Technical Support Document: Proposed Site-Specific Selenium Criterion, Sage and Crow Creeks, Idaho

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LIST OF ACRONYMS

AMSL	Above Mean Sea Level
ANOVA	Analysis of Variance
AOC	Administrative Order on Consent
BAF	Bioaccumulation Factor
BCF	Bioconcentration Factor
CADDIS	Causal Analysis/Diagnosis Decision Information System
CCC	Criterion Continuous Concentration
cfs	Cubic Feet per Second
CPUE	Catch Per Unit Effort
CSE	Channel Stability Evaluation
EC/EC_x	Effect Concentration
ELS	Early Life Stage
EMP	Environmental Monitoring Program
EPT	Ephemeroptera, Plecoptera, Trichoptera
ERED	Environmental Residue Effects Database
FCETL	Fort Collins Environmental Toxicology Lab
GMCV	Genus Mean Chronic Value
GSI	Graduated Severity Index
HS	Hoopes Spring
HSI	Habitat Suitability Index
HQI	Habitat Quality Index
HUC	Hydrologic Unit Code
IDEQ	Idaho Department of Environmental Quality
IDFG	Idaho Department of Fish and Game
K_ds	Enrichment Factors
KW	Kruskal-Wallis
MATC	Maximum Allowable Toxicant Concentrations

LIST OF ACRONYMS (Continued)

MCT	Multiple Comparison Test
NOEC	No Observed Effect Concentration
ODA	Overburden Disposal Area
OLS	Ordinary Least Squares
ORP	Oxidation-Reduction Potential
PHABSIM	Physical Habitat Simulation
SeGSHpx	Glutathione Peroxidase
SETAC	Society of Environmental Toxicology and Chemistry
SFSC	South Fork Sage Creek
SFSCS	South Fork Sage Creek Springs
SFTC	South Fork Tincup Creek
SHI	Stream Habitat Index
SI	Site Investigation
SMI	Stream Macroinvertebrate Index
SRI	Stream Reach Inventory
SSD	Species Sensitivity Distribution
SSSC	Site-Specific Selenium Criterion
TMDL	Total Maximum Daily Load
TRAP	Toxicity Relationship Analysis Program
TTF	Trophic Transfer Factor
USFS	United States Forest Service
USEPA	United States Environmental Protection Agency
WDEQ	Wyoming Department of Environmental Quality
Wr	Relative Weight
WSS	Winter Stress Syndrome
YCT	Yellowstone Cutthroat Trout

1.0 INTRODUCTION

A chronic site-specific selenium criterion (SSSC) is proposed for Hoopes Spring and South Fork Sage Creek (SFSC) and the downstream receiving waters including Sage Creek and Crow Creek upstream of the Idaho and Wyoming State Line. Hoopes Spring is located in Sage Valley near the J.R. Simplot Company (Simplot) Smoky Canyon phosphate mine in Southeastern Idaho (Figure 1-1). Investigations to date, at the nearby Smoky Canyon Mine, have identified elevated concentrations of selenium in surface water being discharged via Hoopes Spring and South Fork Sage Creek Springs, which ultimately discharges to lower Sage Creek. The selenium is released from historical operating areas (known as overburden disposal areas [ODAs]) at the mine.

Source controls have already been implemented at the Pole Canyon ODA and at Panel E. The effects of the Pole Canyon actions are anticipated to be observable at Hoopes Spring approximately 10 years after the diversion of Pole Canyon Creek diversion (NewFields TM, 2007)[1]. The effects of recent backfilling, covering, and reclamation at Panel E are anticipated to take place within a shorter time period; however, the time frame for observable reductions in the selenium concentrations in Hoopes Spring and South Fork Sage Creek springs due to these actions is not certain. The groundwater investigation being conducted for the Remedial Investigation/Feasibility Study (RI/FS) will provide additional information needed to refine previous estimates of the selenium transport times from these different source areas to the springs. The need for and types of additional source controls are also being evaluated through the RI/FS project.

Development of this SSSC has been conducted through a collaborative effort of State and Federal Agency representatives and Simplot, collectively known as the SSSC Workgroup, which includes scientists and resource managers from Simplot, Idaho Department of Environmental Quality (IDEQ), US Forest Service (USFS), US Environmental Protection Agency (USEPA), Idaho Department of Fish and Game (IDFG), and the Wyoming Department of Environmental Quality (WDEQ). This collaborative approach was done as required by IDAPA 58.01.02, Section 275.01(b). Work Plans have been developed with agency review and input to ensure that acceptable and relevant methods were utilized.

A draft document was submitted to the SSSC Workgroup, titled *Draft Interpretive Findings for Field and Laboratory Studies and Literature Review in Support of a Site-Specific Selenium Criterion* (Interpretive Report). This Technical Support Document (TSD) is the final version of

[1] NewFields TM, 2007. Technical Memorandum No. 2, Evaluation of Recent Water Quality Trends at Hoopes Spring and South Fork Sage Creek Springs, Smoky Canyon Mine - Area A, prepared for J.R. Simplot Company, February 2007.

the Interpretative Report and incorporates the comments received from the Workgroup in a December 2, 2010 meeting and the following additional feedback:

- IDEQ (Don Essig, Michael Rowe, Lynn Van Every) via memos -11/23/2010 and 12/17/2010;
- Wyoming DEQ (David Waterstreet), 12/20/2010;
- USFS (Steve Bauer) - 9/8/2010, 12/21/2010; and
- EPA (draft comments) - 12/21/2010, verbal comments – 3/30/2011.

1.1 Background

A number of studies and reports have been generated during this process. In addition, a project web page was developed to provide a centralize location for Workgroup members to access Work Plans, meeting minutes, data reports, and a wide range of selenium literature.

Completed work includes:

- Formation of the SSSC Workgroup;
- Work Plan - Field Monitoring Studies for Developing a Site-Specific Selenium Criterion (NewFields 2006 [Draft]; NewFields 2007a [Final]);
- Fall 2006 Field Monitoring and Interim Data Report (NewFields 2007b);
- Summary of Approach for Developing a Site-Specific Selenium Criterion (NewFields 2007c [Draft]; NewFields 2008 [Revised Draft]);
- Technical Memorandum – Justification and Rationale for Fish Size Selection and Water Quality Analyses for Selenium (NewFields 2007);
- Technical Memorandum – Site Boundaries and Applicability of Site-Specific Selenium Criterion (NewFields 2007);
- Technical Memorandum – Methods for Testing Adult Brown Trout Reproductive Success (NewFields 2007);
- Spring 2007 Field Monitoring and Spring 2007 Interim Data Report (NewFields and HabiTech 2007);

- Fall 2007 Field Monitoring and Fall 2007 Interim Data Report (NewFields and HabiTech 2008a);

- Field collection and laboratory testing for adult brown trout reproductive success (Fall 2007);

- Spring 2008 Field Monitoring and Spring 2008 Interim Data Report (NewFields and HabiTech 2008b);

- Collection of wild cutthroat trout adults for laboratory toxicity studies (Spring 2008);

- Laboratory testing of Yellowstone Cutthroat Trout (YCT) reproduction (egg viability) and early life stage (ELS) (Spring 2008);

- Fall 2008 Field Monitoring and Fall 2008 Interim Data Report (NewFields and HabiTech 2009a);

- Final Data Report – Fall 2006 - Fall 2008 Field Monitoring Studies for Developing a Site-Specific Selenium Criterion (NewFields and HabiTech 2009b);

- Draft Brown Trout Report Laboratory Reproduction Studies Conducted in Support of Development of a Site-Specific Selenium Criterion (NewFields 2009a);

- Draft Final Brown Trout Report Laboratory Reproduction Studies Conducted in Support of Development of a Site-Specific Selenium Criterion (NewFields 2009b); and

- Final Brown Trout Report Laboratory Reproduction Studies Conducted in Support of Development of a Site-Specific Selenium Criterion (Formation 2011).

Study data and reports presented in this document as appendices include the following:

- Yellowstone Cutthroat Trout - Laboratory Adult Reproduction Studies Conducted in Support of a Site-Specific Selenium Criterion; and

- Yellowstone Cutthroat Trout - Laboratory Early Life Stage Studies Conducted in Support of a Site-Specific Selenium Criterion.

This TSD provides an integration of the field and laboratory studies and the literature review.

1.2 Purpose and Objectives

Data collected to date from the monitoring efforts for the SSSC project for Hoopes Spring, Sage Creek, and Crow Creek indicate that Hoopes Spring and Lower Sage Creek exceed the chronic water quality standard for selenium. Data collected as part of Simplot's ongoing Environmental Monitoring Program (EMP) for South Fork Sage Creek also indicate that selenium in surface waters there exceed the chronic water quality standard for selenium. While concentrations of selenium exceed the surface water standard, there is no explicit indication that the aquatic community is impaired or that some exceedances of the standard represent toxic conditions. This is recognized in Idaho's Water Quality Standards (IDAPA 58.01.02 - Section 275) where it states that the water quality criteria adopted in these standards may not always reflect the toxicity of a pollutant in a specific water body. National surface water quality criteria adopted by states as standards, as is the case for the current State of Idaho water quality standard for selenium, do not always take into account site-specific conditions. Many factors influence the in-stream toxicity of selenium including the bioavailability of the form of selenium, tolerance of resident species (e.g., acclimation), and/or other factors that may enhance or ameliorate toxicity. As such, modification of the selenium water quality standard is being investigated for several reasons, including the following:

- The bluegill toxicity data used to derive the current Idaho State Standard and the USEPA (2004) Draft National Criterion are based on bluegill[2] effects in a lentic (still water) environment. Streams near the Smoky Canyon Mine are lotic (flowing water).

- Bluegill sunfish, a warm water species, is not found in Idaho cold mountain streams.

- Recently published literature[3] suggests that the trout species may be less sensitive to selenium than species used to derive the current standard (e.g., bluegill sunfish).

- Data collected during the SI suggested that relatively healthy aquatic communities were present at locations where selenium concentrations exceeded the current chronic surface water standard (0.005 mg/L).

- As indicated in USEPA's Draft Selenium Criteria (2004), diet is the primary route of exposure for chronic selenium toxicity in fish. The current State of Idaho water quality standard for selenium (0.005 mg/L) is based on a concentration in water that is not consistent with the state of the science on aquatic life exposure.

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[2] EPA has repeated the Lemly (1993) study on bluegill which is the basis for the Idaho State Standard. This study is discussed in more detail in the literature review section of this document (Section 3).

[3] The literature sources referenced here are discussed in the literature review section of this document (Section 3).

The intent of this multi-faceted study is to develop a proposed chronic selenium criterion that is protective of the aquatic community based on site-specific species and their responses to ambient exposure levels, and the physical and chemical characteristics of the streams monitored. The approach relies on the relationship between three primary lines of evidence (Figure 1-2): field studies, laboratory studies and a comprehensive literature review. Data collected through field monitoring studies has been used to characterize the exposure environment, the condition of the aquatic community, and the physical habitat. Laboratory studies have been utilized to characterize reproductive and developmental effects in two trout species. A literature review was utilized to guide the design for and augment the findings of the field and lab studies. Utilizing these lines of evidence, the objectives of this report are as follows:

- Summarize data that characterizes the exposure environment and assess, when possible, relationships of exposure conditions (i.e., chemical quality of different media) to biological conditions;
- Summarize laboratory data that characterize effects from brown trout and YCT studies and provide relevant analyses for effects thresholds;
- Summarize current literature to define effects;
- Develop and integrate the lines of evidence for exposure and effects information; and
- Derive and identify a proposed chronic SSSC.

A derived chronic threshold that is protective of the two trout species tested must also be protective of the larger aquatic community. To achieve this, the laboratory study results must be examined in the context of the exposure environment, the biological species present, the aquatic populations and communities, and the most current scientific literature. The three lines of evidence are then brought together to develop a water quality criterion. While the laboratory testing characterizes effects for site species, the field data provide verification on whether or not measureable effects are occurring in the field. Effects information from the literature provides an additional level of effects data for similar and different species that may or may not be covered by the laboratory studies and puts field and laboratory data into context. The laboratory effects threshold developed for Site species is also compared to literature values for other species. After completion of these steps, a criterion is identified for consideration by the state of Idaho.

1.3 Existing Selenium Standard and Draft Criterion

Idaho's current water quality standard for selenium is 0.005 mg/L, which is a chronic standard based on USEPA's 1987 Ambient Water Quality Criteria for selenium. The standard is based on aqueous exposure only and includes no dietary component of exposure. When EPA published a recommended freshwater aquatic life criterion for selenium in 1987, it considered both field data on chronic toxicity from Belews Lake in North Carolina and laboratory toxicity data showing chronic effects. A limited comparison of these data sets indicated that selenium was more toxic to aquatic life in the field than in standard laboratory toxicity tests. Consequently, to ensure that the criterion would protect aquatic life, EPA derived a chronic criterion, or Criterion Continuous Concentration (CCC), of 0.005 mg/L for total selenium from the field data. Because the Belews Lake study did not distinguish between selenite, selenate, and any other form of selenium, and because some forms of selenium can convert to other forms over time (U.S. EPA 1987), EPA established a single CCC for selenium rather than a separate CCC for selenite and/or selenate (Federal Register 1996).

In deriving the Draft Aquatic Life Water Quality Criteria for Selenium – 2004 (USEPA 2004), USEPA recognized that diet is the primary route of exposure that controls chronic toxicity to fish, the group considered to be the most sensitive to chronic selenium exposure (Coyle et al. 1993; Hamilton et al. 1990; Hermanutz et al. 1996). Furthermore, USEPA recognized that the chronic criteria procedure, explicitly set forth in th*e Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses* (Guidelines) (Stephan et al. 1985), was not well suited to bioaccumulative contaminants for which diet is the primary route of aquatic life exposure. Consequently, that procedure was not used for deriving the 2004 draft chronic criterion for selenium.

Instead, the available studies where diet and/or water were used to expose fish to selenium were ranked similarly to the process used in the Guidelines, and the lowest genus mean chronic value (GMCV) was selected. USEPA's (2004) draft chronic criterion value is a tissue-based concentration for selenium of 7.9 mg/kg dw, which is based on the response of bluegill sunfish, again from Belews Lake in North Carolina. To put the bluegill threshold into context, consider that the GMCVs for two trout genera included in the USEPA 2004 draft criterion were 10.7 and 12.8 mg/kg dw.

USEPA (2004) recommends that if whole-body fish tissue concentrations exceed 5.85 mg/kg dw during the summer or fall, fish tissue should be monitored during the winter to determine if selenium concentrations exceed 7.91 mg/kg dw in whole body fish tissues. This draft criterion is based on a study by Lemly (1993a) which observed that juvenile bluegill sunfish experienced what was hypothesized as Winter Stress Syndrome (WSS). Winter conditions of low water temperature (4°C) exacerbated the toxicity of selenium, indicated by increased mortality, decreased condition factor, and decreased energy (lipid) stores.

Given its importance in setting the 2004 draft chronic criterion, USEPA commissioned a study of WSS in bluegill to simulate Lemly's work and attempt to confirm his results. The study (McIntyre et al. 2008) of WSS in bluegill found that bioaccumulation differed based on the diet and form of selenium fed. The study also found that mortality in bluegill was higher when temperature was lowered to 4°C versus 9°C. The effects concentration (EC_{20} and EC_{10}), estimates for the exposure, in which temperature decreased from 20°C to near 4°C, were 10.16 and 9.56 mg/kg dw, respectively, while the EC_{20} and EC_{10} estimates for the exposure that began at 20°C and was systematically lowered to 9°C were 14.02 and 13.29 mg/kg dw, respectively.

Since the release of the 2004 draft criterion, several new studies have been completed. These new studies have augmented the state of the science regarding selenium toxicology. At present, USEPA is revising its national criterion, which includes McIntyre et al.'s (2008) reassessment of Lemly's WSS study for bluegill sunfish, among others[4]. Early information from USEPA is that the revised draft criterion will have two tiers, with tier one being a water quality value and tier two being a tissue-based criterion (egg or ovary). The tier one value is expected to be a surface water value, that if exceeded, triggers monitoring for fish tissues. Egg/ovary concentrations of selenium would be collected to evaluate against the tissue criterion. Expected release of the Revised Draft National Criterion is late 2012.

1.4 Regulatory and Scientific Rationale for Modification and/or Development of a Site-Specific Criterion

Both Federal and State laws permit the development and/or modification of water quality criteria or standards. The Federal water quality standards regulation (at 40 CFR Section 131.1 l(b)(l)(ii)) provides states with the opportunity to adopt water quality criteria that are " . . .modified to reflect site-specific conditions." IDAPA 58.01.02 - Section 275 of Idaho's Standards indicates, "[t]he water quality criteria adopted in these standards may not always reflect the toxicity of a pollutant in a specific water body. These criteria also represent a limited number of the natural and human-made chemicals that exist in the environment which may pose a threat to designated or existing beneficial uses. Thus, it may be possible in some water bodies to develop new water quality criteria or modify existing criteria through site-specific analyses which will effectively protect designated and existing beneficial uses."

[4] Whether or not the USEPA is using this study as a replacement or augmentation to the bluegill response data will not be clear until the Revised Draft National Criterion is released. Presentation of these data here simply indicates that in revisiting Lemly's WSS studies, different results were found for Bluegill sunfish responses.

According to IDAPA 58.01.02 - Section 275 of Idaho's Standards, the following are acceptable conditions for developing site-specific criterion:

1) Resident species of a water body are more or less sensitive than those species used to develop a water quality criterion.

2) Natural adaptive processes have enabled a viable, balanced aquatic community to exist in waters where natural background levels of a pollutant exceed the water quality criterion (i.e., resident species have evolved a greater resistance to higher concentrations of a pollutant).

3) The composition of aquatic species in a water body is different from those used to derive a water quality criterion (i.e., more or less sensitive species to a pollutant are present or representative of a water body than have been used to derive a criterion).

4) Biological availability and/or toxicity of a pollutant may be altered due to differences between the physicochemical characteristics of the water in a water body and the laboratory water used in developing a water quality criterion (e.g., alkalinity, hardness, pH, salinity, total organic carbon, suspended solids, turbidity, natural complexing, fate and transport water, or temperature).

5) The effect of seasonality on the physicochemical characteristics of a water body and subsequent effects on biological availability and/or toxicity of a pollutant may justify seasonally dependent site-specific criteria.

6) Water quality criteria may be derived to protect and maintain existing ambient water quality.

7) Other factors or combinations of factors that, upon review of the Department, may warrant modifications to the criteria.

Each of these conditions, and how Site conditions relate to these, is discussed in more detail in the Approach document (NewFields 2008). By examining the current Site conditions, and those conditions acceptable for considering development of a site-specific criterion, the Approach document found that Condition 7 above fits within the acceptable conditions for the modification or development of a Site-specific criterion, as defined in IDAPA 58.01.02 - Section 275 of Idaho's Standards. The combination of factors present at the Site, present in the derivation of the existing criterion for water quality, and present in the Draft National Criterion all contribute to acceptable reasons for development of a Site-specific criterion.

Acceptable procedures for the modification or development of a site-specific criterion, as defined in IDAPA 58.01.02 - Section 275 of the Standards, include:

- Recalculation Procedure;
- Indicator Species Approach;
- Resident Species Approach;
- Water Effects Ratio; and
- Other Scientifically-Defensible Procedures (such as relevant aquatic field studies, laboratory tests, biological translators, fate and distribution models, risk analyses or available scientific literature).

Of the five acceptable procedures noted above, Other Scientifically-Defensible Procedures (such as relevant aquatic field studies, laboratory tests, biological translators, fate and distribution models, risk analyses or available scientific literature), are utilized as part of this study to develop a SSSC. Section 1.5 below identifies the components of the approach, or lines of evidence that comprise the scientifically-defensible procedures.

1.5 Lines of Evidence

The approach to developing the SSSC was based on developing the relationships between the three primary lines of evidence described above (Figure 1-2) and outlined below in more detail.

- Field studies to define exposure conditions and the condition of the aquatic community.
    - Field studies aid in understanding the relationships between aqueous, sediment, and tissue selenium concentrations (fish, benthic, and periphyton) across the Site.
    - They provide data to evaluate the overall condition of aquatic communities.
        - They allow for analysis and comparison of population and community structure at target locations to existing habitat quality and conditions at reference locations.
    - Results from these studies are used herein to help identify species of ecological significance, aid in the assessment of sensitive species for the Site, and put the laboratory study results in perspective.

- Laboratory studies to characterize toxic effects.
    - These studies aid in understanding mechanisms and potential magnitude of toxicity.
    - They provide site-specific data to assess the strength of relationships between tissue concentrations (egg and/or whole body), various media (e.g., water quality or biological media), and various endpoints (e.g., survival, growth, reproductive metrics, or deformities).
    - Results from these studies identify a chronic threshold for each species tested which can then be evaluated relative to fish populations and communities, and benthic communities that exist at the Site.
- Literature reviews to augment Site-specific data with potentially applicable data for other sites, species, and conditions.
    - Existing literature has aided in the overall design of site-specific studies and has provided a range of species toxicity data for selenium. Understanding the potential range of toxicity, or lack thereof, is important for criterion development.
    - Along with the field studies, the literature base contributes to the evaluation of whether the site-specific studies are representative of the more sensitive, ecologically significant species.
    - Results of continuing literature review augment field and laboratory studies and may reinforce Site-specific interpretations to be made regarding toxicity.

Integrated analysis of the information compiled under the three primary lines of evidence listed above allows for interpretation and consideration of all the lines of evidence, as opposed to basing an evaluation on a single line of evidence. Effects thresholds defined from the laboratory studies need to be validated against field observations. McDonald and Chapman (2007) discussed the need for further evaluating observations of toxicity from laboratory-based studies, through the use of population studies, to understand if individual levels of effects propagate to population-level effects. The laboratory and field findings should be compared with the literature base to assess if the Site-specific findings make sense in terms of the larger body of scientific information.

A recent Society of Environmental Toxicology and Chemistry (SETAC) Pellston workshop, which brings together a variety of experts from private, government, and academic disciplines, reinforced the need to investigate the linkage of individual levels of effects to population-level effects. While there is evidence of population level effects at locations such as Belews Lake and Hyco and Kesterson Reservoirs, exposure to elevated selenium is not a guaranteed indicator of effects. Chapman et al. (eds) 2009 indicate that the inability to observe population-level effects in the field can occur even when the species exposed in the field are the same or closely related to those for which adverse effects have been demonstrated in laboratory settings at lower selenium tissue concentrations. They go on to state that several studies of aquatic ecosystems with naturally elevated selenium concentrations have reported unaffected aquatic communities. These examples illustrate the critical importance of considering ecological and environmental factors when investigating potential selenium toxicity in aquatic ecosystems (Chapman et al. (eds) 2009).

2.0 SITE SETTING

The Site is located in Caribou County, in the southeast corner of Idaho, approximately 10 miles west of Afton, Wyoming and 23 miles east of Soda Springs, Idaho (Figure 2-1). The active mining and milling operations are located along the eastern face of the Webster Range above Sage Valley. The Webster Range is a generally north-south trending mountain range that extends for about 33 miles from Lanes Creek on the north to the Preuss Range on the south. Freeman Ridge and Snowdrift Mountain are prominent ridges on the west limb of the Webster Range. Elevations in the mine area range from approximately 8,300 feet above mean sea level (AMSL) on the ridges west of the mine to approximately 6,300 feet AMSL at Crow Creek on the Meade Peak Ranch. Site streams are cold water streams that are east trending or north to north east trending, ranging from moderately high to low gradients.

2.1 Site Definition

In the general context of site-specific criterion, a “Site” may be a state, region, watershed, water-body, or segment of a water body. The site-specific criterion is to be derived to provide adequate protection for the entire Site however the Site is defined (USEPA 1994 – WQS Handbook). The water bodies being investigated are found within the Salt Sub basin, Hydrologic Unit Code (HUC) 17040105, of the Upper Snake River Basin. Two water body units of the Salt Sub basin are potentially affected, including water body US-9 (Sage Creek) and water body US-8 (Crow Creek) as defined by the Idaho Administrative Code’s Water Quality Standards (IDAPA 58.01.02). These two subunits are defined as:

- US-9 Sage Creek - source to mouth; and

- US-8 Crow Creek - source to Idaho/Wyoming border.

Within these two subunits, the following streams are listed in Idaho’s 2010 Integrated Report (IDEQ 2011) as impaired due to elevated levels of selenium: North Fork Sage Creek, Pole Canyon Creek, South Fork Sage Creek, and Sage Creek from its confluence with the North Fork Sage to its mouth (see Section 2.4 for more information on stream segments).

Initial planning for this study was based on bracketing the source areas at Hoopes Spring and SFSC to the apparent 2005 downstream limit of water quality standard exceedances at LSV-4 (Sage Creek just upstream of confluence with Crow Creek) (Figure 2-2). Locations on Crow Creek downstream of Sage Creek were selected to assess downstream effects and to collect additional information as to whether selenium concentrations in Crow Creek exceeded the state water quality standard. Locations upstream on Crow Creek and in upstream tributaries (i.e., Deer Creek) were selected to represent water quality conditions unimpacted by mining activities. This upstream information was also considered to be useful for the Environmental Impact Studies and future monitoring being planned for Smoky Canyon Mine Panels F and G. On this basis, a total of ten locations were initially selected in the office.

In the fall of 2006, the first field effort was implemented to characterize the chemical, biological, and physical conditions of these locations as part of a multi-seasonal monitoring effort. Locations scoped in the office were evaluated in the field. Not all locations were accessible or usable because of private land access restrictions and size limitations, so some locations were either modified or eliminated. Some locations previously accessed for the 2003/2004 Site Investigation were on private property. However, with this round of new work some landowners did not grant access and the locations were moved to locations where access was available. In addition, in the spring of 2007, a reference location from a different watershed (Tincup Creek) was identified and included.

The primary areas potentially affected by discharge of Hoopes Spring and the SFSC spring include: Sage Creek from its confluence with the Hoopes Spring discharge channel to its confluence with Crow Creek, South Fork Sage Creek below the spring complex, and Crow Creek from its confluence with Sage Creek to the Idaho and Wyoming state line. A site-specific criterion for selenium developed based on data from these areas is anticipated to be applicable to Hoopes Spring, Sage Creek (including South Fork Sage Creek), and Crow Creek. Thus, the Site is defined as Hoopes Spring and its discharge channel, Sage Creek downstream of Hoopes Spring to its confluence with Crow Creek, South Fork Sage Creek below the SFSC spring complex, and Crow Creek downstream of the Sage Creek confluence to the Idaho state line (Figure 2-2). Background locations on Crow Creek and Deer Creek are also included as part of the definition of the Site.

The configuration of selected locations is sufficient to characterize a range of selenium concentrations in biotic and abiotic media, aquatic community conditions, and physical habitat. The Site contains upstream background locations, near field exposure areas, and far field exposure areas.

2.2 Study Area Boundaries

The study area boundaries and monitoring locations are shown on Figure 2-2. The boundary is simply the upstream and downstream limits of the area that encompasses all of the monitoring locations. The monitoring locations were presented in the agency-reviewed April 2007 Final Work Plan – *Field Monitoring Studies for Developing a Site-Specific Selenium Criterion* (NewFields 2007a).

The study area includes four background locations, one reference location, and six downstream locations. All of the locations represent a lotic system. The four background locations characterize non-mining conditions and provide a comparative basis for Hoopes Spring and downstream waters. Background locations include the following:

- **CC-75** – Crow Creek upstream of Wells Canyon;
- **CC-150** – Crow Creek upstream of Deer Creek;
- **CC-350** – Crow Creek downstream of Deer Creek; and
- **DC-600** – Deer Creek upstream of its confluence with Crow Creek.

The remaining six locations were selected to evaluate decreasing trends in aqueous selenium concentrations with distance from the source. Downstream receiving water locations include the following:

- **HS** - Hoopes Spring (near discharge);
- **HS-3** - Hoopes Spring channel near its confluence with Sage Creek;
- **LSV-2C** – Sage Creek downstream of Hoopes Spring and upstream of South Fork Sage Creek;
- **LSV-4** – Sage Creek downstream of SFSC and upstream of the confluence with Crow Creek;
- **CC-1A** – Crow Creek downstream of Sage Creek confluence; and
- **CC-3A** - Crow Creek downstream of CC-1A on the Meade Peak Ranch.

Correspondingly, study area boundaries for this project have been set as follows: just upstream of CC-75 on Crow Creek, at Hoopes Spring above Sage Creek, just upstream of DC-600 on Deer Creek, and immediately downstream of CC-3A on Crow Creek (Figure 2-2).

2.3 Background and Reference Locations

2.3.1 Background Locations

Idaho's Water Quality Standards define background as the biological, chemical or physical condition of waters measured at a point immediately upstream (up-gradient) of the influence of an individual point or nonpoint source discharge. EPA's 2002 *Guidance for Comparing Background and Chemical Concentrations in Soil for CERCLA Sites* defines background as substances or locations that are not influenced by the releases from a site and are usually described as naturally occurring or anthropogenic: (1) Naturally occurring substances present in the environment in forms that have not been influenced by human activity; and (2) Anthropogenic substances are natural and human-made substances present in the environment as a result of human activities (not specifically related to the CERCLA site in question).

In this watershed, background locations not influenced by mining at the Site, do include impacts from grazing and other disturbances such as roads. These locations are not pristine, but they are not influences by mining at the Smoky Canyon Mine. As such, habitat quality may be influenced by non-mining impacts. Initial selection and subsequent field verification of monitoring location availability and appropriateness yielded four background locations. Background locations were selected to provide a potential range of conditions, based on surface water concentrations, outside the influence of mining impacts. Each of these four locations is upstream of Sage Creek which conveys discharge from Hoopes Spring. No known mining influences are present to influence water quality at these locations[5]. Based on the above definitions, the four locations selected upstream of Sage Creek meet the criteria of background locations. These locations are identified above in Section 2.2.

2.3.2 Reference Location

Due to the connectivity of background locations with downstream locations influenced by Hoopes Spring, the SSSC Workgroup collectively agreed that a reference location was also needed. IDEQ's water quality standards define a reference location as "a water body which

[5] Since the inception of this project, Deer Creek has been used as a background location with naturally elevated background conditions due to exposed Meade Peak member rocks that can be naturally leached in the environment. Recent observations indicate the Georgetown Mine has a 3 acre footprint in the extreme ephemeral headwaters of the South Fork Deer Creek (SFDC). Three locations along SFDC, downgradient of the Georgetown Canyon waste rock pile and upstream of background location DC-600 on the main stem of Deer Creek, were monitored between 2002 and 2010, with total selenium concentrations ranging from below detection limits to 0.002 mg/L. Four fish tissue samples were collected from DC-100 (upstream of SFDC), and the average fish selenium concentration was 6.09 mg/kg dw. The average selenium concentration from three fish samples from SFDC-100 was 2.3 mg/kg. One SFDC sediment selenium concentration was found and was 0.76 mg/kg. These data, together with monitoring data collected during this study, indicate that the Georgetown Canyon Mine waste rock pile on SFDC does not impact selenium concentrations on South Fork Deer Creek, the main stem of Deer Creek, or background location DC-600.

represents the minimum conditions necessary to fully support the applicable designated beneficial uses as further specified in these rules, or natural conditions with few impacts from human activities and which are representative of the highest level of support attainable in the basin." (IDAPA 58.01.02) IDFG (as part of the multi-agency selenium tissue protocol workgroup) defines reference locations as those locations outside the phosphate mining district and mining influences. The selected reference location has been agreed upon as an appropriate reference for this study.

The reference location designation and description follows:

- **SFTC-1** – South Fork Tincup Creek upstream of its confluence with Tincup Creek (Figure 2-3).

South Fork Tincup Creek is not in the phosphate mining district, and while some anthropogenic impacts are evident at this location, largely due to grazing, no known mining influences are present. In addition, this stream does not intersect the phosphate-ore body which may lead to a naturally elevated level of selenium. This location, therefore, serves as a reference area for selenium levels in a non-phosphate ore bearing local, but with similar non-mining disturbances to those found in Crow and Sage Creeks. IDFG and IDEQ had previously conducted monitoring in South Fork Tincup Creek, thus some previous data for water quality, physical characteristics, and fish community were available.

2.4 Scope and Applicability of the SSSC

Section 2.1 above defines the Study Area boundaries and the Site boundaries. State-recognized water body segments were identified and used as management units to which a modified criterion could be applied. However, the scale of these waterbody segments is large, and a more refined scale is needed to define the geographic scope to which the proposed SSSC is applied. IDEQ's 2010 Integrated Report (IDEQ 2011) provides one mechanism of readily available and identifiable stream segments useful for this effort. IDEQ's Integrated Report identifies specific stream segments as being limited by one or more parameters that affect use attainment (e.g., sediments, habitat, selenium, etc.). These stream segments are identified using an alpha numeric coding system. The stream segment numbers pertinent to this Site are presented below and should aid in providing definition to narratives that describe stream segments to which the SSSC is applicable. The proposed SSSC is applicable to all or portions of the stream segments identified.

Sage Creek and its tributaries would include the following stream segments:

- ID17040105SK009_02 North Fork Sage Creek (12.41 miles);

- ID17040105SK009_02c Sage Creek (1.81 miles);
- ID17040105SK009_02d Pole Canyon Creek (3.6 miles);
- ID17040105SK009_02e South Fork Sage Creek (7.93 miles); and
- ID17040105SK009_03 Sage Creek - confluence with North Fork Sage Creek to mouth (3.22 miles).

For Crow Creek, only one stream segment is applicable. For this segment, the reach defined would extend from Sage Creek to the Idaho and Wyoming state line. The applicable stream segment falls within the larger segment identified as:

- ID17040105SK008_04 Crow Creek - Deer Creek to border (10.42 miles).

Monitoring locations characterized as part of these site-specific studies are representative of streams in the area; therefore, while some specific streams were not characterized, the proposed SSSC is still applicable and appropriate given the common sources, water quality, and proximity within the basin.

At the time of identifying the study area, the focus was on water quality impacts downstream of Hoopes Spring and framing those impacts through inclusion of upstream areas. As a result, both the field and laboratory efforts provide information to fully characterize the study area because data have been collected from upstream, non-mining influence areas, as well as downstream mining-influenced areas.

The characterization information from the study (field, laboratory, and literature) is representative of both upstream and downstream areas. However, as noted above, the proposed criterion is being targeted to those streams affected by source areas. Hoopes Spring is the only tributary not identified above that is also within the geographic scope of applicability for this proposed SSSC. The State of Idaho will make the final determination regarding applicability, but Simplot believes that the recommended scope identified above is represented by the studies included as part of this TSD.

The technical basis used to develop a SSSC for Crow Creek in Idaho is expected to be representative for the aquatic community in Wyoming as well. Given that the potential applicability of a SSSC developed for this Site was expected to extend to the Idaho and Wyoming state line, the issue of protecting Wyoming water quality and designated uses was raised by the SSSC Workgroup. A practical starting point is to examine how adoption of a SSSC for Crow Creek in Idaho that is higher than the current Wyoming and Idaho standard (0.005 mg/L) would affect Wyoming water quality.

Data for Crow Creek upstream of the Wyoming border (at the Meade Peak Ranch) indicates only a few measured concentrations of total selenium above the chronic standard (0.005 mg/L) between 2006 to 2008 since monitoring has been implemented (Figure 2-4). The May 2006 measurement occurred at Crow Creek downstream of Sage Creek when Pole Canyon Creek flowed through the ODA and connected to Sage Creek, due to higher than usual snowmelt runoff. The duration of the event was short-lived and Removal Actions conducted at Pole Canyon since that time preclude this condition from occurring in the future. For September and November 2008, the measured values in Crow Creek during 2008 were less than 0.007 mg/L. Aqueous selenium concentrations measured in Hoopes Spring and SFSC springs were elevated during this time frame. In November 2009, aqueous selenium was measured at 0.007 mg/L, while in August and November 2010 aqueous selenium was measured at 0.0085 and 0.0081 mg/L, respectively. These increases correspond to increases at Hoopes Spring and South Fork Sage Creek springs.

Wyoming DEQ provided monitoring data beginning in 2008, for Crow Creek at the state line. In June and October 2008, aqueous selenium was measured at 0.004 mg/L during both time periods. For similar time periods in 2009, aqueous selenium at the state line measured 0.004 and 0.005 mg/L. In June 2010, aqueous selenium at the state line measured on two dates equaled 0.0052 mg/L and 0.0058 mg/L, while for two dates in October aqueous selenium was 0.01 mg/L and 0.009 mg/L.

Wyoming DEQ's 303(d) list does not identify Crow Creek or the Salt River as being impaired or not meeting their designated uses due to selenium; however, recent information from WDEQ indicates Crow Creek at the state line may be listed as impaired on the State's 303(d) list. Likewise, Idaho DEQ indicates it is likely that Crow Creek will be listed as impaired due to selenium on the State's 303(d) list, although as of IDEQ's 2010 Integrated Report (IDEQ 2011), Crow Creek is not listed for selenium. Both of these listings would be based on not meeting the existing standard (0.005 mg/L). Selenium concentrations in Crow Creek downstream are primarily influenced by Hoopes Spring and South Fork Sage Creek spring discharges to Sage Creek.

These recent increases indicate that while the majority of surface water measurements of selenium in Crow Creek are typically less than the current standard both in Idaho and Wyoming, there are occasions when the standard is exceeded. The potential for exceedances at the Wyoming-Idaho state line therefore may exist, warranting application of the SSC to the state line. Potential impacts in Wyoming and possible approaches to address these impacts are addressed in Section 7 of this document. One possible approach may include proposal of this criterion to Wyoming DEQ as well.

