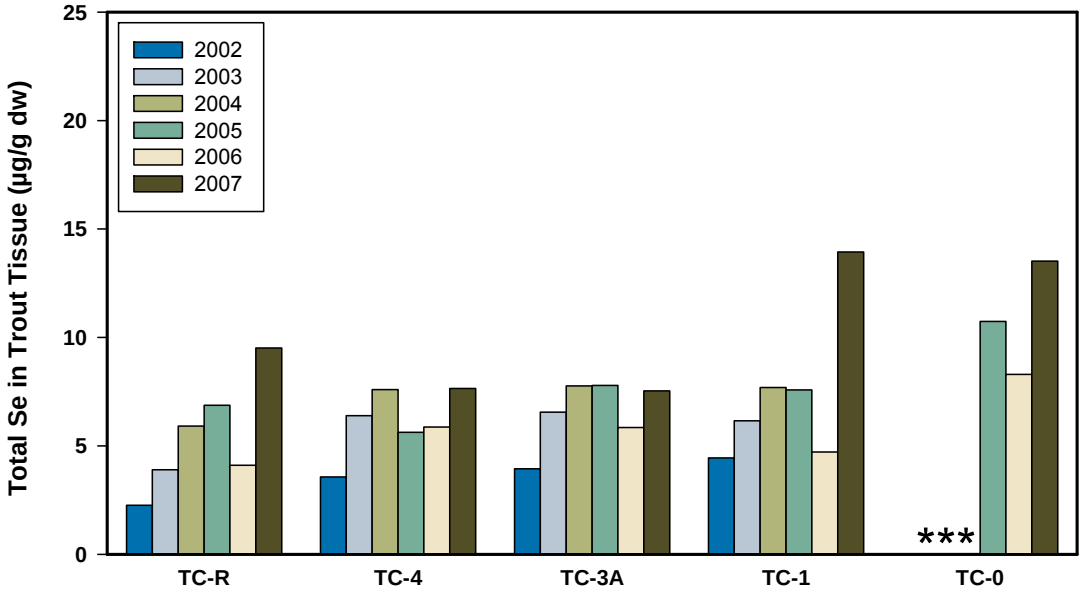


Figure 32: Summary of historic geometric mean Se concentrations (µg/g dry weight) in whole-body trout samples collected from Thompson Creek, 2002 - 2007 (* = no data collected).

A negative relationship, although not statistically significant, was found between mean whole-body sculpin Se concentration and ln transformed sculpin density (p = 0.1195; Figure 33). The opposite relationship, although weak, was seen in trout (p = 0.8479; Figure 33).

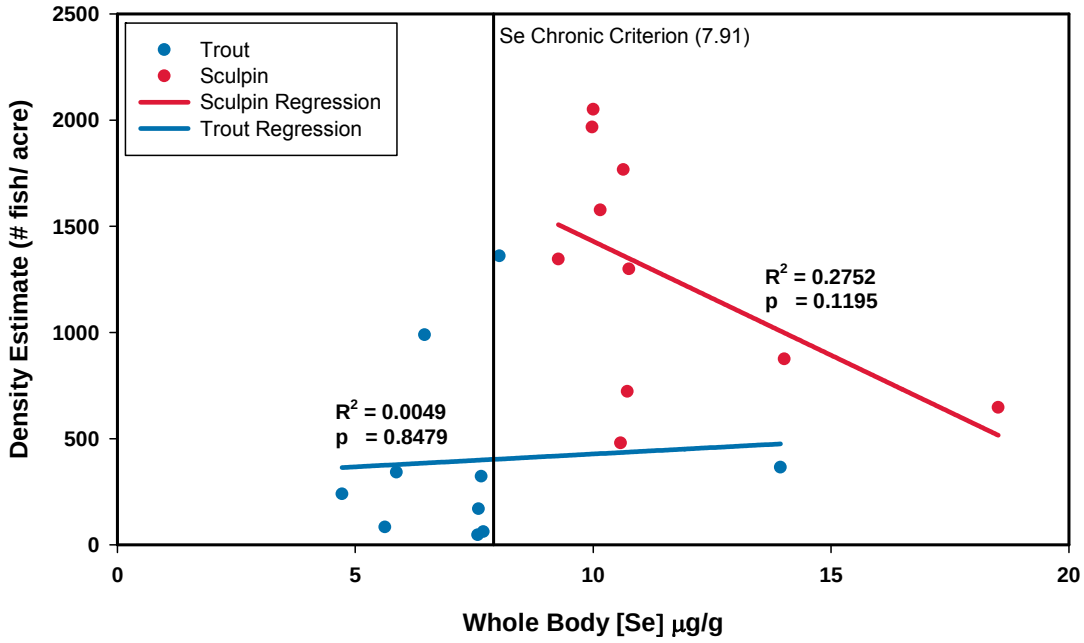


Figure 33: Relationship between trout and sculpin mean whole-body Se concentrations and corresponding species density estimates. Draft EPA (2004) criterion of 7.91 µg/g provided for comparison.

Increasing temporal trends in Se concentrations were present at some or all sites in all components except FPOM.

The data also indicate that there is a dietary transfer (bioaccumulation) of Se from FPOM to macroinvertebrates and to sculpin and trout. However, the dietary pathway is less apparent in trout, possibly because they could be consuming terrestrial insects and are less likely to feed exclusively on drifting stream macroinvertebrates than sculpin.

While Se concentrations above threshold values at some Thompson Creek sites, particularly the downstream sites, indicate some potential risk to aquatic biota, the long-term community monitoring in Thompson Creek continues to show healthy macroinvertebrate, sculpin, and trout populations in the area. Trout densities were lower at all sites in 2004 and 2005, when Se levels in macroinvertebrate, sculpin, and especially trout tended to be higher than they were in most years. However, there was no correlation with macroinvertebrate density and Se bioaccumulation levels at any site, and sculpin population estimates do not appear to be correlated with Se levels in macroinvertebrate or sculpin tissue. Trout population estimates in 2007 were higher than they were in 2005 and 2006 despite the fact that Se levels were higher throughout the system in 2007 than in past years.

The cold, fast-flowing nature of Thompson Creek may limit the sediment-macroinvertebrate pathway for the transfer of Se to fish. Total organic carbon levels in the sediment at all sites have been less than 3 percent since 2002, a level that indicates a low potential for bioaccumulation.

2.2.2 Snake River (Van Kirk and Hill 2007) ID

2.2.2.1 Study Background and Methods

Using population modeling, the authors address the issues associated with predicting population-level effects from the response of individual organisms. More specifically, the population-wide effects of Se on growth and survival of juvenile cutthroat trout (*Oncorhynchus clarkii*) were studied.

The authors compiled data from several studies on cutthroat trout populations and the effects of Se (presented as whole-body concentrations) on various coldwater fish. Juvenile mortality, considered the primary cause of population size decreases, was the authors' focus. Survival rates for fry from the swim-up stage and the onset of winter, as well as the length of juveniles at the end of their first summer of growth, were the endpoints used to assess impacts of Se exposure. To account for variation known to occur during the first 30 to 60 days of life, mortality and growth data for newly emergent fry lasting between 90 and 150 days were used. Both resident and migratory life histories were examined, and the authors' model incorporated various factors such as abundance, survival between stages and age classes, density dependence (for juvenile winter survival), effects of Se on survival (for juvenile summer survival), and environmental stochasticity. Furthermore, the model was

calibrated using published juvenile survival rates for aquatic systems without known Se toxicity. Model simulations investigated population size as a function of both time and Se concentration.

2.2.2.2 Results

Van Kirk and Hill (2007) reported no observable effect concentrations (NOEC) for resident and migratory populations at 7.0 µg/g and 10.0 µg/g dry weight whole-body Se, respectively. A 50 percent population decline in resident and migratory populations was predicted at 13 µg/g and 15 µg/g, respectively. A 90 percent population reduction was predicted to occur at 17 µg/g and 18 µg/g for resident and migratory populations, respectively. Resident and migratory populations showed similar patterns, with both life history types predicted to exhibit a 90 percent population decline at Se concentrations exceeding approximately 17 µg/g dry weight whole-body Se. Above Se concentrations of 15 µg/g, the median and range of modeled juvenile survival rates showed significant declines.

Model results also indicate that juvenile cutthroat trout survival is strongly density-dependent. Because modeled Se-induced mortality occurred prior to density-dependent effects, modeled populations were able to compensate for increased toxicity-related mortality as long as mortality remains below a critical level.

The authors recommend the modeled NOEC for resident populations of 7 µg/g dry weight whole-body Se as the maximum allowable whole-body Se concentration. At the current chronic tissue-based standard of 7.91 µg/g, the model suggests reductions in juvenile growth and survival will occur, potentially resulting in significant population declines.

Van Kirk and Hill's (2007) final conclusions included the following statements: (1) regulatory criteria and risk assessments based on individual-level effects may not be relevant to the population-level; (2) long-term population size, rather than population growth rates, may provide more information about a population's response to Se exposure; and (3) environmental stochasticity must be a consideration for regulatory purposes, management actions, and when performing assessments.

2.3 Lake Studies

2.3.1 Hyco Reservoir (Crutchfield 2000) NC

2.3.1.1 Study Background and Methods

Hyco Reservoir (Hyco) was created in 1964 to provide a source of cooling water and serve as receiving waters for the Roxboro Steam Electric Plant in North Carolina. Following the installation of two electrical generating units in 1973 and 1980, declines in largemouth bass (*Micropterus salmoides*) and bluegill (*Lepomis machrochirus*) reproductive success and abundance were observed in the reservoir during Carolina Power and Light Company

monitoring. Crutchfield evaluated long-term water quality, sediment, invertebrate and fish tissues, and invertebrate and fish population data collected from Hyco to document the recovery of the aquatic community following the 1990 installation of a dry fly ash pollution abatement system. Since 1973, data have been collected from six Hyco sites varying in fly ash exposure.

2.3.1.2 Results

Crutchfield reported a significant decline in water Se concentrations; prior to installation of the dry fly ash system, Se concentrations were 7-14 µg/L at or below the outfall and 0.8-6.5 µg/L at upstream sites. The first year after the system was installed, all concentrations were less than 5 µg/L (most were between 1-3 µg/L). Since 1993, concentrations of Se throughout the reservoir have been ≤ 1-2 µg/L.

The author did not observe a significant decline in sediment Se concentrations following system installation. However, this was expected, as the reservoir had a low sedimentation rate and mass balance modeling had predicted the sediment Se concentration decline to be slow.

A significant decline in benthic invertebrate Se concentrations was also observed following system installation. Prior to system installation, benthic invertebrates had mean Se concentrations of 35-88 µg/g dry weight. Following system recovery, mean concentrations were 8.7-55 µg/g dry weight.

Similarly, the author observed significant declines in fish tissue Se concentrations following system installation. Bluegill muscle Se concentrations decreased from 22-68 µg/g dry weight prior to recovery to < 20 µg/g dry weight after 1994, a decline of 61-84 percent. Largemouth bass muscle Se concentrations declined by 51-65 percent from 1992-1997, with ending Se concentrations < 15 µg/g dry weight.