2.5 Site Characteristics

2.5.1 Source of Selenium

Hoopes Spring was identified as the primary source of aqueous selenium to downstream waters, including Sage Creek and Crow Creek in the 2005 Smoky Canyon Mine Site Investigation Report (NewFields 2005). Elevated selenium concentrations have also been observed in the lower portions of the South Fork Sage Creek, which discharges to Sage Creek downstream of Hoopes Spring. The source of selenium in South Fork Sage Creek is discharge of Wells Formation groundwater at the SFSC Spring complex. Both the Hoopes Spring complex and the SFSC Spring complex are surface expression of groundwater from the Wells Formation aquifer. The primary source of selenium to the aquifer is mining at the nearby Smoky Canyon Mine.

2.5.2 Historic Surface Water Quality

Based on the last three years of SSSC monitoring and routine monitoring that is conducted as part of the mine's environmental compliance work, which has been conducted for more than 20 years, there is a substantial water quality data set to examine temporal and spatial aqueous selenium concentrations. Selenium data are summarized in Table 2-1 and Figure 2-5 for a number of locations from 2000 to just prior to Fall 2006 when the SSSC studies began. No exceedances of the State of Idaho water quality standard have been observed in Crow Creek upstream of Sage Creek, and no selenium concentrations have been detected above 0.0013 mg/L since 2000.

The longest temporal data record available is for Hoopes Spring, which has been monitored since 1979. From 1979 to 1999, selenium concentrations ranged from 0.001 to 0.02 mg/L. It is not clear if detection limits were sufficient to accurately measure concentrations in the earliest sampling efforts. From 2000 to 2006, aqueous total selenium has averaged 0.0125 mg/L. More recently, concentrations at Hoopes Spring have been as high as 0.050 mg/L.

The range of water quality at the locations being used as part of this Site-specific investigation represents a gradient from the source area at Hoopes Spring to the downstream receiving waters. Prior to fall 2006, near the primary source area at Hoopes Spring (HS and HS-3) and immediately downstream of Hoopes Spring in Sage Creek (LSV-2C), selenium concentrations have typically been elevated and exceeded the State Standard. During the pre-fall 2006 period, aqueous selenium in Crow Creek downstream of Sage Creek averaged 0.002 mg/L, and while higher than Crow Creek upstream of Sage Creek, concentrations were typically below the state standard (Table 2-1 and Figure 2-5).

Selenium concentrations in surface water control partitioning of selenium to sediments. Both the sediment and water selenium concentrations are related to biological uptake, which ultimately affects bioaccumulation in the aquatic community, whose most sensitive receptor is expected to be fish. The additional biological tissue and sediment monitoring data, together with the water quality data described above and the data collected more recently, allow for an evaluation of how changes in the selenium concentrations relate to uptake in the biological system.

2.5.3 Surface Water Selenium Speciation

Different species of selenium can exert potentially different bioaccumulation and toxicity in receptors. In lotic (flowing waters such as streams and rivers) systems, well-oxygenated water is typically present, thus selenates, which are less bioavailable (USEPA 2004; Chapman 2000), tend to be the predominant inorganic forms of selenium present. Adams et al. (2000) indicates that “in lotic environments, selenium in the water column is most often found in the form of selenate and migration to sediments is limited. In lentic environments, selenate is less prevalent, selenite is more common, and both forms are biologically and chemically reduced to elemental and organo-selenium forms. These reduced forms are prevalent in lentic sediments and form the basis for uptake by benthic invertebrates and subsequent food-chain bioaccumulation.”

Simmons and Wallshlager (2005) conducted a critical review of the literature to evaluate the differences in biogeochemistry and toxicology of selenium in lotic and lentic systems and found that the data for lotic systems are lacking to definitively conclude that differences exist. However, evidence suggests that lentic systems bioaccumulate dissolved selenium to a greater extent than lotic systems (Simmons and Wallshlager 2005). These authors further corroborate that different selenium species predominate in lentic and lotic habitats, as suggested above. The physical characteristics of the streams adjacent to the mine indicate that a lotic, cold water salmonid community is present. When oxygen is present in solution, organic carbon is low, and water is flowing, selenate will be the dominant form of selenium in solution, and selenate reduction to selenite or elemental selenium will not take place unless the stream conditions change. Thus, geochemically, selenate is the selenium species favored by the conditions present (e.g., high dissolved oxygen, low temperature and low carbon content) in the streams adjacent to the Smoky Canyon Mine.

Oxidation-reduction potential (ORP) and selenium redox-speciation data have been collected during the baseline studies recently conducted for the Panels F and G Environmental Impact Statement (BLM and USFS, 2007) and as part of the Site Investigation conducted to evaluate effects of historical mining operations at the Smoky Canyon Mine (NewFields 2005). ORP measurements for groundwater and surface water samples collected in the vicinity of Smoky Canyon Mine indicate oxidizing conditions. Surface water contains selenate as the dominant redox species. Selenate is also the dominant form of selenium in groundwater from the regional Wells Formation aquifer, springs that discharge from the Wells Formation aquifer and surface water receiving spring discharge from the Wells Formation. The existing data are sufficient to demonstrate that selenate is the dominant form of selenium in the surface waters being evaluated.

2.5.4 Physical Habitat Quality

Physical characteristics of the reaches selected for this study were evaluated during each monitoring event. Habitat conditions are variable across the reaches. Table 2-2 summarizes some of the habitat characteristics evaluated to provide an initial context of the conditions for each reach. Tables 2-3 and 2-4 present summary statistics for long-term temperature monitoring (1.5 to 2 years) for each location. A more thorough habitat quality characterization is provided in the Final Data Report (NewFields and HabiTech 2009b) and analysis of these results is presented later in this document. One common physical quality issue affecting these locations is the consistent observation of present or recent-past grazing activity near stream banks. All locations are impacted to varying levels by this management practice. Grazing has limited near-stream willow growth and caused trampled/failing banks, reducing the quantity and quality of undercut banks, overhead cover, and shading, all important trout habitat features. Some of the more general characteristics of each locale are described below.

The background locations on Crow Creek and Deer Creek, as well as the reference location on South Fork Tincup (SFTC) Creek, were selected to provide a range of conditions that exist in non-mining-impacted areas. Stream gradients and average flows at each location indicate the steepness and size of each stream. Deer Creek and SFTC Creek are the steeper reaches of the background or references locales with fairly similar mean flows. The background Crow Creek reaches are flatter compared to Deer Creek and SFTC Creek, and CC-150 and CC-350 are larger based on mean stream flows. Of the background locations, the CC-350 reach channel is the most unstable with more bank erosion. Mean year-round temperature data for background and reference locations are fairly consistent, except for Deer Creek, which on average is typically colder likely due to its elevation and heavy near-stream cover. This observation is true for mean summer temperatures as well, where Deer Creek is consistently colder by several degrees as compared to background Crow Creek and SFTC Creek locations.

Hoopes Spring (HS) has the lowest overall mean flow, as flow is derived entirely from the spring. Low stable flows contribute to a very stable channel condition and low observed bank erosion. Year-round temperature at the HS location on average is higher than most locations (other than at LSV-4), but the difference between minimum and maximum temperatures spans less than about 2 degrees during summer months (Table 2-4). During summer months, HS has the next lowest mean summer temperature after Deer Creek with very little change between the minimum and the maximum. This is expected due to the groundwater source at the spring. Hoopes Spring is the most markedly different reach from the other stream reaches evaluated.

Hoopes Spring near its mouth (HS-3) has a higher gradient similar to SFTC-1, with mean flows more than double that of HS. The channel is relatively stable, but shows moderate to high levels of bank erosion. Year-round water temperatures on average are lower than the spring location (HS), while the mean summer temperatures more closely resemble stream temperatures at other locations.

Of the two locations on Sage Creek, LSV-2C has a slightly higher gradient and lower flow than does LSV-4. Some differences in channel stability exist, with LSV-2C typically being the more stable of the two. However, accelerated erosion due to high spring runoff flows and intense grazing was evident at LSV-2C in Fall 2008 with nearly a tenfold increase in eroding bank from Fall 2006. Limited data at LSV-4 indicate moderately low bank erosion. Inflow of Hoopes Spring water to Sage Creek moderates Sage Creek water temperature at LSV-2C, as the overall mean summer temperature is only slightly higher than that observed for HS-3, and lower than mean summer temperatures observed further downstream at LSV-4.

Crow Creek downstream of Sage Creek (CC-1A and CC-3A) exhibits the lowest overall gradient, but the highest flows of the downstream most locations. Both locations have flows more than double the Sage Creek inflow on average. Channel stability is rated as fair with multiple factors contributing to overall channel instability. Bank erosion between the two locations is markedly different, with low erosion at CC-1A and moderate to high erosion at CC-3A. Other than the reference location at SFTC-1, the two Crow Creek locations (CC-1A and 3A) downstream of Sage Creek have the highest mean summer temperatures due to their lower elevation, while the year-round mean temperatures are only slightly higher than background.

2.5.5 Biological and Physical Characteristics

Aquatic life in Crow Creek and its tributaries is typical of mid-elevation Rocky Mountain streams. Crow Creek has a healthy population of YCT, as well as mountain whitefish and brown trout (IDFG 2007). As a non-native species, brown trout have been stocked in streams and lakes throughout the west. In Crow Creek, IDFG records for the period from 1967 to 2011 indicate brown trout were stocked twice (in 1993 and 1994). No other records were found indicating that brown trout have been stocked in other streams covered by the Site. By comparison, cutthroat trout were stocked in Crow Creek for a 10 year period from 1969 to 1979 and again between 1984 and 1987. Wyoming Fish and Game shows no records for brown trout stocking in Crow Creek or its tributaries. In the Salt River drainage, there are actually very few stocking records for brown trout. Cottonwood Lake was stocked with brown trout twice in 1935 (18,000 fish), Swift Creek was stocked once in 1935 (9,000 fish) and the Upper Greys River was stocked once in 1933 (25,000 fish) (Rob Gibson, Area Fisheries Manager, e-mail communication April 22, 2011).

During monitoring conducted for this study, as many as 10 different species have been found to be present in Crow Creek, with a slightly higher species richness found in the lower segments of Crow Creek downstream of Sage Creek (n=9) versus upstream of Sage Creek (n=8). Tributaries, including Sage Creek and Deer Creek, have lower numbers of species with four and two species, respectively. Table 2-5 shows the species observed from 2006 to 2008 during the spring and fall monitoring.

Brown trout are found at all Crow Creek and tributary locations except in Deer Creek, where only YCT are found. YCT are found at all locations except at Hoopes Spring, and typically in lower abundance when compared to brown trout. Mountain whitefish are found primarily in Crow Creek at both upper and lower locations, and in lower Sage Creek at the LSV-4 location. IDFG surveys in 1979 reported mountain whitefish present at this location, with greater abundance in the fall as opposed to the summer months (Heimer 1979). This species, however, was not reported in Sage Creek upstream of this location.

Two species of cottids, Paiute *(Cottus beldingi)* and mottled sculpin *(Cottus bairdi)*, are present in the Crow Creek drainage. Sculpin are relatively ubiquitous in the Crow Creek drainage and their numbers vary with the type of habitat. Paiute sculpin have been identified at each of the monitoring locations during nearly every monitoring event, while mottled sculpin have been intermittently identified in voucher collections. Mottled sculpin are infrequently identified, and have only been positively identified in voucher samples from location DC-600 and CC-1A, but in proportionately low numbers compared to the number submitted for taxonomy. During the first monitoring event in 2006, sculpin were not differentiated to species; however, comments received by SSSC Workgroup members following presentation of the 2006 data to the group prompted species identifications for sculpin for all subsequent events. Other than the two trout species selected for testing under the Approach for these Site-specific studies, only sculpin are found at most if not all of the locations monitored.

Both longnose and speckled dace are found at most of the Crow Creek locations, except the upper most Crow Creek location (CC-75); however, these species are largely absent from Sage Creek, Hoopes Spring, and Deer Creek based on the monitoring data. Baseline monitoring conducted by in 1979 by IDFG on Sage Creek at the LSV-4 location reported that in a two-pass depletion, only sculpin and trout were collected. Similar monitoring in Hoopes Spring found no dace, although sculpin were abundant (Heimer 1979).

Utah sucker has routinely been found in Crow Creek at the lower two locations (CC-1A and CC-3A), but is rarely found in the upper Crow Creek locations. No Utah suckers were found in Sage and Deer Creeks, or in Hoopes Spring.

Benthic communities found at monitoring locations included a diverse mixture of aquatic invertebrate families and were typically similar across locations except at the HS location which was ecologically different than the stream locations due to it being a spring headwater area. Total numbers of taxa were consistent across most locations, but not across time periods, as a general reduction in taxa numbers was observed during Fall 2008. Density followed a similar trend.

3.0 LITERATURE REVIEW

Review of published and unpublished gray literature has been conducted at several intervals as it has been and continues to be a very important part of the overall process. Most notably, the fairly consistent input of new science is increasing the overall knowledge of selenium toxicity to aquatic organisms. The literature has guided the development of the approach and design for this study. Initially, the literature was reviewed to examine the various approaches that have been used to evaluate selenium toxicity to aquatic life. Information from the literature has also been used to identify sensitive species of aquatic life, sensitive life stages, the most relevant pathways for evaluating potential effects, and effective measurement endpoints for evaluating toxicity. In the analysis phase of the evaluation, the literature continues to be reviewed to assess how results from this study compare to those of others. This step provides an important "reality" check in making determinations about data applicability, accuracy, and representativeness.

This literature review is focused on relevant studies of cold water species and evaluates: how selenium exerts toxicity, what life stage is the most sensitive, and how toxicity is expressed (i.e., endpoints). The literature review summarizes particularly relevant studies for potential comparison. Furthermore, because it is not practical to conduct laboratory tests for each species found to be present in the Crow Creek drainage, studies found in the literature for different species provide additional useful data.

3.1 Selenium Toxicity

Numerous studies are available that characterize selenium toxicity to aquatic organisms. Most have characterized acute toxicity, while a smaller proportion of those studies characterize chronic toxicity. Most however, focus on aqueous exposures. A smaller percentage of studies focused on the dietary pathway and even fewer focus on maternal transfer. This somewhat iterative approach in defining the modes and mechanisms of toxicity has resulted in a more thorough understanding of selenium effects to aquatic organisms.

Selenium is an essential micronutrient important in the enzyme glutathione peroxidase (SeGSHpx). This enzyme converts peroxide into water and catalyzes the scavenging of free radicals by glutathione, thereby preventing oxidative damage to biological tissues (Simmons and Wallshlager 2005). When this dietary requirement is exceeded, selenium can become toxic. Excess selenium disrupts enzyme and protein formation and function, resulting in disruptions in cell differentiation which can lead to, among other effects, malformations in developing young. Hodson and Hilton (1983) and Lemly (1997a) both suggest that developmental malformations are reliable indicators of chronic selenium toxicity to fish.

Lemly (1997a) described the sequence of selenium toxicity to larval fish: parental exposure, maternal deposition of selenium into eggs during vitellogenesis, and subsequent exposure during yolk resorption in developing larvae. It has been suggested that selenium does not exert its toxic effects until a developing fish absorbs its yolk and accumulated selenium (Lemly 1997a; Holm et al. 2005 as cited in Rudolph et al. 2008). Holm et al. (2005) reports that although egg selenium is present in the yolk throughout development, it may affect larval development rather than egg development because it is mobilized to a greater degree after hatch. Hatchability of eggs is not affected by elevated selenium even though there may be a high incidence of deformities in resultant larvae and fry, and many may fail to survive (Gillespie and Baumann 1986; Coyle et al. 1993).

3.2 Maternal Transfer Studies on Cold Water Species

The prevailing scientific evidence supports the theory that chronic selenium toxicity in fish results in deformities of young fish if exposure concentrations are sufficiently high. More importantly, the exposure is linked to maternal exposure, where the female has accumulated and transferred selenium to forming eggs. Presented below are the relevant maternal transfer studies conducted for cold water species, several of which are included in EPA's revision of the 2004 draft selenium criterion. The complete studies are presented on the project webpage and Table 3-1 summarizes effects data for each of these studies.

Rudolph et al. (2008) sampled cutthroat trout from two areas of active coal mining in British Columbia. Pertinent findings include:

- Egg selenium concentrations ranged from 12.3 to 16.7 and 11.8 to 140 µg/g dw from fish collected at the reference and exposed locations, respectively.
- Muscle selenium concentrations ranged from 6.61 to 8.74 and 7 to 50.4 µg/g dw from fish collected at the reference and exposed locations, respectively.
- Eggs with selenium concentrations greater than 86.3 µg/g dw were not successfully fertilized or were non-viable at fertilization.
- A significant relationship between egg selenium concentration and alevin mortality was observed; however, the mechanism underlying mortality was unknown.
- Larval fish that developed from eggs with selenium concentrations ranging from 46.8 to 75.4 µg/g exhibited some deformities, but died before yolk absorption.

- Deformities were analyzed in surviving fry that developed from eggs with selenium concentrations between 11.8 and 20.6 µg/g dw, but no relationship between selenium concentration in eggs and deformities or edema was found in this range.

- The no-effect threshold for deformities or edema was > 20.6 µg/g dw. The EC_{10} and EC_{20} values for alevin mortality[6] were 24.2 and 28.4 µg/g dw selenium in egg tissue, respectively.

Hardy (2005) and Hardy et al. (2010) examined selenium effects via dietary uptake on the reproductive performance of cutthroat trout from Henry's Lake, Idaho. Pertinent findings include:

- Henry's Lake fish experienced virtually no maternal pre-exposure to selenium, so Hardy et al. (2010) fed seleno-methionine dosed feed to maturing fish over 2 to 3 years.

- Adult whole-body selenium concentrations ranged from 1.2 to 11.4 µg/g dw and egg selenium concentrations ranged from 1.6 to 16.0 µg/g dw in fish ages 2.5 to 3 years. Dietary levels ranged from control, 2, 4, 6, 8, and 10 µg/g selenium as nominal concentrations.

- After week 80 of the study, no effect of dietary intake was observed at the highest diet level (10 µg/g).

- The percentage of deformed fish was highest at the 4 µg/g selenium dietary treatment, but was near control levels (5.6 percent) in the 8 µg/g selenium (7 percent) and 10 µg/g selenium (6.8 percent) treatments.

- The no observed effect concentration (NOEC) for mortality and growth based on whole body selenium concentration was conservatively estimated as > 11.52 µg/g dw (the lower 95 percent confidence bound of the mean no effects value [12.5 µg/g dw]).

- The NOEC for larval deformities based on egg selenium concentration was >16.0 µg/g dw.

- Hardy et al. (2010) suggests that cutthroat trout may not be as sensitive to selenium as other trout species such as rainbow trout.

[6] Mortality effects concentrations were derived from the data provided in published supporting information for Rudolph et al. (2008) using EPA's TRAP software and the non-linear threshold sigmoidal regression function.

Holm et al. (2005) examined reproductive effects in two salmonid species, brook trout and rainbow trout, from coal mine runoff exposed locations and reference locations over a three-year period from 2000 to 2003. Pertinent findings include:

- For rainbow trout, 58 fish were collected from a range of exposure and reference locations.
- Rainbow trout muscle selenium concentrations ranged from 0.5 to 1.5 µg/g wet weight, and egg selenium concentration ranged from 3.5 µg/g to 9.9 µg/g wet weight.
- A significant relationship between egg selenium concentrations and craniofacial deformities, skeletal deformities, and edema was observed in rainbow trout.
- For brook trout, 63 fish were collected from a range of exposure and reference locations.
- Mean brook trout muscle concentrations ranged from 0.6 to 3.8 µg/g wet weight, while egg concentrations ranged from 1.3 to 7.8 µg/g wet weight.
- An association between the occurrence of deformities and egg selenium concentrations was not evident for brook trout.
- For brook trout, the EC_{06} for larval deformities based on egg selenium concentration was 20 µg/g dw. For rainbow trout, the EC_{10} and EC_{20} for larval deformities were 23 and 29 µg/g dw based on egg selenium concentrations, using an assumed 61 percent moisture to convert wet weight data to dry weight.

Kennedy (2000) examined reproductive effects of selenium on wild cutthroat trout from coal mining-exposed areas and reference areas in Canada. Pertinent findings include:

- Egg samples were collected from 37 fish from exposure and reference locations.
- Despite elevated selenium concentrations in eggs (range 8.7 to 81.3 µg/g dw), there was no significant effect on fertilization; time to hatch; percent hatch; or egg, larvae, and fry deformities or mortalities.
- The frequency of deformities in the exposed population was < 1 percent. Some egg clutches suffered 100 percent mortality, but there was no statistical correlation with egg selenium content.
- The authors suggest that the lack of any toxic response in this study may be due to an evolved tolerance to higher tissue selenium concentrations in a population of fish living in a seleniferous river system.

- The NOEC for larval deformities or mortality based on the mean egg selenium residues from the exposure site was > 21 µg/g dw.

Muscatello et al. (2006) examined maternal transfer effects of selenium to young in northern pike collected downstream of a uranium milling operation and from a reference location (total n = 15). Pertinent findings include:

- Pike eggs for this study were reared in site water sent to the laboratory.

- Muscle selenium concentrations ranged from 1.64 µg/g to 38.3 µg/g dw for fish collected from the reference location and "high" location, respectively. Egg selenium concentrations ranged from 3.19 µg/g to 48.23 µg/g dw for fish collected from the reference location and "high" location, respectively.

- No significant differences were observed between reference location and exposed locations in the cumulative time to 50 percent eyed embryos, 50 percent hatch, or 50 percent swim-up.

- Significant linear relationships were observed between selenium concentrations in northern pike eggs and the percentage of fry exhibiting edema, skeletal deformities, craniofacial deformities, and fin deformities.

- The EC_{10} and EC_{20} for larval deformities based on egg selenium tissue concentrations were 20.4 and 33.55 µg/g dw. Effects concentrations for whole body tissues derived from egg concentrations were EC_{10} (9.36 µg/g dw) and EC_{20} (15.56 µg/g dw).

Muscatello and Janz (2009) collected northern pike and white suckers from a lake system receiving treated uranium mill effluent and from a reference location (n = 9 for each species). Pertinent findings include:

- Eggs were reared in site water that was collected and sent to the laboratory.

- Selenium concentrations in northern pike and white sucker eggs (8.02 and 4.89 µg/g dw, respectively) from the exposure location were approximately two- to threefold higher than from the reference location (2.35 ± 0.20 and 1.94 ± 0.25 µg/g dw, respectively; [mean ± standard error]).

- Among all evaluated deformities (skeletal curvatures, craniofacial deformities, fin deformities, and edema), only edema in white sucker fry from the exposure location was slightly elevated (3 percent) compared to the reference location.

- Both fish species displayed strong linear relationships between the selenium concentrations in eggs and other tissues (muscle, liver, kidney, and bone), suggesting that selenium concentrations in eggs could be predicted from selenium concentrations in adult tissues.

- The lack of a clear, toxic response is in agreement with selenium thresholds for ELS deformities reported in other studies, with egg selenium concentrations in northern pike and white sucker collected at the exposure location being less than the 10 µg/g dw threshold associated with the presence of deformities.

de Rosemond et al. (2005) examined selenium effects to white sucker *(Catostomus commersoni)* in a northern Saskatchewan lake downstream of a uranium mine (n = 4). Pertinent findings include:

- Fertilized eggs were reared in the laboratory in clean lake water.

- Egg selenium concentrations ranged from 8.4 to 48.3 µg/g dw. Only 200 eggs from each female were reared for the assessment of deformities.

- Total development deformities ranged from 5.9 to 16.4 percent, although there was no correlation observed between selenium concentrations in eggs and developmental deformities.

- The EC_{13} for larval deformities based on an egg tissue concentration of selenium was 26 µg/g dw. The authors concluded that the low percentage of deformities observed and lack of correlation with embryo selenium concentrations indicate that selenium in the exposure system may be having at most a slight effect on the resident white sucker population.

Golder (2008) provides a review of an unpublished study by Minnow (2006) who evaluated longnose suckers *(Catostomus catostomus)* from the Elk River watershed, British Columbia. Pertinent findings include:

- Selenium concentrations in eggs from Elk River Upper Oxbow females ranged from 6.0 to 12.2 µg/g dw, whereas those from Goddard Marsh females ranged from 15.5 to 65.4 µg/g dw.

- 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 selenium content.

- No significant correlations were found between egg selenium concentrations and embryo-larval mortalities or deformities. The authors concluded that if longnose suckers are sensitive to selenium concentrations in the observed range of 15.5 to 65.4 μg/g dw in eggs, the effects were masked in this study by the influence of other unknown factors.

Golder (2009)[7] developed a site-specific selenium threshold for Dolly Varden char from northwestern British Columbia. Pertinent findings include:

- Six char were collected from high and moderate selenium exposure areas and three char were collected from a low selenium exposure area (an upstream reference area not affected by mine activities). Fertilized eggs were also removed from two redds found in a collection ditch that collects seepage waters from a mine's waste rock storage area. Fertilized eggs were sent to the laboratory for rearing in dechlorinated municipal tap water.

- Muscle tissue selenium concentrations in fish collected from the high and moderate selenium exposure areas ranged from 39.5 to 58.3 mg/kg dw and in the low exposure area (reference area) tissue selenium concentrations ranged from 2.8 to 6.6 mg/kg dw.

- Egg selenium concentrations from the high and moderate selenium exposure areas ranged from 32.6 to 65.8 mg/kg dw, while egg selenium concentrations from the low exposure area (reference area) ranged from 5.4 to 11 mg/kg dw. Eggs from redds found in the collection ditch ranged in selenium concentration from 10.3 to 24.7 mg/kg dw.

- Percent survival of eggs to swim-up for the high and moderate exposure areas ranged from 59 to 100 percent, while from the low exposure area (reference area), survival ranged from 3 to 96 percent. Survival of eggs collected from redds found in the collection ditch ranged from 60 to 80 percent.

- Deformity frequency was the test endpoint utilized for effect threshold derivation. EC_{10} and EC_{20} values for deformity frequency based on egg selenium concentrations were 54 and 60 mg/kg dw, respectively.

- The authors concluded that there is a high degree of confidence in these derived effects thresholds because a robust QA/QC program was implemented for the larval deformity assessment.

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[7] Published as McDonald et al. (2010) in Environmental Toxicology and Chemistry.

Nautilus Environmental (2011) (also presented in Elphick et al. [2009]) conducted studies on maternal transfer in Westslope cutthroat trout to resolve discrepancies between studies conducted by Kennedy et al. (2000) and Rudolph et al. (2008) using the same species. Pertinent finding include:

- Approximately 250 eggs from females from lentic (n = 33) and lotic (n = 26) environments considered high selenium exposure environments were utilized. In addition, eggs from four females from Connor Lake, a non-mine impacted locale were also tested. Eggs were reared to swim-up, and then one half of each replicate were used to assess deformities, while the other half of the swim-up fry were reared for an additional 28 days in order to evaluate the potential of latent adverse effects.

- Fifteen of 21 samples that exceeded 75 µg/g dw selenium did not survive to within 24 hours of fertilization.

- The mean selenium for lentic location Clode Pond eggs was 72 (range: 3.3 – 122.3) µg/g dw. The mean selenium for lotic locations were as follows: Clode Pond outlet was 39 (range: 5.3 – 91.7) µg/g dw, Clode Pond exfiltration was 45.6 (range: 9.5 to 128.3) µg/d dw, and Fording River was 20.3 (range: 9.3 – 67.4) µg/g dw. Connor Lake eggs had a mean selenium of 4.5 (range: 3.2 – 5.4) µg/g dw.

- There was no evidence of significant differences in sensitivity to selenium in offspring from fish collected from lentic and lotic environments with respect to survival or rates of deformity.

- There was a low incidence of adverse effects on survival in eggs containing up to 22.1 µg/g dw selenium, with substantial adverse effects (i.e., > 40 percent mortality) observed in eggs containing 31.5 µg/g dw selenium, and higher; no eggs were tested that had egg selenium concentrations falling between these values. The EC_{10}, EC_{20} and EC_{50} (with 95 percent confidence intervals) for larval survival at the swim-up stage were 19.0 (range: 6.8 – 22.7), 22.8 (range: 16.3 – 26.6) and 29.9 (range: 26.1 – 33.6) µg/g dw selenium in eggs. A revised EC_{10} value was presented in Nautilus Environmental (2011) due to a revision of data included in the derivation of the original EC_{10}. The new EC_{10} is 24.8 mg/kg dw selenium in eggs.

- There was no evidence of selenium-related deformities in fry that produced good survival (i.e., > 60 percent) to the point of swim-up, and containing up to 22.1 µg/g dw selenium. After rearing for an additional 28 days, these fry had good survival (i.e., > 90 percent) and growth rates, and a very low frequency of deformities (i.e., < 5 percent), supporting the conclusion that there were no latent adverse effects due to selenium exposure.

3.3 Other Species Sensitivity

Different fish species exhibit different sensitivities to selenium. This is clearly illustrated in the USEPA's (2004) draft criterion, as well as reviews by Hamilton (2003, 2004); Lemly (1998); DeForest et al. (2006); Brix et al. (2005); and others. The wide range of toxicity studies that have been conducted have yielded important information about life stages most affected and the exposure route that results in the highest levels of effects. The range of species mean chronic values (EC_{20}s and NOECs)[8] in fish tissue presented in USEPA 2004 spans from 5.85 mg/kg dw for rainbow trout to 51.4 mg/kg dw for fathead minnow. These studies, however, cover a wide range of exposures, life stages, and tissues. Among studies focused on maternal transfer, egg development where young alevins are evaluated, and ovary or egg tissue, the NOEC range is much narrower, ranging from 17 to 26 mg/kg dw egg or ovary tissue. The exception to this finding is the recent finding for Dolly Varden char which had an EC_{10} for larval deformities of 54 mg/kg dw. All endpoints are either larval deformities or larval mortality.

To date, no toxicity studies for cold water cyprinids such as dace or shiners have been found. Muscatello et al. (2006) and de Rosemond et al. (2005) tested northern pike and white suckers, respectively, both of which appeared to be within the range of sensitivities (based on larval deformities) for brook and rainbow trout effects reported by Holm et al. (2005).

No toxicity data (i.e., effects) for sculpin exposure to selenium have been published to date. Carmichael and Chapman (undated) report slimy sculpin tissue concentrations for fish found inside of and outside of a coal zone. While no effects data are reported, they illustrate that sculpin inside the coal zone had a wide range of selenium tissue concentrations ranging from less than 2 mg/kg dw to > 12 mg/kg dw. Calls were made to researchers who either may potentially be investigating or may possess data for selenium toxicity to sculpin, but no toxicity data were available (personal communication with Dr. John Besser, USGS and Dr. Charles Delos, USEPA Headquarters).

Long-term studies of benthic macroinvertebrate response to selenium exposure are limited. Swift (2002) conducted long term (> 1 year) experimental dosing studies on stream mesocosms and found no significant effect on benthic community abundance, diversity, or richness in the high (30 µg/L nominal) and moderate (10 µg/L nominal) experimental units, but Tubifex and Isopod numbers were reduced. DeBruyn and Chapman (2007) examined the literature to

[8] The USEPA 2004 Draft National Criterion, Table 4, presents compiled chronic test data that includes EC_{20} values, maximum allowable toxicant concentrations (MATCs), and NOECs for the endpoints measured, all of which are used in the derivation of species and genus mean chronic values. As noted in the 2004 Draft document, "When the data from an acceptable chronic test met the conditions for of the logistic regression analysis, the EC_{20} was the preferred chronic value. When data did not meet the conditions, best scientific judgment was used to determine the chronic value. In this case the chronic value is the geometric mean of the NOAEC and LOAEC and termed the maximum allowable toxicant concentration (MATC). But when no treatment concentration was an NOAEC, the chronic value is less than the lowest tested concentration. And when no treatment concentration was a LOAEC, the chronic value is greater than the highest tested concentration."

assess selenium sensitivity of macroinvertebrates and found that some invertebrates may be sensitive at body burdens similar to those protective of fish. Conley et al. (2009) suggests that mayfly fecundity due to maternal transfer of selenium to eggs is reduced when organisms are fed diets higher than approximately 11.0 µg/g selenium where selenium was introduced to periphyton as selenite. Using the bioaccumulation factor provided in the document (2.2), this translates into an adult tissue selenium concentration in mayflies equal to 24.2 µg/g dw.

The prevailing scientific evidence supports the current thinking that effects to developing fish are among the most sensitive aquatic biological indicators of excessive selenium exposure (USEPA 2004; Lemly 1996; Ogle and Knight 1996; Skorupa et al. 1996; Janz et al. 2010). This would suggest that if the biological response of fish is considered a very sensitive indicator of effects, fish species would be considered a sensitive aquatic receptor. Studies outlined as part of this approach to assess potential site-specific effects of selenium assume that fish are the most sensitive species, which is consistent with the current literature. However, benthic invertebrates are not dismissed as potentially sensitive species.

3.4 Population and Community Studies

Some long-term studies and/or wide-ranging basin-wide studies of fish populations and communities have been conducted in response to selenium exposure. Review of the studies listed below does not imply that these environments are similar to the cold water environment of this Site, but rather suggests that few large-scale population studies have been undertaken to evaluate selenium effects. These studies do provide evidence that site-specific conditions play a role in the level of effects observed using the metrics investigated by the individual authors. Results and conclusions presented for these studies are those reported in the individual reports reviewed.

May et al. (2001) provides a succinct summary of theoretical population level effects in the presence of excessive selenium exposure.

> “Fish reproductive failure is expected to result in altered population structures such as absence of year classes or increases in the average age of the population. The ultimate effects of reproductive failure depend upon intrinsic demographic factors such as fecundity, survival, and reproductive life-span in addition to extrinsic factors such as immigration/emigration rates (Berryman 1981).
>
> Fish in general produce high numbers of young in excess of numbers necessary to sustain the population. Analysis of survival curves of most species indicates rapid mortality of fishes due to predation, starvation, and environmental fluctuations such that only a few individuals survive to eventually reproduce.

> These demographic patterns, however, can be altered due to contaminants which result in localized mortality or in severe cases total absence of a year class of fishes. However, populations usually persist due to immigration or alternating years of strong year classes. Selenium, however, can result in repeated loss of year classes which can ultimately deplete fish populations. Population structure in these cases shifts to a population dominated by mature adults, which is the reverse of population structure normally observed in exploited fish populations (Gillespie et al. 1986). Thus, population-level effects can be analyzed by sampling of fish populations and comparing population structures among high selenium and low selenium areas."

May et al. (2001) conducted a large-scale assessment, spanning 3 years and 46 locations, in the Republican River Basin of Kansas and Nebraska (a warm-water fishery). Water, sediment, benthic invertebrates, and/or fish were collected from all locations in the Basin and were analyzed for selenium to determine the potential for food-chain bioaccumulation, dietary toxicity, and reproductive effects of selenium in biota. Results were compared to existing thresholds for biological effects for selenium hazards (Lemly 1993b, 1995). Findings include the following:

- Water from 38 percent of the locations (n = 18) contained selenium concentrations exceeding 5 µg/L, which is reported to be a high hazard for selenium accumulation into the planktonic food chain. Concentrations in 1997 and 1998 ranged from < 1 to 27.3 µg/L. In preliminary work conducted in 1994 and 1995 surface water concentrations ranged from < 1 to 40 µg/L.

- An additional 12 locations (26 percent of the locations) contained selenium in water between 3 and 5 µg/L.

- Selenium concentrations in sediment indicated little to no hazard for selenium accumulation from sediments into the benthic food chain.

- Ninety-five percent of benthic invertebrates collected exhibited selenium concentrations exceeding 3 µg/g; a level reported as potentially lethal to fish and birds that consume them according to (Lemly 1993b, 1995). Selenium concentrations in benthic invertebrates ranged from 2.8 to 14.4 mg/kg dw.

- Seventy-five percent of fish collected in 1997, 90 percent in 1998, and 64 percent in 1999 exceeded 4 µg/g selenium (Lemly 1993b), a level suggested to indicate a high potential for toxicity and reproductive effects. Selenium concentrations in fish tissues ranged from 2.04 to 19.1 mg/kg dw. Species differences did exist, but insufficient information was presented to assess species differences among sites.

- The authors concluded from these data that selenium has bioaccumulated in the invertebrate-fish food chain of the Republican River Basin to the extent that adverse reproductive impacts **should** exist for fish and possibly resident aquatic bird populations. However, an analysis of individual fish collections revealed significant numbers of very small fish for numerous species and from a variety of locations. This discovery, coupled with no significant loss of year classes in any major sport fish species in the Basin reservoirs, suggested that fish reproduction was successfully occurring in spite of the dangerous levels of selenium bioaccumulation.

GEI Consultants (2007) investigated naturally elevated levels of selenium in the Arkansas River and select tributaries near Pueblo, Colorado. Studies were conducted over more than two years at ten locations. Studies conducted included assessments of fish populations and age structure, habitat quality, selenium concentrations in various biotic and abiotic media, water quality, and deformities in fish. Findings of this study are presented below:

- Total selenium water column concentrations were generally elevated throughout the study area, above the Colorado chronic standard of 4.6 µg/L dissolved selenium, ranging from means of 7.05 to 10.6 µg/L in the Arkansas River, 3.4 to 12.1 µg/L in Fountain Creek, 3.1 to 20.3 µg/L in St. Charles River, and 418 µg/L[9] in Wildhorse Creek.

- Mean benthic tissue concentrations of selenium ranged from 8.74 to 16.8 µg/g dw in the Arkansas main stem, 45.5 µg/g dw in Wildhorse Creek, 7.9 to 14.8 µg/g dw in Fountain Creek, and 6 to 19.7 µg/g dw in the St. Charles River.

- Mean selenium tissue concentrations were measured and presented for three cyprinids (central stoneroller (range: 3.7 to 66.7 µg/g dw), sand shiner (range: 6.1 to 29.7 µg/g dw), and red shiner (range: 18 to 80.5 µg/g dw)), one catostomid (white sucker (range: 4.3 to 61.9 µg/g dw)), and three centrarchids (green sunfish (range: 12.8 to 30 µg/g dw), smallmouth bass (range: 6.2 to 24.6 µg/g dw), and largemouth bass (range: 8.7 to 42.9 µg/g dw)). Selenium tissue concentrations varied noticeably by fish family. Mean concentrations in all cyprinids were greater (21.1 µg/g dw) than either centrarchids (19.7 µg/g dw) or catostomids (17.5 µg/g dw). Most mean whole-body selenium concentrations were well above the draft chronic tissue criterion of 7.91 µg/g.

- Observations of fish deformities revealed that most locations had few or no fish with any observable deformities.

[9] Selenium concentrations measured during the duration of the biological study at the WHC are 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) is 7 times greater than the maximum selenium concentration measured at other study locations (43.6 µg/L at SC5). Groundwater from Pierre Shale-influenced zones is the primary source for selenium in this system.

- Individual observations of fish sensitivity were not made in this study, although central stonerollers and white sucker appeared to be particularly tolerant based on presence of multiple age classes at a range of selenium concentrations.

- Total fish density, when weighted by habitat availability, was not significantly related to tissue selenium. 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, a high silt percentage may be an indicator of high primary production that could influence food availability, or diverse habitat that contains slow backwater or eddy refugia.

- Overall, the results of the study indicate no consistent relationships between selenium concentrations in water, sediment, and fish tissues, to fish taxa richness or density for the range of concentrations observed. Although some locations exhibited lower numbers of fish species (e.g., Wildhorse Creek), which may indicate an adverse effect of selenium concentrations, those concentrations are based on natural sources. In addition, the selenium tissue concentrations in fish were no higher at that location than other locations with many more species.

- Data were used to develop site-specific criteria, resulting in chronic water quality values of 17.4 µg/L for Segment 3 of the middle Arkansas River, 597 µg/L[10] for Segment 4a, and 14.4 µg/L for Segment 1a of the lower Arkansas River. For Segment 2b of Fountain Creek, the chronic value was 28.1 µg/L. All values were based on the 85th percentile of ambient conditions. Values were adopted by the Colorado Water Quality Control Commission in 2007.

[10] Existing ambient conditions are caused by natural sources. Ambient standards are adopted where natural or irreversible man-induced conditions result in water quality levels higher than table value standards. The Commission adopted a site-specific ambient-based selenium standard for this segment based upon information documenting both the natural sources of selenium in the basin and the lack of anthropogenic activity that might potentially exacerbate in-stream selenium loads.

4.0 FIELD MONITORING – EXPOSURE CHARACTERISTICS

As described in the Work Plan, the intent of the seasonal monitoring events was to characterize the selenium exposure conditions and productivity (or health) of the aquatic community within the study area. During each event, locations were sampled for a range of chemical, biological, and physical characteristics. Activities conducted to document and evaluate existing conditions included collection of water, sediment, periphyton, benthic invertebrates, and fish tissues for chemical analyses of selenium concentrations. Surface water and sediments collected for selenium analyses were collected on the same day (within 24 hours) as collections conducted for periphyton, benthic invertebrates, and fish tissues. Benthic community, fish population and community, and physical habitat quality assessments were conducted. Fish communities were sampled to characterize their density and diversity. Physical habitat attributes were measured to document the qualities of habitat conditions that exist at each location. The sections that follow present a summary of the exposure conditions, an assessment of biological populations, and an evaluation of physical habitat relative to trout populations. The primary data for these evaluations are those collected during 2006 to 2008.

Selenium exposure conditions across relevant media and resultant receptor concentrations are described in a spatial context (Appendix A). In particular, changes in concentrations in these media with distance from source and by relationships to receptor concentrations are described. Receptor population/community data and their relationship to exposure conditions and selenium tissue concentrations are examined (Appendix B). Finally, the physical setting/habitat is evaluated at the locations where population/community data were collected to account for the differences between selenium concentrations and the role of habitat (Appendix C). These steps are being performed to generally evaluate whether there are obvious impacts to the aquatic biological system due to selenium and correspondingly to provide a "reality check" on the laboratory data (Appendix D through Appendix F) and literature (Section 3.0) lines of evidence.

4.1 Exposure Environment – Surface Water, Sediment, Biological Tissues

Exposure media (surface water, sediment, prey) show a consistent spatial trend of selenium concentrations. Exposure media have the highest concentrations nearest to the source area at Hoopes Spring, and concentrations decrease at distance downstream from the source area. Sage Creek exposure media concentrations are elevated but are typically lower than those observed at Hoopes Spring. In Crow Creek downstream of Sage Creek, periphyton and benthic tissue concentrations as well as surface water concentrations typically are slightly higher, but within the range of variability of those concentrations measured in Crow Creek and Deer Creek locations upstream of Sage Creek. Key observations for each media are described below.

4.1.1 Surface Water Observations

- The highest aqueous selenium exposure occurs in Hoopes Spring nearest the spring discharge. At its mouth (HS-3) prior to discharge to Sage Creek, Hoopes Spring surface water concentration is about 27 percent lower than at the spring. Sage Creek aqueous selenium concentration immediately downstream of Hoopes (LSV-2C) is on average about 50 percent or less than the average selenium concentrations measured at Hoopes Spring (HS). In Sage Creek, downstream of Hoopes Spring and SFSC springs near its mouth (LSV-4), aqueous selenium concentration is about 66 percent or less than the average selenium concentrations measured at Hoopes Spring (HS). On average, Crow Creek aqueous selenium concentrations are about 90 percent lower than average selenium at HS and about 70 percent lower than selenium in Sage Creek (LSV-4) (Figures 4-1 and 4-2).

- Overall, total aqueous selenium concentrations at downstream Crow Creek locations fall within the range of background concentrations measured during 2006 to 2008, but higher concentrations have been measured in surface waters during fall 2009 and 2010. Significant differences were found between some, but not all, background locations and downstream Crow Creek locations (Appendix A). Elevated aqueous selenium concentrations occur in Hoopes Spring and Sage Creek, based on comparisons to the reference and background location data.

- Aqueous selenium exposure decreases rapidly with distance from the primary source area, and while the SFSC springs contribute selenium to the watershed, the contribution does not appear to significantly alter Sage Creek selenium concentrations as measured at LSV-4 (Figure 4-2).

- Aqueous selenium concentrations are seasonally variable and not necessarily related to the flow regime (Figures 4-1 and 4-3), but related to discharge from Hoopes Spring.

- Comparison of average aqueous selenium concentration prior to 2006 and after 2006 shows similar trends of decrease with distance from Hoopes Spring. While more recent selenium concentrations have been on average higher post-2006 than prior to 2006, concentrations decrease such that average values in Crow Creek downstream of Sage Creek are largely unaffected post-2006 (Figure 4-4).

- Sulfate concentrations are positively and strongly correlated to aqueous selenium concentrations, increasing with increased selenium concentrations (Figure 4-5). Sulfate has been suggested to potentially reduce selenium uptake, thereby reducing selenium bioaccumulation, toxicity, and other effects of selenium exposure (see Appendix A for more discussion on sulfate and selenium relationships observed from various studies).

4.1.2 Sediment Observations

- Sediment selenium concentrations follow the spatial trends of surface water concentrations, although the highest selenium in sediment is observed at HS-3 (Figure 4-6).

- Sediment selenium concentrations are positively correlated to aqueous selenium concentrations (R^2 = 0.67) (Figure 4-7). This relationship suggests that reductions in aqueous selenium concentrations will likely result in corresponding decreases in sediment selenium concentrations.

- Statistical evaluations of sediment selenium concentrations at different locations are described more fully in Appendix A.

4.1.3 Tissue Observations

- Natural background selenium concentrations in trout range from 5.6 to 8.8 mg/kg dw (Figure 4-8). When background Crow Creek and Deer Creek trout tissue data were grouped from the individual locations and compared to trout tissue data from the reference location, background concentrations were significantly different (Appendix A, Table 4). This difference may be attributed to the low sample size for tissue samples from the reference location as compared to the larger sample size for tissue samples from the grouped background locations. When trout tissue data from reference and background locations were evaluated by individual location, concentrations of selenium in trout tissues from each of the background Crow Creek locations were not significantly different from the reference location (SFTC-1) tissue concentrations, except at Deer Creek, where trout tissue selenium concentrations were significantly different from the reference location (Appendix A, Table 5).

- Comparison of mean total trout selenium concentrations from sampling conducted during Fall 2006, Fall 2007, and Fall 2008 with mean total trout selenium concentrations from locations that overlap from Fall 2009 and Fall 2010 indicates that selenium concentrations are similar (Figure 4-9).

- Concentrations of selenium in sculpin tissue from background Crow Creek and Deer Creek locations are not significantly different from reference location (SFTC-1) tissue concentrations (Appendix A, Table 7 and Table 8). Sculpin natural background tissue selenium concentrations range from 5.7 to 8.1 mg/kg dw (Figure 4-10).

- Similar to observations of trout mean tissue selenium concentrations, mean sculpin selenium tissue concentrations from Fall 2006, Fall 2007, and Fall 2008 are similar to mean sculpin tissue selenium concentrations from Fall 2009 and Fall 2010 (Figure 4-11).

- Concentrations of selenium in both trout and sculpin tissue in Hoopes Spring, Sage Creek, and Crow Creek below Sage Creek are elevated above tissue concentrations found at the reference and background locations (Figures 4-8 and 4-10). However, trout tissue concentrations in Crow Creek downstream of Sage Creek are typically significantly lower than respective species tissue concentrations in Hoopes Spring and upper Sage Creek (LSV-2C) (Figures 4-8 and 4-10) and tend to not be significantly different than concentrations observed at the background locations (Appendix A, Table 5). Similar trends are observed for sculpin tissues (Appendix A, Table 8).

- Brown trout make up the largest percentage of the two trout species collected at most locations. A similar analysis of potential differences for tissue concentrations of brown trout only was also conducted. Background locations were significantly lower than locations near the source (HS, HS-3, and LSV-2C) (Appendix A, Table 6). Only CC-75 has significantly lower trout tissue concentrations than those at Sage Creek and downstream Crow Creek locations. Locations CC-150, CC-350, LSV-4, CC-1A, and CC-3A were not significantly different from one another (Appendix A, Table 6).

- Both trout and sculpin tissue concentrations of selenium are strongly related to concurrently-collected surface water selenium concentrations, with sculpin showing a slightly better relationship to surface water than trout (Figure 4-12).[11]

- Trout and sculpin tissue concentrations are not significantly different from one another at a location when all seasons are considered, except at SFTC-1. Some within-location differences may exist on a seasonal basis (Appendix A, Table 9).

- A strong relationship between the selenium concentrations in trout and sculpin tissue exists (R^2 = 0.8). Juvenile trout were the target age class captured, suggesting similar uptake and accumulation based on dietary intake (Figure 4-13).

- Selenium concentrations in tissues of adult brown trout and YCT collected for the reproduction studies had tissue concentrations similar to those observed during the routine monitoring which included smaller, younger fish (Appendix A, Figures 10 and 11).

[11] Sculpin used for tissue analysis generally ranged from 45-120 mm (average 91 mm) and trout generally ranged from 50 to 150 mm (average 126 mm) with some larger trout also being collected (i.e., <300 mm).

- Adult brown trout tissue data (collected in 2007) compared to juvenile brown trout tissue data (collected in 2006 and 2007) were not significantly different (Kruskal-Wallis [KW] one-way Analysis of Variance [ANOVA], p = 0.37). The findings were similar when only 2007 data were compared between the two groups (p = 0.15).

- Comparison between YCT adult tissues collected in 2008 and juvenile YCT tissues collected during 2008 indicate that adult tissue concentrations were significantly different than juvenile tissue concentrations (one-way ANOVA, p = 0.018). Examination of the data distribution for the two data sets indicate overlapping ranges, with a large proportion of the juvenile data collected in Deer Creek and upstream Crow Creek.

- Cyprinid (minnow) and catostomid (sucker) selenium tissue concentrations where available (n = 3 locations on Crow Creek), were not significantly different between the locations upstream (CC-350) and downstream of Sage Creek (CC-1A and CC-3A) (Figure 4-14).

4.1.4 Prey Observations

- Benthic invertebrate selenium concentrations typically mirrored patterns in surface water and sediment selenium concentrations (Figure 4-15).

- Invertebrate tissue concentrations of selenium were significantly different across the monitoring locations (KW ANOVA, p = 0.00649) when compared to one another (Appendix A, Table 13).

- Invertebrate tissue concentrations from the reference and background (SFTC-1, CC-75, CC-150, CC-350, and DC-600) were not significantly different from one another. All of these locations, except DC-600, had significantly lower tissue selenium concentrations than benthic tissues from LSV-2C. SFTC-1 and CC-75 were significantly lower than the Hoopes Spring locations and LSV-2C. Only SFTC-1 was significantly lower than CC-1A. Benthic tissue selenium concentrations at downstream locations (LSV-2C, CC-1A, and CC-3A) were not significantly different from one another, nor were they different from the locations nearest the source (HS and HS-3). Variability in selenium concentration from each fall period influences the findings of this analysis (Appendix A, Table 13).

- Periphyton selenium concentrations typically mirrored patterns in surface water and sediment selenium concentrations (Figure 4-16).

- Selenium in periphyton was significantly different (ANOVA, p << 0.05) across sampling locations. Location LSV-4 was excluded from this analysis due to an inadequate number of samples from that location.

- Tukey-Kramer multiple-comparison test (MCT) results (Appendix A, Table 14) show that selenium in periphyton from locations SFTC-1, CC-75, CC-150, CC-350, and DC-600 were not significantly different from one another, but these locations (except DC-600) had significantly lower periphyton selenium concentrations as compared to the Hoopes Spring location. Concentrations in periphyton tissues from Sage Creek (LSV-2C) were significantly higher than concentrations at SFTC-1 and concentrations at the two furthest upstream Crow Creek locations (CC-75 and CC-150), but no differences were observed when LSV-2C was compared to CC-350 or DC-600. Locations CC-1A and CC-3A were not significantly different from the upstream Crow Creek locations or Deer Creek, but were different from SFTC-1.

- Across the three biological tissues (fish, benthos, and periphyton) for which selenium concentration data were collected, the strongest relationships that exist are between the biological tissue and aqueous selenium. Sediment selenium concentrations are related to biological tissue concentrations; however, selenium in sediment is not as strongly related to tissue concentrations as aqueous selenium concentrations.

4.2 Aquatic Communities

4.2.1 Fish Communities

The fish communities at the monitoring locations vary, and are influenced by a number of factors including the quality and quantity of water, food, and habitat. Fish in Hoopes Spring and Sage Creek are exposed to elevated levels of selenium in water and prey items (benthic tissues, periphyton, prey fish) above those levels observed at background locations and the reference location. If selenium is having a toxic effect, even on a chronic basis, monitoring over a period would be expected to indicate some relative reduction in fishery potential, when other factors are considered. For example, differences in the quality of the physical habitat and water temperature would ultimately need to be considered when comparing population data between locations. These relationships are discussed further in Section 4.3. Appendix B presents a more thorough assessment of population and community data.

Population estimates were conducted for trout and sculpin. Estimates were derived individually for brown trout and YCT, and as a composite of all trout. Population estimate values were derived based on density (#/unit area) and on biomass (kg/unit area). Due to the large differences of the physical habitat of Hoopes Spring at its source (HS) relative to other stream locations, population data from location HS are not included as part of the following comparisons.

- Based on mean standing crop data, brown trout biomass exceeds YCT biomass at all locations except SFTC-1, CC-350, and DC-600. Only YCT are found at SFTC-1 and DC-600 (Figures 4-17).

- Based on mean density, brown trout abundance exceeds YCT abundance at all locations except SFTC-1, CC-350, and DC-600. Only YCT are found at SFTC-1 and DC-600 (Figures 4-18).

- On average, total trout standing crop (kg/Ha) is highest at LSV-2C where selenium concentrations in aqueous and biological media were second only to concentrations observed in Hoopes Spring.

- On a density basis (#/m^2), YCT density was highest at Deer Creek (DC-600) followed by SFTC-1, whereas for brown trout, density was greatest at HS-3, followed by LSV-2C.

- Age class structure for brown trout indicates multiple age classes are present at location LSV-2C, where elevated selenium concentrations are present in aqueous and dietary media. The most diverse age classes for YCT are found at DC-600 and CC-350, where brown trout populations are low or non-existent (see Appendix B).

- In addition to the 2006 to 2008 data, data collected in 2009 and 2010 were utilized to evaluate trout populations by location (refer to Appendix B, Section 3.3.1). Total trout standing crop was found to be significantly higher at location LSV-2C relative to CC-1A, HS-3, and CC-350. Figure 4-19 shows individual standing crop estimates for locations from 2009 and 2010 that overlap with 2006 to 2008 locations.

- For sculpin, population density (#/m^2) was highest at the CC-150 location followed by HS-3, LSV-4, and LSV-2C (Figure 4-20).

- Mean sculpin population density estimates indicate greater than 1,000 sculpin/km at all locations except CC-1A and CC-3A. In fact, at several locations, the mean density is greater than 4,000/km (Figure 4-21).

- Sculpin population density and age class structure suggests that this species is not being limited by elevated selenium (Appendix B).

For the comparisons described below, the sculpin and total trout population density values (#/km) are compared to aqueous selenium and tissue selenium concentrations. Location HS is not included as part of these analyses because the habitat at that location is substantially different from the remainder of the lotic areas evaluated (Figure 4-22, also see figures in Appendix B).

- Increasing selenium concentrations in surface waters show no corresponding trend of decreasing sculpin population density. Likewise, comparison of selenium in sculpin tissues shows no corresponding trend of reduced sculpin density

- The relationship of trout density to aqueous selenium concentrations was generally consistent with that of sculpin, with increasing population density at locations with higher selenium concentrations. Comparison of trout density to trout selenium concentrations in tissues also showed a similarly trend, suggesting no biologically relevant relationship of tissue selenium concentration to trout density.

Population estimates were not derived for mountain whitefish (*Prosopium williamsoni*). Ten or fewer mountain whitefish were observed during one or more sampling events at South Fork Tincup Creek location SFTC-1, upstream Crow Creek locations CC-150 and CC-350, and Sage Creek location LSV-4. Downstream Crow Creek locations CC-1A and CC-3A had, on average, approximately 40 mountain whitefish each per sampling event, and generally more individuals in spring sampling events than during fall events. No mountain whitefish were observed or captured at any of the other monitoring locations. At locations in this Study, mountain whitefish were found where pool habitat was suitable (CC-150, CC-350, CC-1A, and CC-3A) or where a smaller order stream confluenced a larger order stream (e.g., SFTC-1 and LSV-4). The presence or absence of mountain whitefish at various locations does not suggest any level of sensitivity or tolerance to selenium.

Whitefish are generally found further downstream in the watershed in larger rivers compared to other stream-dwelling salmonids (Meyer et al. 2009), likely because smaller headwater streams do not furnish suitable habitat such as adequate pool size (Sigler 1951). However, they are a widely-distributed, hardy species, and are generally more tolerant of warm water and high turbidity than trout (Behnke 2002). Meyer et al. (2009) conducted a large-scale study across the Snake River basin, including reaches on Crow Creek and Stump Creek in southeast Idaho, and found that stream size was the key environmental factor influencing both abundance and distribution of mountain whitefish. Whitefish were rarely present in streams where the mean wetted width was less than 30 feet wide, but were abundant in low-gradient, main stem streams at least 50 feet wide. During base flow in Utah streams, Sigler (1951) reported that upstream movement of whitefish ceases when streams are less than 16 feet wide and pool depths are less than 4 feet deep. The field distribution of mountain whitefish observed during monitoring for this project support this, with higher numbers of whitefish found in the largest stream reaches with the biggest pools.

Population estimates were not derived for cyprinids (minnows including redside shiners and dace species) or catostomids (suckers). The relative density of these fish families was derived based on counts and projected over the unit area sampled. The following evaluations focus on those locations where these families were found. For example, cyprinids and catostomids were not present at all locations (Figure 4-23). Both fish families were absent at locations CC-75, DC-

600, HS-3, LSV-2C, and LSV-4. Several environmental variables may affect why neither of these families were been found at these locations, including water quality, water quantity, and habitat quality, among other factors.

On an individual species basis, a similar trend was observed for redside shiners and both dace species, as shown in Figure 4-23 (which includes all cyprinids and catostomids), with increasing individual species abundance increasing as the size of the water body increased. Sucker species were somewhat similar in this trend. Of the three primary cyprinid species present (i.e., redside shiners [(*Richardsonius balteatus*], Speckled Dace [*Rhinichthys osculus*], and longnose dace [*Rhinichthys cataractae*]), speckled dace were the most frequently collected species, on average, followed by longnose dace, and redside shiners. At the downstream Crow Creek locations, CC-1A and CC-3A, speckled dace were the most abundant cyprinid species. Infrequent captures of dace (i.e., fewer than 15 per sampling event) were made at South Fork Tincup Creek location SFTC-1 and CC- 150 on Crow Creek. No dace or redside shiners were observed or captured at any of the other monitoring locations. Redside shiners prefer slack water habitats where abundant aquatic vegetation is present. Both adult and juvenile longnose dace prefer riffles with coarse substrate, fast-moving water, and abundant cover, using the large substrates such as boulders, large rocks, logs, or debris as shelter from the current (Edwards et al. 1983, Mullen and Burton 1995). They tend to avoid depositional reaches with low current velocity and large amounts of fine sediment (Thompson et al. 2001). Speckled dace live in a variety of habitats, but normally prefers the shallow, cool and slower moving waters rather than the swift riffles preferred by the longnose dace (Idaho AFS, URL: http://www.idahoafs.org/fishes.php?id=27).

Two sucker species, Utah sucker (*Catostomus ardens)* and Mountain sucker (*Catostomus platyrhynchus*) were collected during the study although mountain suckers were only collected at the SFTC-1 location. Utah suckers were captured at SFTC-1, CC-150, and CC-1A in low numbers, while at CC-3A Utah suckers were captured during each sampling event and at varying abundance that always exceeded upstream capture numbers. Utah suckers are highly tolerant of different habitat conditions, flow regimes, and water temperatures (Idaho AFS, URL: http://www.idahoafs.org/fishes.php?id=27). Based on capture locations in this study, Utah suckers preferred deep pools with moderate flow.

Examining cyprinids and catostomids collectively, the following observations were made:

- Aqueous selenium concentrations do not appear to be a factor affecting the absence of these species from the above-mentioned Crow Creek tributaries, given the range of water quality present, from low background concentrations to more elevated concentrations. Where cyprinid and catostomids were collected, the density distribution shows no obvious trend, although the highest density for these families occurs at locations downstream of Sage Creek in Crow Creek (Figure 4-24).

- General observations of fish densities suggested that there may be a link between brown trout and sculpin density and catostomid and cyprinid density (Figure 4-25a).

- Absence of cyprinid and catostomid species at some locations was generally believed to be due to physical factors. Relating cyprinid and catostomid density to mean summer temperature, flow, and stream gradient yielded stronger relationships than either aqueous selenium or brown trout (R^2 = 0.248 or greater) (Figures 4-25b-d), where higher densities of cyprinids and catostomids are found at locations with higher mean summer temperatures, lower stream gradients, and higher flows (refer to Appendix B, Section 3.8).

- In light of the various relationships, it does not appear that aqueous selenium concentrations are a primary determinant affecting the density of cyprinids and catostomids.

To examine the fish community as a whole, the number of taxa was evaluated. Figure 4-26 shows the number of fish taxa across all monitoring periods collected at each location. The number of fish taxa found at each location is lowest at Hoopes Spring and Deer Creek locations, while the highest numbers of fish taxa are observed at SFTC-1 and Crow Creek locations downstream of Sage Creek. Given the diversity of physical habitats (stream gradients, channel sizes, and stream temperatures, among others), variations in the fish community composition is to be expected.

- When compared to aqueous selenium concentrations, the number of fish taxa appears to decline as aqueous selenium increases (Figure 4-27a). Examining different metrics for fish populations and communities relative to aqueous selenium concentrations alone indicates no distinct or strong correlations suggesting diminished populations or communities. More important factors affecting populations and communities appear to be physical conditions such as temperature, gradient, flow (used here as surrogates for a host of other physical characteristics), and predator concentrations (i.e., brown trout).

- Flow was regressed against the number of fish taxa, where flow was considered a surrogate for basin size and position of location within the basin, with greater amounts and diversity of habitat expected at the more downstream locations (where flows are higher). A strong correlation was found indicating that as flows increase, fish diversity increases (Figure 4-27b).

- Stream temperature and gradient were also evaluated (Figures 4-27c-d). Mean summer stream temperatures were strongly related to increased number of fish species, where higher mean summer temperatures were correlated to higher numbers of taxa. Mean numbers of species were weakly related to stream gradients, although the expected trend of lower numbers of species with increasing gradient was observed.

4.2.2 Benthic Invertebrate Communities

Benthic macroinvertebrate community characteristics were evaluated by examining various functional and structure metrics versus selenium concentrations in water and other media (Appendix B). Community samples were collected during each fall event. Several individual structural and functional metrics were evaluated graphically for locations across the three fall periods. Numbers of benthic taxa and abundance per square meter, along with numbers of species in different benthic families as well as their abundance were all evaluated, as well as the popular ephemeroptera, plecoptera, trichoptera (EPT) metric. Functional feeding groups, including predators, shredders, filterers, scrapers, omnivores, and collector-gatherers, were also evaluated (see Appendix B figures). The length of a benthic organism's life cycles (i.e., voltinism) was also independently assessed. Finally, a composite metric used by the State of Idaho to assess beneficial use attainment, the Stream Macroinvertebrate Index (SMI), was also evaluated relative to aqueous selenium concentrations for the 2006 to 2008 data.

Following is a summary of findings for the benthic community.

- Figures 4-28 and 4-29 show benthic density and taxa numbers for all locations, which appear to be relatively similar between background locations and locations downstream of Hoopes Spring in Sage Creek and Crow Creek.

- Of the 22 individual structural and functional metrics evaluated relative to aqueous selenium concentrations, four resulted in significant relationships to aqueous selenium: the number of benthic taxa (R^2 = 0.26, p = 0.0061) (Figure 4-30), number of trichoptera taxa (R^2 = 0.19, p = 0.024), number of EPT taxa (R^2 = 0.21, p = 0.017) (Figure 4-31), and the number of predator taxa (R^2 = 0.318, p = 0.002). For most of these evaluations, the relationships are weak; alternative evaluations were considered.

- Benthic total taxa numbers for each location across the three fall periods were compared using ANOVA procedures (α = 0.05) on log-transformed data. Benthic taxa numbers were not significantly different among locations (p = 0.92). This analysis excluded location HS.

- A comparison of benthic taxa numbers was also conducted between background and reference (SFTC-1, CC-75, CC-150, CC-350, and DC-600) and downstream (HS-3, LSV-2C, LSV-4, CC-1A, and CC-3A) locations using ANOVA procedures (α = 0.05) on log-transformed data. Across the three fall periods, benthic invertebrate taxa numbers were not significantly different (p = 0.16).

- Comparison of numbers of benthic taxa data using smaller location groupings, including upstream (SFTC-1 and DC-600), upstream Crow Creek (CC-75, 150, and 350), Hoopes and Sage Creek (HS-3, LSV-2C, and LSV-4), and downstream Crow Creek (CC-1A and

3A). One-way ANOVA found no significant differences between the number of benthic taxa from these grouped locations ($p = 0.386$).

- Comparisons of composition metrics (Appendix B) from upstream and downstream locations were conducted using one-way ANOVA on log-transformed data. The numbers of ephemeroptera ($R^2 = 0.022$, $p = 0.4601$), plecoptera ($R^2 = 0.062$, $p=0.2169$), and dipteran ($R^2 = 0.023$, $p = 0.4486$) species were not significantly related to aqueous selenium concentrations. The numbers of trichoptera ($R^2 = 0.189$, $p = 0.024$) and EPT taxa ($R^2 = 0.207$, $p = 0.017$) were both significantly related to aqueous selenium concentrations, with decreasing taxa numbers found at increasing selenium concentrations.

- However, when compared between background and downstream locals, both the number of trichoptera taxa and ephemeroptera, plecoptera, trichoptera (EPT) taxa were not significantly different ($p = 0.365$ and $p = 0.541$, respectively).

- Similar comparisons were evaluated again with the addition of 2009 and 2010 data added to the 2006 to 2008 data. Because these metrics are inclusive, the focus was on EPT taxa and total number of taxa. The total number of benthic taxa was significantly greater at background locations (SFTC-1 was excluded) versus downstream locations (One way ANOVA, $p = 0.04$). Similarly, the number of EPT taxa was significantly greater at background locations than at downstream locations (One Way ANOVA, $p = 0.0007$). The shift in no significant differences to the finding of differences could be due to the addition of data for the analysis, which may have reflected changes in water quality or changes in habitat quality.

- Six types of functional feeding group metrics were evaluated based on numbers of taxa in each group and abundance of organisms in each group. Of the many upstream versus downstream comparisons, only numbers of predator taxa was significantly different between upstream and downstream grouped locations ($p = 0.00088$).

- The length of benthic organism life cycles (i.e., voltinism) was independently assessed between upstream and downstream locations. No significant differences were found between the percentage of multi-, semi-, and uni-voltine abundance (parametric one-way ANOVAs, $p = 0.40$, $p = 0.24$, and $p = 0.95$, respectively).

- The multi-metric stream macroinvertebrate index (SMI) was compared between grouped background and downstream locations. One-way ANOVA indicates that the SMI scores were significantly different between upstream and downstream locations ($p = 0.0068$), with SMI scores at upstream locations higher than those from downstream locales. Similar findings resulted when adding SMI data from Fall 2009 and Fall 2010 to existing data and reanalyzing all of the data. Background location data had significantly higher

SMI scores than those SMI scores observed for downstream locations (ANOVA, p=0.0068).

- Comparing SMI scores to aqueous selenium concentrations indicated a significant relationship of decreasing SMI scores with increasing aqueous selenium concentrations (R^2 = 0.32, p = 0.002, Appendix B, Figure 45). This same relationship augmented with available 2009 and 2010 data resulted in a similar finding (R^2 = 0.32, p = 0.0002) (Figure 4-32).

- Recognizing that many factors affect benthic communities, mean percent fines (substrate size < 2 mm) from pebble counts were evaluated relative to mean SMI scores. Mean SMI scores were significantly and negatively related to increased frequency of substrate particles less than 2 mm (R^2 = 0.63, p = 0.011, Appendix B, Figure 46). The same regression was run with the addition of 2009 and 2010 data, and was again significant (R^2 = 0.52, p = 0.0192) (Figure 4-33). That is, SMI scores decreased with increasing fines less than 2 mm, with the highest mean percent fines < 2 mm observed at downstream locations (HS-3 and LSV-2C).

- The addition of new data from Fall 2009 and 2010 indicates that there are differences in benthic taxa metrics between upstream and downstream locations that were not detected based on the 2006 to 2008 data. This could be due to water quality or habitat differences. Likewise, addition of data from 2009 and 2010 for percent fines indicates a strong relationship of percent fines to changes in SMI scores. Benthic community metrics show relationships to both changes in selenium concentrations and physical characteristics that result in significant differences between background locations and downstream locations. Deciphering the causal factors is not practical given the data currently available. Most likely, detectable differences are a function of both increasing selenium (2009 and 2010) and increasing percentages of fines during the same time period.

4.3 Trout Population and Habitat Relationships

Because of the influence of physical habitat quality and quantity on the quality of the aquatic community, trout populations were evaluated relative to habitat quality. Habitat is a strong determinant in fish abundance and diversity; therefore, it is incumbent on investigations evaluating effects of contaminants to also consider the habitat effects.

The physical trout habitat present at 10 of the 11 monitoring locations was evaluated in 2007 and 2008 using the PHABSIM (Physical Habitat Simulation) approach. This effort was undertaken to complement other habitat evaluation metrics measured at each location and to provide species and life-stage specific information regarding physical habitat availability. Appendix C describes the PHABSIM data collection, modeling and findings.

For this study, trout populations are being evaluated to assess whether there are any obvious negative impacts to those populations due to selenium. This section evaluates the relative influence of physical habitat quality on trout populations and provides a basis for better identifying any effects from elevated selenium concentrations. Objectives of this assessment are to: 1) investigate the relationship of available trout habitat over several seasons and years to the standing crops of trout supported at these stream locations; and 2) compare available stream habitat and trout standing crops between background locations and those downstream locations potentially impacted by selenium.

For data consistency between locations and years, two locations (HS and LSV-4) and one sampling season (Fall 2006) were omitted from this analysis. Location HS is a source location for selenium and was excluded because: 1) it is a spring originating location with no watershed influences, and as such its habitat is not directly influenced by highly variable fluvial processes such as stream flow and sediment transport; 2) the dense aquatic vegetation present during all field visits prevented effective fish sampling of the total wetted surface area; 3) vegetation removal efforts to facilitate electrofishing likely re-distributed fish into the remaining wetted marsh-like habitat which could not be effectively sampled; and 4) fish movement into and out of the area is limited by the lack of surface flow upstream of the location and a rock outcrop just downstream that is a likely fish barrier. Location LSV-4 was omitted because it could only be sampled in fall 2006 and spring 2007 due to landowner access restrictions. Fall 2006 data were not included because: 1) no data were collected at SFTC-1 (it was not added to the study until 2007); 2) no water temperature data other than instantaneous measurements were available, which resulted in high Habitat Quality Index (HQI) scores at many locations; and 3) backpack electrofishing at locations CC-1A and CC-3A due to deep water and unwadeable conditions was not as effective as the remaining sampling periods, when a larger bank-based unit was used to more effectively electrofish those locations. All other locations and sampling times were included within the analyses, as appropriate, for the metrics being evaluated (e.g., HQI is based on late summer conditions only and does not include spring measurement data).

Linear regression was used to investigate relationships between fall trout standing crop estimates (kg/Ha) and habitat quality scores obtained from HQI, the Stream Reach Inventory and Channel Stability Evaluation (SRI/CSE), Stream Habitat Index (SHI), and Habitat Suitability Index (HSI) for both brown and cutthroat trout.

Based upon the results and analyses (Appendix C and Tables 4-1 to 4-5), the preliminary findings to-date include:

1. No differences in total trout standing crop were found between spring and fall sampling seasons for Fall 2007 and Fall 2008.

2. The four habitat models (i.e., scoring methods) applied varied widely in their relationship to estimated trout standing crop, with the HSI cutthroat trout model and the SRI/CSE exhibiting the strongest relationships to trout standing crop. These habitat quality models (other than HQI) do not predict standing crop, but rather, they rate habitat quality.

3. Stream habitat quality at the nine study locations varied over a fairly broad spectrum, with CC-350 and HS-3 generally near the lower end of the range and DC-600 near the higher end.

4. Comparison of trout standing crop between background and downstream locations indicated no differences in total trout and YCT standing crops. Brown trout standing crop was different between background and downstream locations (higher at downstream locations).

5. Comparison of habitat quality between background and downstream locations found no significant differences in HQI, SRI/CSI, and brown trout HSI scores. Differences were observed for SHI scores and cutthroat trout HSI scores (higher at background locations).

6. Individual stream habitat attributes identified as potentially limiting to trout populations include the lack of riparian shading, high water temperatures, low levels of woody debris recruitment, and lack of cover for all trout life stages, extensive bank erosion, and elevated fine sediment levels in likely spawning gravels. The degree of each habitat deficiency varies by location within the watershed.

7. The habitat deficiencies observed are symptomatic of stream reaches affected by livestock grazing in the riparian zone, extensive non-engineered road development, stream crossing and off-road vehicle use, and irrigation diversion and return flows in the watershed. Overall, while trout habitat quality is quite good at most study locations and supportive of naturally reproducing trout populations, watershed-based land-use impacts are likely limiting the affected reaches from achieving their full potential.

Analysis of trout populations and habitat quality data overall indicates that trout populations from downstream areas affected by Hoopes Spring discharge are not substantially reduced when compared to background trout populations. While some specific habitat features are limiting the full potential of the fishery, the quality is not diminished substantially enough to negatively alter trout populations.

4.4 Trout Population Comparisons to Ecoregional, Regional, and Historical Data

4.4.1 Ecoregional Data

The trout population data used in this analysis included electrofishing data from the 10 study locations on South Fork Tincup Creek, Crow Creek, Sage Creek, Deer Creek and Hoopes Spring in the fall of 2007 and fall of 2008. For purposes of regional comparisons, these analyses closely followed the methods described by Brouder et al (2009), Chapter 15 in Bonar et al., editors (2009). Site brown trout and YCT data were compared to Ecoregion 6 population data, inclusive of all of the Site study streams, among others. These comparisons included electrofishing catch per unit effort (CPUE, #/hr) and length-frequency distribution. Relative weight (Wr) was calculated for each trout of sufficient length collected from the Site streams as the ratio of its field-measured weight to that estimated for a fish of the same length using a length-weight relationship developed from species data collected across North America. Thus, a Wr of less than 1.0 indicates the sample fish weighed less than a typical North American fish of that same species and length.

Using the dataset described above, comparisons were also made for trout standing crop estimates (kg/Ha) from the Site streams to a sample of 44 such estimates made on a suite of Wyoming trout streams by Binns (1979). Appendix B presents the full analysis, which is summarized below.

CPUE at the Site streams (Table 4-6) compares favorably with that for other Ecoregion 6 streams (Table 4-7). For brown trout, CPUE at the Site streams having brown trout populations exceeded the Ecoregion 6 mean CPUE in all cases except at location HS-3 in Fall 2007. Brown trout CPUE was greatest at location LSV-2C for both sampling times, with location CC-150 ranking second. Results for cutthroat trout were similar to those for brown trout. Thirteen of 14 Site samples containing cutthroat trout (using 2007 and 2008 data) exceeded the Ecoregion 6 mean CPUE, while all 14 Site samples exceeded the regional median. Cutthroat CPUE was greatest at DC-600 for both sampling events, while location LSV-2C ranked second.