Successful bluegill and largemouth bass reproduction resumed within two years following system installation at the least impacted site and within four years at the outfall and other impacted sites. During this time, bluegill and largemouth bass muscle and liver samples collected from the least impacted site had Se concentrations ranging from 6-24 µg/g dry weight and 9-35 µg/g dry weight, respectively; a 1992 sample of three bluegill ovaries had Se concentrations ranging from 9-24 µg/g dry weight. These values are higher compared to Lemly (1993), who reported that Se concentrations of 8 µg/g dry weight in muscle and 12 µg/g dry weight in liver were threshold values for impaired health and reproductive success in fish. However, it should be noted that 1997 was the most reproductively successful year for bluegill and largemouth bass; Se concentrations in muscle and liver samples collected in 1997 were 6-7 µg/g dry weight and 11-12 µg/g dry weight, respectively.

During the 1982-1983 monitoring seasons, significant rainfall resulted in decreased water Se concentrations of 1-2 µg/L. Although bluegill muscle and ovary Se concentrations were 10-46 µg/g dry weight and 15-55 µg/g dry weight, respectively, during this time, reproductive success was higher during this period than any other pre- or post-recovery time of the study.

Prior to installation of the pollution abatement system, the fish community was dominated by green sunfish (*Lepomis cyanellus*), eastern mosquitofish (*Gambusia holbrooki*), gizzard shad (*Dorosoma cepedianum*), and satinfin shiner (*Cyprinella analostana*). In 1984, redbelly tilapia (*Tilapia zilli*) and blue tilapia (*T. aurea*) were introduced into Hyco, resulting in complete elimination of aquatic vegetation in the reservoir. Subsequent golden shiner, eastern mosquitofish, and green sunfish population declines were observed; Se is not thought to be the cause, as declines were likely due to loss of aquatic vegetation habitat and interspecific competition. Following tilapia introduction, green sunfish, gizzard shad, and satinfin shiner remained the dominant fish species in the reservoir.

Green sunfish and satinfin shiner populations declined 95 percent within five years of system installation. During the recovery period, populations of these species and gizzard shad decreased, possibly due to competition with increasing populations of bluegill, largemouth bass, crappie (*Pomoxis* spp.), and yellow perch (*Perca flavescens*). This observed switch to a bluegill-dominated community during system recovery is expected for this region.

2.3.2 Belews Lake (Barwick and Harrell 1997) NC

2.3.2.1 Study Background and Methods

Belews Lake was created in 1972 to provide a source of cooling water for Duke Power in North Carolina. Beginning in the fall of 1975, this lake was also used to hold power plant discharge from coal fly ash ponds. Following the installation of a dry ash system in 1984-1985, discharge to the lake ceased.

Barwick and Harrell evaluated fish population monitoring and tissue Se data to document the recovery of Belews Lake in the ten years following cessation of Se discharge. From 1983 to 1994, Se concentrations were determined in the muscle of catfish (*Ameiurus* spp. and *Ictalurus* spp.), green sunfish, and bluegill collected from five Belews Lake sites with varying Se exposure. Fish diversity and biomass data were collected from 1977 to 1994 (with the exception of 1978-1979 and 1982-1983) at two sites on the lake.

2.3.2.2 Results

The authors reported mean fish tissue Se concentrations of up to 21.7 µg/g wet weight at the four sites closest to the discharge point. The site furthest from the discharge, Station 5, had a much lower tissue Se concentrations that rarely exceeded 3.0 µg/g wet weight.

Se concentrations in catfish, green sunfish, and bluegill muscle were highest in sites closest to the discharge; maximum Se concentrations in these species were 10.4, 21.7, and 16.5 µg/g wet weight, respectively. Maximum Se concentrations in these same species collected from Station 5 were 1.8, 14.9, and 3.2 µg/g wet weight, respectively. The green sunfish value of 14.9 µg/g wet weight appears to be an outlier, as it was much higher than other values found at Station 5; the authors suggested that this value may represent a fish that moved to Station 5 from a more highly contaminated part of the lake.

By 1987-1988, mean Se concentrations had decreased at contaminated sites, and by 1992 maximum concentrations in catfish, green sunfish, and bluegill were 2.6, 3.8, and 3.2 µg/g wet weight, respectively. After 1992, fish muscle Se concentrations all remained below 5 µg/g wet weight.

Over the course of the study, the number of taxa significantly increased ($p < 0.005$) from 7 to 22 and biomass significantly increased ($p < 0.011$) from 5.67 to 79.66 kg/ha. Diversity and biomass values from 1984 were significantly greater than those from 1977-1981; while values remained similar until the end of the study, there was a considerable amount of change in species composition. Common carp (*Cyprinus carpio*) and channel catfish (*I. punctatus*) were the dominant fish species in 1977, comprising 87 percent of the biomass. Fathead minnows and eastern mosquitofish dominated the fish community in 1980 and 1981, representing 62 percent and 81 percent of the biomass, respectively. The authors suggest this may be an indication of Se tolerance in these species. By 1984, red shiner (*Cyprinella lutrensis*), common carp, fathead minnow, and green sunfish were dominant, representing 87 percent of the lake's biomass. By 1987-1988, red shiner and fathead minnow populations had decreased, potentially due to increased competition and predation by the expanding fish community, including increased numbers of recovering piscivores, such as green sunfish. In 1989-1990, however, green sunfish populations decreased and kept decreasing until 1994, when this species made up only 1 percent of the lake's biomass. In 1990-1994, gizzard shad, channel catfish, bluegill, and largemouth bass populations increased. Overall, there was a decrease in biomass from 1993-1994; the authors believe this may be due to increased largemouth bass recruitment and associated increases in predation.

The authors point out that even though fish diversity and biomass did increase from 1977-1994, these parameters are still lower than values reported for 1972-1975 in other studies. However, this is to be expected, as new lakes are known to have higher biodiversity and biomass rates which gradually decrease over time. Overall, from the mid-1970s to the early 1980s, Se concentrations in fish decreased while diversity and biomass increased. In addition, diversity and biomass values from 1991-1994 were found to be similar those reported for other North Carolina and South Carolina lakes and reservoirs.

3.0 Summary Discussion

It is difficult to find comprehensive studies evaluating the potential adverse effects of elevated Se on fish populations that include measurement of other potential physical habitat factors or stressors. This lack of comprehensive studies may be a result, in part, of the seeming need for better tissue threshold data, requiring most of the research effort to be focused on effects studies on individual fish species. However, when thresholds are developed, it is clear that predictive qualities to fish populations in natural waters would be useful.

This chapter evaluated studies that did include a complete evaluation of fish populations in environments with widely varying levels of Se and also varying habitat conditions. These studies appear to indicate that Se may be a factor in structuring fish communities, especially with regard to specific fish families, such as centrarchids. However, based on these available studies, it is also apparent that habitat quality is a major factor to consider when trying to discern Se effects on fish in streams, especially warmwater streams. Habitat constraints to fish communities cannot be accounted for in a lab setting when developing tissue thresholds. While the "winter stress" syndrome has garnered attention with regard to Se effects (EPA 2004), the field studies also indicate that warm summer temperatures can restrict fish use of waters, regardless of Se concentrations.

In warmwater streams, this analysis indicates major species-specific differences to consider, both in terms of potential Se impacts, but also with regard to habitat constraints or preferences. In fact, some families appear to decline with increasing Se, whereas others are unaffected in the same exposure range, while some appear to actually increase in density (possibly due to Se effects on their competitors or reduced predation, or co-variance with habitat). Many of these differences appear to be related to fish size, as large-bodied fish, such as members of the Centrarchidae family, showed significant influences of Se concentrations and presence of suitable (deep water) habitats. Smaller fish, such as cyprinids, appeared to thrive in the shallow warmwater streams, despite Se.

Natural history factors, such as migratory versus non-migratory populations, replacement (immigration) or loss (emigration) of fish, competition between fish species, and the balance between terrestrial (assumed low Se) versus aquatic (potentially high Se) food sources, are also important when determining if patterns observed in fish populations are Se-related. These factors may be more important for salmonids than warmwater fish or non-trout coldwater fish like sculpin.

Despite modeling of Van Kirk and Hill, their threshold for cutthroat trout of 7 µg/g was frequently approached or exceeded in the Thompson Creek trout populations with no measurable effect on the population – even with more than 25 years of sampling

(certainly long enough to measure the predicted effects). This may be a result of their predicted compensatory mechanisms or simply evidence that their predictions are not transferable to other systems.

Lastly, long-term Se-toxicity studies in lakes reveal that populations of bluegill and largemouth bass have the ability to resume normal reproduction and exhibit reduced Se tissue concentrations within two to seven years of the cessation of Se exposure from fly ash discharge.

4.0 References

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Recommendations

The evaluations presented in this report support that larval deformities and mortality resulting from the maternal transfer of Se to the ovaries and eggs is the critical exposure pathway and endpoint for Se toxicity in fish. Accordingly, the Se concentration in the egg is the most relevant tissue for linking Se exposures to adverse effects in the development of a criterion or threshold.

Inter-species and inter-study relationships between Se concentrations in the eggs and other tissues are highly variable, suggesting that other tissues are not a broadly useful predictor of Se toxicity. Further, collection of adult fish for egg Se analysis is a useful biomonitor because the adults are relatively insensitive to Se. Although egg Se is recommended as a broadly applicable tissue-based criterion, site- and species-specific studies may still be conducted to develop a site- and species-specific relationship between egg Se and other tissues (e.g., whole-body Se).

In terms of documenting Se-related impacts to fish populations in the field, data are too limited to evaluate whether exceedance of various egg Se thresholds is indicative of population-level effects. Fish population evaluations based on whole-body Se concentrations suggest that it is difficult to discern habitat-related effects from Se-related effects, and that species-specific (or even family-specific) differences are important. Perhaps data on the relationship between egg Se and larval deformities and mortality can be used in future field studies to field-truth whether egg Se concentrations are predictive of population-level effects in fish.

In summary, this report makes the following key conclusions and recommendations:

- A broadly applicable Se threshold for fish should be based on the Se concentration in eggs.
- The critical toxicity endpoint is larval deformities and mortality, resulting from maternal transfer of Se to the eggs.
- Collection of adult fish (an insensitive life stage) for egg Se analysis is a good biomonitor of exposure that is directly linked to the sensitive larval deformities and mortality endpoint (a good biomonitor of effects).
- Se thresholds have not been validated as meaningful in predicting effects in stream fish populations, apparently as a result of some combination of the following: (1) overwhelming influence of habitat conditions; (2) lack of meaningful thresholds for species actually predominant in flowing water systems; and (3) potential acclimation processes in systems with historically elevated Se (whether from natural or anthropogenic sources).

- Recommendations for future studies include:
  - A concerted effort to conduct more maternal-transfer larval Se-effects studies on more fish species is necessary.
    - While the database has improved since the EPA (2004) draft criteria document, there are still large gaps in key fish families with no chronic Se effects data.
  - Retesting of species with published Se-effects thresholds based on tissues other than egg/ovary to confirm back-calculated values or provide new values.

Appendix A

Standard Operating Procedure for Evaluating Selenium-Induced Deformities in Early Life Stages of Freshwater Fish

Standard Operating Procedure for Evaluating Selenium-Induced Deformities in Early Life Stages of Freshwater Fish

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Scope

Toxicant-specific responses are extremely useful tools in toxicology since they can mechanistically link cause and effect, and thus (theoretically) simplify risk assessment. Teratogenicity of larvae/fry exposed to Se via maternal transfer is an example of such a response. If possible, this response should be utilized when Se is of concern since it can contribute significantly to a weight of evidence approach. Thus, when a broadly applicable tissue Se criterion is exceeded at a site, evaluation of characteristic terata in larvae/fry should be considered as an integral part of any effects-based investigation. Nonetheless, endpoints other than terata should also be included, and deciding which endpoints to use may be site- and/or species-specific as outlined above. There has not been a consensus on specific methodological details involved in conducting Se deformity evaluations in larval fish, and there is a need to develop a generic protocol for such evaluations. Although there will always be differences among personnel assessing terata due to the qualitative nature of deformity evaluations, a more rigorous set of rules is needed. Our goal is to prepare a standardized operating protocol for conducting deformity evaluations in fish larvae/fry that is applicable to both laboratory and field investigations of potential early life stage Se toxicity. This document and its associated recommendations may assist with Se toxicity comparisons among sites and/or species, and ultimately help build a more robust database of early life stage Se toxicity to fishes.

This guideline describes universal procedures, conditions and recommendations for gamete collection, embryo incubations and evaluation of selenium-induced deformities in freshwater fish. The methods presented in this guideline are based largely on northern pike (*Esox lucius*), white sucker (*Catostomus commersoni*) and lake trout (*Salvelinus namaycush*) embryo incubations and deformity evaluations developed at the Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada. Although this guide is broadly applicable to other freshwater fish species, some modifications from the procedures described here could be justified under special circumstances. In addition, although this guide focuses on embryo incubations carried out under laboratory conditions, the same approach, with modifications, is also applicable to embryo incubations in field settings. Recommendations for experimental design, statistical treatment of data and detailed guidance for performing selenium effects evaluation in fish larvae and fry are provided. Advantages and disadvantages of different approaches are also provided when appropriate. This standard guide does not address safety concerns; it is the responsibility of individual investigators to establish appropriate safety practices.

1. Fish sampling and acquisition of test organisms

Gametes (eggs and sperm) can be obtained from brood fish cultured in the laboratory, hatcheries or wild populations, depending on the experimental design. This document will focus on procedures for obtaining gametes from wild fish, although the same principles apply

to fish raised under more controlled conditions of selenium exposure, such as in research laboratories or fish hatcheries. For wild fish collections, investigators should be aware that local governments usually require permits for wild fish collection and gamete transport (Canadian Council on Animal Care 2003, 2005). Transportation of fish or gametes across state or provincial boundaries will require sufficient lead time to ensure proper regulations are addressed. This will be particularly challenging if gametes obtained from species at risk are being collected. State/provincial fish and wildlife or environment departments are the best avenue for initial inquiries.

1.1. Field collection and handling of adult fish

Many fish species spawn in spring, and the life history of the species being investigated should be reviewed, taking into account latitudinal differences in time of spawning, to ensure proper timing of field collections. Some fish families (e.g., salmonids, esocids, catostomids) are synchronous spawners, where all eggs are at the same stage of development and are ovulated over a short period of time (e.g., 1 - 2 days). Water temperature is the most important environmental cue for synchronous spawning fish species. In contrast, many fish species are asynchronous (also referred to as batch or indeterminate spawners), where eggs at different stages of development (previtellogenic, vitellogenic, and preovulatory) are present in the ovary and spawning occurs over several weeks or even months (Mommsen and Walsh 1988).

Spawning adults can be collected in the field using a wide variety of techniques, including fish traps (e.g., hoop or trap nets), electrofishing or angling in areas close to spawning areas. Gillnets are also effective in capturing fish during spawning migrations but it is essential to monitor these nets constantly to remove fish immediately after capture. If possible, the use of passive capture methods (e.g., hoop or trap nets) is recommended since this is the least stressful capture technique of those listed above. Trap nets are usually set up in creeks, streams or narrows in lakes, although successful fish capture can also occur when these nets are set perpendicular to shore in lentic habitats. Trap or hoop nest can be purchased from fisheries suppliers, or even constructed in creeks and streams using chicken wire, baling wire and reinforcing bar.

Water quality parameters (e.g., pH, dissolved oxygen [DO], temperature) should be recorded daily during fish collection and holding. Once collected, ripe males and females are held separately without food in net-pens (made of durable material, such as synthetic knotless netting, that will not harm fish) or in onshore holding pens (totes) under flow-through conditions. Spawning fish should be held for no more than 5 days prior to gamete collection to avoid potential resorption of eggs (Springate et al. 1984; Kjörsvik et al. 1990; Aegerter and Jalabert 2004). If fish are not expressing eggs or milt after 2 - 3 days of holding, intramuscular administration of a gonadotropin-releasing hormone (GnRH) analogue (e.g., Ovaprim®) may be indicated. Crowded conditions in net pens should be avoided. Net

pens should be covered with dark plastic tarps to avoid direct overhead sun exposure and/or fish jumping out. It is highly recommended to enclose the sides and top of the net pen with wire (e.g., chicken wire) to avoid predation (e.g., by raptors, otters, bears, etc.). Fish should be carefully observed during holding for signs of physical damage, mortality or other sources of stress (Canadian Council on Animal Care 2005). Since any handling of the fish will remove the protective body layer of slime, fish should be handled as little as possible using dip nets and soft material gloves.

1.1.1. Anesthetics

Fish must be individually anesthetized (e.g., MS-222 [3-aminobenzoic acid ethyl ester] or quinaldine sulfate) before sample collection to facilitate handling (Canadian Council on Animal Care 2003, 2005). Eugenol (clove oil) is not recommended. The uptake of the anesthetic depends on the gill surface area and the same anesthetic solution can be used for several fish presenting moderate variations in size (Gordon 1967). The anesthetic solution should be prepared using site water. Since the strength of the solution will vary depending on water characteristics (e.g., hardness), it is recommended to record water quality parameters. Sedation should be monitored closely. When opercular movement rate is slow or irregular, fish can be removed from the anesthetic and researchers can proceed with morphological measurements and gamete extraction (Canadian Council on Animal Care 2003).

1.1.2. Fish euthanasia and post anesthesia recovery

1.1.2.1. Euthanasia

Following fish measurements and gamete extraction female fish should be euthanized for tissue sample collection when possible. Use of lethal levels of anesthetics (e.g., MS-222 concentrations > 0.4 g/L) is recommended followed by stunning blow in the head or cervical dislocation (Canadian Council on Animal Care 2005). Fish carcasses must be disposed of according to appropriate state or provincial regulations.

1.1.2.2. Recovery

Male fish should be fully recovered from the anesthesia before being released back into the water. The use of a well aerated recovery tank is recommended, containing fresh site water and located in a sheltered area with low lighting. The temperature in the recovery tank should closely match the temperature of the lake or stream to avoid temperature shock. Signs of recovery should be closely monitored. Fish should be gently released back into the water (approximately at or close to the collection area) only when normal behavior is observed (e.g., alertness, equilibrium) (Canadian Council on Animal Care 2003, 2005).

1.1.3. Morphological measurements

External health examination (e.g., ulcers, parasites) of fish upon collection is desirable. Weight and length measurements of adult fish must be recorded for estimating fish condition factor (CF) (see Section 4.1). Balances can be used to measure weight in the field; however, spring balances are a good tool for fieldwork involving larger fish species because they are small and durable. Fish should be placed on their side on a hard surface covered with clean paper towels for length measurements. The most common length measurements in fish are total length, fork length and standard length (Anderson and Gutreuter 1983). Investigators should clearly report the chosen type of length measurement (e.g., fork length). Appropriate length units (e.g., cm) should be used depending on the size of the collected fish.

1.1.4. Tissue collection

Although whole-body Se analysis is the most common approach used by previous investigators, determination of egg selenium is recommended as the most relevant tissue type for Se analysis as discussed in Chapter 1. In larger fish species (i.e., > 500 g) it may be possible to collect a sample of eggs and muscle (e.g., using muscle plugs) and determine whole-body Se in the remaining carcass. However if a large proportion of eggs are removed for embryo incubations, then the whole-body selenium may be severely underestimated. If whole-body Se determination is not indicated, collection of multiple tissues is recommended to determine tissue-specific Se accumulation. Liver (weight determined on-site), muscle (caudal region), kidney and bone (spine) should then be collected for trace metal analysis. Teflon coated tools are used for the collection of fish tissues. Tools should be cleaned between individual fish samples to avoid cross contamination (see Section 1.3.1). Tissue samples should be rinsed with distilled and deionized (nano-pure) water, transferred to plastic bags (e.g., Ziploc® or whirl-pack®), and kept frozen until analysis (see Section 1.4.4). In addition to tissue collection, collection of two different ageing structures (e.g., cleithra and scales) is required for age determination. Details for ageing structure collection and preparation can be found in Jearld (1983) and Sjolung (1974). It is recommended that the collection of female tissues be completed after gamete fertilization.

In certain cases nondestructive (nonlethal) sampling may be required, for example when investigating species at risk. Spawning fish can be released back into the area they were collected following gamete collection. In this case, eggs can be collected for embryo incubations and Se analysis without having to euthanize female fish. If fish are large enough, a muscle plug can also be collected for Se analysis if required.

1.2. Gamete collection and fertilization procedures

1.2.1 Gamete collection

Adult fish for gamete collection should be randomly selected from net pens. Eggs or milt should not be in contact with water before fertilization; thus, it is imperative to dry the area surrounding the urogenital opening with paper towels. All the material used for gamete collection should be carefully cleaned (see Section 1.3.2) and dried. Precautions to avoid fecal, blood or urine contamination should be taken. Gametes must be kept covered to avoid direct sun exposure. Collection of gametes from all fish before proceeding with fertilizations is recommended (see Section 1.2.2).

1.2.1.1. Milt collection

Collection of milt from males should be performed prior to egg collection. Male fish should be anesthetized (see Section 1.1.1) and gamete collection should proceed after recording weight and length (see Section 1.1.3). Milt, collected by applying light pressure on the abdomen, should be placed into clean Eppendorf® or Falcon™ tubes (see Section 1.3.2). Milt should be collected from a minimum of three males and kept in separate vials on ice until use (Environment Canada 1998). Care should be taken to prevent the contamination of sperm (e.g., with urine or feces), which can severely reduce sperm viability. However, any urine, feces or blood present in the milt vial after collection should be removed as soon as possible by using a clean (see Section 1.3.2) plastic transfer pipette. Latex gloves should be changed between fish. Male fish can be released back into the water after anesthesia recovery (see Section 1.1.2.2).