Length frequency distributions for brown and cutthroat trout from the Site streams compared favorably with those for other Ecoregion 6 streams. For brown trout, most Site streams tended to have proportionally more “quality” and “preferred” class fish than the Ecoregion 6 streams, while no “trophy” class browns were collected at any of the Site streams. Almost all cutthroat trout collected at the Site streams fell within the “stock” class, as did those from the Ecoregion 6 streams. “Quality” class cutthroat were collected only at locations CC-1A and CC-3A.

Mean Wr for both brown and cutthroat trout were consistently less than 1.0 at all locations and events, with the exception of cutthroat trout at SFTC-1 in fall 2008 (1.03) and LSV-2C in fall

2007 (1.0) (Table 4-8). Substantial differences in mean relative weights do not appear to exist between sample locations and events.

Comparison of Site standing crop estimates (kg/Ha) to those from Wyoming streams showed that both 2007 and 2008 standing crop estimates at locations CC-150, LSV-2C, and CC-3A exceed the Wyoming 75th percentile value of 84 kg/Ha, while the fall 2008 estimate of 277 kg/Ha at LSV-2C exceeds the Wyoming 95th percentile value (data from Binns 1979) (Tables 4-9 and 4-10).

4.4.2 Historical Site Data

A number of entities have collected data at locations in Crow, Deer, and Sage Creeks and tributaries for more than 30 years. Some population data are available from relatively consistent locations at varying intervals. Collectively, these data provide baseline fish population estimates prior to mining and population estimates about 10 years after mining commenced for upper and lower Sage Creek, South Fork Sage Creek, and Hoopes Spring that can be used qualitatively to compare population estimates from the SSSC investigations. Comparisons of current data to historical data are presented in Appendix B. Collectively, these population estimates spanning a lengthy time period suggest that trout populations have remained stable. Variability in these estimates is likely due to a number of factors including sampling methods, conditions during sampling (flows, water quality and quantity), and fish sizes used in the population estimates. While these comparisons are qualitative, they do provide some insights into long-term trends.

4.5 Summary of Field Monitoring Program Findings

Characterization of the exposure environment and ecological receptors indicates the following:

- Selenium exposure in aqueous and dietary media is occurring in surface water downstream of Hoopes Spring.

- Fish population, abundance, density, community and biomass data do not exhibit negative impacts due to exposures that are not accounted for by habitat quality.

- Analysis of benthic invertebrate data (including the 2009 and 2010 data) indicates that taxa abundance metrics (i.e., number of taxa or number of EPT taxa) are significantly lower at downstream locations versus upstream locations. Relationships between these metrics and selenium in aqueous media exist as do relationships to percent fines. It is not clear if reductions in taxa are due to selenium exposure, increases in percent fines, a combination of both, and/or other physical characteristics that may affect availability or stability of benthic habitats.

- When differences in habitat are considered, the trout population data do not indicate negative impacts due to selenium exposure conditions.

- Habitat quality data suggests overall, that good quality habitat is available, but external land uses exist that may limit the full range of the fishery potential.

- Comparisons of Site trout population data to ecoregional and more localized trout population and growth data indicates that Site population and growth data are well within the range of these outside reference sources and often exceed upper percentile ranking when compared to these outside reference sources.

- Qualitative comparisons of select Site trout population estimates to Site historical fish population estimates suggest that trout populations have remained relatively stable.

5.0 LABORATORY STUDIES – EFFECTS CHARACTERISTICS

Three laboratory studies were conducted to assess effects of selenium in trout species present in the Crow Creek watershed. Two reproduction studies focused on maternal transfer of selenium and its effects on developing young. A third early life stage (ELS) study focused on the effects of selenium from aqueous and dietary exposure to developing young YCT that had no maternal selenium transfer. Collectively, these three studies examine two different pathways: (1) maternal transfer of accumulated selenium; and (2) dietary and aqueous exposure of young with no maternal transfer.

The maternal transfer studies evaluated reproduction of adult wild trout from the study area in a controlled laboratory setting. These studies were conducted independently with one study using brown trout *(Salmo trutta)* and the second study using YCT *(Oncorhynchus clarki)*. Trout were collected from different areas in the watershed, covering a range of exposure conditions. Eggs from females were fertilized in the field and returned to the laboratory for rearing. Method controls for the study were obtained from hatchery-raised fish.

The ELS study utilized YCT from an IDFG fish trap located at the Henry's Lake outlet to Henry's Fork, a tributary of the Snake River. Unlike traditional hatchery fish, those from Henry's Lake comprise a natural run of cutthroat trout that move into the river from the lake to spawn. The trap is set near the lake outlet to the river, and pre-spawn trout are captured as a hatchery source for other areas from this location. These wild trout experienced no elevated selenium exposure. Fish from this source have been used in previous studies (e.g., Hardy et al. 2010). Eggs from parent females were fertilized on site and sent to the laboratory for rearing under different levels of aqueous and dietary selenium exposure.

Endpoints for each study were reproductive success based on measurements of survival, growth, and a range of deformities. Fecundity, while noted, was not used as an endpoint in these studies. Variability in fecundity has been related to numerous factors such as environmental conditions (e.g., discharge and temperature), adult fish condition, food availability, species, and reproductive strategy (Bond 1996; Dubuc and Devries 2002; Durham and Wilde 2006; Moyle and Cech 2000). The study design for brown trout was presented in *Technical Memorandum - Methods for Testing Adult Brown Trout Reproductive Success* (October 17, 2007). This Technical Memorandum was subsequently integrated into a larger *Draft Work Plan – Laboratory Toxicity Tests for Developing a Site-Specific Selenium Threshold for Trout* (April 14, 2008) which also included methods for the YCT ELS study. Figure 5-1 shows the overall testing approach and Figure 5-2 shows a diagram of how egg batches were handled through each test.

Analyses for the adult reproduction studies and ELS studies are focused on identifying dose-response relationships. Where those relationships can be identified, effect concentrations (EC_x) can be identified, corresponding to a threshold level of response at a given concentration.

Results and analyses of the brown trout study were presented in the *Draft Report – Brown Trout Laboratory Reproduction Studies Conducted in Support of Development of a Site-Specific Selenium Criterion*, which was submitted to the SSSC Workgroup for review on February 5, 2009. Comments from the SSSC Workgroup were received and integrated into a revised Brown Trout Report which was submitted to the SSSC Workgroup as a Draft Final on June 17, 2009 (Appendix D).

The YCT maternal transfer study and the YCT ELS study report are presented in this TSD as Appendix E and Appendix F, respectively.

5.1 Brown Trout Adult Reproduction

Gametes (eggs and milt) were collected from wild pre-spawn brown trout from different areas of the watershed in November 2007, fertilized in the field, and shipped to ENSR in Fort Collins, Colorado to be reared to 15 days post-swim-up. Brown trout were collected from several locations which had previously been monitored to assess exposure conditions. Collection of wild trout from different exposure conditions yielded adult males and females that had experienced a wide range of selenium exposure. Brown trout were also obtained from two different hatcheries. Eggs from these fish were reared similarly to wild fish and were used as methodological controls.

Multiple test-effects endpoints were measured at different times during the test including: fecundity, fertilization success (egg mortality), hatching success, length, weight, survival, tissue concentrations (egg and whole body), and deformities. Table 5-1 shows the measurement data for each endpoint measured except for deformities. These endpoints were consistent with those of Holm et al. (2005), Hardy (2005), and Kennedy et al. (2000). Feeding success was added as a test endpoint to evaluate the change from endogenous to exogenous feeding post-swim-up. For the deformity assessment, general criteria were adopted from Holm et al. (2003), and included assessments of craniofacial deformities (mostly of the head, eyes, and jaw), vertebral deformities, fin deformities, and edema. More specific definitions for each of the assessment categories were developed to provide consistency across studies, and to aid others in understanding the range of deformities possible.

Selenium concentrations were measured in adult whole body tissues and eggs from each parent. Because the study design reared eggs from each parent separately, eggs and/or whole body selenium concentrations could be evaluated relative to the test endpoints. Selenium concentration in egg tissue was selected as the independent variable to be related to test endpoint data.

All endpoints evaluated in the study were graphed to look for obvious relationships to egg selenium concentrations using scatter plots. Best-fit ordinary least squares (OLS) regressions were used as a preliminary method to assess if relationships existed between individual exposure assessment endpoints (i.e., parental selenium body burdens or egg selenium concentrations) and test-effects endpoints. Significant breaks between no and/or low ranges of effects and observed high ranges of effects were observed based on visual inspection of the data between 20 and 25 mg/kg dw egg selenium concentration for several endpoints. Those relationships that resemble a dose response curve were carried forward for further consideration using dose response regression routines included in USEPA's Toxicity Relationship Analysis Program (TRAP) (Version 1.2; Erickson 2008).

USEPA's TRAP software provides a number of statistical analysis tools, including logistic, threshold sigmoidal, and piecewise linear (i.e., hockey stick) regression models to derive dose-response relationships and predict effect concentrations (EC_x). The dose response model can be used to predict EC_x values, defined as a reduction of some percent in the response observed at control, to estimate thresholds for potential effects for brown trout. The logistic regression approach is consistent with the methods utilized by the USEPA in their assessment of dose-response data for the 2004 draft criterion; however, other methods were also explored (i.e., threshold sigmoidal and piecewise linear). For the brown trout studies, the definition described above was modified as the response observed at background, since true controls for this study were not practical. In other words, the EC_x values derived are based on the distribution of the field-collected data, which includes data from background locations as well as mine-influenced locations and the response is based on effects relative to the background fish response (or those that exhibited no response). The background response was evaluated relative to the hatchery fish and determined to show no differences. Both EC_{10} and EC_{20} values were derived for each relevant relationship developed.

Initial screening and analysis of the various relationships evaluated are described in more detail in the Brown Trout Report (NewFields 2009b) (Appendix D). The results of the regression analyses used to examine the potential for dose-response relationships for these endpoints are discussed in more detail below.

5.1.1 Growth

Growth was measured in swim-up fish at the end of the 15 day feeding trial period. Twenty fish (or fewer if 20 were not available) from each egg batch, were fed for 15 days post-swim-up to examine if there might be differences in the ability of swim-ups to transition from endogenous to exogenous feeding. Morphological or physiological impairments could arise in young fish that may limit successful growth. Average growth of post feeding swim-ups, as measured by dw, was related to egg selenium levels. Except for samples LSV-2C-003 and LSV-2C-010, 20 fish were included in this analysis for every location. The preliminary analyses indicated that while growth was not strongly related to egg selenium concentrations, a relationship was present illustrating decreased growth with increasing egg selenium; therefore, this endpoint was carried forward to the logistic regression model analysis to examine the potential for a dose-response relationship. Figure 5-3 illustrates the logistic regression model derived for growth versus egg selenium concentrations. The R^2 for this model is 0.21 and, as illustrated in Figure 5-3, the predicted dose response curve does not fit these data. Output from the TRAP software indicates a large standard error for the steepness of the slope. Slope steepness, or lack thereof, combined with wide confidence intervals in the predictive ability of the model suggests a poor relationship of growth to egg selenium concentrations. The EC_{10} and EC_{20} for this endpoint are 28.13 and 33.8 mg/kg, respectively. Piecewise linear and threshold sigmoidal models were investigated but did not improve model fit to the data.

5.1.2 Survival

Survival was evaluated for different periods within the study, including the following:

- Total survival through the duration of the test. This all-inclusive survival endpoint measured the total number of fish surviving to the end of the test based on the number of eggs the test began with. The endpoint reflects survival of eggs to hatch, post hatch, swim-up, and through the feeding trial.

- Survival (hatch to test end) was evaluated as a test endpoint due to the range of variability in the survival data prior to eggs hatching. Egg mortality is a normal condition of fish reproduction, due to a number of factors that may not be related to selenium tissue concentration. Eliminating egg mortality through this endpoint allowed for evaluation of young trout survival post hatch.

- Survival of swim-ups at the end of the feeding trial (15-day post-swim-up). This endpoint only examined survival for 15 days post-swim-up, when exogenous feeding began.

Total survival through the duration of the test was related to egg selenium concentrations based on preliminary screening and regression analysis. The logistic regression model for this dose response relationship is shown in Figure 5-4, resulting in a R^2 of 0.31. The error report of the logistic regression model output from TRAP indicates a large standard error for the slope steepness. As illustrated, the fit of the data between observed and predicted values is low and the confidence intervals about the EC_{10} (19.66 mg/kg) or EC_{20} (21.43 mg/kg) values are large, encompassing a large range of the curve. The poor fit is largely driven by high variability in egg survival at the low selenium concentrations, which is not evident at higher selenium concentrations. While the endpoint is relevant, the variability of the overall survival endpoint is not well suited for the logistic function and its predictive ability for EC_x values.

Eliminating egg mortality as part of the survival metric, as described previously, reduced variability in the survival endpoint spanning from hatch to test end. Preliminary observations of this endpoint suggested a strong relationship of post hatch survival to egg selenium concentrations. Figure 5-5 shows the logistic regression for log egg selenium concentrations versus percent survival (hatch to test end). As illustrated, the predicted line fits the data well resulting in an R^2 of 0.89. The confidence intervals for the predicted EC_{10} (17.68 mg/kg) and EC_{20} (21.63 mg/kg) values are narrow and no errors (standard error was small and convergence was met) were reported in the TRAP software output. Reduced variability of the survival term post-hatch reflects the exclusion of factors such as incomplete fertilization or egg viability, which could be affected by egg selenium concentrations as well as other factors. The strong fit of the model prediction to the actual data allows for confident predictions of EC_x values.

Survival of swim-ups at the end of the 15-day feeding trial had a strong relationship in preliminary analyses to egg selenium concentrations. Percent survival measured as part of the 15-day post-swim-up feeding trial had the best fit polynomial regression to egg selenium concentrations. Figure 5-6 shows the logistic regression curve fitted to survival data in the post-swim-up feeding trial versus log egg selenium concentrations. The R^2 for this model is high (0.96) and the fit of the predicted data to the observed data is good. Confidence intervals are also narrow for the predicted EC_{20} (24.52 mg/kg) and EC_{10} (20.0 mg/kg) values. The dose response curve reflected by this model illustrates a similar breakpoint in effects as previously mentioned.

Although EC_x values for the three survival endpoints are very similar, each endpoint represents different stages of development of young fish. Percent survival in the post-swim-up feeding study and percent survival from hatch to test end both appear to provide data that are strongly related to log egg selenium concentrations in terms of a dose response. Both predict similar EC_x values and narrow confidence intervals about the EC_x. Both provide biologically meaningful and relevant measures of effects, although survival during the 15-day post-swim-up feeding trial is more narrowly focused, with pre-swim-up survival eliminated. Survival from hatch to test end is an inclusive endpoint and encompasses the 15-day post-swim-up survival rate. While both endpoints provide useful information to estimate survival effects due to egg selenium concentrations, the endpoint for survival (hatch to test end) appears to be a more representative endpoint to evaluate potential effects due to egg selenium concentrations than the 15-day post-swim-up survival data.

5.1.3 Deformities

The four primary deformity categories examined were: cranio-facial; skeletal; fin fold; and edema. Fish were also scored as part of the Graduated Severity Index (GSI) which is derived as an inclusive metric for all of these deformities. Initial analyses were conducted to derive fractions or percentages of deformed fish relative to the total number of fish evaluated for an egg clutch. However, the TRAP software is sensitive to a declining effects response versus the exposure variable (i.e., the response must be in the form of decreasing response with increasing effects). For the purposes of fitting within the model framework, these data were structured in terms of the fraction of normal fish (number of normal fish/the total number of fish evaluated for an egg clutch). GSI data are not structured for use in the TRAP model as GSI scores increase with increasing egg selenium.

Figure 5-7 shows the logistic function for log egg selenium versus the fraction normal for cranio-facial deformity assessment. The TRAP software error report of the logistic regression model output indicates a large standard error for the slope steepness. Scatter of the observed values relative to the predicted values at lower egg selenium concentrations reduced the fit of this model as reflected in the R^2 (0.70). The predicted EC_{10} (20.37 mg/kg) and EC_{20} (22.31 mg/kg) values have reasonable confidence intervals despite the reduced fit of the model.

Figure 5-8 shows the logistic function for log egg selenium versus the fraction normal for skeletal deformity assessment. Similar to the cranio-facial plot, the observed data do not closely fit the predicted model at lower egg selenium concentrations, although the R^2 value is higher than that of the craniofacial endpoint (R^2 = 0.81). The TRAP software error report of the logistic regression model output indicates a large standard error for the slope steepness, convergence was not reached at the maximum number of model iterations, and the steepness was at a maximum or minimum limit. The predicted EC_{10} and EC_{20} values for this endpoint are 22.29 and 23.3 mg/kg, respectively.

Figure 5-9 shows the logistic function for log egg selenium versus the fraction normal for finfold deformity assessment. The R^2 for this function is low (0.28) probably due to the lack of adequate data at the high end of the egg selenium concentration. The TRAP software error report of the logistic regression model output indicates a large standard error for the slope steepness, and convergence was not reached at the maximum number of model iterations. The errors associated with this model and poor fit reduce the utility of predicted EC_{10} (20.96 mg/kg) and EC_{20} (23.22 mg/kg) values.

Figure 5-10 shows the logistic function for log egg selenium versus the fraction normal for edema deformity assessment. The R^2 for this function is high (0.96) and the observed data fit the predicted model well. No errors were reported for this model from the TRAP software output. The predicted EC_{10} (18.45 mg/kg) and EC_{20} (21.23 mg/kg) values and their confidence intervals intersect the predicted dose response curve at the top end of the curve, with no inclusion of higher effects levels at the lower end of the curve.

Figure 5-11 illustrates the logistic regression for log egg selenium versus total fraction normal. This endpoint is a summed value proportion of the total number of normal fish per egg clutch to the total number of fish examined for that egg clutch. Because an individual fish could have more than one type of deformity and because it is a summation of fractions, it can be greater than one and in fact could be as high as four for a given fish. These predicted functions fit the data well and the confidence limits for the predicted ECs are narrow. Residual error is small and the R^2 is high (0.88). The predicted EC_{10} is 19.33 mg/kg and the EC_{20} is 21.7 mg/kg. For this function, the EC_{20} and its confidence intervals intersect the predicted line, bisecting the observed data where a clear break in effects has been previously discussed for other endpoints. The confidence limits are tight about the predicted EC_{10} and EC_{20} values suggesting not only a good fit, but a low variability as well. Figure 5-12 shows essentially the same relationship; only the mean fraction normal was used as the dependent variable. The R^2 is the same as for sum fraction normal and the EC_{10} and EC_{20}s are nearly identical.

Based on the five logistic regression models for deformities, the overall best fit model is for edema, followed by total fraction normal. Edema can severely hamper young fish survival. However, the condition is reversible and may be a function of other factors not related to egg selenium concentration. Because the fraction normal endpoint takes into account all deformities assessed for any given fish from each of the egg clutches, it appears to be a more representative endpoint to evaluate potential effects due to egg selenium concentrations than the edema deformity alone.

5.1.4 Summary and Update of Brown Trout Maternal Studies

The effects of maternal selenium transfer in wild brown trout were evaluated as part of this study. Eggs from wild female brown trout collected from different locations with varying selenium exposure levels were used to assess a number of reproductive endpoints as part of this study. Initially, the data were plotted and reviewed for any obvious relationships and patterns. In the initial review, a consistent breakpoint was identified where egg selenium concentrations were contrasted with reproduction test endpoints. These observed relationships are consistent with expected dose-response relationships.

Moving forward from these initially-defined relationships, adult whole body and egg selenium concentrations were considered the independent variables in a regression-based analysis approach. The focus of the analysis was narrowed to focus on egg selenium concentration versus growth, survival, and deformity endpoints. Logistic regression was used to develop dose-response relationships and predict EC_x of egg selenium for a measured effect endpoint. A summary of the EC_x values derived is presented in Table 5-2. Post-hatch survival and total deformity frequency (fraction normal) were found to be the most biologically relevant endpoints exhibiting dose response relationships and concurrence of observed data to predicted values. The predicted post-hatch survival EC_{20} was 21.63 (95 percent LCL – 17.77, 95 percent UCL – 26.32) mg/kg dw egg selenium, while the EC_{10} for this endpoint was 17.68 (95 percent LCL – 13.44, 95 percent UCL – 23.25) mg/kg dw egg selenium. For deformities, the sum fraction normal endpoint, the EC_{20} was 21.7 (95 percent LCL – 18.09, 95 percent UCL – 26.02) mg/kg dw egg selenium, while the EC_{10} for this endpoint was 19.33 (95 percent LCL – 15.07, 95 percent UCL – 24.79) mg/kg dw egg selenium.

The brown trout data presented in the *Draft Final Brown Trout Laboratory Reproduction Studies Conducted in Support of Development of a Site-Specific Selenium Criterion* (NewFields 2009) were submitted to USEPA for use in their derivation of the National Criterion. Their subsequent review of these data submitted as part of formal comments (December 21, 2010) on this Interpretive Report suggested some alternative evaluations may be practical. USEPA's review of these data indicates agreement with the selection of the endpoint for survival (hatch to test end). As noted earlier in this section, the TRAP software includes two additional non-linear models, threshold sigmoidal and piecewise linear models. USEPA's comment letter illustrated an investigation of each of these models relative to the logistic model used as part of the brown trout studies presented above, and found that the projected EC_x values are likely conservative. As part of the USEPA's evaluation, another alternative examined exclusion of data points that exceeded 30 mg/kg dw in eggs, due to the fact that effects were already occurring between 15 and 30 mg/kg dw. This approach was investigated as a means of optimizing the model output. By eliminating the three highest data points, the logistic model is able to focus on the region of interest (i.e., between 15 and 30 mg/kg dw egg selenium). Using this approach, the logistic model run using log-transformed exposure data (egg selenium concentrations) versus survival (hatch to test end) results in a model with a R^2 = 0.99 (Figure 5-13). Confidence intervals derived for the estimated EC_x values are also tight about the estimates and the standard error of the model is low. This improved model results in an EC_{20} equal to 23.1 mg/kg dw egg selenium and an EC_{10} equal to 20.8 mg/kg dw.

5.2 YCT Adult Reproduction

Similar to the brown trout adult reproduction studies, wild pre-spawn adult YCT were also captured to assess reproductive potential and young viability. In June 2008, YCT males and females were collected from several locations representing a range of selenium exposures. Fourteen fertilized egg clutches were sent to ENSR in Fort Collins, Colorado for rearing to 15 days post swim-up. Methods for rearing were similar to those utilized for the brown trout studies, and any deviations are presented in Appendix E. Measurement data collected as part of the YCT laboratory studies are presented in Table 5-3. Results and interpretation of the YCT adult reproduction studies are presented Appendix E.

Capture locations of YCT that were ultimately used for the reproduction study included: Sage Creek (LSV-2C), Crow Creek (CC-150 and 350), Deer Creek (downstream of DC-600 near Crow Creek), and South Fork Tincup Creek (SFTC-1). Eggs were collected from 15 females, but only 14 egg batches were included in the test, as one set of eggs (SFTC-1) arrived at the laboratory dead. Pre-spawn Henry's Lake YCT were captured at the Henry's Lake fish trap by IDFG personnel and used as methodological controls. ENSR staff was on site at Henry's Lake to fertilize eggs consistent with the methods used in the field for wild-caught YCT.

5.2.1 Egg Selenium Concentration, Hatch, and Swim-Up

Summary data for the YCT adult studies are presented in Table 5-3 and described below. To put these data in perspective, brown trout data are also presented for comparison purposes where appropriate.

- For YCT from the study area, log egg selenium concentrations are strongly related to log maternal whole body selenium concentrations ($R^2 = 0.76$) (Figure 5-14).

- YCT adult female tissue concentrations from within the study area ranged from 8.17 to 25.7 mg/kg dw. Selenium in egg tissues from these fish ranged from 11.4 to 47.6 mg/kg dw. Adult females in the brown trout study had selenium tissue concentrations ranging from 4.7 to 22.6 mg/kg dw with egg selenium ranging from 6.2 to 40.3 mg/kg dw.

- Selenium in whole body YCT from Henry's Lake fish ranged from 0.23 to 0.91 mg/kg dw. Egg concentrations of selenium ranged from 0.83 to 3.23 mg/kg dw. Hatchery brown trout whole body selenium ranged from 2.5 to 4.3 mg/kg dw with egg concentrations of 0.76 to 1.2 mg/kg dw.

- Some notable differences were observed between the Henry's Lake YCT eggs (non-exposed) and the wild YCT eggs from the study area (varying levels of exposure). For Henry's Lake eggs, the day of first hatch ranged from day 24 to day 28, whereas for eggs from study area fish, the day of first hatch was day 20 to day 21 day. Likewise, the day of swim-up for Henry's Lake fish was day 49 whereas for study area fish, swim-up occurred on days 40 and 41. Fish from the study area hatched and swam up sooner than fish from Henry's Lake. For brown trout, the day of first hatch for fish from the study area ranged from 36 to 43 days and swim-up ranged from 67 to 88 days with some brown trout never reaching the swim-up stage. Hatchery browns hatched from days 40 to 47 and swim-up occurred on day 69.

5.2.2 Survival

Total survival was measured through the duration of the test for YCT. The preliminary findings are as follows:

- Wild YCT total survival at the end of the test ranged from zero to 88.9 percent. The lowest survival rates were not necessarily associated with the highest egg selenium concentrations. For example, the highest egg selenium concentration measured (47.6 mg/kg dw) had an associated total survival rate of 68.7 percent (Figure 5-15). Henry's Lake fish total survival ranged from 0.7 to 83.8 percent (Table 5-3). Conversely for brown trout, the lowest total survival rates were associated with the highest egg selenium concentrations (Table 5-1).

- The brown trout studies revealed an apparent break in effects observed between 20 and 25 mg/kg dw selenium in egg tissues (i.e., variable survival at selenium concentrations less than 20 mg/kg dw and low survival at selenium concentrations greater than 25 mg/kg dw). Survival data for wild YCT does not show a similar break. Although, at egg selenium concentrations greater than about 27 mg/kg dw, the variability in percent survival response was substantially higher than the survival response at egg selenium concentrations less than about 22 mg/kg dw.

- Examination of the YCT survival data showed two data points where egg selenium concentrations were high (> 40 mg/kg dw) and corresponded to very different survival rates (i.e., high egg selenium low survival, and high egg selenium high survival). No evidence suggested that either data point was wrong. However, when the YCT data were plotted together with the brown trout data, the high egg selenium high survival data point was inconsistent with the two data sets and observed trends. The high egg selenium high survival data point was removed and relationships were re-evaluated as a conservative measure.

Percent survival measured from hatch to test end proved to be a valuable threshold for brown trout as it eliminated the variability of egg mortality and focused on eggs that actually hatched and produced swim-up fry. A similar endpoint was also evaluated for YCT.

- Percent survival (hatch to test end) ranged from 0 to 96.8 percent in wild YCT from the study area. At the highest egg selenium concentration (47.6 mg/kg) survival was 88.2 percent, while at the next highest egg selenium level (40.1 mg/kg dw), survival was 0 percent. In this second highest egg selenium batch, egg mortality was low and percent hatch was high (92.7 percent), however no fry reached the swim-up stage (Table 5-1). The lowest survival percentage for brown trout was associated with the highest egg selenium concentration (40.3 mg/kg dw) and the next highest egg selenium concentration (38.8 mg/kg dw) had a survival rate of 24 percent (Table 5-3).

- Henry's Lake YCT percent survival (hatch to test end) ranged from 71.9 to 95 percent. In this range of survival, percent hatch for eggs from Henry's Lake ranged from 10.3 to 87.8 percent. Hatchery brown trout had survival ranging from 95.8 to 100 percent, but hatch ranged from 11.7 to 100 percent.

- Figure 5-16 shows the variability of the survival (hatch to test end) endpoint as compared to brown trout when all YCT data are included. The expected relationship of decreasing survival relative to increasing egg selenium concentration was present for wild fish, but exploratory regression analysis yielded only a weak relationship (R^2 = 0.36) due to the variability in survival at the upper end of the egg selenium concentration range.

- Further evaluation of these data during the preliminary evaluation phase found that exclusion of the high survival and high egg selenium data point yielded the best relationship between percent survival (hatch to test end) and egg selenium concentrations (Figure 5-17), while exclusion of the high egg selenium low survival data point yielded a relatively poor relationship (Figure 5-18).

- Survival during the 15-day post-swim-up feeding trial for YCT (wild fish) ranged from 1.9 to 99 percent with all but one egg clutch having a survival rate during this trial of 66 percent or higher. For brown trout, survival during the feeding trial ranged from 28.1 to 100 percent (Table 5-3).

- Screening of the YCT survival data found that percent survival (hatch to test end) provided the best relationship to egg selenium concentrations. Exclusion of the data point described above allowed for the TRAP model's regression functions to be used to estimate EC_x values (Figure 5-19). The R^2 for this model is 0.64. The TRAP software error report for this model indicates that for these data maximum iterations were reached without convergence, steepness was at maximum or minimum limit, and there was a large standard error for steepness. Confidence intervals are tight about the predicted EC_x values, most likely due to the steepness of the response curve. The EC_{20} for YCT percent survival (hatch to test end) is 36.28 mg/kg dw egg selenium, while the EC_{10} is 35.6 mg/kg dw egg selenium.

- Using a piecewise linear regression model with un-transformed egg selenium data yielded estimated EC_x values (Figure 5-20) with a model R^2 of 0.64. No errors were reported as part of the output. The EC_{20} for YCT percent survival (hatch to test end) is 36.2 mg/kg dw egg selenium while the EC_{10} is 35.8 mg/kg dw egg selenium. The slope of this response is steep, similar to that of the logistic dose response model due to the single response of zero survival at approximately 40 mg/kg dw egg selenium. While this response is not unrealistic, there is adequate variability in the response at the upper egg selenium concentrations to consider that the EC_x values predicted may be overestimating or underestimating effects at a certain level relative to background.

- Effects concentrations derived for these YCT data can only be derived at the cost of removing a data point that could be a real and probable response. Each fish responds differently to selenium exposure and some fish may tolerate higher exposure and resulting bioaccumulation better than others. A "response" of zero survival is the primary driving variable that results in the model to force a sharp dose response, where one may not actually exist.

- Henry's Lake percent survival (hatch to test end) response data were evaluated for those fish with greater than 50 percent hatch to assess the low egg selenium response for survival. Median survival of Henry's Lake eggs was 94.5 percent. Examination of the wild collected YCT indicates a break in the survival data between 22.3 and 27.9 mg/kg dw egg selenium. For those eggs at or below 22.3 mg/kg dw selenium (n = 7 egg batches), median survival was 91.1 percent, a difference of less than 2 percent between wild caught fish and Henry's Lake eggs (Figure 5-21). For eggs equal to or greater than selenium of 27.9 mg/kg dw selenium (n = 7 egg batches), median survival was 80.9 percent. Compared to the wild fish with lower egg selenium concentration, the higher egg selenium fish survival rate was 11.9 percent lower. A non-parametric Kruskal-Wallis one–way analysis of variance (NCSS 2007) verified that the medians are significantly different ($p = 0.015$, $\alpha = 0.05$). Median value were used here due to the extremes in survival rates of the higher egg selenium survival rates (range = 0 to 88.2 percent). Using the mean or median value (equivalent for n = 2) of the egg selenium concentrations for these two groups of wild collected fish indicates a value of 25.1 mg/kg dw egg selenium, suggesting that an EC_{10} for survival is greater than 25 mg/kg dw.

5.2.3 Growth

- Growth, as measured by dry weight measured at the end of the 15-day post-swim-up feeding trial, was obviously different between fry from the Henry's Lake parents and study area parents. Henry's Lake 15-day post-swim-up growth ranged from 15.63 to 26.6 mg/kg dw whereas growth in the study area fish ranged from 6.02 to 14.35 mg/kg dw (Table 5-3). Similar to hatchery fish used for the brown trout study, Henry's Lake YCT may not undergo the same stresses and competition for resources as Site fish. Furthermore, Henry's Lake YCT were substantially larger than Site YCT, where size and age of the parents may lead to larger, stronger young.

- YCT showed a similar growth response to that observed for brown trout growth. Lower growth rates were observed where egg selenium concentrations were highest, but both species also exhibited low growth rates together with high growth rates where egg selenium concentrations were much lower.

- The expected relationship of decreasing growth relative to increasing egg selenium concentration was present, but exploratory regression analysis yielded a weak relationship (R^2 = 0.21) due to the variability of growth at the lower end of the egg selenium concentration range (Figure 5-22). Distribution of the growth data did not lend itself to useful dose-response modeling. An attempted dose-response plot for growth of wild collected fish provided a poor fit using a piecewise linear model with no data transformations (Figure 5-23) (R^2 = -0.2). Model runs using TRAP's other non-linear routines together with and without transformations did not improve the model fit. Despite the poor model fit, the EC_{10} value was 28.9 mg/kg dw and the EC_{20} value was 31.9 mg/kg dw, but the reliability of these estimates are highly uncertain.

- The data distribution illustrates a shift in the growth response at similar egg selenium levels observed to have slightly lower survival. As noted above for survival, there is a clear break in the egg selenium concentrations and a corresponding break in survival responses. For growth, the median growth of alevins from eggs less with less than 22.3 mg/kg dw selenium was 12.3 mg dw, while median growth was 8.1 mg dw in alevins from eggs with 27.9 or more egg selenium.

- A parametric one-way analysis of variance found that growth was significantly different between the low egg selenium group and the high egg selenium group ($p = 0.03$, α = 0.05). Similar to the survival data, the growth EC_{10} also likely lies between the "no effect" and "effect" concentration observed in these data which would result in a value greater than 25 mg/kg dw egg selenium.

5.2.4 Deformities

Deformities were evaluated for YCT alevins and included measuring and ranking cranio-facial, skeletal, finfold, and edema deformities. Ranking included rating a fish as either normal or as having few/slight, several/moderate, or many/severe deformities. Figures 5-24 to 5-27 show the total percentage of all fish from each of the samples from the locations ranked for each of the deformities assessed.

- On average, the percentage of normal fish scored for the craniofacial deformity endpoint from eggs of parents from upper Crow Creek (CC-150, CC-350) and Deer Creek ranged from 76 to 96 percent, from 18.5 to 95.7 percent for fish from Sage Creek, and from 69.2 to 96 percent for Henry's Lake fish (Figure 5-24).

- The percentage of normal YCT scored for skeletal deformities from the upper Crow Creek locations averaged from 17.6 to 35 percent, while for Sage Creek normal fish averaged from 7 to 35.7 percent of the sample. Henry's Lake fish that were normal averaged from 5.6 to 52 percent of the sample. While the percentage of normal fish was lower in all samples as compared to the craniofacial endpoint, the severity of skeletal deformities was not high. A number of fish that were not ranked as normal were ranked as having only slight or few skeletal abnormalities (Figure 5-25).

- Finfold deformities were infrequent, resulting in high numbers of fish that ranked, on average as normal. Upper Crow Creek fish ranked as 95 percent or greater normal. Similarly, fish from Sage Creek were ranked as having high numbers of normal fish in three of the four samples (> 95 percent). One sample however, only had 85 percent normal fish. Henry's Lake fish ranged from 55.6 to 98 percent normal fish for finfold deformities (Figure 5-26).

- Edema was variable across the board for all YCT evaluated. In upper Crow Creek samples, fish ranked as normal ranged from 61.5 to 95.8 percent. Sage Creek fish ranged from 50.5 to 95 percent normal and Henry's Lake fish ranged from 33.3 to 82.3 percent normal (Figure 5-27).

- Across all the individual deformities measured, no consistent trend of effects (i.e., increasing deformity percentage or severity) was observed with increasing egg selenium concentrations based on exploratory regression analysis.

- A preliminary regression run using the endpoint which showed the best relationship for brown trout deformities (fraction normal) (Figure 5-28), shows the expected response of decreasing percentage of normal fish with increasing egg selenium concentrations (R^2 = 0.59).

- A threshold sigmoidal regression run using the TRAP software allowed for the best overall model fit with no errors in prediction of EC_x values (Figure 5-29). For this model run the egg selenium data were log transformed, and the high egg selenium, high normal percentage data point was deleted (shown as open diamond on the figure). The predicted dose response model had a R^2 of 0.57 and confidence intervals about the predicted EC_x values that were fairly tight. The EC_{20} for fraction normal fish was 37.6 mg/kg dw egg selenium, while the EC_{10} was 32.7 mg/kg dw egg selenium. The dose response was re-evaluated using a piecewise linear model using the same variable transformation listed previously, and revealed a model with a lower R^2 (0.51). Brown trout logistic regressions, described previously, found a significant relationship of increasing egg selenium concentrations and decreasing fraction of normal fish.

- Similar to the survival response data, there is a separation in the response data at egg selenium concentrations equal to 22.3 and 27.9 mg/kg dw. For the seven egg batches equal to or less than 22.3 mg/kg dw, the mean percentage of normal fish was 75 percent. To put these data in perspective, mean percent normal alevins for the eight egg batches from Henry's Lake with > 50 percent survival at hatch was 74 percent. Thus, data for wild caught YCT with egg selenium concentrations at or less than 22.3 mg/kg dw have nearly identical percentages of normal fish as those from a reference lake. For egg batches greater than 27.9 mg/kg dw, the mean percentage of normal fish is 68 percent (including all seven egg batches) and 66 percent (excluding the single highest egg selenium egg batch). This apparent difference was evaluated using a one-way ANOVA. Lack of normality prompted use of the Kruskal-Wallis non-parametric ANOVA that found the medians were not significantly different between the two wild-collected groups ($p = 0.074$, $\alpha = 0.05$). Similarity of the response for data less than 22.3 and greater than 27.9 mg/kg dw egg selenium suggests that the deformity EC_{10} value is higher than 27.9 mg/kg dw, however, by how much is not clear as the upper end potential threshold is not bounded.