1.2.1.2. Egg collection

Female fish should be anesthetized (see Section 1.1.1) and gamete collection should proceed after recording weight and length (see Section 1.1.3). Gentle pressure from behind the pectoral fins towards the anus is applied to express the eggs. This process needs to be repeated several times. Check that eggs are released "clean" (e.g., without feces) before starting collection to avoid contamination of the entire egg batch. Eggs should be obtained from a minimum of four females (Environment Canada 1998**)**, although larger sample sizes are desirable, especially if releasing females after gamete collection. Eggs are individually collected into pre-cleaned (see Section 1.3.2) stainless steel bowls and kept covered in a cool place until use. Collected eggs should be closely inspected and eggs with adhered feces, urine or blood discarded by using a clean (see Section 1.3.2) plastic pipette. After gamete collection, female carcasses should be labeled and kept on ice until tissue collection (see Section 1.1.4). It is critical to correctly label the females to further match egg and tissue metal concentrations with frequencies of specific deformities.

1.2.2. Fertilization

Before fertilization, a sub-sample of eggs from each female should be collected using a clean (see Section 1.3.2) plastic spoon, placed into whirl-pack bags for trace metals analysis, and kept frozen until use. The mass of eggs collected will depend on the fish size and analytical technique used to determine trace elements. For larger fish, a 10 - 20 g subsample of eggs is sufficient for trace metal analysis. If small-bodied fish are being collected, it is advised to consult with the analytical personnel responsible for trace element analysis to ensure sufficient egg mass is collected.

Eggs from each female are fertilized separately with pooled milt derived from the captured males at the same site. Alternatively, a "dry fertilization" procedure can be followed where gametes are transported in isolation to the laboratory prior to fertilization (Holm et al. 2005). Collected milt is pooled in one vial and equal amounts (~ 0.5 ml) poured into each individual egg bowl using a clean (see Section 1.3.2) plastic pipette. Milt should be evenly distributed on the egg batch. Eggs are combined with the milt by swirling gently by hand for 1 minute. Enough site water to cover the eggs is then added to activate the sperm and gently mixed for 2 to 3 minutes. After fertilization, a bentonite (potter's) clay solution (~ one teaspoon of clay per 200-ml of site water) is added to the eggs to prevent clumping, and thoroughly rinsed 2 to 3 times after 5 minutes using the same site water to remove any remaining clay. Eggs from each individual female are then transferred to 4-L pre-cleaned Eagle Picher (level 1) plastic jars filled with site water (no head space) for egg hardening. Embryos should be carefully oscillated for 1 to 2 minutes every 20 to 30 minutes for approximately 2 hours to prevent clumping. Egg hardening occurs between 2 to 3 hours after fertilization.

1.3. Tool sterilization and cleaning

1.3.1. Tissue collection

Tools used for tissue collection should be cleaned between samples to avoid cross contamination. It is recommended to rinse all materials with nitric acid (5 percent) followed by several rinses with deionized water.

1.3.2. Gamete collection

All steps involved in gamete collection must include hygienic (sterile) precautions to avoid cross contamination between different fishes. All materials (e.g., bowls, pipette) used during the egg fertilization procedure should be sterilized using a 0.000075 percent (~ 0.04 ml in 5 L of water) betadyne (10 percent povione-iodine, 1 percent free iodine) solution prepared with deionized water and rinsed with deionized water to remove any remaining iodine. The use of latex examination gloves is strongly recommended during

fertilization procedures. Gloved hands should be washed with betadyne solution (see above), rinsed with deionized water and carefully dried before and after egg handling.

1.4. Sample labeling, transport and storage

Location, adult female number, fish species, collection date, and name of researcher should be recorded on the label for each individual sample.

1.4.1 Embryo transport

Embryos (or gametes if conducting dry fertilizations) can be transported to the laboratory, or to field sites if conducting *in situ* embryo incubations. Embryos should be kept cool (generally at < 10°C) and be water hardened before transportation (ASTM 2003). All embryo containers should be packed in cooler(s) with ice or freezer packs to maintain the temperature and avoid severe jolts during transportation. It is recommended that embryos be transported within the first 48 hours after fertilization (Environment Canada 1998; ASTM 2003). Transported embryos should follow the incubation procedure described in Section 2.6 after arrival at laboratory facilities. Unfertilized eggs and milt can be transported for 24 hours after collection. Gametes should be kept in plastic bags on ice for transport (ASTM 2003). If unfertilized gametes are transported, fertilization procedures as described in Section 1.2.2 should follow after arrival at research facilities.

1.4.2 Fish eggs and tissues for trace metal analysis

Eggs and tissues should be kept frozen until analysis. After collection samples should be kept in a container with ice or freezer packs until transfer to a freezer (–20°C) for storage. If the transport period exceeds a few hours, it is recommended to transfer all the whirl-packs containing egg and tissues collected from each individual female into sealed Ziploc® bags to prevent water (from ice melting) entering the sample. Storage time is 6 months to 2 years at –20°C for the majority of trace metals, including selenium (EPA 2000).

1.4.3 Ageing structures

Details for ageing structure storage can be found in Jearld (1983) and Sjolung (1974).

1.5. Field material disposal

All disposable material utilized in the field should be collected in garbage bags for disposal. Acid and iodine solutions utilized for tool cleaning should be collected in plastic jars and disposed according to local regulations.

1.6. Preparation of egg and tissue samples for metal analysis

Egg and tissue samples should be thawed and wet weight recorded for each individual sample. To prevent cross contamination between samples, a plastic foil (e.g., parafilm®) should be placed on the scale and replaced after each weighing. Samples are oven dried at 60°C until constant weight is recorded. It is required to record the moisture content for each individual sample in order to express analytical data on a dry weight basis. Trace element analysis is routinely performed using hydride generation atomic absorption spectrophotometry (HG-AAS) or inductively coupled plasma-mass spectrometry (ICP-MS) and reported on a dry-weight basis.

1.6.1. Egg and muscle

A sub-sample of eggs and muscle is cut from the frozen sample using a scalpel. Samples should be rinsed with nano-pure water and then oven dried in a clean plastic vial. Samples are stored in a dry place until analysis. The remaining sample should be archived at –20°C with collection site location, adult female, fish species, collection date and expiration date recorded on the sample label.

1.6.2. Bone

A sub-sample of bone can be cut from the frozen sample using a scalpel and the remaining sample archived at –20°C with collection site location, adult female, fish species collection date and expiration date recorded on the sample label. Bone sub-samples are dipped into boiling nano-pure water in a clean beaker (see Section 1.6.4). Attached flesh should be removed carefully with Teflon®coated tweezers. Clean bone is then rinsed with nano-pure water, carefully dried with a paper towel, weighed (wet weight) and oven dried in a clean plastic vial. The dried sample is ground to a powder in a clean porcelain mortar with a pestle following dry-weight determinations. Samples are stored in a dry place until analysis.

1.6.3. Liver and kidney

Liver and kidney samples should be homogenized due to the heterogeneous nature of these organs and to ensure even distribution of contaminants through the samples. Samples are partially thawed, rinsed with nano-pure water and cut into pieces using a scalpel. Samples should be homogenized using a Teflon® pestle with a fitted plastic tube and motor homogenizer. Homogenates are oven dried in a plastic vial and stored in a dry place until analysis.

1.6.4. Labware cleaning

Labware (e.g., beakers) should be soaked (~ 12 hours) in detergent and rinsed with deionized water before acid washing. All labware in contact with the samples (except Teflon coated material) should be soaked for a minimum of 3 hours in a nitric acid bath (5 percent, analytical grade), rinsed with deionized water, and thoroughly rinsed with nano-pure water. Plastic vials used for sample drying and storage should be rinsed with ultra-pure nitric acid (5 percent) following acid soak and prior to rinse with deionized and nano-pure water. Teflon® coated materials corrode in acid and therefore a rapid rinse with 10 percent ultra pure nitric acid, followed by several rinses with nano-pure water is advised. All the washed materials should air dry for at least 24 hours before use.

2. Embryo incubation procedures

2.1 Facilities

Several options are available for embryo incubations and depend on available facilities. Ideally, fish hatchery equipment such as Heath trays or Robertson jars can be used although these are not always available and their use in the field is logistically challenging. In this document we describe the use of a simple and inexpensive approach (Environment Canada 1998) that we have used successfully in northern pike, white sucker, and lake trout embryo-alevin-fry tests in the laboratory. This approach is also amenable to field studies, and is described below.

Ideally, embryo incubations should be conducted in an environmental chamber with a set photoperiod and constant temperature. Lighting should be provided by overhead full spectrum fluorescent (or equivalent) tubes with intensity ranging from 500 to 1300 lux. The set photoperiod and temperature will vary depending on the fish species used in the test (e.g., for northern pike and white sucker the set photoperiod and temperature should be 16:8 h light:dark and 10 ± 1°C, respectively, while lake trout should be incubated in the dark at 10 ± 1°C). During embryo incubations, disturbances of test organisms should be minimized to prevent unnecessary stress.

2.2 Materials

All materials or equipment should be cleaned with detergent, rinsed with nitric acid (5 percent, analytical grade) and then thoroughly rinsed with deionized water prior to use. Containers (e.g., Nalgene® HDPE bottles) used for the collection of water samples for metal analysis should be soaked in a detergent bath with lids removed for at least 12 hours, followed by several rinses with double-distilled (ultra-pure) nitric acid (5 percent) and nano-pure water. All the washed materials should air dry for at least 24 hours before use. Pre-cleaned containers (e.g., Eagle Picher, level 1) do not have to be cleaned prior to use, unless

re-used. Materials should not contain substances that can leach into the water, cause sorption of elements from water and/or cause toxic effects in fish embryos. Glass, stainless steel, porcelain, nylon and non-toxic plastics (e.g., polyethylene) may be used (Environment Canada 1998). Materials that have been in contact with test water (or solution) can be re-used after following the cleaning method mentioned above. No dip nets, pipettes or other tools should be used between treatments or incubation chambers without being cleaned or sterilized. All materials used during embryo incubation setup must be sterilized using a 0.000075 percent betadyne (povione-iodine) solution (see Section 1.3.2).

2.3 Incubation chambers

Each incubation chamber can be made from a 4-L white plastic (polyethylene) bucket enclosing a smaller bucket (~ 2-L) (Figure 1). A hole is cut in the lid of the 4-L bucket allowing the suspension of the small bucket by inserting it through the hole. The sides of the small bucket are replaced by a non-metallic mesh screen (leaving ~ 2 cm from the bottom) to allow water circulation. A non-toxic aquarium-approved silicon is used to glue the screen to the bucket. This design allows embryos to be immersed in test water at all times during water renewal. A second hole of smaller diameter (~ 1 mm) is cut in the 4-L bucket lid to enable aeration throughout the duration of the test. Air is gently bubbled through a polyethylene capillary tube which is passed though the 1 mm hole (overhanging close to the bottom of the 2-L bucket) providing continuous aerated water to the embryos.

2.4 Water sources and characteristics

Ideally, water used in the test should be collected in the field (from reference and exposure areas) and shipped to the laboratory, or prepared in the laboratory (e.g., reconstituted water). Since all the wastewater should be discarded following local regulations, it is important to consider the volume of the generated wastewater before starting the test.

2.4.1. Field collected water

Containers for transportation must be thoroughly cleaned and rinsed several times with the water to be collected and should be filled to a maximum without leaving air spaces. Each sample container should be filled, sealed and labeled after water collection. When possible, water should be kept at 4°C during transportation. Upon arrival at the laboratory, a sub-sample for water quality parameters should be collected from each shipped container. Water is then transferred to clean (see Section 2.2) carboys (~ 50-L, previously rinsed with respective site water) and kept aerated in the environmental chamber to reach test temperature for 24 hours prior to its use in the test. The remaining water for subsequent water renewals should be stored in sealed containers in the dark.

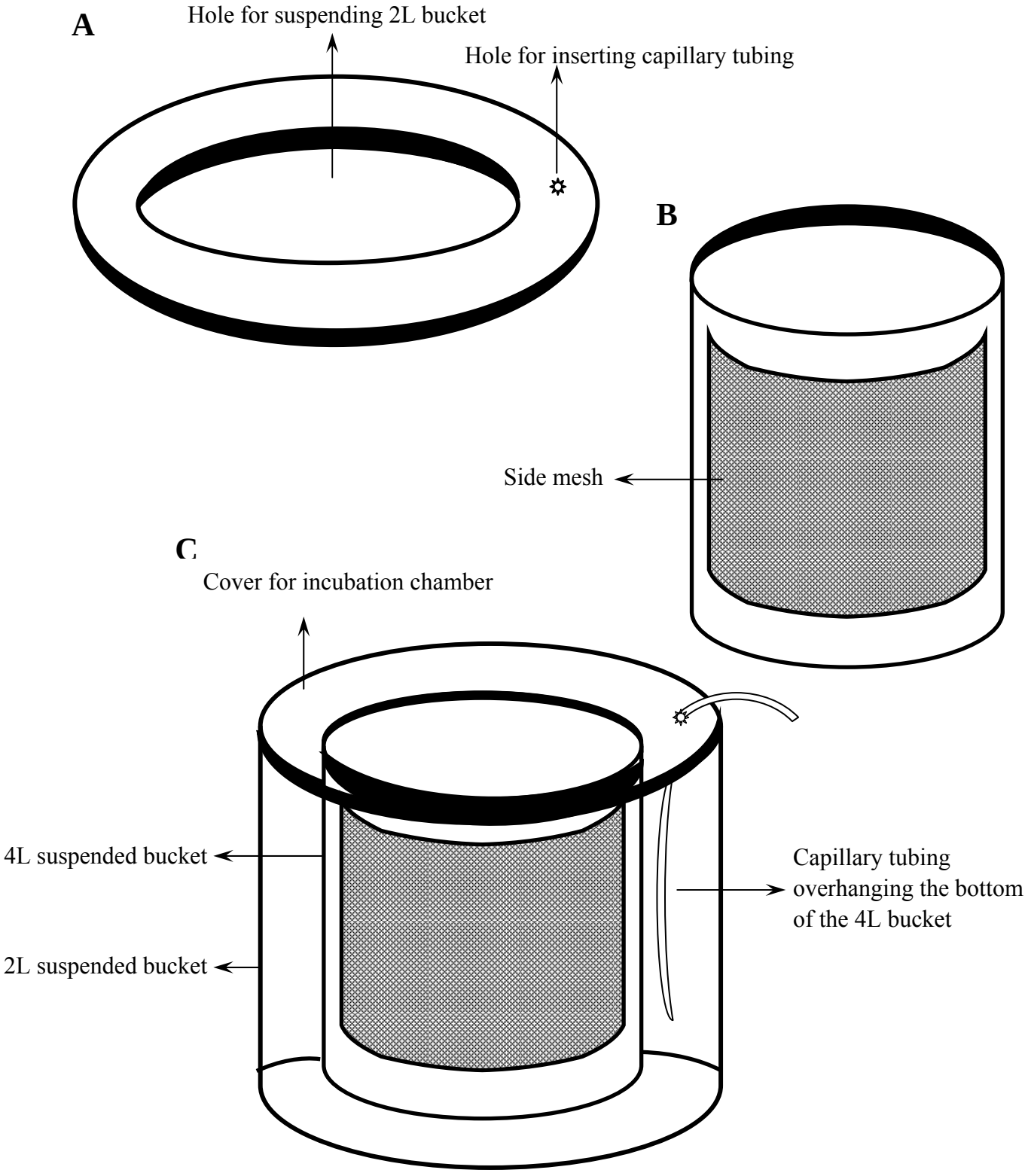

Figure 1: Diagram of proposed incubation chambers for fish embryos. A) Cover lid of the incubation chamber, B) 2L bucket showing side mesh replacement, C) Incubation chamber diagram showing the 2L bucket suspended inside the 4L bucket and capillary tubing.

2.4.2. Reconstituted water

Information on reconstituted solution preparation can be found in Environment Canada (1998). It is recommended that the prepared solutions follow as close as possible field water characteristics. The use of reconstituted water in early life stage tests is not recommended due to the large volume necessary for these tests (ASTM 2003).

2.4.3. Test water characteristics

Water characteristics should be acceptable for the survival and growth of the test organisms. Water must be intensively aerated prior to addition to the incubation chambers. Dissolved oxygen in the water should be at 60 to 100 percent saturation in all the incubation chambers (ASTM 2003). The pH range should normally be between 6.5 and 8.5 (Environment Canada 1998). The temperature of test/site water should be adjusted as required for each fish species (e.g., temperature for pike incubations, 10 ± 1°C).

2.6. Procedures

2.6.1. Experimental design

Ideally, embryos should be incubated using a two-way (cross-over) ANOVA experimental design using water either from reference/control or exposure sites (Muscatello et al. 2006; Muscatello and Janz 2008). Thus, embryos originating from reference or exposure site females are incubated in either reference or exposure site water. Importantly, the two-way ANOVA experimental design allows statistical discrimination between effects due to maternal transfer and effects due to exposure of developing embryos to site water. Although this experimental design may not be appropriate for all studies, it is recommended to allow statistical discrimination between potential effects due to maternal transfer vs. aqueous exposures, especially when testing complex effluents. The minimum desirable number of replicates (incubation chambers) per female is 3. Each chamber should contain at least 40 embryos. The number of organisms per replicate is determined based on the expected size of the larvae at the end of the test and should not exceed 0.5 g/L (total mass of organisms/liter of water) (ASTM 2003). It is recommended to add three extra replicates per female for fry weight and length determinations.

2.6.2. Experimental setup

All materials used during embryo incubation procedures should be sterilized using a 0.000075 percent betadyne (povione-iodine) solution (see Section 1.3.2). Upon arrival at the laboratory, a random sub-sample of approximately 100 embryos from each female should be collected in 20-ml scintillation vials for the determination of fertilization success. (See Section 4.3). Photographs taken with the use of a dissecting microscope are also recommended to quantify egg diameter and document fertilization. Fertilization success

should be calculated as soon as the experimental setup is completed. Water in embryo transport containers should be replaced gradually to achieve test temperature and prevent thermal shock. Before transfer to incubation chambers, embryos should be placed in a 0.000075 percent betadyne (povione-iodine) solution (see Section 1.3.2) for approximately 15 minutes to discourage fungal growth (DeRosemond et al. 2005). After water hardening, egg membranes are impermeable and therefore safe to treat with iodine (Environment Canada 1998). The temperature of the prepared iodine solution should match the water temperature set during the test. Following the iodine treatment, the embryos should be rinsed with test/site water to remove any remaining iodine. Fish embryos should be immersed in water at all times. Embryos are gently transferred to a clean (see Section 1.3.2) glass tray and viable embryos (non-opaque) from each individual female fish randomly transferred (using a plastic pipette with a cut end if necessary) to each replicate incubation chamber. Floating embryos should be gently squirted with water to sink. An identical number of embryos should be added to each incubation chamber. Each replicate should be clearly labeled and randomly allocated in the environmental facility. It should be noted that the pre-eyed stage of embryo development is an extremely sensitive period; thus, it is recommended to not disturb the embryos except for gentle removal of infertile eggs and/or dead embryos (ASTM 2003; Environment Canada 1998). To serve as back-up specimens, the remaining embryos not used in the test should be kept in the environmental chamber provided with aerated and fresh site/test water for at least 7 days.

2.6.3. Test options and water replacement

2.6.3.1. Static-renewal

Water in incubation chambers should be replaced every 2-3 days with new test/site water. The inside bucket (2-L) in the incubation chambers containing viable embryos is gently removed and transferred to a new outer bucket (4-L) containing fresh site/test water (see Figure 1). The inside bucket should be gently re-suspended into the new 4-L bucket to prevent the embryos from floating. Each 4-L bucket should be cleaned (see Section 2.2) before use. This procedure allows water renewal without disturbing developing embryos. The incubation chambers should hold a minimum of 2 L of water.

2.6.3.2. Flow-through

Flow-through tests continually deliver fresh test/site water to incubation chambers. There are several designs and devices to create successive water renewals. Flow rates should be checked daily throughout the test. Flow rate speed will depend on experimental requirements (Environment Canada 1998). It is recommended to adjust flow rates to allow complete water renewal every 2 days. Caution to avoid embryo disturbance due to high flow rates should be taken.

2.6.4. Test measurements and endpoints

2.6.4.1. Water quality variables

Throughout the test, DO and temperature must be recorded daily in all the incubation chambers. Water samples for routine water quality analyses (hardness, alkalinity, conductivity, pH and ammonia) must be collected from three incubation chambers per treatment before and after water renewal.

2.6.4.2. Water collection for trace metal analysis

Water samples for trace metal analysis should be collected from three incubation chambers per treatment before and after water renewal. It is recommended that the concentration of trace metals be measured at least weekly during the test. Samples for dissolved metal analysis are collected using a 20-ml sterilized plastic syringe and placed in pre-cleaned (see Section 2.2) 8-ml Nalgene® bottles following filtration through a 0.45 μm Nalgene® disposable filter. Samples are then acidified with double distilled (ultra-pure) nitric acid to pH < 2 and stored at 4°C until analyzed. Samples for total metals should be acidified without filtration. Metal analysis should be performed using hydride generation atomic absorption spectrophotometer (HG-AAS) or inductively coupled plasma-mass spectrometry (ICP-MS) within 6 months of collection (EPA 1983).

2.6.4.3. Mortality and embryo developmental time

Embryo mortality should be recorded daily in all incubation chambers throughout the study. The criterion for mortality in embryos is usually opaqueness or presenting some fungal growth. For fish larvae the mortality criterion is immobility and/or not responding to gently prodding. Dead eggs and embryos should be gently removed using a plastic pipette. Cumulative time (degree-days) to the 50 percent eyed embryo, 50 percent hatch, and 50 percent swim up stages must be recorded when at least one-half of the embryos reach these stages in each incubation chamber. The time required for hatching depends on the incubation temperature and fish species tested (e.g., pike and white sucker hatching time is ~ 10 days at 10 ± 1°C).

2.6.5. Test duration and fish larva preservation

The experiment is terminated individually for each incubation chamber when the majority (~ 100 percent) of the fry exhibit swim-up and have absorbed the egg yolk. At this stage fish larvae surface and swim actively. Although the yolk sac is no longer visible, its absorption may not be complete. Therefore, fry exhibiting swimming behavior are a more appropriate indicator of attaining the swim up stage than yolk absorption (Environment Canada 1998). Fry must be euthanized with an overdose of MS-222 (~ 0.8 g/L), preserved in

an appropriate fixative (e.g., 10 percent buffered formalin) for 24 hours, and then transferred to 70 percent ethanol for subsequent evaluation of deformities. It is recommended to use double-capped plastic bottles for fry storage to avoid evaporation of ethanol. Fry from extra replicates used for the evaluation of fry condition factor should be euthanized (as previously indicated) prior to weight (wet) and length (either total, fork or standard) evaluations. Individual fish weight is preferred; however, small fish can be weighed in groups and individual weight obtained by dividing by the total number of animals.