5.2.5 YCT Adult Reproduction Results Summary

Clearly, adult YCT females were exposed (via ambient diet and aqueous exposure) for a sufficient duration to bioaccumulate selenium in whole body and eggs. Endpoints for YCT were variable and not always consistent with those observations for brown trout. As a conservative measure, the high egg selenium and high survival data point was removed and relationships were reevaluated. Removal of that single data point for the survival data (hatch to test end) allowed for a data distribution that more closely resembled observations found for brown trout. Growth data for YCT did not show enough differences between low and high egg selenium concentrations to distinguish a dose response. Deformity observations were also variable, with most fish ranking as having no deformities or only slight deformities as in the case of skeletal ratings. Considering all of the deformity data, and using the fraction normal as a total endpoint for this measure did produce a viable dose response relationship, however, the percent survival (hatch to test end) endpoint proved to be a stronger relationship, similar to the response observed for brown trout, albeit at a higher egg selenium concentration.

Of the relationships evaluated for YCT, percent survival (hatch to test end) provided the best relationship to egg selenium exposure. Relying solely on the model output, EC_{10} and EC_{20} values are greater than 35 mg/kg dw egg selenium. Additional analyses were also conducted using piecewise linear and sigmoidal dose response models. Despite the use of multiple approaches and data transformations, clear dose response models using these effects endpoints were few. YCT data showed highly variable responses to egg selenium concentrations. Examination of the data distribution, however, did suggest differences in responses between 22.3 and 27.9 mg/kg dw egg selenium. A decreased response was noted at egg selenium concentrations greater than 27.9 mg/kg dw for survival and growth. Averaging the observed no effect and potential effect concentrations resulted in a value of 25.1 mg/kg dw, which is expected to be lower than a derived EC_{10}. Without a true EC_x value derived from the dose response modeling, effects for egg selenium exposure on survival and deformities are at some concentration greater than 25 mg/kg dw in eggs.

These analyses indicate that there is a species differences in brown trout and YCT responses to selenium exposure. Brown trout are more sensitive in their response to maternally-accumulated selenium and its effects on reproduction than are YCT. This finding is not inconsistent with studies that have utilized several different cutthroat trout species (Hardy et al. 2005, 2010; Rudolph et al. 2009; Nautilus Environmental 2010) that indicate sensitivity differences among similar species.

5.3 YCT Early Life Stage Studies

A study of ELS YCT exposure to selenium via diet and aqueous exposure was also conducted (Appendix F). For this study, no pre-maternal exposure occurred. The preferred approach was to test the influence of diet and aqueous exposure on eggs from pre-exposed female fish. This approach would have required collection of wild parents from different exposure areas, similar to sampling conducted for the adult reproduction studies. Recognizing the difficulties of capturing high numbers of wild spawning fish based on efforts for the brown trout studies, particularly females that are ripe, but not yet spawned out, it was assumed that insufficient numbers of wild YCT spawning females would be captured for both the adult reproduction studies and the ELS studies. The assumption was indeed correct, as three separate periods were evaluated for YCT spawning individuals, and only 14 spawning females were captured. Several females were obtained from the Henry's Lake hatchery run in Idaho and the eggs from which were utilized for this study.

Use of YCT eggs with no history of selenium exposure provides some useful information for a sensitive life stage (eggs through swim-up). It isolates the exposure regimen to focus only on diet and aqueous exposure for this species. In terms of environmental realism, it mimics the potential effects of low or unexposed females that may spawn in tributary streams that may have elevated selenium in aqueous and dietary media. This study improves the understanding of the potential effects of selenium on eggs and young reared in such an environment with no maternal pre-exposure where selenium is transferred to eggs during early egg formation. Appendix E includes the laboratory report for these studies.

Six treatments and a control with five replicates in each treatment were used. Nominal treatment levels were 2.5, 5, 10, 15, 20, and 40 µg/L and mg/kg selenium in aqueous and dietary exposures, respectively. Nominal and empirical concentrations in both the aqueous and dietary media are shown in Tables 5-4 and 5-5. Aqueous exposures began at hatch and continued through the end of the test, while dietary exposures began at swim-up, when alevins begin to lose their yolk sac and begin active feeding. Selenium in the dietary pathway was delivered to trout via *Lumbriculus* that were fed dietary yeast supplemented with selenium. *Lumbriculus* were fed one of the six dietary treatment levels over a specified period of time, allowing them to bioaccumulate selenium and achieve equilibrium in tissue concentrations prior to being fed to trout.

The combination of aqueous and dietary treatments yielded significant selenium bioaccumulation in fry by test termination. Table 5-6 shows the whole body selenium concentrations in fish at day 27 following aqueous only exposure (~21 days) and at day 71 following aqueous and dietary exposure. Treatment replicates during aqueous only exposure had whole body selenium concentrations ranging from 1.47 to 1.84 mg/kg dw. At the end of the test following aqueous and dietary exposure, whole body selenium concentrations in treatment fish ranged from 2.67 to 34.48 mg/kg dw.

Survival was measured at multiple time periods during the test including at hatch, swim-up, a thinning stage, at commencement of the dietary exposure, and at test termination (Table 5-7). A cursory examination of the first four of these survival measurement points suggests little variation between control and treatments and between treatments of different levels.

- Control survival at hatch was lower than expected (mean = 74 percent) but within acceptable limits[12] (ASTM 2006); however, natural spawning run YCT experience environmental stressors that influence hatch even under the best conditions. Control survival at hatch in this study was higher overall than method control survival at hatch of Henry’s Lake eggs in the YCT maternal transfer study which ranged from 0 to 87.8 percent (in 16 different test chambers), thus for the ELS test, the control data are considered acceptable for use as a comparative basis to higher selenium treatments. Survival at the end of the test was variable with a higher mean survival (34.5 percent) at the highest treatment (40) as compared to a mean survival of 28.5 at the next highest treatment (20). Control survival averaged 50.6 percent. In treatments 2 to 15, mean survival ranged from 34.9 to 45.4 percent. In this early phase of analysis, survival does not show promise as an endpoint that is related to increasing exposure (Figure 5-30).

- Percent survival post hatch is higher than overall survival, with mean control survival being higher than all post hatch treatment survival means. This endpoint eliminates the variability of egg hatch success (Figure 5-30).

[12] ASTM E1241-05, Section X.1.2.8 - An early life-stage test with a salmon, trout, or char is unacceptable if survival of the controls is less than 70 % from thinning of the embryos (see 11.5) to test termination.

- Using log transformed survival data (hatch to test end) a significant difference was observed when control survival was compared to treatment survival (one-way ANOVA, $p = 0.009$). The Tukey Kramer MCT identified that only the 20 µg/L treatment was different than controls, and all other treatments were not significantly different from the controls.

Growth was measured based on length and dry weight (Tables 5-8 and 5-9). Growth was derived based on two metrics, per the total test population (i.e., original number of fry in the test chambers), and per the fish alive at the end of the test (i.e., the surviving number of fry in the test chambers) (Figure 5-31). Growth measured based on the total test population takes into account growth and survival, whereas growth based on the number of fish alive at the end of the test only measures growth.

- Based on the total test population, growth in treatments is lower in all treatments when compared with the control; however, there does not appear to be a dose response relationship of growth to increasing aqueous and dietary selenium.

- Based on the fish alive at end of test, growth appears to be similar between treatment levels and between controls and treatment levels, although the highest growth measured was at the 40 µg/L treatment level.

- Length measurement data is consistent among controls and treatments (Table 5-10).

- No significant differences were found for growth between controls and treatments regardless of how growth was measured (ANOVA, $p > 0.05$).

- In addition to growth and survival, deformities were evaluated both for type and severity similar to the deformity assessment conducted for the adult reproduction studies (Table 5-11 and Figures 5-32 to 5-35). Of the four deformities evaluated (craniofacial, skeletal, finfold, and edema), only finfold deformities resulted in fish scored as “moderate” or “several” (i.e., severity score of 2), albeit the percentage was low. All other deformities evaluated resulted in high percentages of normal fish or fish with only slight or few deformities.

The YCT ELS studies provide important information relative to pathway of exposure and life stage effects. Based on these data, pre-maternal exposure is a more important determinant of effects than aqueous and dietary exposure. Conclusive results for effects to survival, growth, or frequency or severity of deformities to ELS YCT despite exposures up to 40 µg/L in water and 40 mg/kg in the diet were not observed. Studies by Vidal et al. (2005) and Hamilton et al. (1990) that used rainbow trout and Chinook salmon, respectively, found widely diverging results in bioaccumulation and effects. An increasing relationship between whole body selenium and reduced growth was observed for Chinook salmon but not rainbow trout. YCT in this study showed no relationship between tissue selenium concentrations and growth or survival.

Whereas relatively consistent concentration-response curves are typically observed for maternal transfer studies, data from juvenile studies are fewer, highly variable, and poorly understood (Parametrix 2009). Based on the review of studies for bluegills and trout, where both maternal transfer and juvenile data were available, Parametrix (2009) concluded that reproductive tissue is the appropriate biomonitoring tissue for selenium effects in fish, while whole body, even at the juvenile stage, is not as sensitive in developing dose-response relationships. This is consistent with USEPA's approach to revision of the 2004 National Draft selenium criterion (Charlie Delos personal communication).

The hypothesis for this test when initially presented to the SSSC Workgroup was that effects to developing young that were not pre-exposed to selenium via maternal transfer would not be as sensitive as those effects levels where maternal transfer had occurred. Results from this ELS study do not contradict that hypothesis. Applications to real world scenarios exist, for example, consider the situation where resident Crow Creek trout travel to Sage Creek to spawn.

5.4 Summary and Conclusions of Maternal Transfer and Early Life Stage Studies

Based on the results from testing for brown trout, several conclusions were drawn from the study and are presented below:

- Significant breaks between no and/or low ranges of effects for brown trout and observed high ranges of effects were observed based on visual inspection of the data between 20 and 25 mg/kg dw egg selenium concentration for several endpoints.

- Post-hatch survival and total deformity frequency (fraction normal) were found to be the most biologically relevant endpoints exhibiting dose response relationships and concurrence of observed data to predicted values for brown trout. The predicted post-hatch survival EC_{20} was 21.63 mg/kg dw egg selenium, while the EC_{10} for this endpoint was 17.68 mg/kg dw egg selenium. For deformities, the sum fraction normal endpoint EC_{20} was 21.7 mg/kg dw egg selenium, while the EC_{10} for this endpoint was 19.33 mg/kg dw egg selenium.

- Model data for survival were reevaluated focusing in on the data range where effects occur. This improved model results in an EC_{20} equal to 23.1 mg/kg dw egg selenium and an EC_{10} equal to 20.8 mg/kg dw.

- Effects thresholds defined for brown trout from this study are consistent with reported effects found for other species in the literature.

Results and analyses for YCT adult reproduction study yield the following conclusions:

- For YCT, egg selenium concentrations were strongly related to whole body selenium concentrations.

- Maternal transfer studies for YCT yielded evidence of decreasing growth, survival, and fraction normal fish relative to egg selenium concentrations. However, these relationships were weak due to variability of each response to egg selenium concentrations. Removal of a single data point that had high egg selenium and high survival improved relationships substantially.

- The test endpoint, percent survival (hatch to test end) for YCT was evaluated using the TRAP software regression routines to evaluate the potential for a dose response relationship. The best model produced using a piecewise linear regression resulted in EC_{10} and EC_{20} values of 35.8 and 36.3 mg/kg dw egg selenium, respectively.

- The test endpoint, fraction normal evaluated for YCT deformities yielded a dose response relationship using logistic regression models similar to that developed for brown trout. The EC_{20} for the mean fraction normal fish was 37.6 mg/kg dw egg selenium while the EC_{10} was 32.7 mg/kg dw egg selenium.

- YCT data showed highly variable responses to egg selenium concentrations. Examination of the data distribution, however, did suggest differences in responses between 22.3 and 27.9 mg/kg dw egg selenium. A decreased response was noted at egg selenium concentrations greater than 27.9 mg/kg dw for survival and growth. Averaging the observed no effect and potential effect concentrations resulted in a value of 25.1 mg/kg dw, which is expected to be lower than a derived EC_{10}. Without a true EC_x value derived from the dose response modeling, effects for egg selenium exposure on survival and deformities are at some concentration greater than 25 mg/kg dw in eggs.

- Comparison of the predicted EC_x values for percent survival (hatch to test end) between brown trout and YCT indicates that brown trout are more sensitive to maternal transfer of selenium to young developing embryos than are YCT.

Results and analyses for the YCT ELS study yield the following conclusions:

- The post hatch survival rate at test termination was significantly different from controls (One way ANOVA, $p = 0.009$) at the nominal treatment of 20 mg/kg diet and 20 µg/L aqueous selenium. At the nominal treatment of 40 mg/kg diet and 40 µg/L aqueous selenium, no difference from control was found. No dose response was observed for dietary treatments[13].

- Growth and deformities in treatments were not significantly different from controls.

- Despite selenium tissue residues up to 34.5 mg/kg dw in young fish with no pre-parental exposure, resulting from dietary and aqueous treatment exposures, no relationships were observed for survival, growth, or fraction normal fish that suggested effects with increasing exposure.

- The lack of maternal exposure may preclude detrimental effects even if the adult spawns in a stream with elevated selenium, since the maternal pathway is the primary route of exposure to induce chronic effects.

5.5 Egg to Whole Body Translator

Effects data for the maternal transfer studies with brown trout and YCT are expressed in terms of egg selenium concentration. The survival end point (hatch to test end) was selected as the best predictor of effects for both species. Egg to whole body translations were developed individually for both species (Figure 5-36). Data for both species were also combined in developing this relationship because of the similarities in the ratios of egg to whole body selenium concentrations. The mean egg to whole body selenium ratios were 1.51 and 1.43 for brown trout and YCT, respectively, suggesting that exposure, bioaccumulation, and deposition of selenium to eggs between the two species was similar. Analysis of variance on the two data sets for egg to whole body ratios indicates that brown trout and YCT ratios are not significantly different (ANOVA, $p > 0.05$). While similarities exist between the two species, it is likely more appropriate to develop translators for each species. Augmenting the species-specific translators should be done when practical.

The equation for derivation of the whole body selenium concentration (mg/kg dw) from egg tissue concentrations (mg/kg dw) for brown trout is is as follows:

$$Log_{10}\ egg\ selenium = 1.1926*Log_{10}\ (whole\ body\ selenium\ concentration) - 0.0071$$

[13] As noted in Appendix F, the lack of a dose-response for survival, although a significant difference was observed at one treatment level may be due to complications encountered during testing ELS YCT where young trout were found to be gorging themselves to death. Competition in the treatment tanks also complicated exposure.

Solving for whole body concentration, the equation is rewritten as:

$$Log_{10}\,(\textit{whole body selenium concentration}) = ([Log_{10}\ \textit{egg selenium}] + 0.0071)/1.1926$$

Using this equation, the whole body selenium concentrations predicted from brown trout egg selenium concentrations that equate to the EC_{10} (20.8) and EC_{20} (23.1) values for survival are 12.9 and 14.1 mg/kg dw selenium, respectively

The egg to whole body translation factor for YCT is as follows:

$$Log_{10}\ \textit{egg selenium} = 0.962 * Log_{10}\,(\textit{whole body selenium concentration}) + 0.2007$$

Solving for whole body concentration, the equation is rewritten as:

$$Log_{10}\,(\textit{whole body selenium concentration}) = ([Log_{10}\ \textit{egg selenium}] - 0.2007)/0.962$$

As noted earlier in this document, the EC_{10} for YCT is expected to be greater than 25 mg/kg dw. At 25 mg/kg dw, the egg to whole body translator indicates at 25 mg/kg dw egg selenium, the whole body concentrations would be 17.6 mg/kg dw.

6.0 INTEGRATING LINES OF EVIDENCE

The *Summary of Approach for Developing a Site-Specific Selenium Criterion* (NewFields 2008) discussed integration of the following three primary lines of evidence to arrive at a proposed SSSC:

- Laboratory reproductive studies using two species of trout collected from differing exposure conditions within and outside of the Study area. Laboratory-based studies that examine maternal transfer of selenium to eggs for Site species provided information to define levels of potential effects to individuals. Laboratory studies using brown trout and YCT provide Site-specific evidence of effects that may occur from aqueous and dietary exposure and the resulting bioaccumulation in the female parent.

- Coincident characterization of selenium concentrations and field monitoring of the abundance and diversity of aquatic biota. Site selenium characterization in biotic and abiotic media defines exposure. Defining population and community characteristics allows for assessment of whether or not negative trends in population and or community structure are occurring, and serves to validate the reproduction studies.

- Review of scientific literature to provide context for the representativeness of the study design and the laboratory and field findings. Consideration of available effects data for different species from the literature allows for evaluating Site-specific effects data relative to other similar studies and other potentially sensitive species.

Consideration of multiple lines of evidence is an accepted approach that is commonly utilized in science as well as in the ecological risk assessment process and other regulatory programs, including development of site-specific criteria. EPA clearly encourages the use of field studies and/or toxicity tests, recognizing that several types of data may be developed to provide supporting information for a lines-of-evidence approach to characterize risks. This approach is far superior to using single studies or tests or measurements to determine whether or not the observed or predicted risk is unacceptable (USEPA 1999).

This section brings together the lines of evidence and related rationale that form the support for a proposed Site-specific chronic selenium criterion. By considering the strength of information provided through each line of evidence, both individually and in combination, the overall confidence in any finding can be gauged. As discussed further below, the findings developed for each line of evidence converge to provide a strong foundation for the identification of a Site-specific criterion for selenium.

6.1 Site-Specific Maternal Transfer and ELS Studies

The correlation between selenium concentrations and several biologically relevant endpoints (e.g., growth, survival at various life stages, deformities, fecundity, etc.) were evaluated as part of the maternal transfer studies conducted using wild collected brown trout and YCT. The strongest (and most well defined) biologically relevant dose-response relationships were used to identify effects thresholds for a species.

For the brown trout studies, two endpoints were identified with strongly defined relationships to egg selenium concentrations:

- *Percent survival* (hatch to test end), representing the number of brown trout that hatched, matured to swim-up, and survived the 15-day post-swim-up feeding trial; and

- *Fraction normal* (number of normal fish/total number of fish), representing the number of normal fish that were not deformed, considering four different types of major deformities, relative to the total number of fish.

Data from both endpoints, when input into a logistic model (TRAP), provided similar predicted levels of effects based on egg selenium concentrations. The EC_{20} values for both were nearly identical, with the survival endpoint EC_{20} equal to 21.63 mg/kg selenium dw, and the fraction normal endpoint EC_{20} equal to 21.7 mg/kg selenium dw. Because the survival endpoint for individual brown trout is slightly more sensitive than the fraction normal endpoint, the survival endpoint is selected as an appropriately conservative endpoint. Relationships for other endpoints evaluated for this species did not yield as well-defined dose response relationships. Further review of the derivation method used for EC_x values for brown trout revealed that the logistic regression approach using all of the data was conservative, as the TRAP logistic function attempted to fit the model to data that exceeded effects (i.e., >30 mg/kg dw egg selenium). Revising the approach and excluding data points that exceeded 30 mg/kg dw egg selenium focused the model to the area of concern (i.e., near the EC_{10} and EC_{20} thresholds) and yielded EC_{10} and EC_{20} values of 20.8 and 23.1 mg/kg egg selenium.

For the YCT maternal transfer studies, the survival (hatch to test end) and fraction normal endpoints also emerged as having the most well-defined relationships to egg selenium concentrations. Both of these dose-response relationships were best quantified after the data were censored to remove results for a clutch that had high egg selenium and high survival. Removal of this data point was considered a conservative step to reflect a pattern of increasing egg selenium with decreasing survival and increasing deformities. Of the two endpoints, survival (hatch to test end) was the better-defined relationship, with predicted EC_{10} and EC_{20} values of 35.8 and 36.3 mg/kg dw egg selenium. The strength of this relationship (R^2 = 0.64) however, was much lower than the dose response curves derived for brown trout. A break or shift in the data was recognized and evaluated further. The difference in lower egg selenium concentrations and higher egg selenium concentrations suggested the two groups of data were significantly different, thus the break points for these two data sets (i.e., NOEC and LOEC) were averaged resulting in a value of 25.1 mg/kg dw egg selenium for YCT. The EC_{10} value for YCT is believed to be greater than 25.1 mg/kg dw.

Of the two species, brown trout results yielded the better-defined relationships to egg selenium concentrations, with effects occurring in individuals at lower concentrations. Comparison of these data indicates that YCT are less sensitive than brown trout to the reproductive effects of bioaccumulated selenium. Indeed, Hardy et al. (2010) suggested that YCT from Henry's Lake, Idaho, tested in a 2.5 year exposure study, showed signs that they were less sensitive than rainbow trout.

An ELS evaluation of selenium accumulation and potential effects to developing YCT embryo-larvae, without maternal exposure, was also conducted. In this study, diet was clearly the dominant factor over water quality in influencing selenium bioaccumulation in ELS. Endpoints evaluated included survival, growth, and deformities. Despite bioaccumulation up to 34.5 mg/kg dw at an average diet of 45.2 mg/kg dw in *Lumbriculus* feedstock and an aqueous exposure concentration of 40.7 µg/L, no significant effects were found when compared to controls or between treatments. Of the four deformities evaluated (craniofacial, skeletal, finfold, and edema), when considered collectively, the mean fraction normal in treatments ranged from 87 percent at the lowest treatment level to 90 percent at the highest treatment level.

The relative lack of consistent effects in the ELS study with increasing exposure confirms that maternal exposure is the primary and more sensitive exposure pathway affecting young developing fish. These ELS test data also suggest that when excessive selenium through maternal transfer does not occur, young are capable of survival and growth in areas of elevated selenium concentrations at the egg, alevin, and swim-up stages. The lack of observed effects further supports the initial hypothesis that chronic selenium effects in fish are due to the deposition of selenium from the parent to the egg, thereby affecting embryo-larval development.

6.2 Literature

The literature has been continually reviewed during this study. Laboratory studies were designed based on previous maternal transfer studies conducted by Holm et al. (2005), Kennedy et al. (2000), and Hardy et al. (2005; 2010). As new studies have become available, they have been reviewed and integrated to increase the understanding of selenium toxicity, sensitivity of different life stages, and range of species sensitivities. Part of the literature review process includes consideration of how the site-specific studies compare to work from other investigators. Another aspect of the literature review is to evaluate the sensitivity of the test species relative to the larger aquatic community.

Comparisons with literature provide an important cross check on the representativeness of the studies conducted for this Site. It is expected that similarly designed tests, with the same or similar endpoints, should provide similar results from the same (or similar) species. If these comparisons exist, as they do here, then confidence in the results obtained from these Site-specific studies increases. Furthermore, species sensitivities can be put into context, specifically whether the important species identified for testing for this Site are significantly more or less sensitive than others.

6.2.1 Maternal Transfer Studies in Fish

Different fish species exhibit different sensitivities to contaminants. This is clearly illustrated in the USEPA's (2004) draft criterion, as well as reviews by Hamilton (2003, 2004), Lemly (1998), DeForest et al. (2006), Brix et al. (2005), and others. Several studies of maternal transfer effects in developing young for different species were previously described (Section 3.2) representing a range of effects. Questions have been raised concerning the sensitivity of different fish species relative to the species selected for testing in this study. Maternal transfer study data are available for brown trout and Yellowstone cutthroat trout (from this study) and northern pike, white sucker, char, and brook, rainbow, Westslope, and Yellowstone cutthroat trout species (from studies previous described in Section 3.2).

No studies for cold water cyprinids, such as dace or shiners, have been found that suggest higher or lower sensitivities. As stated earlier in this document, the various fish species identified and collected to date comprise four families and up to nine genera. Other than the two trout species, only sculpin are found at all of the locations. Sculpin are relatively ubiquitous, although they are found in much lower numbers at the lower Crow Creek locations. Sculpin are likely an important prey species for trout. While trout are the target test species in this study, sculpin would be the next logical choice if another species is needed for testing because of their small home range, which would provide excellent data on exposure, and for other reasons described in previous sections. Unfortunately, no toxicity data for sculpin exposure to selenium has been found to date. Carmichael and Chapman (undated) report slimy sculpin tissue concentrations for fish found in a coal zone and outside this zone. While no effects data are reported, they illustrate that sculpin inside the coal zone had a wide range of selenium tissue concentrations ranging from less than 2 mg/kg dw to greater than 12 mg/kg dw.

Considering the data available, a species sensitivity distribution (SSD) was derived using data from the maternal transfer studies to evaluate the range of effects levels relative to one another and overall sensitivity for fish species (Figure 6-1). The SSD was generated using the Species Sensitivity Distribution Generator, part of USEPA's Causal Analysis/Diagnosis Decision Information System (USEPA 2010) (http://www.epa.gov/caddis/index.html). The SSD-generator is an Excel-based tool that allows creation of custom SSDs.

The SSD provides a statistical estimation and distribution, based on the effects data input, of effects relative to one another and confidence limits on projected effects thresholds. This statistical distribution indicates that the EC_{10} for brown trout (20.8) is at the 31^{st} percentile of the fish species distribution, with northern pike and brook trout data being more sensitive. The separation between the three species is 0.8 mg/kg dw selenium in egg tissues. Brook trout effects represented in this figure are based on EC_{06}, so an EC_{10} value is likely higher. Compared to Westslope cutthroat trout, rainbow trout, and Yellowstone cutthroat trout, brown trout are the more sensitive species. YCT are the least sensitive of the trout species evaluated.

Site Yellowstone cutthroat trout are less sensitive than several of the species evaluated. Species included in Figure 6-1 illustrate a range of feeding strategies including piscivores, invertivores, and omnivores from which selenium accumulation would occur, suggesting that no specific feeding strategy is influencing selenium bioaccumulation and transfer to eggs. Based on these studies, brown trout is a sensitive cold water species that is suitable to reflect the potential for effects in a larger cold water aquatic community.

Effects for YCT from this Site study occurred at higher concentrations than cutthroat trout results reported by Rudolph et al. (2008), Hardy et al. (2005; 2010) and Kennedy et al. (2000). In the Kennedy et al. (2000) study, the mean no effect threshold of > 21 mg/kg dw in eggs is the result of egg tissue concentrations from the impacted site that ranged from 8.7 to 81.3 mg/kg dw. YCT effects from this study clearly fall within the range of egg selenium concentrations considered by Kennedy et al. (2000). In the Rudolph study, the EC_{10} for alevin mortality was 24.2 mg/kg dw in Westslope cutthroat trout eggs, which is more consistent with the findings of YCT effects found in this study. Nautilus (2009) reported an EC_{10} for Westslope cutthroat trout of 19 mg/kg dw selenium in eggs for trout from the same region studied by Rudolph (2008) and Kennedy et al. (2000). In the Hardy et al. (2005; 2010) study, the highest diet fed to YCT resulted in no effects, thus definition of a point estimate of effects was not achieved (NOEC > 16.04 mg/kg dw in eggs).

Because the site-specific selenium sensitivity of all resident species will never be known, a representative species must be utilized. Selection of a criterion based on the protection of an appropriately sensitive species will result in protecting most, if not all, less-sensitive species. For this Site, brown trout are a relatively abundant species found in a wide range of exposure environments, which is an important practical consideration. Brown trout are also predators at the top of the aquatic food chain. For a food chain bioaccumulating constituent such as selenium, a top level predator is a good choice for a representative species. Brown trout utilize different feeding strategies, depending upon life stage, and as shown by these site-specific studies, are more sensitive than the native YCT. As a non-native species, it is possible that brown trout are more sensitive to selenium than the native YCT simply due to genetic adaptation of the YCT. Brown trout are a European species, where historically low levels of selenium are present in crustal soils. As a top-level predator, brown trout response data provide a sensitive endpoint from which to gauge potentially larger aquatic community protectiveness, as discussed below.

6.2.2 Test Species Sensitivity Relative to Aquatic Community

Reproductive effects in fish and aquatic birds are the most sensitive biological indicators of aquatic ecosystem-level impacts of selenium (Lemly 1998; Ohlendorf 1989; Skorupa et al. 1996). Available testing data for selenium indicates that fish are likely the more sensitive aquatic receptor to selenium (Coyle et al. 1993, Hamilton et al. 1990, Hermanutz et al. 1996) (as cited in USEPA 2004). Lemly (1985) suggested that aquatic food organisms of wildlife strongly bioaccumulate selenium hundreds to thousands of times the waterborne concentration, but are unaffected by tissue residues that are high enough to cause reproductive failure when consumed by fish and aquatic birds. Evaluation of this concept, that fish are more sensitive than other aquatic species is necessary to assess the protective value of a criterion derived based on effects in fish.

While brown trout were demonstrated to be an appropriately sensitive fish species, the goal of a site-specific criterion is to be protective of a larger aquatic community. Water quality criteria are designed to protect 95 percent of the aquatic species (Stephan et al. 1985). Sensitive species should be considered in the testing process to define effects, and inclusion of effects data for sensitive species in the criteria derivation process usually provides for adequate protection of most species. The traditional approach for deriving criteria requires toxicity testing of data from eight families, one of which is Salmonidae. Generally, toxicity data for three vertebrate species and five invertebrate species are required.

Long-term studies of benthic macroinvertebrate response to selenium exposure are limited. Swift (2002) conducted long-term (> 1 year) experimental dosing studies on stream mesocosms and found no significant effect on benthic community abundance, diversity, or richness in the high (30 µg/L nominal) and moderate (10 µg/L nominal) experimental units, with the exception of Tubifex and Isopod populations which were reduced. DeBruyn and Chapman (2007) examined the literature to assess selenium sensitivity of macroinvertebrates and found that some invertebrates may be sensitive at body burdens similar to those protective of fish. Conley et al. (2009) evaluated maternal transfer in mayflies and identified a dietary threshold of 11 mg/kg dw as a concentration above which reproductive effects were observed. Studies outlined as part of this approach to assess potential site-specific effects of selenium assume that fish are the most sensitive species, which is consistent with the current literature. However, benthic macroinvertebrates are not dismissed as potentially sensitive species.

To more thoroughly evaluate the sensitivity of brown trout relative to other species, a representative aquatic community which included possible or actual resident species was evaluated using a SSD. Considering the 1985 Guidelines, three salmonids were included along with five or more invertebrates to gauge species sensitivity. In order to equate effects based on selenium concentrations in eggs from salmonids to a more comparable unit for invertebrates, egg to whole body translators from each study were used to derive an equivalent whole body tissue concentration. The Environmental Residue Effects Database (ERED), along with published literature sources for effects of selenium to benthic macroinvertebrates, was used to narrow down a range of representative invertebrate data with a range of selenium tissue concentrations and effects. Of the wide range of tissue based effects data reported, EC_{10} and EC_{20} values were rarely reported. Furthermore, the bulk of data for benthic invertebrates is for short-term acute studies, while a much smaller data set exists for longer-term chronic data.

Table 6-1 shows the species data selected for this analysis. These data reflect a mix of laboratory- and field-derived effects information. Most of these data come from a long-term mesocosm study conducted by Swift (2002) because of the duration of exposure, interaction of field conditions, and longer term effects monitoring (populations). Evaluation of population responses relative to selenium exposure in Swift (2002) provide similar data to responses being evaluated as part of this site-specific study. The Conley et al. (2009) mayfly reproduction study was also included because maternal transfer and reproduction effects are a key component in selenium toxicity. The Dobbs et al. (1996) effects data for rotifers are included to integrate a low-level primary consumer, other than a benthic macroinvertebrate, that feeds on algae. Besser et al. (2006) provides *Lumbriculus* bioaccumulation data from a laboratory study where *Lumbriculus* accumulated selenium from being fed selenized yeast.

Whole body tissue data were available for each of these species and a level of effect or no effect was reported, as presented in Table 6-1. For both the Isopod and Oligochaete data from Swift (2002), a population level effect was reported as the endpoint. Clearly, a population-level effect that is not quantified (i.e., some percentage reduction in population) is not the same as NOECs or EC_{10}s. The data are still useful when put into the context that an EC_x value derived is likely lower than the value reported and provides important information about species sensitivity. If EC_x values could be determined, it is likely that the concentrations reported would be lower; however, as evidenced by dose-response for most selenium studies, the response is typically very sharp, and the difference between no effects concentration and low and moderate effects concentrations are typically small. The Oligochaete tissue data related to the population response level is 1.4 times higher than the EC_{10} for brown trout, while the tissue data related to the Isopod response is 3.3 times higher than the brown trout EC_{10}. The nearest invertebrate effect concentration to the brown trout EC_{10}, other than that for Oligochaetes, is for Centroptilum (mayfly) (1.7 times higher), which is a no effect concentration for reproduction.

The SSD shown in Figure 6-2 was generated using this range of fish and invertebrates species effects data for whole body selenium concentrations. Similar to the fish-only SSD (Figure 6-1), brown trout (*Salmo sp.*) effects data are sensitive. Of the two most sensitive invertebrate species utilized in the SSD, one is found at all locations included within the Site. Oligochaetes are widespread and found at higher abundance in downstream mine-influenced zones than at background areas. The mayfly Centroptilum was only found once at location (CC-3A). Less sensitive species such as Baetids (mayflies) and Chironomids are found at all locations. The distribution of these data indicate that protection of brown trout at the whole body translated level of 12.9 mg/kg dw will also protect a range of aquatic species within a representative community. Considering the SSD, the endpoint for brown trout resides at the 5th percentile of the species evaluated, indicating that for the community represented, protection of brown trout will also protect 95 percent of the species in a representative aquatic community.

6.3 Study Area Conditions

Characterization of the field exposure conditions and the health of the aquatic community provides for a validation, or check, on the laboratory and literature lines of evidence. Population and community data were evaluated to assess whether the observations from the field were consistent with effects data from the other two lines of evidence. Data were also collected on physical conditions (i.e., habitat quality) to complement exposure/chemical condition data because diversity and abundance are strongly controlled by physical conditions.

Characterization of the exposure environment and ecological receptors indicates the following:

- Aqueous selenium concentrations decrease with distance downstream of Hoopes Spring.
- Selenium concentrations in fish and other biota generally follow the pattern of decreasing aqueous selenium concentrations with distance from Hoopes Spring.
- Habitat characterization data indicate that good quality habitat is available, but limitations exist.
- Population and community metrics for fish do not indicate negative impacts due to selenium exposure conditions that are not concomitantly observed when habitat limitations are observed.
- When differences in habitat are considered, the trout population data do not indicate negative impact due to selenium exposure conditions.
- Benthic density and diversity between locations or between grouped upstream and downstream locations are not significantly different.

Field selenium characterization data indicate that elevated aqueous and dietary concentrations are found at locations downstream of Hoopes Spring in Sage Creek, and to some extent in Crow Creek. Trout populations in these reaches do not appear to be reduced when compared to background locations, or to ecoregional trout population data. The overall fish community appears to be more functionally related to physical habitat characteristics than to selenium concentrations.

Putting the field studies in context with the laboratory findings for maternal transfer and effects to individual young brown trout suggests that the laboratory-based EC_{10} is a conservative value. Rationale for this logic includes the following:

- Trout abundance and population structure data do not reflect impacts even though whole body trout tissue concentrations at several locations exceed the predicted EC_{10} effects value for parental tissue (based on egg selenium concentrations).

- Multiple age classes of brown trout are present immediately downstream of Hoopes Spring, at LSV-2C, where selenium concentrations in diet and aqueous media are substantially elevated.

- Trout populations (both brown trout and YCT) in Sage Creek and Crow Creek downstream of Hoopes Spring are very productive compared to regional averages. For example, location LSV-2C exceeds the 95th percentile catch per unit effort for Ecoregion 6 streams.

- Compared to historical trout population data from 1979, 1981, 1986, 1987, 1999, and 2000, trout population estimates in Sage Creek fall within the range of estimates collected prior to mining and those collected about 10 years after mining commenced.

Of the non-trout species, literature and site-specific evidence suggest that local abundance of these species can be more strongly affected by habitat and predator-prey relationships than selenium exposure conditions. High sculpin densities are found at locations with the highest aqueous and dietary selenium concentrations. Cyprinids and catostomids are found sporadically at locations evaluated within the Site. Relating cyprinid and catostomid density to mean summer temperature, flows, and stream gradient yielded stronger relationships than either aqueous selenium or brown trout density (R^2 = 0.38 or greater). Higher densities of cyprinids and catostomids are found at locations with higher mean summer temperatures, lower stream gradients, and higher flows.

6.4 Relationships of Aqueous Selenium Concentrations and Fish Tissue Selenium Concentrations

Monitoring in surface water is a practical and commonly used approach to gauge changes in environmental conditions as well as compliance with State Standards. As noted in preceding sections of this document, a chronic selenium criterion is being developed based on selenium concentrations in fish tissues, specifically egg tissues. Reconciling accepted and practical monitoring approaches (e.g., surface water) with a tissue-based criterion (e.g., egg tissues) is an important consideration. This section discusses whether aqueous selenium concentrations can be accurately related to tissue concentrations of selenium in egg tissues. If an appropriate relationship can be derived, then aqueous selenium concentrations may be utilized as a proxy to evaluate whether conditions exist at the Site that may lead to selenium bioaccumulation greater than the egg criterion.

6.4.1 Approaches for Deriving a Relationship between Aqueous Selenium and Tissue Selenium

Initial attempts to relate aqueous selenium concentrations to tissue concentrations utilized linear regressions to examine mathematical relationships between selenium in egg tissues, whole body tissues, and water. A linear regression approach was being used to predict (based on the empirical data relationship) maternal whole body selenium tissue concentrations from egg tissue concentrations. From this relationship, whole body selenium concentrations were used to predict aqueous selenium concentrations based on the field data collected. Limitations of this approach were observed, most notably in the relationship of whole body tissue selenium to aqueous selenium. Slight shifts in the slope of the regression line relating whole body tissues to aqueous selenium concentrations resulted in widely varying prediction results for aqueous selenium. A simple linear regression back calculation can lead to widely disparate results, and thus is not a practical approach for deriving aqueous selenium concentrations from egg effects threshold values.