3. Evaluation of morphological deformities in fish larvae

All fry should be collected and properly preserved (see Section 2.6.5) before proceeding with the deformity analysis. It is essential to euthanize fry with an overdose of anesthetic prior to preservation to avoid potential artifacts (i.e., spinal curvatures). The same investigator should perform analyses for a given experiment. Evaluation of developmental malformations must be performed in a blinded fashion by covering identification labels on vials of preserved larvae. It is imperative to be consistent with the decision-making rules for identifying specific deformities. If rules change during the analysis, all the preserved fry should be re-evaluated again, taking into consideration the new set of rules. It is highly recommended to perform a preliminary evaluation of all preserved fry (blinded) to develop a plan for the definitive deformity evaluations. The use of a dissecting microscope with an attached camera for recording the different categories of evaluated deformities is ideal. Abnormalities are recorded in four categories: skeletal curvatures, craniofacial, finfold, and edema (Holm et al. 2005; Muscatello et al. 2006).

There are three techniques that have previously been used for evaluation of Se-induced deformities in larval fish: (1) morphometric analysis, which involves measurement of distances between specific landmarks on the body of fry, (2) a graduated severity index, which subjectively evaluates the relative severity of each specific deformity, and (3) frequency analysis, in which each deformity is determined to be either present or absent. The most detailed comparison of these three approaches has been provided by Holm et al. (2003) in the evaluation of Se-induced deformities in rainbow trout and brook trout. The conclusion reached in this study was that frequency analysis provided the most reproducible and meaningful results (Holm et al. 2003). However, in certain situations, which may depend on species-specific responses, the graduated severity index may be appropriate. In terms of simplicity and reduced variation among personnel evaluating deformities, it is recommended that frequency analysis be conducted in most cases, at least as an initial evaluation of deformities.

3.1. Procedures

All pertinent information regarding deformity analysis should be recorded on the appropriate data sheet containing the researcher name, project information, fish species

evaluated and date on the top of each page. Each work sheet should be numbered and have columns with space to record replicate number and label, categories of deformities, and additional notes. The deformity analysis should start with the reference/control group. The investigator should be familiar with all the morphological characteristics of the reference fish before proceeding with deformity identifications in exposure larvae. Deformity evaluations should continue for a minimum of three times before attempting the final analysis. It is recommended that differences between evaluations be less than 10 percent. It is advised to perform statistical analysis to evaluate (e.g., using ANOVA) differences between deformity evaluation attempts.

Fry should be carefully transferred to a Petri dish and the remaining ethanol solution kept in an Erlenmeyer (see Section 2.6.5). Fry should always be covered by ethanol during analysis. Fish larvae must be inspected from lateral, dorsal and ventral views. It is recommended to increase the magnification of the dissecting microscope to detect any slight deformities present in the operculum and jawbones. Tools for larvae handling should be used carefully to prevent damage to the sample; thus, it is recommended the use of tweezers with a round end and a plastic pipette with a cut end during larva inspections. When larvae inspections are finished, fry should be transferred into their respective storage vial (see Section 2.6.5) and the rest of the ethanol solution kept in the Erlenmeyer added. It is recommended to analyze the presence of deformities one vial at a time.

3.2. Graduated severity index

The severity of the evaluated deformities can be recorded using the graduated severity index described in Holm et al. (2003, 2005). Significant differences among personnel in evaluating a graduated severity index are likely to occur; therefore, the applicability of this index is controversial. As mentioned above, it is recommended to conduct frequency analysis (i.e., presence or absence of specific deformities) in order to simplify the deformity evaluations.

4. Calculations and statistical considerations

Statistical analyses should be performed with a 95 percent ($\alpha = 0.05$) level of confidence. Analysis of variance (ANOVA) should be used when more than two treatment groups are being compared. Differences between two treatment groups should be evaluated by *t*-test. Data that fail tests for normality or homogeneity of variance are transformed (e.g., log(10) or arcsine square root) prior to use of parametric statistical tests. If data fail the parametric assumptions (normality and/or homogeneity of variance) after transformation, a suitable non-parametric test (e.g., Kruskall-Wallis) should be used on the non-transformed data. Post-hoc tests for parametric (e.g., Tukey's) or non-parametric (e.g., Dunn's) data should then be used when appropriate.

4.1. Adult fish age, condition factor (CF) and hepatosomatic index (HSI)

Differences in age between adult fish should be evaluated using ANOVA or a t-test. Analysis of covariance (ANCOVA) should be used to compare condition factors (body weights of adult fish with body length as covariate) and hepatosomatic index (liver weight with body weight as covariate) between adult fish. If a significant interaction is present between variable and co-variable (treatment factor), differences should be evaluated using ANOVA or *t*-test (or equivalent non-parametric test) as body weight/(length)^3 x 100 and liver weight/body weight x100 for CF and HSI, respectively.

4.2. Egg and tissue metal concentrations

Differences in egg and tissue metal content among sites or treatments should be evaluated using one-way ANOVA (> 2 treatments) or *t*-test (2 treatments) followed by a post-hoc test when appropriate. Non-parametric statistical tests should be used if data fail assumptions after transformation. Post-hoc evaluations should follow the statistical analysis when appropriate. Best-fit relationships between the incidence of deformities and selenium concentrations in eggs and muscle, and between muscle, bone, kidney, liver and egg selenium concentrations should be evaluated using regression analysis.

4.3. Egg size and fertilization success

Fertilization success is calculated as the number of fertilized eggs divided by the total number of eggs. Fertilization success and egg diameter differences between treatment/sites should be evaluated using ANOVA or a *t*-test. If assumptions for parametric tests are not fulfilled an equivalent non-parametric test should be used. Post-hoc comparisons of means should be used when appropriate.

4.4. Embryo mortality

Total percentage of embryo mortality (calculated as number of dead organisms at the end of the test divided by the total number of organisms per incubation chamber) should be analyzed by two-way ANOVA with egg origin and water source as the two factors of variability. However, percentage of mortality is a nominal variable and thus it is recommended to transform the data using arcsine square root before the statistical analysis. Post-hoc comparisons of means should be used when appropriate.

4.5. Embryo developmental time and fry condition factor

Significant differences among treatments in cumulative time to the 50 percent eyed embryo, 50 percent hatch and 50 percent swim-up stages are evaluated using two-way ANOVA with egg origin and water source as the two factors. Fry condition factor is

calculated as body weight/length^3 x 100 and differences between treatments/groups evaluated using two-way ANOVA (if two factors of variability are significant). If only one of the factors is significant, fry can be grouped and differences in condition factor evaluated using ANOVA or a *t*-test. Post-hoc comparisons of means for embryo developmental time and condition factor should be used when appropriate.

4.6. Percentages of deformities

The frequencies of each category of deformity should be expressed as percentages and calculated as the total number of fry from each individual female pike that exhibited one category of deformity, divided by the total number of fry preserved for that female. It is also recommended to calculate the total number of deformed fish as the total number of abnormal fish (i.e., exhibiting at least one category of deformity) divided by the total number of fry for each individual female. Percentages of deformed larvae should be recorded for each treatment including control. Fry deformities are evaluated using two-way ANOVA with egg origin and water source as the two factors. Since percentage of deformed fish larvae is a nominal variable, transformation of data using arcsine square root is advised. Post-hoc comparisons of means for embryo developmental time and condition factor should be used when appropriate. It is recommended to calculate effective concentration (EC) values (i.e., Se concentration in tissue corresponding to a percent increase in deformities above control value) using appropriate programs (e.g., EPA-TRAP) and to report EC10 and EC20 when possible, with associated 95 percent confidence intervals.

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Appendix B

A Critical Evaluation of Winter Stress Syndrome

A Critical Evaluation of Winter Stress Syndrome

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Introduction

Maintenance of a healthy fish population is primarily influenced by recruitment of new individuals into the population, which is closely linked to survival of juvenile fish beyond their first year. For freshwater fishes in north temperate environments, winter represents a major challenge to young-of-the-year (YOY) survival (Hurst and Conover 1998). The major factors that can increase overwinter mortality of fishes are low temperature (thermal stress), low oxygen (hypoxia/anoxia), reduced food availability (starvation), increased predation, disease, and parasitism (reviewed in Hurst 2007). Most of these factors act more severely in smaller fish due to size-dependent changes in surface: volume relationships (Post and Parkinson 2001). In addition, there can be significant interactions among factors that influence overwinter mortality, as well as annual variation in the severity of winter conditions. It has long been known that freshwater fishes have differing abilities to survive the overwinter period, largely due to species-specific tolerances of these factors (Moore 1942; Mooreman 1957). The complexity of abiotic and biotic factors that can influence overwinter mortality and the logistical difficulty of investigating freshwater fish during the overwinter period (e.g., fish are under ice) have challenged fish biologists and fisheries scientists for many years (Hurst 2007). Thus, it is not surprising that very little research has investigated impacts of overwinter mortality on population dynamics, resulting in imprecise life-history models for many fish populations (Shuter et al. 1980; Shuter and Post 1990).

It is surprising that a recent comprehensive review on causes and consequences of winter mortality in fishes (Hurst 2007) did not discuss the impact of anthropogenic contamination of aquatic ecosystems. In addition to possibly causing mortality due to direct toxic effects, many contaminants have the potential to interact synergistically with some of the factors described above to increase overwinter mortality. In aquatic ecotoxicology, the term "winter stress syndrome" has been used to describe the potential for contaminants to potentiate overwinter mortality (Lemly 1993, 1996). The winter stress syndrome hypothesis is based on a single laboratory study in which juvenile bluegill sunfish (*Lepomis macrochirus*) were exposed for 180 days to dietary and waterborne selenium (Se) under either summer or winter conditions (Lemly 1993). Winter conditions of low water temperature (4°C) exacerbated the toxicity of Se, indicated by increased mortality, decreased condition factor, and decreased energy (lipid) stores. Recently, the EPA (2004) incorporated the concept of winter stress syndrome into a draft tissue-based Se criterion, reducing the criterion from 7.91 to 5.85 µg Se/g dry whole-body weight if fish are sampled prior to winter. Lemly's (1993) laboratory study has recently been replicated, with similar results overall (Great Lakes Environmental Center 2008). Although the concept of winter stress syndrome is a scientifically sound hypothesis, it has rarely been tested under field conditions with individuals exposed to elevated Se or other toxicants. The objective of this manuscript is to provide a critical review of the evidence for winter stress syndrome occurring under natural (field) conditions in freshwater fishes.

1. Known Causes of Overwinter Mortality

1.1 Starvation

It is generally believed that many fish species remain relatively motionless and exhibit reduced or no feeding during the winter (Sayer and Davenport 1996; Pratt and Fox 2002; Bauer and Schlott 2004). Cool water temperatures of approximately 6°C result in lower metabolic demands for fish, while at temperatures near 0°C, fish will generally become torpid (Matthews 1998). Reduction in light due to a shorter photoperiod as well as ice cover decreases primary and secondary productivity and impedes visual predators. Although decreased temperature also causes reduced metabolism in fish, if overall metabolism exceeds energy intake through feeding, then an energy deficit (negative scope for growth) occurs. This is a critical aspect of Lemly's winter stress syndrome hypothesis, since any additional metabolic stressor(s) may enhance the energy deficit. The three conditions noted by Lemly (1996) for winter stress syndrome to occur are: (1) the presence of a metabolic stressor (natural or anthropogenic); (2) cold water temperatures; and (3) reduced activity and foraging by fish. Importantly, the potential of a given stressor to lead to winter stress syndrome depends on its propensity to increase metabolism. Potential metabolic stressors include exposure to inorganic or organic toxicants, parasites, altered pH, and high levels of suspended sediment. The presence of multiple metabolic stressors likely increases the probability of winter stress syndrome (Lemly 1996).

During the first summer of life, it is important for YOY fish to allocate energy to growth as well as energy storage for overwinter survival (Adams 1999; Sogard and Olla 2000; Figure 1). Body size influences an animal's energetic requirements, its potential for resource exploitation, and its susceptibility to natural enemies (Werner and Gilliam 1984). Smaller fish have relatively higher metabolic rates than larger individuals (Love 1980), and therefore have an increased demand for stored energy over the winter compared to larger, older individuals (Cunjak 1988). Since smaller fish have a higher mass-specific metabolic rate and lower energy density than larger fish, size-dependent overwinter mortality may occur (Peters 1983; Post and Parkinson 2001; McCollum et al. 2003). Numerous laboratory and field studies with various fish species have found that smaller YOY fish indeed suffer higher mortality over the winter than larger individuals (Oliver et al. 1979; Toneys and Coble 1980; Post and Evans 1989; Johnson and Evans 1991; Griffiths and Kirkwood 1995; Foy and Paul 1999; Gotceitas et al. 1999; Kristiansen et al. 2000; Sogard and Olla 2000; Grant and Tonn 2002; Biro et al. 2004). In addition, size-dependent predation can regulate winter mortality, as smaller fish are more vulnerable to gape-limited predators (Werner and Gilliam 1984; Kristiansen et al. 2000). Therefore, YOY fish are under intense selective pressure to reach a sufficient size in order to avoid predation, attain resources (e.g., lipid energy stores), decrease relative metabolism, and ultimately, survive the first winter of their lives.

Lipids, stored primarily as triacylglycerols (triglycerides), are the primary source of energy in fish (Figure 1) and are important for both survival and fitness (Adams 1999).

Winter conditions present significant stress for fish as fasting, cold temperatures, and reduced activity are generally accompanied by diminishing stored lipid levels over time (Toneys and Coble 1980; Cunjak 1988; Lemly 1996; Berg and Bremset 1998; Sogard and Olla 2000; Post and Parkinson 2001). During winter, age-0 fish rely on stored energy and some energy intake to meet metabolic demands (Johnson and Evans 1991). Therefore, it is critical for fish to attain sufficient lipid stores before the relative inactivity of winter.

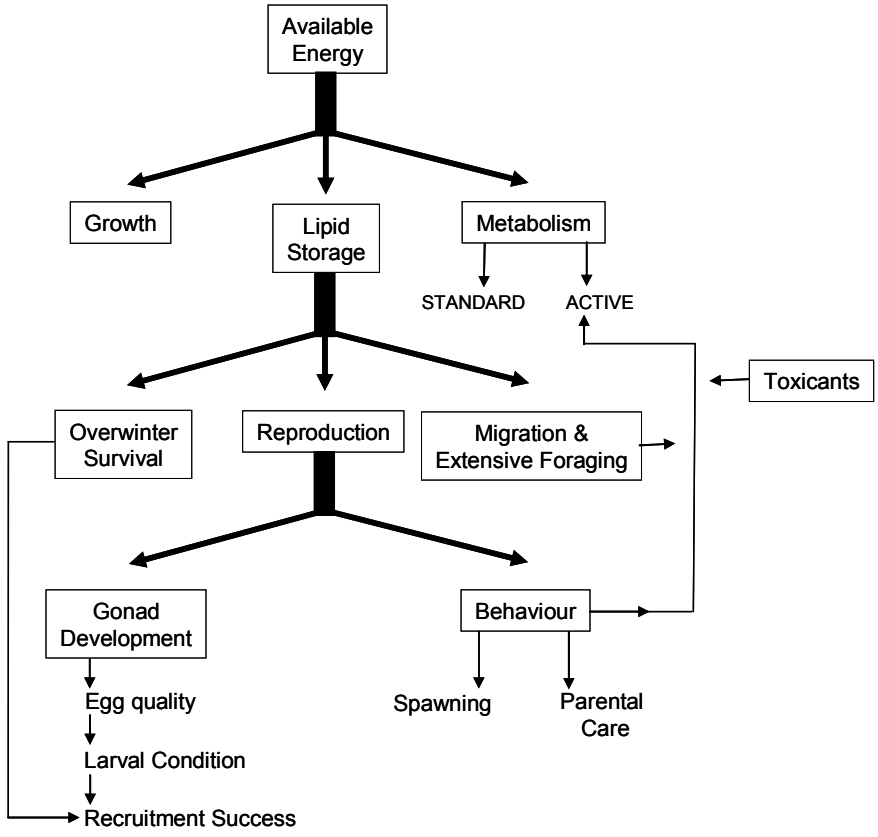

Figure 1. General allocation strategy of available (assimilated) energy into the main functional processes of growth, metabolism, lipid storage, and reproduction for a typical temperate or north-temperate fish species (modified from Bennett 2008, with permission).

Indeed, fish that obtain sufficient fat reserves before winter have a higher probability of winter survival (Thompson et al. 1991; Lemly 1996; Post and Parkinson 2001). Decreased lipid levels can lead to starvation as well as compromised osmoregulation, particularly in YOY fish (Lemly 1996; Adams 1999). However, not all studies have demonstrated a decrease in lipid stores during the overwinter period. Eckmann (2004) reported that YOY perch (*Perca fluviatilis*) had decreased lipid levels after the winter, whereas ruffe (*Gymnocephalus cernuus*) actually had higher lipid levels following winter. This difference was attributed to differences in predation; ruffe use sensory ability to forage, while perch are a visually-oriented predator (Eckmann 2004). Since winter feeding conditions are

characterized by low light due to ice cover and reduced photoperiod, a species that relies primarily on vision for feeding will experience a reduction in foraging success compared to a sensory and olfactory predator. Similar results were reported in YOY burbot (*Lota lota*), a tactile predator, and northern pike (*Esox lucius*), a visual predator, where burbot consistently had higher total lipid and triglyceride levels in spring compared to the previous autumn (Bennett and Janz 2007a,b).

The concept that fishes must rely on stored energy to survive winter months is related to low food availability (Johnson and Evans 1991; Foy and Paul 1999) as well as decreased food digestion associated with low temperatures (Toneys and Coble 1980). However, Hurst and Conover (1998) noted that many details related to winter feeding ecology of the majority of fish species remain unstudied. Indeed, various reports have challenged the idea that fish are unable to feed or assimilate energy from food during winter (Sogard and Olla 2000; Biro et al. 2004; Parrish et al. 2004). Bauer and Schlott (2004) reported that common carp (*Cyprinus carpio*) were relatively active in the winter, despite previous beliefs that the fish were inactive. McCollum et al. (2003) reported that winter food availability regulated the energetic condition of age-0 white crappie (*Pomoxis annularis*) entering the spring. Biro et al. (2004) reported that fed YOY rainbow trout (*Oncorhynchus mykiss*) in a laboratory setting under simulated winter conditions were capable of doubling their lipid content over a 100-day period. Similarly, juvenile Atlantic salmon (*Salmo salar*) provided with shelter from predators in a setting with high food availability experienced increased growth and survival over the winter, as there was an advantage for fish to expend energy to feed for growth and weight maintenance (Parrish et al. 2004). Clearly, lipid depletion following winter does not occur with all fish species and may vary depending on various ecological variables including foraging strategy, predator density, prey abundance, and competition.

1.2 Thermal stress

The influence of water temperature on virtually all aspects of fish physiology has been studied extensively (reviewed in Crockett and Londraville 2006). In general, low temperature tolerance in fishes has not been as well studied as upper lethal temperatures (Hurst 2007). The effect of water temperature on the acute lethality of selected toxicants has been reviewed (Cairns et al. 1975). In general, most toxicants exhibit greater toxicity (i.e., lower LC50s) at higher temperatures. Major exceptions to this rule include pyrethroid and certain organochlorine insecticides (e.g., DDT, methoxychlor), where lower temperatures cause neuronal sodium channels to remain open longer, exacerbating toxicity (Narahashi 2000). The majority of toxicological research in fish acclimated to different temperatures has been conducted using short-term exposures in the laboratory (Gordon 2005). To date, very few studies have investigated the potential interactions of winter conditions (i.e., prolonged low temperature) and toxicity in the laboratory or field.

At extremely low temperatures near 0°C (or below), freezing of body fluids can occur unless "antifreeze" proteins, such as those in certain marine fishes, are expressed. In fact,

many polar fish species are believed to be "metabolically cold adapted" (Scholander et al. 1953), although the occurrence of this phenomenon in north temperate fish species is not known. Under more common conditions of low temperature in temperate freshwater systems (i.e., 2-4°C), the main mechanism of thermal stress is impaired cellular homeostasis due to altered membrane fluidity. This can lead to osmoregulatory failure due to inability to maintain ionic gradients across cell membranes (Morris and Bull 1968; Johnson and Evans 1996). Ultimately, impaired nervous transmission in the central nervous system (CNS) and periphery leads to mortality. Smaller fish may be more prone to osmoregulatory failure since smaller individuals have a larger gill area, on a per gram basis, than larger individuals (Johnson and Evans 1996). Thus, osmoregulatory failure due to low winter water temperatures may have a proportionally higher impact on YOY fish compared to older age classes. Whether organic or inorganic toxicants can exacerbate the effects of osmoregulatory impairment during winter in juvenile fish remains unknown.

1.3 Predation

In certain circumstances there may be a greater risk of predation during winter. In shallow freshwater systems that freeze over, ice thickness may reduce the ability to escape predation or find refugia. Swimming performance is decreased at low temperatures (Bennett 1990), and predation risk may increase if predators are less affected by temperature than prey. This may be particularly important in areas where there is distributional overlap of thermal guilds. Schooling behavior may also be compromised by low water temperature (McLean et al. 1985). Fish with a higher predation risk employ a less active feeding regime and forage sub-optimally (Post and Parkinson 2001), which may exacerbate overwinter energy depletion. Field-based experiments have shown that the presence of a predator will reduce growth and survival of YOY fish (Landry et al. 1999; Biro et al. 2003). Larger fish are more likely to survive than smaller fish due to size-dependent predation (Kristiansen et al. 2000). As mentioned previously, this is due to the fact that small fish are more vulnerable to gape-limited predators (Werner and Gilliam 1984) and larger fish have relatively more energy and therefore would be in better condition to actively escape predators (McCollum et al. 2003).