Other modeling approaches proposed by various researchers include the use of multiple regression techniques that incorporate significant co-variants along with fish tissue concentrations. This approach has been shown to be somewhat successful with data for Westslope cutthroat trout. Dr. Adrian Debruyn of Golder Associates has been using a linear mixed effects model to examine lentic and lotic relationships for modeled predicted tissue concentrations versus water concentrations of selenium. Length and/or weight factors have shown promise as co-variants thus far. An empirical BAF approach requires less data, but it can only extrapolate to similar conditions, so this approach has limited utility in other areas. Predicted selenium concentrations in ovaries for Westslope cutthroat trout using this approach yield an initial steep slope followed by a nearly flat slope for much of the prediction range. Model fit for lotic Westslope cutthroat trout was weaker and there was some indication of bias among tissues; the much smaller range of tissue selenium in the lotic dataset may have contributed to the reduced model fit (Golder Associates 2010).

Orr et al. (2010) used a series of linear relationships for water-to-periphyton enrichment factors and trophic transfer factors (TTF) for higher-order food chain modeling based on the empirical data to develop relationships. The models are integrated to use as a predictive tool for modeling the relationship between tissue selenium concentrations and water selenium concentrations. The model, however, results in a relatively flat line slope following the initial steep slope at the low end of the selenium concentration relationship.

Debruyn compared outputs of the two models (Orr's and his). He found that for the lotic models, results were fairly comparable and tracked each other in a similar manner, but neither model was as strong as their corresponding lentic models. For both lotic models, the flat slope of the relationships suggests that at concentrations greater than about 8 mg/kg dw tissue, small changes in tissue concentration resulted in large changes in the predicted aqueous selenium concentration.

Presser and Luoma (2010) introduced a biodynamic model that quantifies each of the influential processes that links source inputs of selenium to toxicity. The USEPA has indicated in its updated Draft National Criterion for selenium that the Presser and Luoma approach will be cited as the method to derive the relationship between aqueous selenium and a tissue-based criterion. This model approach begins with the enrichment of selenium from surface water into the base of the food chain (algae/periphyton). Subsequent steps derive trophic transfer factors for higher order food chain relationships (Figure 6-3). Conventional methodologies relate dissolved or water column selenium concentrations and tissue selenium concentrations through simple ratios (i.e., bioconcentration factor [BCF], bioaccumulation factor [BAF]), regressions, or probability distribution functions (DuBowy 1989; Peterson and Nebeker 1992; McGeer et al. 2003; Toll et al. 2005; Brix et al. 2005; DeForest et al. 2007). Presser and Luoma (2010) state that none of these approaches adequately accounts for each of the important processes that connect selenium concentrations in water to the bioavailability, bioaccumulation, and toxicity of selenium.

The mechanistic modeling approach developed in Presser and Luoma (2010) makes use of biological processes and allometric equations similar to wildlife models used in ecological risk assessment to model uptake of chemicals via food chain bioaccumulation and predict a concentration in a receptor of concern. In lieu of available field data for bioaccumulation, one could use these allometric equations as well as some limited surface water, sediment, and algal data to derive enrichment and trophic transfer factors to predict concentrations of selenium in various components of the food chain. This approach becomes appealing when data are limited but a need to understand selenium in the environment exists. As a process, however, the model has applications that can inform less generic and more site-specific evaluations when utilized together with existing data for a site.

As noted above, the linear regression approaches appear limited in their accuracy for deriving relationships of aqueous selenium to tissue selenium. For this site-specific work at Smoky Canyon, empirical field data were collected over a series of seasonal conditions and multiple trophic levels, so a large data set is available for use. It is anticipated that the Presser and Luoma (2010) trophic model will be recommended by USEPA as part of their Revised Draft

National Criterion as a tool for deriving aqueous selenium to tissue relationships. As such, site-specific data and an accepted modeling approach provide the best tools available to investigate these relationships. This approach is described in more detail below.

6.4.2 Site-Specific Derivation of Aqueous Selenium and Tissue Selenium Relationships

The Presser and Luoma (2010) modeling approach is similar to a wildlife dietary uptake model. It allows for selenium to be modeled up from an aqueous concentration to higher trophic levels with some basic information on selenium concentrations in ambient surface waters, sediments, and algae. It provides approaches to derive the basic enrichment factors (K_ds) and TTFs if site data are limited. This project, however, has generated data for nearly every level of the food chain as well as concentrations in abiotic media. As such, the data for this Site are abundant and only the modeling processes (as opposed to more generic data) used in the Presser and Luoma (2010) modeling approach are needed to derive K_ds and TTFs used in the model as inputs. While Figure 6-3 shows a hypothetical selenium trophic bioaccumulation curve, Figure 6-4 shows an actual curve using Site-specific data. The sections that follow describe the methods used to derive the K_ds and TTFs presented in Figure 6-4.

Monitoring was conducted across two spring seasons and three fall seasons, providing a range of selenium concentration data in abiotic and biotic media, and covering an array of potential exposure conditions (Table 6-2) available for the derivation of K_ds and TTFs. South Fork Tincup Creek (SFTC-1) and Hoopes Spring (HS) data were not considered because: (1) data from SFTC-1 were collected from a location outside the phosphate mining area and thus do not represent exposures to varying ranges of selenium specific to the Crow Creek watershed, and (2) data from location HS were excluded as those data were collected near the primary spring, with no surrounding watershed, and represent very different conditions than those found in free flowing stream environments. The HS environment is not comparable to environments of the remainder of the watershed for which this criterion is being developed. Data from HS-3 at Hoopes Spring were used, as this location receives direct discharge from the spring about three quarters of a mile downstream of HS. The data from HS-3 incorporates selenium concentrations as well as watershed processes, providing a source area monitoring location with some comparability to other monitoring locales.

Effective use of the available site-specific information requires integration of the data into representative model inputs (K_ds and TTFs). Initially, the available data across all sites (except SFTC-1 and HS) and sampling periods (i.e., spring and fall) were integrated using geometric means. A geometric mean, unlike an arithmetic mean, tends to dampen the effect of very high or low values, which might bias the mean if a more common arithmetic average was calculated. However, the endpoint being evaluated (e.g., brown trout survival, the most sensitive endpoint developed) prompted consideration of potential seasonal differences, as well as differences between background and areas downstream of the source. The following subsections describe how the data were integrated to derive representative K_ds and TTFs.

6.4.3 Derivation of K_ds

Presser and Luoma (2010) define K_d as a partition descriptor for selenium from the aqueous to particulate fractions (e.g., algae, detritus, and sediments). Phase transformation reactions from dissolved to particulate selenium are of toxicological significance because particulate selenium is the primary form by which selenium enters food webs (Cutter and Bruland 1984; Oremland et al. 1989; Luoma et al. 1992). The different biogeochemical transformation reactions also result in different forms of selenium in particulate material—organo-selenium, elemental selenium, or adsorbed selenium—which in turn affects the bioavailability of selenium to invertebrates depending on how an invertebrate processes the complex water, sediment, and particulate milieu that composes its environment (Presser and Luoma 2010). Field observations and empirical data were used to quantify this relationship, which is expressed as:

$$K_d = C_{particulate}/C_{water}$$

Where

$C_{particulate}$ = selenium concentration in algae (periphyton), detritus, and/or sediments

C_{water} = selenium concentration in surface water (dissolved concentration)

Field data collected from 2006 to 2008 include selenium concentrations in both sediments and periphyton; therefore, K_ds can be derived for sediment and periphyton. Presser and Luoma (2010) suggest that if the data are available, averaging concentrations of selenium in sediment, detritus, biofilm, and algae may help to define K_d and take into account partitioning in different media and best represent the dynamic conditions present in an aquatic system. Bed sediments are the least desirable choice for calculating K_ds, especially if the sediments vary from sands to fine-grained materials, due to possible dilution of selenium concentrations from the high mass of inorganic materials (resulting in artificially low K_ds). For this site-specific assessment, however, selenium concentrations in sediments and surface waters from the same locale were strongly related, suggesting that there is some degree of partitioning of selenium to sediments from surface water that warrants its inclusion in deriving the K_d. Further, the K_ds derived using sediments for this site-specific assessment are not always lower than the K_ds derived for periphyton. Because periphyton is the primary selenium accumulator at the base of the food chain and some partitioning of selenium from the aqueous phase to sediments occurs, K_ds for both periphyton and sediment were developed.

Of the 42 analyses conducted for periphyton, a single sample resulted in no data due to insufficient material. A regression relationship of dissolved aqueous selenium to periphyton selenium concentration was used to predict the missing periphyton concentration at the measured aqueous selenium concentration (Figure 6-5, R^2 = 0.68). No sediment data were missing; therefore, regression modeling was not needed to predict missing data.

6.4.4 Integrating K_ds

Presser and Luoma (2010) recommend performing calculations with several alternatives that represent plausible, site-specific choices for K_d to elucidate and constrain the uncertainty around the introduction of K_d. For this effort, three different K_d values were derived, including: mean K_d = mean of periphyton K_d and sediment K_d for a location; 75:25 K_d = 75 percent periphyton, 25 percent sediment for a location; and periphyton K_d = 100 percent periphyton for a location (Table 6-3). Location-specific K_ds were then averaged using a geometric mean to derive K_ds for each of the scenarios presented above for background Crow Creek and Deer Creek areas, Hoopes Spring and Sage Creek, and downstream Crow Creek areas (Table 6-3).

For periphyton data in this site-specific study, K_ds derived from the different locations were: Sage Creek and Hoopes Spring (647), Deer Creek (1,826), and Crow Creek upstream (1,562) and Crow Creek downstream (1,373) of Sage Creek. The geometric mean of all of the individual K_ds was 1,226, with an overall range from 187 to 6,214. For comparison, data provided in Presser and Luoma (2010) indicate K_ds for four locations in the Crow Creek watershed: Sage Creek (494), Deer Creek (2,250), and Crow Creek upstream (1,818) and downstream of Sage Creek (657). The geometric mean value for these data is 1,073. It appears that these K_ds were derived for periphyton as the sole contributor to the particulate fraction.

Integrating sediment and periphyton K_ds can be accomplished through derivation of a simple average of K_ds. Due to the number of samples collected during the nearly 3-year monitoring effort, a range of K_ds are available that vary within a location as well as between locations. To integrate this range of potential exposure conditions, the average site K_ds (periphyton and sediments) were averaged across each location and time period to generate a mean K_d for the overall site. The overall mean K_d was derived as a geometric mean of the site K_ds. Location-specific K_ds were as follows: Sage Creek and Hoopes Spring (516), Deer Creek (1,527), and Crow Creek upstream (1,239) and Crow Creek downstream (997) of Sage Creek. The geometric mean of all of the individual K_ds was 939.

Presser and Luoma (2010) state that sediment may dilute selenium in the particulate fraction. Site-specific data indicate a relationship of aqueous selenium to sediment selenium, suggesting that selenium does, to some degree, partition to the sediments. Another alternative to using a straight average of periphyton and sediment concentrations to derive K_d is to utilize a ratio of selenium partitioning. These K_ds were derived as a percentage of periphyton and sediments, where 75 percent of the particulate selenium was attributed to periphyton and 25 percent was attributed to sediment. Because selenium in periphyton is the primary factor influencing selenium uptake in the food chain, it was given more weight. Each of these fractions was then summed to arrive at a location and time-period-specific K_d. Using this approach, location-specific K_ds ranged as follows: Sage Creek and Hoopes Spring (588), Deer Creek (1,709), and Crow Creek upstream (1,409) and Crow Creek downstream (1,254) of Sage Creek. The geometric mean of all of the individual K_ds was 1,092.

6.4.5 Derivation of Trophic Transfer Factors (TTFs)

A key aspect of selenium risk is bioaccumulation (i.e., internal exposure) in prey and predators (Luoma and Rainbow 2005). Just as the K_ds were used to describe partitioning of selenium at the basal layers of the food chain, TTFs are derived to describe the accumulation of selenium from lower trophic levels to upper trophic levels. They link particulate, invertebrate, and predator selenium concentrations. TTFs differ from traditional BAFs in that BAFs are almost always implemented as the selenium concentration in an animal relative to selenium in water, whereas the TTF is the selenium concentration in the animal relative to the selenium concentration in its prey.

Due to the large amount of data collected for this project, measured concentrations of selenium in organisms from different trophic levels provide the most direct data available for selenium bioaccumulation. Benthic macroinvertebrates, sculpin, and trout were collected within 24 hours of one another at each location during each of the seasonal monitoring events. These data are also paired with the site-specific surface water, sediment, and periphyton data.

Invertebrates

For benthic invertebrates, composite community samples were collected, representing a cross-section of the resident benthic invertebrate community. Of the possible 42 data points (i.e., benthic community selenium tissue samples), 4 were missing and one was eliminated as an outlier. These five data points were predicted using a linear regression of the selenium concentration of the assumed diet for invertebrates (90 percent periphyton and 10 percent sediment) to benthic tissue selenium concentrations. The regression relationship (R^2 = 0.51) was used to predict missing benthic tissue concentrations (Figure 6-6). Selenium concentrations were derived from a multi-species sample. From these field collected data, a site-specific $TTF_{invertebrates}$ was derived as followed:

$$TTF_{invertebrate} = C_{invertebrate}/C_{particulate}$$

Where

$C_{invertebrate}$ = selenium concentration (mg/kg dw) in benthic macroinvertebrates

$C_{particulate}$ = selenium concentration (mg/kg dw) in particulate materials

The $C_{particulate}$ term is the sum of 10 percent sediment selenium and 90 percent periphyton selenium concentrations. As noted previously, the average of sediment and periphyton concentrations were used in the derivations of K_d. For benthic invertebrates, an assumption was made that the bulk of their selenium intake was through ingestion of selenium-containing periphyton. Using this approach, a range of $C_{particulate}$ for invertebrates was derived for each location and seasonal sample. Benthic invertebrate tissue selenium concentrations were divided by the particulate fractions of selenium in periphyton and sediment as indicated above. Again, similar to derivation of the K_ds, geometric means for $TTF_{invertebrate}$ were derived for different locations in the drainage (e.g., Hoopes and Sage Creek, and downstream Crow Creek, etc.). Site-specific $TTF_{invertebrate}$ derived for composite invertebrate tissues ranged from 0.83 to 9.92. The geometric mean $TTF_{invertebrate}$ varied among drainages depending upon differences in selenium exposure: Sage Creek and Hoopes Spring (1.9), Deer Creek (2.5), and Crow Creek upstream (3.9) and Crow Creek downstream (2.6) of Sage Creek. The geometric mean of all of the individual $TTF_{invertebrate}$ is 2.7.

For comparative purposes, Luoma and Presser (2010) presented a range of $TTF_{invertebrate}$ for freshwater invertebrates including amphipod (0.9), zooplankton (1.5), daphnia (1.9), stonefly (2.6), mayfly (2.7), chironomid (2.7), average aquatic insects (2.8), caddis fly (3.2), and aquatic insect composite (3.2).

Sculpin

Sculpin are ubiquitous throughout the monitoring locations, but are more abundant at some locations than others. Sculpin are important components of fish assemblages in the Western US (Quist et al. 2004), are native species, and often numerically dominate fish assemblages of streams of the interior Rocky Mountain region (Baily 1952; Jones 1972 [cited from Quist et al 2004]). They represent a secondary consumer in the food chain and are primarily benthic invertivores, although they have been documented to be cannibalistic (Johnson 1985). For this assessment, the $TTF_{sculpin}$ was derived using benthic invertebrates as the primary food source. The $TTF_{sculpin}$ was derived as follows:

$$TTF_{sculpin} = C_{sculpin}/C_{invertebrate}$$

Where:

$C_{sculpin}$ = mean selenium concentration in sculpin from a location and time period (mg/kg dw)

$C_{invertebrate}$ = selenium concentration in benthic invertebrates (mg/kg dw) from the same location and time period

The $TTF_{sculpin}$ was derived by dividing the measured selenium concentration in sculpin tissues (arithmetic average for a location) by the selenium concentrations in benthic invertebrates from the same location for each seasonal sample. Geometric means for $TTF_{sculpin}$ were derived for different grouped locations as presented in Table 6-3.

Presser and Luoma (2010) presented some limited data for mottled sculpin in Idaho (Upper Blackfoot River), with $TTF_{sculpin}$ ranging from 1.23 to 1.66 and a mean value of 1.45. Derived site-specific $TTF_{sculpin}$ are as follows: Sage Creek and Hoopes Spring (1.2), Deer Creek (1.0), and Crow Creek upstream (1.4) and Crow Creek downstream (1.2) of Sage Creek. The geometric mean of all of the individual $TTF_{sculpin}$ is 1.2.

Trout

Finally, TTF_{trout} was derived to describe the transfer of selenium via the dietary pathway to a top-level predator, in this case brown trout. Two different types of TTF_{trout} variables were derived, including one for the transfer of selenium via the diet to adult trout whole body tissues, and one for the transfer of selenium via diet in maternal adults to eggs tissues. Both represent similar transfers from the dietary component, albeit with different tissue types (i.e., whole body or egg tissues).

Adult brown trout are opportunistic feeders. The diets of brown trout have been described as “diversified,” and their food habits range broadly with variation in size and age, spatial and temporal variability in food availability, behavior, and habitat characteristics (Simpson and Wallace 1982, Bachman 1991, Baxter and Stone 1995, Bridcut and Giller 1995). Adult brown trout are considered to be primarily piscivores as adults, and while they continue to consume macroinvertebrates, size selection of the prey items increases as the fish matures. With the exception of extremely productive systems that produce dense populations of aquatic invertebrates, most larger brown trout (> 310 mm [12.2 inches]) inhabiting large streams, rivers, and lakes are thought to transition from a diet composed predominately of invertebrates to one comprised mainly of fish and crayfish (Bachman 1991). Certainly, the ratio of forage fish to invertebrates in the brown trout diet will vary with fish size, brown trout gape size, and prey type and availability, among other factors. As trout size increases, the proportion of fish in the diet would logically be expected to increase. By the time adults reach a size of about 16 to 18 inches or larger, one would expect that the proportion of fish in their diet to exceed 50 percent, especially if fish as prey are readily available. The brown trout HSI (Raleigh et al. 1986) states that at 25 cm (~8 inches), fish as prey items will begin to enter the adult brown trout diet. Other considerations for the trout diet include the proportion of the invertebrates in the diet that are terrestrial, crustaceans such as crayfish, and/or freshwater shrimp.

The literature base describing brown trout diets is as varied as the different diets reported. To account for this, TTF_{trout} includes a mixed diet proportion of sculpin (i.e., forage fish) and invertebrates for adult brown trout. The equation to derive TTF_{trout} is described below:

$$TTF_{trout} = C_{trout}/\left(0.5 \times C_{sculpin}\right) + (0.5 \times C_{invertebrate})$$

Using this approach, the TTF_{trout} for adult brown trout ranged from 0.6 to 1.82.

TTF_{trout} can also be derived based on the dietary transfer of selenium to egg tissues (hereafter referred to as $TTF_{trout\ eggs.}$ Yellowstone cutthroat trout data from Hardy's (2005, 2010) study were used to develop $TTF_{trout\ eggs}$ based on the diet-to-egg trophic transfer factor. These data were developed from a 2.5 year study of maternal selenium transfer using YCT. Because the diet was controlled, and bioaccumulation was measured during the course of this study for a range of exposures, a representative TTF for the diet to eggs can be derived. The geometric mean value for the $TTF_{trout\ eggs}$ equals 1.0 (Table 6-4).

6.4.6 Derivation of the Aqueous Selenium Concentration from Tissue Concentrations

The previous sections identified model components, described how each component was derived, and used site-specific data to derive each model component. Below, the equation for derivation of an aqueous value based on effects in eggs is presented.

$$C_{water} = \frac{C_{trout\ tissue}}{K_d \times [(TTF_{sculpin}\ x\ 0.5) + (\ TTF_{invertebrate}\ x\ 0.5)] \times TTF_{trout\ eggs}}$$

Where

C_{water} = dissolved aqueous concentration of selenium (mg/L)

$C_{trout\ tissue}$ = target egg selenium threshold for brown trout (20.8 mg/kg dw)

K_d = selenium concentration (mg/kg dw) in particulate materials / dissolved selenium concentration in water [$C_{particulate}$ (periphyton, detritus, sediments) / C_{water}] (L/kg dw)

$TTF_{sculpin}$ = 50% weighted diet proportions for adult trout (0.5 * $TTF_{sculpin}$)

$TTF_{invertebrate}$ = 50% weighted diet proportions for adult brown trout (0.5 * $TTF_{invertebrate}$)

$TTF_{trout\ eggs}$ = TTF representing the transfer of dietary selenium to trout tissues ($TTF_{trout\ eggs} = C_{trout\ tissue} / C_{diet}$).

$C_{trout\ tissue}$ is the concentration of selenium in the top predator fish that is the final bioaccumulator in the food web. This term is based on tissue concentrations in trout and can be tissue-specific (i.e., whole body, muscle, egg/ovary, etc.). To derive an aqueous selenium concentration based on an egg effects threshold, the tissue concentration represents the target threshold in eggs for brown trout. Based on the work previously presented, the target egg threshold is an EC_{10} = 20.8 mg/kg dw.

To derive the aqueous selenium concentration using the above equation, several different input variables are possible depending upon how data are integrated. As indicated earlier, three different types of K_ds were derived to evaluate the range of possibilities, including: a periphyton-based K_d, K_d derived using the ratio of 75 percent periphyton and 25 percent sediment, and K_d derived as the arithmetic average of the sediment only K_d and periphyton only K_d.

In addition, a variety of different approaches exist to integrate seasonal data collected from a range of different locations and exposure conditions. Careful consideration of logical food web linkages, critical exposure periods, and exposure locations must be made in order to derive a representative aqueous value. As noted previously, diets used for the various TTFs derived were based on logical and reported information about species dietary preferences. To account for seasonal differences, the fall data were used to focus on periods when brown trout are forming eggs and being exposed to selenium concentrations that may ultimately affect maternal transfer of selenium to developing embryos. Locations evaluated also affect how data are integrated, in that data from both background and downstream areas were available. Because data from the downstream areas have selenium concentrations that are elevated due to source inputs, the data integration process focused on downstream areas. Table 6-5 shows the range of aqueous selenium values using the above equation.

6.4.7 Validation of Model Inputs

In an attempt to validate the realism of the model (i.e., observed versus predicted), the model was used to predict aqueous selenium concentrations using the site-specific input data. Two variables were substituted in the equation shown above. First, in the validation model, $C_{trout\ tissue}$ was set equal to the concentration of selenium in whole body trout tissues from each location. Recall that $C_{trout\ tissue,}$ as used above, was equal to the egg effects threshold for selenium derived from the site-specific brown trout studies. Second, instead of using $TTF_{trout\ eggs}$, which was the transfer factor for diet to eggs, it was substituted with TTF_{trout}, the transfer factor for diet to whole body trout tissues. By substituting these two variables into the equation, aqueous selenium concentrations for each location and time period sampled can be predicted based on the field-derived data to evaluate if the model inputs are representative in predicting an aqueous selenium concentration that approximates the measured actual aqueous selenium concentration. The model takes the following form:

$$C_{water} = \frac{C_{trout\ tissue}}{K_d \times [(TTF_{invertebrate} \times 0.5) + (TTF_{sculpin}\ x\ 0.5)]\ \mathrm{x}\ TTF_{trout}}$$

Where

C_{water} = dissolved aqueous concentration of selenium (μg/L)

$C_{trout\ tissue}$ = mean trout tissue selenium concentration, includes brown trout and YCT (μg/g dw)

K_d = selenium concentration (mg/kg dw) in particulate materials / dissolved selenium concentration in water [$C_{particulate}$ (periphyton, detritus, sediments) / C_{water}] (L/kg dw)

$TTF_{sculpin}$ = 50% weighted diet proportions for adult trout (0.5 * $TTF_{sculpin}$)

$TTF_{invertebrate}$ = 50% weighted diet proportions for adult brown trout (0.5 * $TTF_{invertebrate}$)

TTF_{trout} = C_{trout} / ([$C_{sculpin}$ * 0.5 + $C_{invertebrate}$ * 0.5])

As noted previously, K_d can vary depending upon how data for sediment, periphyton, and other potential components of the particulate selenium fraction are integrated. In Table 6-5, three different approaches for deriving K_d were presented. Ultimately, the K_d selected was based on 100 percent periphyton, primarily because as the base of the food chain, selenium is entering the food web primarily through algal uptake. And, while sediment selenium concentrations clearly show a strong relationship to dissolved aqueous selenium concentrations, suggesting that there is some attenuation to sediments and microbial processes that may be affecting selenium accumulation, as Presser and Luoma (2010) point out, there is the potential for a sediment “dilution” effect. To minimize this dilution effect, the simplest solution is to use a K_d based solely on periphyton uptake.

Another factor in the derivation of an aqueous selenium concentration using the Presser and Luoma model is consideration of the spatial and temporal composition of the data. For these site-specific data, both background and downstream locales were monitored. Because data from the downstream areas have selenium concentrations that are elevated that are more likely to pose effects, than selenium found in background, the data integration process focused on downstream areas. Finally, although two seasonal periods were monitored, key exposure periods for brown trout prior to and during spawning occur in the fall. Data were focused to inclusion of the fall data only.

Using this approach, an aqueous selenium concentration was predicted and compared to the actual measured concentration for each location via a linear regression. Logically, if observed values equal predicted values, the R^2 would be 1.0. In this case, using the model described, the predicted selenium in surface water versus the observed selenium in surface water yields a R^2 of 0.9 (Figure 6-7). The strength of this relationship suggests that the model assumptions of diet for the top predator, uptake fractions for benthic invertebrates, and periphyton as the primary K_d provide representative model inputs. Additional validation was conducted by limiting the data utilized in the regression to downstream data which resulted in an $R^2 = 0.87$ (Figure 6-7). Finally, the data were limited to only the fall data from the downstream areas which resulted in an $R^2 = 0.87$ for predicted aqueous selenium versus measured aqueous selenium (Figure 6-8).

Another validation test was performed by using the model input variables to predict trout concentrations using the bioaccumulation data and comparing those concentrations to observed mean trout concentrations for a particular location. The correlation coefficient (R^2) for the measured trout concentration relative to the predicted trout concentration was 0.83 (Figure 6-9).

6.4.8 Aqueous Selenium Concentration Derived from Tissue Selenium Concentrations

An aqueous selenium concentration was derived using a biodynamic food web model based on site-specific data for food web components. It included a sensitive species (brown trout), and a sensitive effects threshold (EC_{10}). The effect threshold utilized is an EC_{10} value for egg selenium concentrations for brown trout alevin survival, an endpoint that has been demonstrated to be sensitive (i.e., survival was as sensitive as deformities in predicting effects). This approach translates to an aqueous selenium value of 15.4 µg/L. The aqueous selenium value is conservative not only due to the use of an EC_{10} as the basis for derivation, but additional conservatism is included in the derivation of this value by using the 100 percent periphyton K_d. Higher K_d values tend to lower aqueous selenium concentrations using this calculation method and K_ds derived based on the assumption that the particulate fraction is comprised completely of periphyton increases the K_d.

A unique issue presents itself with the use of an aqueous trigger value. It should not be too sensitive such that tissue monitoring is triggered unnecessarily nor should it be insensitive such that if tissue monitoring is triggered, the whole body or egg tissue thresholds are already exceeded. As noted previously, the trigger value is inherently conservative since it is derived based on an EC_{10} value and uses 100 percent periphyton as the K_d. Through the validation process, it was noted that the variables used to derive an aqueous selenium concentration using the Presser and Luoma model tended to predict aqueous selenium concentrations that were generally higher than the actual measured aqueous selenium concentrations. Recall that the validation model did not specify a target threshold (20.8 mg/kg dw), but used the actual measured mean trout tissue concentrations from the field. The average predicted aqueous selenium minus actual dissolved selenium equals 4.4 µg/L (for positive values only, two values were negative indicating under prediction). A linear regression of log transformed predicted aqueous selenium versus actual aqueous selenium concentrations of fall only data for downstream areas yields a R^2 = 0.87 (Figure 6-8). As shown the quality of the relationship suggests that a practical predictive relationship could be used to derive the actual measured aqueous selenium from a predicted concentration. Substituting the aqueous trigger value (15.4 µg/L) into the equation shown in Figure 6-8 and solving for x results in a value of 10.8 µg/L. Using two different approaches, two similar values emerge (11 and 10.8 µg/L), essentially yielding a value of 11 µg/L. At an aqueous selenium concentration of 11 µg/L, the trigger value should be adequately conservative to trigger more in depth tissue monitoring prior to the actual target value of 15.4 µg/L without prematurely triggering tissue monitoring.

The derived aqueous value is based on a dissolved selenium concentration, and site-specific data indicates that dissolved selenium typically comprises nearly 100 percent of the total selenium fraction in surface water; therefore, no translation of dissolved to total selenium is warranted.

6.5 Proposed Chronic Value

Throughout this TSD, reference has been made to two particular effects thresholds, the EC_{10} and EC_{20}. The first draft of this TSD (i.e., Interpretive Report) proposed an EC_{20} as the site-specific criterion for this project. The USEPA has made it clear that they intend to propose an EC_{10} in their Draft National Criterion, which includes the brown trout data developed as part of this study. In comments received from USEPA on an earlier draft of this Interpretive Report, EPA stated that for this project, the EC_{10} is a more appropriate endpoint than the proposed EC_{20} in developing a site-specific criterion for the Smoky Canyon site. Their primary rationale was that as a bioaccumulative pollutant that accumulates in fish tissue, concentrations in fish tissue are more stable over time than aqueous selenium concentrations. This stability may lead to concentrations that are just below the criterion for extended periods of time. A number of strong arguments were presented as to why an EC_{20} was appropriate for this Site. However, to accommodate USEPA policy and to provide an additional margin of safety, the proposed criterion for this site is based on the EC_{10}.

The brown trout EC_{10} (20.8 mg/kg dw egg selenium) for survival (hatch to test end) is proposed as the Site-specific criterion. The proposed value is supported by the three primary lines of evidence evaluated for the study: Site-specific laboratory studies, field studies of aquatic communities and populations, and literature on toxicity of selenium to cold-water fish. Rationale for selection of this value is provided below.

1. The life stage for the criterion is appropriate. Embryo-larval effects resulting from maternal transfer of selenium from the parent fish have been shown to be the most sensitive life stage, as opposed to juvenile or adult life stages.

2. A tissue-based criterion is consistent with the state of the science. Early on, investigators pursued and actively researched relationships of a variety of different fish tissues from different life stages of fish. The mounting body of evidence supports the tissue threshold approach. Whole body tissues were initially advocated as a basis for USEPA's 2004 national criterion. USEPA has since revised its position. Based on new research, its current position is that the best predictor of selenium toxicity is the egg/ovary tissue.

3. The species tested is sensitive. Based on the current Site-specific studies and literature for similarly conducted maternal transfer studies, brown trout young are sensitive to the effects of selenium. Of the cold-water species tested, brown trout are one of the more sensitive species, with ECs falling below those for most other trout species (Figure 6-1). Compared to benthic invertebrates, brown trout also tend to be more sensitive relative to effects of selenium (Figure 6-2). The brown trout is an appropriately sensitive species protective of the larger aquatic community assemblage.

4. The endpoint is biologically relevant. Survival directly affects individuals and populations. Selenium literature has focused on larval deformities as a sensitive endpoint, rationalizing that individuals with severe enough deformities ultimately do not survive, affecting population dynamics. In the brown trout studies, the survival endpoint, measured from hatch to test termination, was slightly more sensitive in terms of predicted effects levels than the deformity endpoint.

5. Site and species specificity requires no extrapolation. Effects levels and criteria are often based on species and conditions that may not represent site conditions (e.g., laboratory studies of surrogate species, warm- versus cold-water environments and species, non-representative exposure pathways). The site and species specificity of this study eliminates many, if not all, of these variables.

6. Field observations of abundance, population structure, and diversity over the same range of exposure conditions utilized for the laboratory studies indicate that the EC_{10} derived for individual brown trout survival is conservative with regard to population effects and is protective of other aquatic species.

7. The EC_{10} provides a margin of safety for aquatic communities present at this Site. Consideration of the effects data generated for both brown trout and YCT, as well as the population and community data for these species and other species present at the Site, indicates that the EC_{10} is a protective threshold for the aquatic community. The brown trout EC_{10} is lower than the EC_{10} for YCT derived from Site studies.

In combination, the three primary lines of evidence considered for this Site-specific study provide a very large and detailed body of information upon which to select a criterion. Importantly, the three lines of evidence converge to support the proposed criterion. The information comprising these lines of evidence provides the appropriate level of scientific assurance that the proposed criterion is conservative and thereby provides an adequate margin of safety for the aquatic community.

7.0 IMPLEMENTATION CONSIDERATIONS

The preceding sections of this report have laid a foundation for a chronic selenium criterion that is different than the current State of Idaho selenium criterion (0.005 mg/L) in surface water and the current Draft National Criterion (7.91 mg/kg dw) in whole body fish tissue. In developing this Site-specific selenium criterion, three lines of evidence were considered, including field monitoring results, laboratory toxicity studies, and relevant literature that describes the varying levels of effects of selenium on different species. Based on these lines of evidence, Site-specific effects concentration were derived in the form of effects concentrations (EC_{10} and EC_{20}) using brown trout eggs exposed to selenium via maternal transfer from wild-collected females. Quantitative measures for selenium effects to eggs and developing young trout were derived resulting in EC_{10} and EC_{20} values of 20.8 and 23.1 mg/kg dw, respectively. These egg effect thresholds are based on a survival endpoint of eggs through the duration of a 75 day test. To provide a margin of safety in the effects thresholds derived, the EC_{10} was selected as the site-specific chronic selenium criterion.

While egg tissue has been demonstrated to be one of the most important tissues in fish when evaluating selenium effects, it is not the most practical tissue to monitor. Collecting egg tissue requires that fish be monitored at a specific time of year (i.e., during or just prior to spawning), and requires excision of eggs from maternal fish. In addition, while the general spawning period may be known, capture of pre-spawn female fish requires a larger effort than, for example, simple collection of fish for whole body tissue analysis. Monitoring egg tissues is not a practical long-term assessment approach to gauge compliance or non-compliance with a criterion, although occasional monitoring of egg tissue may be required. A sustainable and more practical monitoring approach is needed in order to effectively utilize a criterion based on effects in eggs. Given the issues and complexities associated with monitoring egg tissues, other viable monitoring alternatives were evaluated that could be used as thresholds or triggers for implementation of a more involved egg tissue monitoring. Alternatives examined included relationships of selenium in eggs to selenium in whole body tissues, and relationship of selenium in eggs to aqueous selenium concentrations.

In developing the egg effects threshold, concentrations of selenium in whole body maternal fish were collected for analyses to correspond to their egg tissue selenium analyses. This was done for both brown trout and YCT. Section 5.4 of this TSD derived the relationship of egg selenium from their maternal parent to the maternal parent whole body selenium concentrations. The resulting relationship showed a strong relationship (R^2 = 0.79) of the log-transformed data for brown trout and a similar relationship for YCT (R^2 = 0.76). However, there is a certain amount of variability (i.e., 20 percent) in these estimates that is not explained by the relationships. An egg to whole body translation can be used as a predictive tool to estimate whether the egg tissue criterion is being achieved using this relationship; however, the ultimate criterion is the derived egg tissue value. While whole body fish tissue is a more practical monitoring tool than egg tissue, it is still a less efficient evaluation mechanism, due to demands on personnel to gather data and over-utilization of the fish resources (i.e., repeated monitoring of fish tissues may diminish the resource in small streams).

Current State of Idaho standards for toxics are typically represented by numeric threshold values which represent not-to-be-exceeded values based on some averaging period associated with the specific type of standard, either chronic or acute. For metals and metalloids, the standard for a specific parameter is based on either dissolved or total concentrations of that parameter in ambient surface waters. Virtually all State of Idaho standards, excepting mercury, are water quality-based standards. The mercury standard is a fish tissue-based standard that targets a threshold for human health (i.e., methylmercury) that is deemed protective of aquatic life as well. Thus, at the State level, compliance with standards is predominantly based on comparison of monitoring data to a numeric aqueous concentration. Likewise, for selenium, the most practical and direct means of monitoring selenium in the environment and gauging compliance will be through development of an aqueous value, but direct relationships between selenium in tissues and water lack adequate precision.

At the National level, the soon to be released USEPA Draft National Criterion for selenium is based on an egg/ovary concentration effects threshold. It is expected to include (as reported by Dr. Charles Delos, at USEPA headquarters) a surface water value designed to be a trigger value that indicates when more involved tissue monitoring should take place. The actual criterion will be based on effects as measured in eggs or ovaries. Because pre-spawn monitoring of eggs or ovaries is not always practical, the National criterion will also include a translator to derive a whole body tissue value from the egg tissue criterion.

Using surface water selenium concentrations as a routine monitoring approach will allow for a less destructive form of monitoring (i.e., as compared to fish tissue sampling) to initially gauge compliance with regulatory mechanisms. During the course of monitoring aqueous selenium concentrations, if the aqueous selenium trigger value is not exceeded, then one would conclude that the egg selenium concentrations do not exceed the site-specific egg selenium criterion. Aqueous selenium measurement in ambient surface waters used as a monitoring tool effectively becomes a trigger for additional monitoring for fish tissues if needed.

7.1 Implementation Approach

Consistent with our understanding of the soon to be released National Criterion, the criterion proposed herein is based on effects in young developing fish relative to the egg tissue selenium concentrations. To implement the proposed brown trout egg criterion of 20.8 mg/kg dw, a tiered approach is described that will provide clear targets by which to assess compliance, and will provide regulated entities with clear requirements for monitoring. Because of the level of effort required to monitor egg selenium concentrations, two relationships will be useful to the implementation process, including: (1) a process to derive an aqueous selenium concentration or trigger value from the egg criterion value; and (2) translation from egg selenium concentrations to whole body selenium concentrations. An aqueous trigger value is proposed for selenium (based on the egg effect criterion) as a measure by which rapid evaluations can be made to assess whether more in-depth monitoring is needed. An egg to whole body translation relationship is also provided if more in-depth monitoring is needed. Subsequent steps are included in a decision tree procedure should the aqueous trigger value be exceeded. Figure 7-1 graphically illustrates this decision tree. Monitoring environmental media to gauge compliance includes identifying where, when, and what types of samples will be collected. Consideration of location, the frequency of sampling, number of samples, and how the data are integrated all affect how the data are used to gauge compliance and ultimately protect downstream uses.

The subsections that follow present key components of how to effectively implement this chronic selenium criterion that is based on an egg effects threshold. Important considerations included in these components include:

- Monitoring and assessment strategies;
- Protection of beneficial uses including downstream uses; and
- Geographic range of applicability.

7.1.1 Routine Aqueous Selenium Monitoring

In Section 6.4 of this document, the relationship between aqueous selenium and tissue selenium concentrations was evaluated, and a numeric aqueous selenium concentration was derived based on those relationships. This concentration (15.4 µg/L) is based on the proposed EC_{10} egg tissue criterion for brown trout alevin survival. The Presser and Luoma (2010) biodynamic food web model was used to integrate trophic transfer of selenium through the aquatic food web to derive a concentration of selenium in aqueous media. In effect, if concentrations of selenium are less than 15.4 µg/L in surface water, then egg concentrations should be less than 20.8 mg/kg dw. Through the validation process, where observed versus predicted values were regressed against one another, the aqueous value was back calculated by inserting 15.4 µg/L as a predicted value into the regression equation to predict an actual value. Based on this linear regression approach and a general observation that the model slightly over predicts the aqueous concentration, the aqueous trigger value proposed is 11 µg/L. Use of this aqueous trigger value (i.e., 11 µg/L) serves as the first level of routine monitoring from which to assess compliance with the proposed Site-specific criterion.

7.1.1.1 Monitoring Locations and Purpose

Water quality varies within each of the Site drainages. The purpose of the monitoring locations described below is to provide representative selenium measurement data in aqueous media. Due to the record of selenium measurement data in surface waters at several locations, and consistent with current Mine permit monitoring, the following locations are proposed as routine monitoring locations for surface water. Locations at Hoopes Spring (HS) and the South Fork Sage Creek Springs complex (SFSCS) will be monitored on a monthly basis consistent with the mine monitoring permit to provide continued measurement data on changing conditions associated with these springs. Locations downstream of these springs will be used as compliance monitoring points.

- **HS-3:** Hoopes Spring channel near the confluence with Sage Creek – Monitor surface water downstream of the primary source area at HS.

- **LSS:** Lower South Fork Sage Creek – Monitor surface water downstream of the secondary source area at the SFSCS complex.

- **LSV-2C:** Sage Creek downstream of Hoopes Spring – Monitor receiving waters from the primary source area at HS.

- **LSV-4:** Sage Creek near confluence of Sage Creek and Crow Creek – Monitor Sage Creek receiving waters downstream of both Hoopes Spring and SFSC.

- **CC-350:** Crow Creek upstream of Sage Creek and downstream of Deer Creek – Monitor potential influence of Deer Creek on Crow Creek (Panels F and G).

- **CC-1A:** Crow Creek downstream of Sage Creek – Monitor Crow Creek selenium concentrations downstream of Sage Creek as an indication of whether exceedances are occurring in Crow Creek.

- **Crow Creek at the State Line:** Monitor a location to be determined immediately upstream of the Idaho-Wyoming state line to evaluate whether selenium concentrations in Crow Creek are exceeding the trigger value prior to crossing the Idaho-Wyoming state line.

7.1.1.2 Timing and Frequency of Measurements

Quarterly surface water monitoring is proposed as part of this implementation plan. Two key time periods are proposed: spring (April or May) and fall (August or September), with the other two monitoring events spaced between the spring and fall events.

Currently, a chronic criterion exceedance occurs when the four-day average exceeds the numeric chronic value. For a bioaccumulative chemical such as selenium, a short-term exposure (e.g., days) at or below the aqueous trigger value is not likely to be of sufficient duration for selenium to bioaccumulate to a level that is adverse. Thus, the time frame for gauging compliance needs to be lengthened in order to produce meaningful results. If selenium concentrations in surface water exceed the trigger value at a particular location, monitoring at each location where an exceedance occurred would be immediately followed by four weeks of monitoring (once per week). During quarterly monitoring, normal laboratory turnaround times are acceptable. During monitoring triggered by the initial exceedance, laboratory turnaround times would be seven days or less.

7.1.1.3 Aggregation of Data to Assess Compliance

Surface water quality data for selenium measured during the four-week period will provide the analytical data to assess whether or not the aqueous trigger value has been exceeded. A single grab sample at a location does not constitute a viable measure of exceedance given the time necessary for elevated selenium concentrations in surface water to bioaccumulate. The average of the four consecutive week grab samples will be used as a measure of exceedance.

If the aqueous trigger value is exceeded in Hoopes Spring, South Fork Sage Creek, Sage Creek, or Crow Creek, managers can decide to proceed to tissue monitoring for whole body selenium concentrations, egg tissue monitoring, or to corrective action. The decision to move forward to fish tissue monitoring or straight to corrective action will be dependent on the magnitude of the exceedance. For example, if a high level exceedance of the surface water trigger value occurs during fall monitoring, the decision tree allows for moving directly to egg tissue monitoring, rather than wasting budget resources to monitor whole body tissues only to discover that egg tissue monitoring is warranted. If there is no exceedance of the aqueous trigger value, then compliance has been achieved and routine surface water monitoring would continue.

7.1.2 Whole Body Tissue Monitoring

If the aqueous trigger value is exceeded, based on the approach presented above, additional monitoring to collect whole body brown trout tissues to compare with a translated egg to whole body tissue concentration can be conducted. This step allows for tissue monitoring to be conducted at any time during the year. In Section 5.5 of this document, a translator to derive whole body tissue selenium based on egg tissue selenium was presented. The relationship of egg selenium concentrations to selenium in whole body tissue can be used to translate egg concentrations to whole body concentrations. While this relationship is fairly strong (R^2 = 0.8), the proposed criterion is still based on egg tissue concentrations. It does, however, provide a means by which to gauge selenium concentrations in the field, using a more readily obtainable tissue.

Details for monitoring whole body fish tissue will be compiled in a Work Plan prepared prior to field efforts. Details of the monitoring plan should be fully vetted and approved by Agency personnel so there is no delay in monitoring, should it be necessary.

7.1.2.1 Monitoring Locations and Purpose

Whole body fish tissues would be collected if the aqueous trigger value is exceeded at each location where the exceedance occurs. These locations were identified in Section 7.1.1.1 above.

7.1.2.2 Timing and Frequency

High flow monitoring is difficult, can be unsafe, and can result in low capture efficiency. If the aqueous trigger value is exceeded during the spring monitoring event, tissue monitoring during high flow periods may have to be postponed until flows subside to a workable level. Fall quarterly monitoring is planned such that fish tissue monitoring could be conducted in October or November to check whether whole body or egg selenium concentrations exceed the whole body tissue levels derived from the criterion, or if eggs are sampled, the criterion itself.

7.1.2.3 Fish Size and Gender

For whole body tissue analyses, the size of the fish should be consistent with the Interagency Fish Tissue Protocol, which focuses on collection of juveniles (< 100 mm and, if need be, up to 150 mm). This size class is expected to be resident to the locale where collected. No gender specificity is required for juvenile fish destined for whole body analysis. Juvenile fish can be sampled during any time period.

7.1.2.4 Number of Measurements

From each location where the aqueous trigger value is exceeded, ten (10) brown trout should be collected for selenium tissue analysis. All ten trout should be the same species.

7.1.2.5 Aggregation of Data to Assess Compliance

Whole body fish tissues will be analyzed for individual specimens. Analytical data for tissue measurements will be aggregated using typical summary statistics (mean, median, maximum, minimum, standard deviation, and upper and lower 95 percent confidence intervals).

If the lower 95 percent confidence interval of the whole body selenium mean indicates a tissue concentration greater than the whole body tissue level translated from the egg criterion, then there is a possibility that the egg tissue criterion is exceeded (based on whole body tissue selenium concentrations). If the whole body tissue selenium value is exceeded, egg tissue monitoring could be conducted or corrective action could be implemented, depending on the magnitude of exceedance. If no exceedance of the whole body selenium tissue value occurs, then compliance is achieved and routine water quality monitoring would continue.

7.1.3 Egg Tissue Monitoring

If field-collected whole body fish tissue selenium concentrations are lower than the translated egg to whole body concentration, no further monitoring would be necessary, and routine surface water monitoring would continue. The stream would be considered compliant with the egg selenium criterion. If whole body selenium concentrations exceed the translated egg to whole body value, an option is available to target egg tissue and further assess the state of compliance.

Details for monitoring egg tissue will be compiled in a Work plan prepared prior to field efforts. Details of the monitoring plan should be fully vetted and approved by Agency personnel so there is no delay in monitoring, should it be necessary.

7.1.3.1 Monitoring Locations

If the whole body tissue concentration of selenium in juvenile trout indicates exceedance of the translated egg to whole body value at a particular location, then egg tissue monitoring should be conducted during the fall spawning period. The location will include a reach of stream inclusive of the aqueous selenium monitoring location, and should focus on locations that include a mixture of habitats including favorable spawning gravels with appropriate water velocity and nearby deep pools or cover.

7.1.3.2 Timing and Frequency

Fall quarterly monitoring is planned such that fish tissue monitoring could be conducted in October or November to determine whether egg selenium concentrations exceed the criterion.

Collection of fish to extract egg tissues may require multiple trips to a location in order to correctly time the presence of pre-spawn females.

7.1.3.3 Fish Size and Gender

If egg tissues are being collected, brown trout is the target species. Fish size should be greater than 300 mm or larger. Obviously, if eggs are sought, female fish in pre-spawn, ripe condition are needed. Adult females in ripe and running condition can only be sampled during the late fall period (late October/November). Each female would need to be checked for the availability of eggs.

7.1.3.4 Number of Measurements

Collection of egg tissue samples is highly destructive sampling because it removes eggs from the next year's age class. Therefore, if egg tissues are to be collected, it is suggested that eggs from ten or fewer adult females be collected.

Eggs from each female can be expressed to collect a number of eggs for selenium residue analysis. Five grams is adequate for selenium tissue concentration analysis. Of these ten fish, three parent fish should be retained for whole body tissue analysis and complete egg stripping. Eggs and whole body maternal fish should be sent to the laboratory as two separate samples for analysis for each fish.

7.1.3.5 Aggregation of Data to Assess Compliance

Egg tissues will be analyzed for individual specimens. Analytical data for tissue measurements will be aggregated using typical summary statistics (mean, median, maximum, minimum, standard deviation, and upper and lower 95 percent confidence intervals). For egg tissues, if the lower 95 percent confidence interval of the mean indicates a tissue concentration greater than the egg criterion, then corrective action should be implemented.

7.2 Reporting

Reporting surface water quality data, whole body tissue data, and or egg tissue data to IDEQ will require that laboratory analytical results be obtained in a timely fashion. Typically, results can be obtained within 2-3 weeks. Result of monitoring would be due to the Agencies within 30 days of the initial monitoring. The reporting format will discussed with IDEQ.

7.3 Protection of Beneficial Uses

A water quality standard defines the water quality goals for a water body or portion thereof, in part, by designating the use or uses to be made of the water. The designated beneficial use of a water body must consider its actual use, the ability of the water to support a use in the future that is not currently supported, and the basic goal of the Clean Water Act that all waters support aquatic life and recreation where attainable (IDEQ Web Page URL www.idaho.deq.gov). The State of Idaho designates its aquatic uses accordingly.

Streams in the Crow Creek drainage do not have specific designated beneficial uses. As such, the default beneficial uses apply, including: Aquatic Life Cold and primary or secondary contact recreation.

7.3.1 Aquatic Life Cold

The Aquatic Life Cold beneficial use requires water quality appropriate for the protection and maintenance of a viable aquatic life community for cold water species. The criterion proposed herein has been demonstrated to be protective of a range of aquatic species that occur in the area defined as the Site. An appropriately sensitive species that is nearly ubiquitous in the Crow Creek drainage is the basis for the site-specific criterion, and a conservative EC was selected. The combination of species sensitivity and use of a conservative EC lends itself to a criterion protective of aquatic life that occurs at this Site.

7.3.2 Recreation

Recreational uses are divided into primary contact and secondary contact recreation. Each of these uses is defined below:

- **Primary contact recreation** applies to waters where people engage in activities that involve immersion in, and likely ingestion of, water, such as swimming, water skiing, and skin diving.

- **Secondary contact recreation** applies to waters where people engage in activities where ingestion of water may occasionally occur, such as fishing, boating, wading, and infrequent swimming.

In the Crow Creek drainage, the following human uses have been observed: swimming, fishing, and wading. Streams of this drainage flow through a mix of public and private lands, and therefore are accessible at certain points to members of the general public. Idaho State Standards for protecting the recreation use is 4,200 µg/L selenium in surface water (for human consumption of organisms only). The highest concentration measured has been slightly greater than 50 μg/L. Implementation of a criterion for protection of aquatic life will also be protective of the recreation beneficial use.

7.3.3 Protecting Downstream Designated Uses

The Crow Creek drainage flows north into Wyoming. Idaho has a responsibility to ensure that water quality standards are being met at the State Line. Actions being taken under CERCLA at the Smoky Canyon Mine to reduce selenium source inputs will ultimately address downstream issues. Achieving the water quality trigger value at the source areas (i.e., HS and SFSCS) will result in downstream criteria being met, particularly at the State Line. Wyoming DEQ has expressed concern that recent monitoring has shown selenium concentrations in Crow Creek at the State Line exceed the Wyoming State Standard (0.005 mg/L).

Using a selenium load-based model to predict selenium concentrations at downstream locations, based on concentrations and flows from Hoopes Spring and SFSC springs, it can be demonstrated that the surface water concentration of selenium will not exceed 0.005 mg/L at the State Line. This load-based model assumes that selenium concentrations discharged at HS and SFSCS equal 0.011 mg/L (i.e., the aqueous trigger value). The flow downstream of Hoopes Spring is 8.5 cubic feet per second (cfs) at HS-3 and downstream of SFSC springs is 6 cfs (at water quality sampling location LSS). No attenuation is assumed along the flow path, and the flows downstream are assumed to represent base flow conditions, since base flows would encompass more of the load comprised by the spring's discharges. From these assumptions, the model is used to derive the selenium load. At the following locations, the load is back calculated to an aqueous selenium concentration.

Using this initial information and existing relationships for surface water quality and flows, the projected selenium concentration at LSV-3 or LSV-4 is ~ 0.010 mg/L at 15.8 cfs in Sage Creek, and the projected selenium concentration at CC-1A downstream of Sage Creek 0.004 mg/L at ~40 cfs in Crow Creek.

At the Idaho-Wyoming State Line, flows during October 2008, 2009, and 2010 ranged from 41-44, 48-53, and 43-44 cfs, respectively. In September and November 2008, the measured selenium concentrations in Crow Creek at CC-1A were less than 0.007 mg/L. Wyoming DEQ provided recent monitoring data beginning in 2008 for Crow Creek at the State line and in October 2008, aqueous selenium was 0.004 mg/L. Aqueous selenium concentrations measured in Hoopes Spring (> 0.04 mg/L) and SFSC springs (0.011 mg/L) were elevated during this time frame.

In November 2009, aqueous selenium was measured at 0.007 mg/L in Crow Creek at CC-1A. WDEQ reported that in 2009, aqueous selenium at the State Line was 0.005 mg/L. Aqueous selenium concentrations measured in Hoopes Spring (> 0.041 mg/L) and SFSC springs (0.010 mg/L) were again elevated during this time frame.

Finally, in August and November 2010, aqueous selenium concentrations measured 0.0085 and 0.0081 mg/L, respectively, in Crow Creek at CC-1A. Aqueous selenium concentrations at the State line measured on two dates in October were 0.01 and 0.009 mg/L. Aqueous selenium concentrations measured in Hoopes Spring (0.047 mg/L) and SFSC springs (0.012 mg/L) were also elevated during this time frame.

Clearly, recent empirical data indicates that elevated selenium concentrations in Crow Creek at both the CC-1A location and at the State line correspond to concentrations observed at Hoopes Spring and South Fork Sage Creek Springs and the duration that selenium concentrations remain elevated. Reductions at these source areas will have corresponding reductions in Crow Creek. The loading model described above appears to be conservative in its estimates. This conservatism should provide a margin of safety that is protective of downstream uses provided source control is achieved to the aqueous trigger value.

7.3.4 Interim Measures for Protecting Downstream Uses

Representatives of Wyoming DEQ have suggested three alternatives to managing exceedances of the selenium criterion at the State Line as part of protecting downstream uses:

- Address the exceedances upstream of the State Line;

- Carry the SSSC science forward into Wyoming (this option would involve a three-year time frame to adopt a similar Site-specific criterion in Wyoming and could be viewed as a long-term solution); or

- List Crow Creek at the State Line for a Total Maximum Daily Load (TMDL).

Source-control actions are currently underway at the Smoky Canyon Mine as part of the CERCLA process to address exceedances of the current selenium standard. The Pole Canyon Diversion project has been implemented to route Pole Canyon Creek through a pipeline and isolate flows from infiltrating the Pole Canyon ODA, which has resulted in significantly improved water quality downstream of the diversion in Pole Creek. Simplot has also been evaluating various treatment options for source controls at Hoopes Spring to remove selenium from the Hoopes Spring discharge. Ongoing remediation of currently exposed pits is being conducted to limit infiltration into the Wells Formation aquifer which feeds Hoopes Spring. Each of these actions is aimed at lowering selenium contributions to the surrounding watershed.

The first alternative of addressing exceedances upstream of the State Line is already underway. A process of adaptive management can be employed to provide assurances that corrective actions are being implemented through CERCLA and mining permits. Adaptive management is a six-step process for defining and applying management policies for environmental resources under conditions of high uncertainty concerning the outcome of management actions. A well-structured adaptive management plan contains the following interactive steps:

a. Assessing the problem;

b. Designing a management plan;

c. Implementing the plan;

d. Monitoring;

e. Evaluating results obtained from monitoring; and

f. Adjusting the management plan in response to the monitoring results.

Adaptive management puts into place a plan that involves current and future mitigation and corrective actions, regular monitoring to meet regulatory requirements, and evaluation of results to gauge effectiveness of the effort.

The second alternative essentially involves development of a site-specific selenium criterion in Wyoming. While a large amount of information has already been developed on the Idaho side for Crow Creek that is potentially applicable to Crow Creek in Wyoming, the process for developing a site-specific criterion in Wyoming is at least a three-year endeavor. The potential advantage of taking on such a long-term effort is that a site-specific criterion in Wyoming could result in a value that is higher than the current State standard (0.005 mg/L). Costs to develop another criterion, however, are likely a disadvantage.

The third alternative involves Wyoming DEQ listing Crow Creek at the state line on the 303(d) list, triggering requirements for a TMDL to be developed for Crow Creek. This regulatory action will have consequences in Idaho, and require that upstream loads be reduced to meet the current Wyoming standard. Listing a water body on the 303(d) list typically relegates a water body to Category 5 which is defined as “available data and or information indicate that at least one designated use is not being supported or is threatened, and a TMDL is needed.” However, an alternative listing, termed Category 4B, is available. Category 4B indicates that a TMDL is not needed because other pollution control requirements are expected to result in the attainment of an applicable water quality standard in a reasonable period of time. USEPA’s supporting regulations recognize that alternative pollution control requirements may obviate the need for a TMDL (refer to 40 CFR 130.7(b)(1), Monschein and Mann 2007).

To qualify for Category 4B status, the State, in this case Wyoming, must submit a plan that demonstrates each of the following six elements:

1. Identification of segment and statement of problem causing the impairment;

2. Description of pollution controls and how they will achieve water quality standards;

3. An estimate or projection of the time when water quality standards will be met;

4. A schedule for implementing pollution controls;

5. A monitoring plan to track effectiveness of pollution controls; and

6. Commitment to revise pollution controls, as necessary.

Current CERCLA actions and mining permits at the Smoky Canyon Mine ensure that appropriate response actions and/or reclamation activities are being conducted as part of a pollution control plan. Working with the Wyoming DEQ to adopt a Category 4B status for Crow Creek in lieu of a Category 5 listing on the 303(d) list provides for a regulatory mechanism and plan that would allow response actions currently in place to fulfill their intended purpose.

If selenium concentrations in Crow Creek surface waters continue to exceed the State of Wyoming water quality standard, then meeting the downstream designated uses will require that one of the options described above be implemented in order for the State of Idaho to meet its obligations.

Essentially, the first alternative is currently being implemented and can be developed into an adaptive management process. The second alternative of carrying the SSSC science into Wyoming has merit, but will be costly to conduct the additional monitoring needed to address conditions in Crow Creek downstream of the State line. The third alternative is quite practical, and very closely resembles the first alternative. Development of a Category 4B demonstration document, and subsequent approval of that document by USEPA, would allow time for attainment of the site-specific criterion in a reasonable period of time and provide, similar to an adaptive management approach, and plan by which to gauge the efficacy of actions taken.

Adoption of one or more of these measures will ensure that downstream designated uses are ultimately met. The proposed SSSC will ensure that aquatic life is protected once source control actions reduce selenium inputs. Combining the process of a science-based effects criterion, with a management plan put into effect to control sources and gauge effectiveness through monitoring, will provide for protection of the aquatic resources surrounding the Smoky Canyon Mine.

7.4 Geographic Range of Criterion Applicability

The Final Field Monitoring Studies Work Plan (NewFields 2007a) and Draft Approach document (NewFields 2007c) define the "Site" as Hoopes Spring and its downstream receiving waters. State-recognized water body segments were identified and used as management units to which a modified criterion could be applied. In particular, the Approach document states:

> In the general context of site-specific criteria, a "site" may be a state, region, watershed, water-body, or segment of a water body. The site-specific criterion is to be derived to provide adequate protection for the entire site, however the site is defined (USEPA 1994 - WQS Handbook). The water bodies being investigated are found within the Salt Subbasin, HUC 17040105, of the Upper Snake River Basin. Two water body units of the Salt Sub basin are potentially affected, including water body US-9 (Sage Creek) and water body US-8 (Crow Creek) as defined by the Idaho Administrative Code's Water Quality Standards (IDAPA 58.01.02). These two subunits are defined as follows:
>
> - US-8 Crow Creek - source to Idaho/Wyoming border
> - US-9 Sage Creek - source to mouth
>
> Of these two subunits, only Sage Creek is listed in Idaho's 2002 Integrated 303(d)/305(b) Report (IDEQ 2005) as impaired due to elevated levels of selenium.
>
> Hoopes Spring is the primary source of aqueous selenium to its downstream receiving waters, Sage Creek and Crow Creek. The primary areas potentially affected by discharge of Hoopes Spring include Sage Creek from its confluence with the Hoopes Spring discharge channel to its confluence with Crow Creek, and Crow Creek from its confluence with Sage Creek to the Idaho and Wyoming state line. A SSSC developed based on data from these areas is anticipated to be applicable to Hoopes Spring, Sage Creek, and Crow Creek. Thus, the Site is defined as Hoopes Spring and its discharge channel, Sage Creek downstream of Hoopes Spring to it confluence with Crow Creek, and Crow Creek downstream of the Sage Creek confluence to the Idaho state line.

A Technical Memorandum was provided to the SSSC Workgroup in October 2007 that discussed Site Boundaries and Applicability of Site-Specific Selenium Criterion. That memo concluded the following:

> Clearly, at the time of identifying the study area and initial thinking on the area of applicability for any newly developed criterion, the focus was on water quality

> impacts downstream of Hoopes Spring and framing those impacts through inclusion of upstream areas. As a result, both the field and laboratory efforts will provide information to fully characterize the study area.
>
> It is anticipated that this characterization will ultimately provide the information from which the area of criterion application can be identified. For example, if it is determined that the characterization information from the study (field, laboratory, and literature) is representative of both upstream and downstream areas, then it would likely be the case that the area of criterion application could cover both the areas upstream and downstream of Hoopes Spring. However, it will be up to the State of Idaho to make the final determination regarding applicability.

Few site-specific criteria have been developed in Idaho. In the Coeur d'Alene basin, site-specific criteria were developed for cadmium, lead, and zinc in the upper South Fork, but there was a need to apply the criteria to lower sections of the South Fork and the main stem. Authors of the site-specific document argued that part of the rationale for expanding site-specific criteria to downstream reaches of the South Fork is based on the application of site-specific chemical, biological, and toxicological data to factors affecting metals toxicity in freshwater (Bergman and Dorward-King 1997). The ecological principle of the stream continuum provides a context for understanding watershed biogeochemistry and species distributions, and factors into the evaluation (Vannote et al. 1980).

By all accounts, this argument also holds true for Crow Creek. The data developed as part of this Interpretive Report suggest that the Site-specific criterion is applicable to locations both upstream and downstream of Hoopes Spring. The SSSC is applicable to Crow Creek and its tributaries in Idaho based on the application of Site-specific chemical, biological, and toxicological data to factors affecting selenium toxicity in this system.

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TABLES

Table 2-1
Summary Statistics for Aqueous Selenium Concentrations from 2000 to Pre-Fall 2006

Location	Count	Minimum	Maximum	Mean	Median	Standard Deviation	UCL	UCL Method	Distribution	Notes	25th percentile	75th percentile	Med-Max	Med-Min	95 CI	Regulatory Standard
CROW_US	28	0.00028	0.0013	0.001	0.00086	0.0003	0.00086	95% Student's-t UCL	normal		0.00051	0.001	0.00044	0.00058	9.11971E-05	0.005
HS	21	0.003	0.0189	0.013	0.0126	0.0037	0.014	95% Student's-t UCL	normal		0.01	0.015	0.00630	0.00960	0.00142381	0.005
LSV-2_2C	8	0.0052	0.0252	0.010	0.0082	0.0063	0.014	95% Student's-t UCL	non-parametric	Small sample size	0.007	0.0088	0.01700	0.00300	0.0042	0.005
LSS	25	0	0.0042	0.002	0.002	0.0010	0.0022	95% Student's-t UCL	normal		0.001	0.0021	0.00220	0.00200	0.0003448	0.005
LSV-3	11	0.0029	0.0232	0.007	0.006	0.0057	0.0102	95% H-UCL	lognormal		0.00365	0.0068	0.01720	0.00310	0.003409091	0.005
LSV-4	16	0.0023	0.0146	0.006	0.005	0.0033	0.00729	95% Student's-t UCL	lognormal		0.004	0.006125	0.00960	0.00270	0.00145875	0.005
CC-1A_1	8	0.0003	0.0054	0.002	0.00165	0.0016	0.00305	95% Student's-t UCL	normal	Small sample size	0.0009075	0.00225	0.00375	0.00135	0.00109375	0.005

Note: CROW_US includes Crow Creek locations upstream of Sage Creek (CC-7, SW-CC-100, SW-CC-300, SW-CC-50, AWI012-29, CC-300, CC-350, CC-75, and CC-150); all locations are upstream of SSSC location CC-350.
LSV-2_2C includes locations LSV-2 and LSV-2C;
LSV-3 is on Sage Creek between LSV-2C and LSV-4; and
CC-1A_1 includes location CC-1A at the Meade Peak Ranch and nearby historical location CC-1.

Table 2-2
Summary of the Stream Reach Inventory/Channel Stability Evaluation Scores and Comparison of Seasonal Conditions

Location	Reach	D/S End of Reach Elevation	Gradient	Flow (all sampling events)			Channel Stability Score	Fall 2008 Channel Condition	% Eroding bank (Fall 2006)	% Eroding bank (Fall 2007)	% Eroding bank (Fall 2008)
		(ft amsl)	(%)	Average (cfs)	Min (cfs)	Max (cfs)	Average (Range)		(linear feet/total reach length *2)		
Reference											
SFTC-1	South Fork Tin Cup Creek near mouth	6,125	1.48	9.8	0.1	21.0	76.3 (70-81)	**Fair**	-	18 (167/940)	2 (20/940)
Upstream of Sage Creek											
CC-75	Crow Creek u/s of Wells Canyon	6,736	0.67	6.7	2.5	15.3	79.5 (69-90)	**Good**	11 (78/700)	13 (93/700)	29 (201/700)
CC-150	Crow Creek u/s of Deer Creek	6,663	0.51	13.3	3.2	27.5	74.3 (72-76)	**Good**	5 (53/1000)	5 (55/1000)	5 (55/1000)
CC-350	Crow Creek d/s of Deer Creek	6,552	0.73	24.5	16.4	36.0	95 (87-103)	**Fair**	35 (414/1200)	24 (294/1200)	46 (546/1200)
DC-600	Deer Creek u/s of Crow Creek, d/s of NFDC	6,742	2.05	7.0	2.0	20.0	58.3 (55-63)	**Good**	0 (0/630)	<1 (1/630)	0
Hoopes Spring and Sage Creek											
HS	Hoopes Spring	6,654	PHABSIM not conducted	2.1	1.6	2.5	55.3 (52-60)	**Good**	6 (20/350)	1 (4/350)	0
HS-3	Hoopes Spring (Discharge Channel)	6,585	1.58	5.7	5.1	6.8	72.8 (64-77)	**Good**	25 (182/720)	28 (203/720)	61 (442/720)
LSV-2C	Lower Sage Creek d/s Hoopes Spring	6,575	0.70	9.9	6.6	15.1	67.8 (65-72)	**Good**	5 (38/800)	8 (64/800)	50 (403/800)
LSV-4	Lower Sage Sage Creek u/s Crow Creek	6,428	0.59	13.8	12.3	15.3	84.5 (80-89)	No Data Collected	15 (124/830)	-	-
Downstream of Sage Creek											
CC-1A	Crow Creek d/s Sage Creek	6,398	0.22	38.7	21.6	61.0	80.3 (72-90)	**Fair**	11 (130/1200)	8 (102/1200)	14 (172/1200)
CC-3A	Crow Creek d/s Sage Creek and CC-1A	6,341	0.32	43.1	25.1	65.2	87.3 (81-92)	**Fair**	43 (692/1620)	25 (406/1620)	50 (806/1620)

Score Ranges: <38 Excellent, 39-76 Good, 77-114 Fair, >115 Poor

Table 2-3
Temperature Logger Summary Statistics (degrees C)

Location	Count	Minimum	Maximum	Mean	Median	Standard Deviation	Start Date	End Date
SFTC-1	43,326	0.05	25.02	7.13	5.15	6.81	5/7/2007	8/1/2008
CC-75	73,814	1.75	18.27	7.20	5.92	4.27	5/8/2007	7/7/2009
CC-150	73,940	-0.12	22.42	7.62	6.99	4.94	5/9/2007	7/9/2009
CC-350	73,820	0.00	24.48	7.44	6.79	5.73	5/8/2007	7/9/2009
DC-600	73,453	-0.06	17.82	6.83	6.69	3.39	5/13/2007	7/9/2009
HS	35,424	9.61	12.90	11.63	11.61	0.32	5/14/2007	5/17/2008
HS-3	73,978	0.52	24.17	10.22	9.68	3.31	5/12/2007	7/9/2009
LSV-2C	74,067	0.00	25.33	9.23	8.47	4.25	5/12/2007	7/9/2009
LSV-4	11,028	5.44	23.21	13.47	12.61	4.18	5/9/2007	9/1/2007
CC-1A	73,655	-0.03	22.87	8.02	7.14	5.74	5/10/2007	7/9/2009
CC-3A	66,150	-0.28	23.76	7.65	6.36	6.34	5/11/2007	4/26/2009

Table 2-4
Summer (July 1 - September 15) Temperature Logger Summary Statistics (degrees C)

Location	Count	Minimum	Maximum	Mean	Median	Standard Deviation
SFTC-1	10,452	6.86	25.02	15.88	15.93	3.49
CC-75	15,418	6.43	18.15	12.23	12.07	2.25
CC-150	14,424	6.71	22.42	13.17	12.36	3.59
CC-350	14,507	5.62	24.48	14.31	13.95	3.84
DC-600	14,712	5.72	17.82	10.63	10.12	2.22
HS	7,390	11.13	12.53	11.90	11.88	0.20
HS-3	15,590	7.12	24.17	13.15	12.12	3.48
LSV-2C	15,591	6.18	25.33	13.53	12.61	3.84
LSV-4	5,997	7.32	23.21	14.24	13.38	3.91
CC-1A	15,612	7.14	22.87	15.00	14.84	3.27
CC-3A	14,783	7.54	23.76	15.66	15.61	3.15

Table 2-5
Presence/Absence of Fish Species by Location, Fall 2006 - Fall 2008

	Location										
Family/Common Name (Species)	**SFTC-1**	**CC-75**	**CC-150**	**CC-350**	**DC-600**	**HS**	**HS-3**	**LSV-2C**	**LSV-4**	**CC-1A**	**CC-3A**
Salmonidae											
Brook Trout *(Salvelinus fontinalis)*											√
Rainbow Trout *(Oncorhynchus mykiss)*	√										
Brown Trout *(Salmo trutta)*		√	√	√		√	√	√	√	√	√
Cutthroat Trout (*Oncorhynchus clarki bouvieri*)	√	√	√	√	√		√	√	√	√	√
Mountain Whitefish *(Prosopium williamsoni)*	√		√	√					√	√	√
Cottidae											
Paiute Sculpin *(Cottus beldingi)*	√	√	√	√	√	√	√	√	√	√	√
Mottled Sculpin *(Cottus bairdi)*	√									√	
Sculpin (*Cottus spp*.)		√	√	√	√	√	√	√	√	√	√
Cyprinidae											
Leatherside Chub *(Snyderichtys copei)*				√							
Longnose Dace *(Rhinichthys cataractae)*	√		√	√						√	√
Speckled Dace (*Rhinichthys osculus*)	√		√	√						√	√
Redside Shiner *(Richardsonius balteatus)*	√		√	√						√	√
Catostomidae											
Utah Sucker *(Catostomus ardens)*	√		√							√	√
Mountain Sucker *(Catostomus platyrhynchus)*	√										

Table 3-1

Effects Endpoints from Maternal Transfer Studies for Cold Water Species

Species	Reference	Adult Exposure	Endpoint	Tissue	Selenium Concentration (µg/g dry weight)			
					NOEC	LOEC	EC_{10}	EC_{20}
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; Hardy 2010	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	-	-	24.13	28.75
	Nautilus 2011; Elphick et al. 2009	Field	Alevin mortality	Egg			24.8	
Dolly Varden char	Golder 2009*; McDonald et al. 2010	Field	Larval deformities	Egg	-	-	53.6	59.7
Rainbow trout	Holm et al. 2005	Field	Larval deformities	Egg	17	25	26	29
Northern pike	Muscatello et al. 2006	Field	Larval deformities	Egg	3.8	31.28	20.4	33.55
Northern pike	Muscatello and Janz 2009	Field	Larval deformities/mortality	Egg	>8.02	-	-	-
White sucker	Muscatello and Janz 2009	Field	Larval deformities/mortality	Egg	>4.89	-	-	-
White sucker	de Rosemond et al. 2005	Field	Larval deformities	Egg	-	-	26 (EC13)	-

Original table Source: Selenium Tissue thresholds - Tissue Selection Criteria, Threshold Development Endpoints, and Potential to Predict Population or Community Effects in the Field (NAMC 2009).