1.4 Disease and Parasites

There is ample evidence from laboratory studies and aquaculture literature that fungal and bacterial infections are exacerbated by low temperatures, but much less information is available in natural fish populations (Hurst 2007). Parasite infections have enhanced negative effects on host survival at low temperatures, mainly via an increase in metabolic rate (Lemly and Esch 1984). Winter survival was reported to be directly related to parasite load (rate of infection) (Cunjak and McGladdery 1991). Energy stores were reported to be inversely correlated with the rates of parasitism in northern pike inhabiting areas receiving metal mine discharge (Kelly and Janz 2008). More work is needed in aquatic ecotoxicology investigating the potential interactions among disease, parasitism and overwinter mortality.

2. Consequences of Overwinter Mortality on Population Dynamics

Aquatic ecotoxicologists are primarily interested in protecting the sustainability of native populations and communities of aquatic species such as fishes. Thus, toxic effects such as enhanced overwinter mortality that have potential negative impacts on population dynamics are a major concern. The rate of overwinter mortality can affect population dynamics by two main mechanisms. First, and perhaps most relevant to the topic of winter stress syndrome, overwinter mortality of early life stages (e.g., YOY) can regulate cohort strength, producing a "recruitment bottleneck" (Hurst 2007). As mentioned previously, survival of fish beyond their first year is a critical determinant of future year-class strength. Depending on the sources of mortality, the overwinter period can result in both density-dependent and density-independent population regulation (Cunjak et al. 1998). As described in detail above, size-dependent winter mortality may be important in certain fish populations (Post and Parkinson 2001). In this case, winter can act as a density-dependent regulator of population size (Hurst 2007). Exposure to toxicants that cause increased metabolism can potentially increase this size-dependent mortality. Secondly, large winterkills that often occur during abnormally cold winters can cause severe density-independent reductions in all age-classes. Depending on the cause of mortality, toxicant exposure may or may not exacerbate overwinter mortality resulting from winterkills. In either case, it is clear that overwinter mortality can have a significant influence on population dynamics. Thus, a scenario where toxicant exposure can exacerbate the rate of winter mortality has the potential to negatively affect fish populations.

3.0 Does Winter Exacerbate Toxicity in Fishes?

A surprisingly limited number of laboratory studies, and even fewer field studies, have directly investigated the influence of winter conditions (i.e., prolonged cold water temperature and reduced photoperiod) on toxicity of organic or inorganic contaminants. As described previously, Lemly (1993) formulated the winter stress syndrome hypothesis in his study investigating the effects of dietary and waterborne Se to juvenile bluegill sunfish. Rainbow trout exposed to copper exhibited increased oxygen consumption compared to unexposed fish when forced to swim against high water velocities, indicating increased metabolism (McGeer et al. 2000). These same fish were found to have increased appetite compared to control fish, but showed no difference in growth rates. Similarly, common carp responded to various concentrations of sublethal copper exposure with increased metabolic demand and reduced feeding (De Boeck et al. 1997). Higher lipid metabolism and lower triglyceride levels in fish experiencing chronic metal exposure compared to fish inhabiting uncontaminated lakes has been previously reported in a fall field study (Levesque et al. 2002). Clearly, further research is needed to address the potential influence of winter conditions on toxicological responses in fish.

Toxicants may act directly or indirectly on fish condition. Previous studies have reported that fish exposed to trace metals exhibit decreased condition factor (weight-at-

length) compared to unexposed fish (Laflamme et al. 2000; Sherwood et al. 2000; Levesque et al. 2002; Rajotte and Couture 2002). A contaminant could indirectly alter the operative environment in an aquatic system such as temperature, predation risk, resource availability, or cover (Congdon et al. 2001). For example, the release of industrial effluent may substantially increase water flow within a stream ecosystem, which could result in removal of organic debris, algae, macrophytes, invertebrates, spawning gravel, eggs, and the crowding of fish into refugia or stranding fish in temporary pools (Matthews 1998). Indirect, food web-mediated effects of metal contamination have been shown to impact yellow perch bioenergetics, as metal-contaminated lakes created gaps in prey size structure which resulted in slow-growing perch populations (Iles and Rasmussen 2005). In addition, contaminant exposure can adversely affect fish behaviors involving sensation, perception, cognition, coordination, and motor function (Atchison et al. 1987; Døving 1991; Scott and Sloman 2004). Avoidance behavior has been observed in lake whitefish (*Coregonus clupeaformis*) exposed to copper, lead and zinc (Scherer and McNicol 1998). Exposure to cadmium inhibited predator avoidance behaviors in juvenile rainbow trout through accumulation in the olfactory system (Scott et al. 2003). An organism can respond to changes in their environment at all levels of biological organization, from the molecular to the population levels. In theory, energetically expensive changes in physiology or behavior resulting from contaminant exposure can reduce the allocation of energy to storage and growth, which are critical for winter survival and recruitment. Juvenile fish would be most sensitive to these changes.

Our group has recently investigated aspects of the winter stress syndrome hypothesis in several native fish species inhabiting areas receiving complex metal mine effluents containing elevated selenium (Bennett and Janz 2007a,b; Kelly and Janz 2008; Driedger et al. in press). In these studies, juvenile fish were collected just prior to ice-on (i.e., late September/early October) and immediately following ice-off (i.e., May) from lakes and creeks receiving discharges from uranium mining (Bennett and Janz 2007a,b) and base metal (copper/nickel) mining (Driedger et al. in press) operations in northern Saskatchewan and Ontario, respectively. Measures of growth (length, weight, condition factor, muscle RNA:DNA ratio, muscle proteins) and energy storage (whole-body lipids, whole-body triglycerides, liver triglycerides, liver glycogen) were determined in fish collected along gradients of exposure and from reference sites. Based on the winter stress syndrome, we hypothesized that fish collected in spring from all sites (exposure and reference) would exhibit decreases in growth and energy storage measures compared to the previous autumn, and that these measures would be decreased to a greater extent in juvenile fish collected from exposure sites. In contrast to our hypotheses, juvenile northern pike, burbot, fathead minnow (*Pimephales promelas*), creek chub (*Semotilus atromaculatus*), and white sucker (*Catostomus commersoni*) collected from exposure sites generally exhibited similar or greater growth and energy stores in spring when compared to the previous autumn and reference sites (Bennett and Janz 2007a,b; Driedger et al. in press). Only slimy sculpin (*Cottus cognatus*) exhibited changes in energy stores (whole-body triglycerides) that were

consistent with the winter stress syndrome hypothesis (Bennett and Janz 2007b). In contrast, most of these species collected from reference sites exhibited overwinter decreases in energy stores that were consistent with the overwinter fish biology literature. We also determined Se residues in selected species. At the two highest exposure sites in Junction Creek, ON, whole-body Se concentrations in juvenile fathead minnows and white sucker ranged from 11 to 43 µg/g dry weight (Driedger et al. in press). In northern Saskatchewan, Se concentrations in muscle of juvenile northern pike ranged from 17 to 23 µg/g dry weight at exposure sites (D.M. Janz, unpublished data). At both study locations, Se was the predominant element consistently elevated in fish tissues.

These studies led us to formulate hypotheses that might explain these differences between juvenile fish collected from reference and exposure sites. It is important to note that we did not directly determine overwinter mortality in these studies. In other words, we may have been collecting fish in the spring that represented the individuals having greater growth and lipid stores the previous autumn, thus biasing our results. This would indicate that size-dependent overwinter mortality was occurring. Further work is needed using mark-recapture techniques (e.g., passive integrated transponder or fluorescent elastomer tagged fish) or direct sampling of fish over time (and likely under ice) during winter.

Direct and/or indirect effects of the metal mining effluents may have been responsible for the responses we observed in these studies. Since the effluents contain sources of nitrogen (including ammonia and nitrates) and phosphorus, there may have been an indirect nutrient enrichment effect occurring in the receiving environment. This potential increase in productivity could result in increased prey quantity or quality, although further work by us (Kelly and Janz 2008) and previous work by others (Jaagumagi and Bedard 2002; Golder 2005) at these same sites did not confirm this possibility. In fact, the opposite effect may be occurring (Iles and Rasmussen 2005). Another possible explanation is related to the presence of elevated levels of all major physiological ions (e.g., Ca^{2+}, Na^{+}, K^{+}, Mg^{2+}, Cl^{-}) in receiving environments, which may have reduced the energetic costs associated with maintaining osmotic homeostasis that occur in freshwater teleosts (Hurst and Conover 2002). There may be differences in the risk of predation at exposure sites that allow juvenile fish to optimize foraging. It is also possible that parasitism rates are lower at exposure sites, resulting in a decreased energetic burden associated with parasite infection (Kelly and Janz 2008). There also exists a potential direct toxicological effect of trace element exposure on intermediary metabolism associated with lipid and carbohydrate homeostasis (e.g., enzymes involved in lipogenesis and lipolysis) (Levesque et al. 2002). Finally, it is possible that fish develop tolerance to certain toxic components of these effluents following chronic exposure. All of the potential direct and indirect effects of complex metal mining effluents mentioned above may be involved in the responses we observed in our field research, and further work is required to identify factors of greatest importance.

These studies further illustrate the difficulty of extrapolating laboratory-based experiments exposing fish to single elements such as Se to field studies where Se is but one of a myriad of components in complex effluents. Although it is clear from the laboratory studies in juvenile bluegill sunfish (Lemly 1993; Great Lakes Environmental Center 2008) that Se causes increased mortality during simulated winter conditions in this species, recent field studies where Se was a major component of metal mining discharges did not provide similar results in other fish species (Bennett and Janz 2007a,b; Kelly and Janz 2008; Driedger et al. in press). The studies using bluegill sunfish (Lemly 1993; Great Lakes Environmental Center 2008) indicated that Se likely causes increased metabolism, which may occur, in part, through oxidative stress (Spallholz and Hoffman 2002; Palace et al. 2004). Thus, Se appears to be an element that has the potential to cause winter stress syndrome in field settings. Further work is needed to evaluate the possible occurrence of winter stress syndrome in fish species exposed to elevated levels of Se in the field.

4.0 Conclusion

Winter stress syndrome represents a scientifically sound hypothesis, although to date only laboratory studies exposing fish to Se have provided support for it. Much more work is needed to investigate whether winter stress syndrome occurs in the field, whether other aquatic contaminants can cause it, and whether it is species-specific. As Lemly (1996) noted, basic knowledge of life history characteristics and feeding ecology, particularly for YOY fish, would allow identification of potentially vulnerable fish species in temperate regions of the world. This would be equally important for adults, larvae, and eggs. Unfortunately, there are few studies with direct observations and concrete conclusions regarding feeding ecology in juvenile fishes. It is possible that winter stress syndrome is most important in species at the northern limit of their ranges, and future studies should focus on this aspect. It may also help to identify Se interactions with other toxicants in a particular environment under winter stress conditions. Knowledge of local fish community ecology is essential when assessing the potential importance of overwinter mortality in aquatic ecotoxicological investigations of Se or other aquatic contaminants.

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