Table 4-1
Summary of Trout Standing Crop Estimates (kg/Ha) from the Nine Locations Used for Trout and Habitat Analyses

		Dependent Variables											
	Location	All Trout Standing Crop (kg/Ha)				Brown Trout Standing Crop (kg/Ha)				Cutthroat Trout Standing Crop (kg/Ha)			
		Fall 2007	Fall 2008	Spring 2007	Spring 2008	Fall 2007	Fall 2008	Spring 2007	Spring 2008	Fall 2007	Fall 2008	Spring 2007	Spring 2008
Control	SFTC-1	62.7	27.9	56.7	93.4	0.0	0.0	0.1	0.0	62.7	27.9	56.6	93.4
	CC-75	74.5	62.8	73.3	27.7	72.5	45.0	67.5	22.3	2.0	17.8	5.8	5.4
	CC-150	105.9	114.7	82.2	135.6	86.6	83.8	67.0	90.2	19.3	30.8	15.2	45.4
	CC-350	43.1	49.2	11.6	39.4	14.2	18.3	3.9	2.8	28.9	30.9	7.7	36.6
	DC-600	76.2	126.9	93.3	54.7	0.0	0.0	0.0	0.0	76.2	126.9	93.3	54.7
Test	HS-3	95.1	47.1	35.1	100.6	95.1	45.8	31.2	86.7	0.0	1.2	3.9	13.9
	LSV-2C	197.0	277.0	262.7	162.0	154.1	231.1	146.3	109.3	42.9	45.9	116.4	52.7
	CC-1A	69.3	73.1	49.2	48.6	40.2	48.0	30.5	24.0	29.1	25.1	18.7	24.7
	CC-3A	118.6	87.5	53.9	107.5	56.3	58.6	38.1	54.7	62.3	28.9	15.8	52.8

Table 4-2
Summary of HQI, SRI/CSE, SHI and HSI Scores for the Nine Locations Used for Trout and Habitat Analyses

	Location	Independent Variables							
		Habitat Quality Index_Total Score (HQI - kg/Ha)		Stream Reach Inventory/Channel Stability Evaluation-Total Score (SRI)		IDEQ Stream Habitat Index-Total Score (SHI)		Habitat Suitability Index-Total Score (HSI)	
		2007	2008	Fall 2007	Fall 2008	2007	2008	Brown Trout	Cutthroat Trout
Control	SFTC-1	1.9	30	70	81	49	58	0.76	0.85
	CC-75	197	173	89	70	50	56	0.75	0.90
	CC-150	30	93	75	76	47	56	0.76	0.83
	CC-350	44	92	90	103	47	39	0.74	0.83
	DC-600	265	22	55	59	59	71	0.36	0.80
Test	HS-3	73	32	77	75	32	45	0.10	0.35
	LSV-2C	47	73	68	66	41	55	0.67	0.78
	CC-1A	132	13	76	83	50	51	0.68	0.75
	CC-3A	105	79	81	85	44	51	0.75	0.75

Table 4-3

Results of Linear Regression Anaylsis Relating Log-Transformed Standing Crop (kg/Ha) to HQI, SRI/CSE and SHI Habitat Metrics for the Nine Locations Monitored in Fall 2007 and 2008

Model	Attribute	Sample Size	R^2	p
HQI	Overall Score	18	0.0001	0.9760
	Cover	18	**0.3152**	**0.0153**
	Width	18	0.0231	0.5469
	Velocity	18	0.0562	0.3435
	Macro Abundance	18	0.0022	0.8542
	Eroding bank	18	0.0034	0.8194
	Max Temp	18	0.0163	0.6142
	Nitrate as N	18	0.0119	0.6662
	Food Index	18	0.0004	0.9393
	Shelter Index	18	0.1278	0.1452
SRI/CSE	Total Score	18	**0.2110**	**0.0551**
	Upper Banks	18	0.0787	0.2595
	Lower Banks	18	**0.1901**	**0.0705**
	Channel Bottom	18	0.0778	0.2624
SHI	Total Score	18	0.0017	0.8696
	%Cover	18	0.0631	0.3148
	Large Organic Debris	18	0.0206	0.5699
	% Fines	18	0.0011	0.8962
	Embeddedness	18	0.0652	0.3063
	# Wolman Classes	18	0.0062	0.7557
	Channel Shape	18	0.0074	0.7337
	% Bank Vegetation	18	0.0247	0.5330
	% Canopy Cover	18	0.0752	0.2708
	Disruptive Pressure	18	0.0214	0.5624
	Zone of Influence	18	0.0041	0.8009

Values in Bold Indicate Significance at $p = 0.10$

Table 4-4

Results of Linear Regression Anaylsis Relating Log-Transformed Brown and Cutthroat Standing Crop (kg/Ha) to HSI Component Scores for the Nine Locations Monitored in Fall 2007 and 2008

Year	Species	HSI Component	N	R^2	p
2007	Brown Trout	Overall	9	0.0020	0.9087
		Adult	9	0.0012	0.9301
		Juvenile	9	0.0186	0.7261
		Fry	9	0.0035	0.8801
		Other	9	**0.4090**	**0.0636**
2008	Brown Trout	Overall	9	0.0233	0.6952
		Adult	9	0.0123	0.7761
		Juvenile	9	0.0575	0.5342
		Fry	9	0.0004	0.9575
		Other	9	**0.3809**	**0.0766**
2007 and 2008	Brown Trout	Overall	18	0.0097	0.6976
		Adult	18	0.0014	0.8812
		Juvenile	18	0.0353	0.4553
		Fry	18	0.0004	0.9395
		Other	18	**0.3948**	**0.0052**
2007	Cutthroat Trout	Overall	9	0.2906	0.1342
		Adult	9	**0.4304**	**0.0550**
		Juvenile	9	**0.3845**	**0.0749**
		Fry	9	0.1916	0.2387
		Other	9	0.0052	0.8543
2008	Cutthroat Trout	Overall	9	**0.6662**	**0.0073**
		Adult	9	**0.6334**	**0.0103**
		Juvenile	9	**0.6103**	**0.0129**
		Fry	9	**0.4945**	**0.0346**
		Other	9	0.3260	0.1083
2007 and 2008	Cutthroat Trout	Overall	18	**0.4307**	**0.0031**
		Adult	18	**0.5066**	**0.0009**
		Juvenile	18	**0.4699**	**0.0017**
		Fry	18	**0.3032**	**0.0179**
		Other	18	0.0846	0.2417

Values in bold indicate significance at $p = 0.10$.

Table 4-5

Results of Wilcoxon Rank Sum Tests Comparing Trout Standing Crop (kg/Ha) and Habitat Metrics for Spring and Fall 2007 and 2008 Sampling Periods between Background and Downstream Locations

Comparison	Background		Downstream		Two-tailed p
	N	Mean Rank	N	Mean Rank	
Total Trout - All Seasons	20	16.2	16	21.3	0.1519
Total Trout - Fall 2007 and 2008	10	8.0	8	11.4	0.2592
Total Trout - Spring 2007 and 2008	10	8.6	8	10.6	0.4873
Brown Trout - All Seasons	20	13.8	16	24.4	**0.0028**
Cutthroat Trout - All Seasons	20	19.9	16	16.8	0.3900
HQI Score - Fall 2007 and 2008	10	9.6	8	9.4	0.9310
SRI/SCE Score - Fall 2007 and 2008	10	9.5	8	9.6	0.9846
SHI Score - Fall 2007 and 2008	10	11.3	8	7.3	**0.0894**
HSI Score - Brown Trout	5	6.1	4	3.6	0.1587
HSI Score - Cutthroat Trout	5	7.0	4	2.5	**0.0079**

Values in bold indicate significance at p = 0.10.

Table 4-6
Catch Per Unit Effort (CPUE) for Brown Trout and Cutthroat Trout (YCT) for the 10 Locations Sampled in Fall 2007 and Fall 2008

Location	2007		2008	
	Brown Trout (#/hr)	YCT (#/hr)	Brown Trout (#/hr)	YCT (#/hr)
SFTC-1	0.0	6.2	0.0	2.7
CC-75	28.3	0.0	36.5	9.1
CC-150	28.4	8.7	72.8	17.3
CC-350	13.6	0.0	25.9	29.2
DC-600	0.0	23.7	0.0	36.8
HS	8.1	0.0	16.9	0.0
HS-3	5.7	0.0	12.4	0.0
LSV-2c	48.5	15.4	118.9	34.0
CC-1A	18.0	11.0	18.0	10.0
CC-3A	16.4	10.0	31.7	10.3

CPUE = #/hour of browns >150 mm and YCT >200 mm.
Based on 1st pass electrofishing data.

Table 4-7
Catch Per Unit Effort (CPUE) Statistics for Brown Trout and Cutthroat (YCT) Trout by One Pass Electrofishing in Ecoregion 6 Streams

Statistic	Brown Trout (#/hr)	Cutthroat Trout (#/hr)
Sample size	18	27
Mean	7.5	3.2
Standard Error	1.4	0.7
Percentiles:		
5	1.6	0.1
25	3.3	0.7
50	5.0	2.0
75	12.1	4.3
95	19.5	10.6

CPUE = #/hour of browns >150 mm and YCT >200 mm.
Data are from Brouder et al. 2009.

Table 4-8

Summary of Relative Weights (W_r) for Brown Trout and Cutthroat Trout (YCT) Collected at the 10 Locations in Fall 2007 and Fall 2008

Location	2007						2008					
	Brown Trout			YCT			Brown Trout			YCT		
	N	Mean	Range	N	Mean	Range	N	Mean	Range	N	Mean	Range
SFTC-1	0			36	0.97	0.81-1.21	0			25	1.03	0.79-1.22
CC-75	11	0.96	0.88-1.06	2	0.91	0.83-1.00	16	0.91	0.80-1.06	5	0.88	0.71-1.04
CC-150	18	0.96	0.82-1.49	8	0.95	0.85-1.03	25	0.92	0.77-1.08	14	0.87	0.78-1.00
CC-350	6	0.93	0.85-1.12	27	0.90	0.57-1.20	10	0.86	0.82-0.92	38	0.93	0.82-1.15
DC-600	0			31	0.92	0.8-1.08	0			34	0.96	0.84-1.14
HS	1	0.90	-	0			2	0.87	0.75-0.99	0		
HS-3	5	0.92	0.86-0.99	0			12	0.88	0.65-1.00	1	0.85	-
LSV-2c	30	0.93	0.82-1.18	9	1.00	0.91-1.15	43	0.91	0.82-1.03	12	0.96	0.88-1.07
CC-1A	25	0.88	0.69-0.98	18	0.98	0.74-1.12	29	0.87	0.71-1.05	15	0.93	0.85-1.09
CC-3A	44	0.90	0.76-1.01	28	0.92	0.64-1.15	52	0.86	0.75-0.97	17	0.92	0.81-1.05

Brown trout ≥ 140mm long
YCT ≥ 130mm long

Table 4-9

Trout Standing Crop Estimates (kg/Ha) for 10 Locations Sampled in Fall 2007 and Fall 2008

Location	Fall 2007	Fall 2008
SFTC-1	62.8	27.9
CC-75	74.5	62.8
CC-150	105.9	114.7
CC-350	43.1	49.2
DC-600	76.2	126.9
HS	48.6	26.4
HS-3	95.5	47.1
LSV-2c	197.1	277.0
CC-1A	69.3	73.1
CC-3A	118.6	87.5

Table 4-10

Summary Statistics of 44 Trout Stand Crop Estimates (kg/Ha) for Wyoming Streams Reported by Binns (1979)

Statistic	kg/Ha
Mean	73.4
Standard Error	15.4
Minimum	0
Maximum	634
Percentiles	
5	0
25	26
50	52
75	84
95	238

Table 5-1
Adult Reproduction Summary Data for Brown Trout

Location	Treatment	# Eggs in Study	Total # Eggs	Adult Fish Total Length (mm)	Adult Fish Wt (g)	Se - Whole-Body (mg/kg dw)	Se - Egg (mg/kg dwt)	Hatch (%)	Egg Mortality (%)	Swim-up (%)	Survival at Swim up (%)	Survival (%) in 15-d PSU Study	Total Survival (%)	Survival (hatch-test end) (%)	Day of Test Term.	Day of 1st hatch	Day of swim-up	Avg Std Length (mm)	Avg Dry wt (mg)
Brown Trout																			
Saratoga Hatchery Fish	SC-001	600	4173.4	498	1,393	3.6	0.76	23.8	76.2	22.8	22.8	97	22.7%	98.9	84	42	69	21.45	18.34
	SC-002	600	4005	420	826	4.1	0.94	22.8	77.2	22.5	22.5	99	22.3%	99.5	84	41	69	22.15	18.47
	SC-003	600	5120	520	1,553	3.7	0.83	56.7	43.3	55.7	55.7	98.9	54.7%	98	84	40	69	22.85	22.60
	SC-004	600	1247.68	562	2,500	4.3	0.92	31.1	68.9	27.3	27.3	100	27.3%	96.2	84	40	69	22.20	23.25
	SC-005	600	5448	558	2,187	3	1.2	11.7	88.3	10.7	10.7	100	10.7%	99	84	42	69	21.80	19.39
	SC-006	600	3175.8	439	842	3.1	1.2	93.2	6.8	92.8	92.8	98	92.7%	99.5	84	40	69	21.80	18.24
	SC-007	600	3223.5	449	1,175	2.7	1	30.5	69.5	26.8	26.8	96	26.3%	95.8	84	47	69	21.05	18.57
	SC-008	600	4004.8	494	1,446	2.5	0.96	66	34	65	65	100	65.0%	99	84	46	69	21.15	15.12
Spring Creek Hatchery Fish	SPC-001	600					0.73	99.3	0.7	98	98	100	98.0%	98.7	49	11	34	24.35	22.56
	SPC-002	20					0.73	100	0	100	100	100	100%	100	49	13	34	22.85	20.85
	SPC-003	600					0.73	97.5	2.5	94.2	94.2	100	94.2%	96.7	49	11	34	23.30	21.81
	SPC-004	21					0.73	100	0	100	100	100	100%	100	49	13	34	23.45	21.56
	SPC-005	600					0.73	98.8	1.2	97.5	97.5	96	96.8%	98	49	11	34	23.00	20.79
	SPC-006	600					0.73	99.7	0.3	96	96	100	96.0%	96.3	49	9	34	23.15	22.39
Sage Creek	LSV2C-002	600	1,096	304	221	8.9	12.8	99	1	96.7	96.7	100	96.6%	97.6	83	36	68	20.10	11.07
	LSV2C-003	400	474	300	217	13.8	40.3	93.5	6.5	0	8	28.1	2.3%	8.8	88	41	88	21.33	9.71
	LSV2C-004	500	766	290	219	17.9	36	50.6	49.4	0	30.2	55.6	16.8%	66.2	88	40	88	21.20	9.98
	LSV2C-005	300	476	294	217	13.6	26.8	71.3	28.7	0	37	62.2	23.0%	51.7	88	40	88	20.25	10.97
	LSV2C-006[1]			346	335	17.2	26.9	---	---	---	---	---	---	---	---	---	---	---	---
	LSV2C-007[1]	500	773	315	243	6.7	18.6	---	---	---	---	---	---	---	---	---	---	---	---
	LSV2C-008	300	372	296	194	9.6	17.7	88.3	11.7	86.7	86.7	98.2	84.2%	95.9	83	39	68	20.45	9.34
	LSV2C-010	100	161	311	278	22.6	38.8	87	13	0	25	44	11.0%	24	88	42	88	19.91	8.09
	LSV2C-012	600	1,031	360	341	7.2	13.2	98.3	1.7	95.7	95.7	100	95.7%	97.4	88	42	73	22.00	13.26
	LSV2C-016	500	826	300	198	9.2	13.4	95	5	91.7	91.7	100	91.7%	96.7	88	39	73	21.80	15.84
	LSV2C-017	300	447	341	275	13.2	20.5	71.3	28.7	64	64	96.3	60.5%	89.2	88	41	73	23.70	18.88
	LSV2C-019	500	693	330	282	8.6	12.5	94.2	5.8	89.8	89.8	100	88.9%	94.7	88	38	73	23.65	19.32
	LSV2C-020	400	525	280	198	11.3	11.2	89.2	10.8	88	88	100	86.8%	97.6	83	40	68	21.75	11.57
	LSV2C-021	600	1,208	307	246	20	28.1	69.3	30.7	0	21.7	68.5	14.8%	45.5	88	38	88	20.20	10.66
Crow Creek	CC-150-009	600	1,215	324	520	8.4	12.8	28.5	71.5	27.2	27.2	99	27.0%	98.3	87	39	72	21.85	13.19
	CC-150-011	300	488	342	303	5.6	8.4	96	4	95.3	95.3	100	95.3%	98.7	82	42	67	20.10	9.25
	CC-150-012	350	556	317	232	6.7	8.5	88.8	11.2	86.8	86.8	97	86.0%	98.1	87	40	72	21.45	12.84
	CC-150-013	600	1,234	332	283	5.9	8.4	66.7	33.3	59.7	59.7	97.3	57.5%	93	87	39	72	22.55	15.58
	CC-150-015	600	1,003	313	213	6	9.1	78.3	21.7	77.8	77.8	98	77.5%	98.9	82	40	67	22.55	14.49
	CC-150-016	600	1,658	391	468	7	7.5	14.8	85.2	14.3	14.3	100	15.4%	99.7	87	43	72	22.80	16.74
	CC-150-017	250	414	265	150	5.6	6.6	89.2	10.8	86.4	86.4	100	84.3%	97.2	82	42	72	20.95	10.65
	CC-150-018	600	959	308	224	4.7	6.9	87.2	12.8	84.3	84.3	100	84.3%	96.5	87	41	72	21.30	13.24
	CC-150-020	600	1,332	310	238	7.2	6.2	97.3	2.7	96.3	96.3	100	96.2%	98.9	82	38	67	20.20	11.25
	CC-350-006	600	1,154	370	373	9.2	14	71.7	28.3	68.0	68.0	98	68.0%	96.3	87	41	72	21.40	12.34
	CC-350-007	600	1,174	350	321	5.5	6.9	29.7	70.3	28.7	28.7	98.8	26.0%	98.6	82	38	67	21.30	11.01
	CC-350-008	600	922	335	254	8.5	9.5	67.7	32.3	64.5	64.5	98.6	62.6%	97.1	82	37	67	20.55	12.09

Table 5-2

Effects Concentration (EC_x) Values for Egg Selenium Tissue Residues Versus Different Biological Endpoints for Brown Trout

Biological Endpoints	(EC_x)			R^2
	50	20	10	
Growth and Survival				
Growth	46.23	33.79	28.13	0.21
95% LCL	27.05	22.84	13.09	
95% UCL	79.01	50.00	60.44	
15-Day Post Survival	34.73	24.52	20.00	0.96
95% LCL	33.11	22.26	17.37	
95% UCL	36.42	26.99	23.02	
Total Survival	24.83	21.43	19.66	0.31
95% LCL	19.27	13.60	10.75	
95% UCL	32.00	33.77	35.98	
Survival Hatch -Test End	30.52	21.63	17.68	0.89
95% LCL	27.58	17.77	13.44	
95% UCL	33.78	26.32	23.25	
Survival Hatch -Test End (revised)	27.43	23.10	20.80	0.99
95% LCL	27.09	22.37	23.77	
95% UCL	27.78	19.89	21.83	
Deformities				
Fraction normal	26.43	21.70	19.33	0.88
95% LCL	23.94	18.09	15.07	
95% UCL	29.19	26.02	24.79	
Fraction normal (revised)	25.96	23.41	22.03	0.87
95% LCL	24.40	20.11	27.25	
95% UCL	27.61	17.88	27.16	
Cranio-facial deformity fraction normal	26.04	22.31	20.37	0.68
95% LCL	22.06	15.91	12.79	
95% UCL	30.75	31.27	32.47	
Skeletal deformity fraction normal	25.13	23.30	22.29	0.81
95% LCL	19.89	15.01	12.68	
95% UCL	31.76	36.18	39.20	
Fin deformity fraction normal	27.65	23.22	20.96	0.28
95% LCL	24.27	17.85	14.30	
95% UCL	31.49	30.19	30.73	
Edematous Tissue fraction normal	26.98	21.23	18.45	0.96
95% LCL	25.31	18.96	15.82	
95% UCL	28.76	23.77	21.52	

Rationale for and derivation of the revised values is described in the text.

Table 5-3
Adult Reproduction Summary Data for Yellowstone Cutthroat Trout

Location	Treatment	# Eggs in Study	Total # Eggs	Adult Fish Total Length (mm)	Adult Fish Wt (g)	Se - Whole-Body (mg/kg dw)	Se - Egg (mg/kg dwt)	Hatch (%)	Egg Mortality (%)	Swim-up (%)	Survival at Swim up (%)	Survival (%) in 15-d PSU Study	Total Survival (%)	Survival (hatch-test end) (%)	Day of Test Term.	Day of 1st hatch	Day of swim-up	Avg Std Length (mm)	Avg Dry wt (mg)
Yellowstone Cutthroat Trout																			
Henry's Lake	HL001	600	**2,114**	489	1,329	0.4	1.65	0	100		---	---	---	---	---	---	---	---	---
	HL002	600	**1,597**	387	667	0.45	2.03	11.5	88.5	9.8	9.8	100	9.8	85.5	64	28	49	24.65	19.31
	HL003	600	**2,999**	400	770	0.44	2.48	56.8	43.2	54.0	54.0	97.9	53.7	94.4	64	24	49	25.70	20.74
	HL004	600	**2,452**	438	1,160	0.36	1.36	76.0	24.0	72.8	72.8	99	72.7	95.6	64	26	49	27.85	26.62
	HL005	600	**2,108**	451	1,165	0.5	2.33	0	100	---	---	---	---	---	---	---	---	---	---
	HL006	600	**2,162**	368	674	0.36	0.83	61.0	39.0	44.0	44.0	99	43.8	71.9	64	27	49	24.50	15.63
	HL007	600	**2,734**	470	1,528	0.44	2.26	73.7	26.3	70.7	70.7	100	70.7	95.9	64	27	49	28.15	26.41
	HL008	600	**2,985**	476	1,265	0.28	1.87	78.2	21.8	72.2	72.2	99	72.0	92.1	64	28	49	24.60	16.12
	HL009	600	**1,906**	406	775	0.44	1.98	0	100	---	---	---	---	---	---	---	---	---	---
	HL010	600	**3,791**	527	1,945	0.43	1.34	0.7	99.3	0.7	0.7	100	0.7	100	64	27	49	---	---
	HL011	600	**4,668**	476	1,468	0.31	3.23	56.3	43.7	52.8	52.8	99	52.7	93.5	64	25	49	26.85	25.08
	HL012	600	**2,735**	470	1,500	0.23	1.58	83.5	16.5	79.3	79.3	98	79.0	94.6	64	26	49	26.50	25.74
	HL013	600	**2,420**	457	1,340	0.72	1.93	87.8	12.2	83.8	83.8	100	83.8	95.4	64	28	49	25.45	20.63
	HL014	600	**3,676**	508	1,650	0.73	1.79	0	100	---	---	---	---	---	---	---	---	---	---
	HL015	600	**2,322**	445	1,580	0.91	2.06	10.3	89.7	9.3	9.3	100	9.3	90.3	64	27	49	22.60	15.79
	HL016	600	**3,876**	508	1,560	0.85	1.74	0	100	---	---	---	---	---	---	---	---	---	---
Sage Creek	LSV2C-001	600	**1,290**	362	429	19.4	40.1	92.7	7.3	0	0	---	0	0	---	21	---	---	---
	LSV2C-002	550	**1,068**	322	257	21.0	30.0	80.7	19.3	67.8	67.8	66.0	61.6	80.9	55	20	40	20.35	7.66
	LSV2C-003	650	**1,358**	340	363	18.6	35.6	99.2	0.8	80.6	80.6	83.2	78.0	78.8	55	21	40	20.05	8.70
	LSV2C-004	600	**1,072**	345	347.1	22.5	30.5	95.2	4.8	85.5	85.5	83.0	82.7	87.5	55	20	40	21.10	8.12
Deer Creek	DC001	600	**1,017**	343	461.9	8.17	22	54.2	45.8	50.2	50.2	93.9	49.2	95	56	20	41	23.85	14.36
	DC002	600	**1,539**	360	293	9.07	15.4	85.2	14.8	81.0	81.0	99.0	80.8	95.6	56	22	41	23.75	12.65
	DC003	450	**846**	458	684.5	8.63	11.4	97.6	2.4	95.3	95.3	70.4	88.9	91.3	56	20	41	21.10	7.39
	DC004	100	**242**	343	369	16.6	12.7	64.0	36	60.0	60.0	68.3	41.0	77	56	20	41	23.15	14.28
Crow Creek	CC150-Nates-001	300	**600**	263	180.2	16.3	17.6	78.3	21.7	74.7	74.7	77.6	67.3	89	56	21	41	20.40	7.55
	CC-350-001	400	**748**	284	194.5	20.7	27.9	40.5	59.5	35.8	35.8	1.9	10.5	30	55	21	40	20.50	6.03
	CC-350-002	750	**1,209**	325	343.6	19.4	29.7	94.3	5.7	85.1	85.1	85.6	83.2	89	55	20	40	20.00	8.65
	CC-350-003	500	**929**	348	326	17.0	22.3	77.2	22.8	73.8	73.8	80.4	70.0	92.8	56	20	41	22.00	12.27
	CC-350-004	600	**1,294**	345	357.7	16.7	14.6	86.5	13.5	85.2	85.2	88.8	83.3	96.8	56	21	41	22.30	8.07
	CC-350-005	600	**1,160**	316	292.5	25.7	47.6	80.5	19.5	70.3	70.3	89.6	68.7	88.2	56	20	41	19.35	8.43
SF Tincup Creek	SFTC1-FT0012[1]	300	**1,472**		1131	2.56	3.43	---	---	---	---	---	---	---	---	---	---	---	---

[1] Eggs from these parents either died during transport or were not successfully fertilized and were not continued in the test.

Table 5-4
Measured Aqueous Selenium Concentration in Trout Dietary Study

Nominal Selenium Treatment (µg/L)	TWA Total Recoverable Selenium	% of Nominal	TWA Dissolved Selenium	% of Nominal	Diss/TR (%)
Control	0.1 U	--	0.1 U	--	--
2.5	2.8	112	3	118	107
5	5.3	107	5.9	119	111
10	10.3	103	11.5	115	116
15	15.8	105	17.8	118	113
20	20.3	102	23.6	118	116
40	40.7	101	47.4	118	116

Table 5-5
Selenium Concentration in Yeast, *Lumbriculus* (worms) and Trout during Dietary Study

Nominal Selenium Treatment (µg/g)	Average Selenium Concentration in Yeast (µg/g dwt)	Average Selenium Concentration in Lumbriculus (µg/g dwt)	Average Selenium Concentration in Trout (µg/g dwt)
Control	<0.05	1.69 ± 0.39	1.438 ± 0.420
5.0 / 2.5	1.115 ± 0.39	3.82 ± 0.39	2.654 ± 0.617
10 / 5.0	4.34 ± 0.20	5.94 ± 0.14	4.462 ± 0.570
20 / 10	11.53 ± 6.5	10.6 ± 0.81	5.404 ± 1.720
30 / 15	23.95 ± 7.1	17.3 ± 3.1	14.774 ± 6.827
40 / 20	37.1 ± 6.8	22.0 ± 0.28	12.900 ± 1.845
80 / 40	85.15 ± 11.2	45.2 ± 2.7	34.480 ± 7.382

Note: Target selenium concentration in yeast was 2X higher to achieve the target concentration in *Lumbriculus* .

Table 5-6

Whole Body Selenium Concentration in Trout Prior to Dietary Study and at Test Termination

Nominal Selenium Treatment (µg/L)	Whole Body Fish Concentration	
	Average Selenium Concentration (µg/g dwt)	Average Selenium Concentration (µg/g dwt)
	Day 27	Day 71
Control	1.503 ± 0.0513	1.438 ± 0.420
2.5	1.467 ± 0.2346	2.654 ± 0.617
5	1.700 ± 0.0200	4.462 ± 0.570
10	1.673 ± 0.0709	5.404 ± 1.720
15	1.840 ± 0.2081	14.774 ± 6.827
20	1.713 ± 0.0289	12.900 ± 1.845
40	1.710 ± 0.1453	34.480 ± 7.382

Note: Day 27 analysis was prior to start of dietary study (i.e., aqueous exposure only).

Table 5-7

Percent Survival of Trout at Different Stages during the Aqueous-Dietary Study

Nominal Selenium Treatment (µg/L)	% Survival				
	Hatch	Swim-Up	Thinning	Selenium Diet Start	Termination
	(Day 6)	(Day 22)	(Day 27)	(Day 38)	(Day 71)
Control	72.1	70.1	70.1	67.1	50.6
2.5	84.9	80.3	79.3	73.6	42.7
5	82.6	81.6	78.5	71.9	45.4
10	72.9	67.9	67.9	60.9	34.9
15	80.9	78.1	77.1	69.2	40.7
20	79.5	76.9	76.3	71.6	28.5
40	76.1	73.5	73.5	65.1	34.5

Table 5-8
Summary of Dry Weight Measurement (per Original) for Trout at Test Termination

Nominal Selenium Treatment (µg/L)	Dry Weight (mg per original)						
	Rep A	Rep B	Rep C	Rep D	Rep E	Overall Mean	Std. Dev.
Control	40.87	43.35	37.09	43.67	60.87	45.17	9.17
2.5	39.22	25.12	23.59	45.35	34.51	33.56	9.25
5	26.38	38.3	30.49	37.63	59.34	38.43	12.7
10	23.01	20.19	33.86	27.39	89.8	38.85	28.9
15	27.09	33.39	31.79	40.49	41.97	34.94	6.21
20	18.02	14.14	24.22	34.17	29.48	24	8.16
40	22.47	44.99	28.49	29.51	53.22	35.74	12.8

Table 5-9
Summary of Dry Weight Measurement (per Surviving) for Trout at Test Termination

Nominal Selenium Treatment (µg/L)	Dry Weight (mg per surviving)						
	Rep A	Rep B	Rep C	Rep D	Rep E	Overall Mean	Std. Dev.
Control	90.82	78.81	82.41	79.4	81.17	82.52	4.85
2.5	71.31	71.76	78.64	64.78	77.65	72.83	5.59
5	87.94	69.64	76.22	68.42	81.59	76.76	8.2
10	65.73	80.75	67.72	78.25	89.8	76.45	9.89
15	90.3	74.19	70.63	67.48	73.45	75.21	8.84
20	90.09	94.25	69.19	62.13	88.43	80.82	14.2
40	89.89	74.98	81.41	84.31	106.4	87.4	11.9

Table 5-10
Summary of Standard Length Measurement for Trout at Test Termination

Nominal Selenium Treatment (µg/L)	Standard Length (mm)						
	Rep A	Rep B	Rep C	Rep D	Rep E	Overall Mean	Std. Dev.
Control	34	32.6	31.8	31.6	34.2	32.8	1.22
2.5	33.7	30.9	32.2	30.7	35.2	32.5	1.94
5	33.3	31.3	32.5	31.6	34.2	32.6	1.24
10	30.4	33.6	31.2	32.1	34	32.3	1.53
15	34.7	31.8	32.2	31	33	32.5	1.4
20	31	34.3	31.6	31.6	34.7	32.6	1.72
40	33	33.3	33.6	33.6	35.3	33.8	0.91

Table 5-11
Summary of Deformity Measures for Early Life Stage YCT

Treatment	Deformity Type and Severity									Mean Fraction Normal	Total n all reps
	Cranio-facial		Skeletal		Finfold			Edema			
	0	1	0	1	0	1	2	0	1		
Control	97.73%	2.27%	100.00%	0.00%	72.73%	25.00%	2.27%	100.00%	0.00%	93.00%	44
2.5	89.19%	10.81%	100.00%	0.00%	81.08%	16.22%	2.70%	72.97%	27.03%	87.00%	37
5	97.44%	2.56%	100.00%	0.00%	71.79%	28.21%	0.00%	97.44%	2.56%	91.00%	39
10	89.66%	10.34%	96.55%	3.45%	89.66%	6.90%	3.45%	100.00%	0.00%	94.00%	29
15	94.29%	5.71%	100.00%	0.00%	88.57%	11.43%	0.00%	82.86%	17.14%	89.00%	35
20	100.00%	0.00%	100.00%	0.00%	56.52%	43.48%	0.00%	52.17%	47.83%	79.00%	23
40	93.10%	6.90%	96.55%	3.45%	82.76%	17.24%	0.00%	79.31%	20.69%	90.00%	29

Severity Score: 0 = normal, 1 = slight or few, 2 = moderate or several, 3 = severe or many.

Table 6-1
Aquatic Community Data Used in the Species Sensitivity Distribution to Evaluate Brown Trout Sensitivity to Other Species

Taxa	Common Name	Whole Body Selenium (mg/kg dw)	Proportion Taxa	Effect Threshsold	Reference
Salmo Sp.	Brown trout	12.9	5%	EC_{10}	Formation Environmental (2011)
Oncorhynchus sp 1	Yellowstone cutthroat trout	17.6	14%	EC_{10}	Formation Environmental (2011)
Oncorhynchus sp 2	Rainbow trout	19.96	23%	EC_{10}	Holm et al. (2005)
Oligochaete	Tubifex	20	32%	Pop Eff	Swift (2002)
Centroptilum sp.	Mayfly	24.2	41%	NOEC	Conely et al. (2009)
Baetis sp.	Mayfly	35.2	50%	NOEC	Swift (2002)
Enallagma sp.	Damselfly	42	59%	NOEC	Swift (2002)
Brachionus sp.	Rotifer	42.4	68%	EC_{20}	Dobbs et al. (1996)
Caecidotea	Isopod	46.8	77%	Pop Eff	Swift (2002)
Chironomid sp.	Midge	54.4	86%	NOEC	Swift (2002)
Lumbriculus sp.	Worm	140	95%	NOEC	Besser et al. (2006)

Table 6-2
Site-Specific Data Used to Develop K_ds and TTFs

Stream	Location	Monitoring Event	C_{water} Dissolved Aqueous Selenium (mg/L)	$C_{particulate}$ Total Selenium Sediment (mg/kg dw)	$C_{particulate}$ Total Selenium Periphyton (mg/kg dw)	$C_{Invertebrate}$ Total Selenium Invertebrate (mg/kg dw)	$C_{sculpin\ (3)}$ Total Selenium Sculpin (mg/kg dw)	$C_{trout\ (4)}$ Total Selenium Trout (mg/kg dw)
Upstream of Sage Creek								
Crow Creek	CC-75	Fall 2006	0.00057	0.61	1.01	3.11	5.58	4.05
	CC-75	Spring 2007	0.00046	0.6	0.68	**6.59 (1)**	5.03	5.35
	CC-75	Fall 2007	0.00033	0.34	1.1	**6.93 (1)**	3.77	3.18
	CC-75	Spring 2008	0.0012	0.54	2.7	4.45	7.19	10.32
	CC-75	Fall 2008	0.0008	0.48	0.55	3.49	7.08	6.60
	CC-150	Fall 2006	0.00067	0.88	1.2	4.94	6.01	5.83
	CC-150	Spring 2007	0.00092	0.43	1.37	4.46	5.04	8.67
	CC-150	Fall 2007	0.00068	0.54	0.77	1.90	5.14	5.20
	CC-150	Spring 2008	0.0014	0.63	2.4	7.03	10.73	10.14
	CC-150	Fall 2008	0.0016	0.79	0.65	**6.58 (2)**	7.35	7.83
	CC-350	Fall 2006	0.00082	1.3	1.5	2.11	6.47	6.28
	CC-350	Spring 2007	0.0011	0.52	3.3	4.20	**8.1**	8.53
	CC-350	Fall 2007	0.00026	0.55	0.77	**6.66**	5.28	5.78
	CC-350	Spring 2008	0.00089	0.7	3.4	10.60	**11.23**	11.50
	CC-350	Fall 2008	0.0013	0.81	0.59	12.30	9.53	7.95
Deer Creek	DC-600	Fall 2006	0.0013	2.8	1.2	10.71	8.50	8.54
	DC-600	Spring 2007	0.0015	1.4	7.44	6.35	7.87	6.20
	DC-600	Fall 2007	0.002	2.6	**2.94 (1)**	**8.76 (1)**	7.63	5.85
	DC-600	Spring 2008	0.0014	0.98	8.7	8.65	7.96	12.83
	DC-600	Fall 2008	0.0034	0.95	1.65	7.01	8.62	10.54
Hoopes Spring and Sage Creek								
Hoopes Spring	HS-3	Fall 2006	0.0092	7	6.5	12.47	21.85	20.60
	HS-3	Spring 2007	0.018	6.2	12.00	11.40	18.57	18.83
	HS-3	Fall 2007	0.0161	7.5	6.20	15.41	26.63	17.89
	HS-3	Spring 2008	0.026	2.1	28.5	28.4	23.93	23.68
	HS-3	Fall 2008	0.0375	8.1	24.2	24.7	23.68	28.97
Sage Creek	LSV-2C	Fall 2006	0.0093	4.6	2.6	22.62	17.47	19.45
	LSV-2C	Spring 2007	0.0135	4.5	8.09	8.26	11.38	12.78
	LSV-2C	Fall 2007	0.0143	5.4	18.50	31.74	18.85	22.67
	LSV-2C	Spring 2008	0.0141	1.1	11.6	30.00	25.95	19.53
	LSV-2C	Fall 2008	0.0234	5.7	4.38	23.90	20.32	20.96
	LSV-4	Fall 2006	0.0068	3.3	7.42	10.00	20.01	16.20
	LSV-4	Spring 2007	0.0101	3.9	11.70	9.08	18.28	15.18
Downstream of Sage Creek								
Crow Creek	CC-1A	Fall 2006	0.0027	1.8	3.64	3.53	9.94	10.51
	CC-1A	Spring 2007	0.0012	1.1	3.39	12.9	**9.31**	9.33
	CC-1A	Fall 2007	0.0022	0.67	3.20	12.24	7.78	9.95
	CC-1A	Spring 2008	0.0029	1.2	7.10	15.50	**17.13**	16.85
	CC-1A	Fall 2008	0.0067	1.7	5.86	11.60	**12.41**	14.03
	CC-3A	Fall 2006	0.0029	1.45	3.10	5.48	14.45	10.44
	CC-3A	Spring 2007	0.0014	0.74	1.89	5.41	11.65	9.20
	CC-3A	Fall 2007	0.0018	0.83	3.80	**9.30 (1)**	**9.07**	11.25
	CC-3A	Spring 2008	0.0026	0.66	14.9	17.80	**13.16**	15.38
	CC-3A	Fall 2008	0.0058	1.3	1.67	11.20	**13.01**	19.68

[1] No data for this location/time period and value shown is predicted by linear regression.

[2] Value was an apparent outlier (21.5 mg/kg dw) and was deleted and replaced with a predicted value from the linear regression.

[3] All values are mean selenium concentrations for sculpin tissue except highlighted (bold) values which also include tissue data for other forage fish captured at the location (redside shiner and dace species).

[4] Mean of whole body selenium concentrations for brown trout and when tissue data were available YCT. Deer Creek is comprised only of YCT tissues.

Table 6-3
Derived Site-Specific K_ds and TTFs

Stream	Location	Monitoring Event	$C_{particulate}$ Invertebrates	Mean Periphyton and Sediment K_d	K_d (75:25 Periphyton: Sediment)	$K_d$sediment	$K_d$periphyton	$TTF_{invertebrate}$	$TTF_{sculpin}$	TTF_{trout}
Crow Creek	CC-75	Fall 2006	0.97	1421.05	1596.49	1070.18	1771.93	3.21	1.79	0.93
	CC-75	Spring 2007	0.67	1391.30	1434.78	1304.35	1478.26	9.81	0.76	0.92
	CC-75	Fall 2007	1.02	2181.82	2757.58	1030.30	3333.33	6.77	0.54	0.59
	CC-75	Spring 2008	2.48	1350.00	1800.00	450.00	2250.00	1.79	1.62	1.77
	CC-75	Fall 2008	0.54	643.75	665.63	600.00	687.50	6.43	2.03	1.25
	CC-150	Fall 2006	1.17	1552.24	1671.64	1313.43	1791.04	4.23	1.22	1.07
	CC-150	Spring 2007	1.28	978.26	1233.70	467.39	1489.13	3.50	1.13	1.82
	CC-150	Fall 2007	0.75	963.24	1047.79	794.12	1132.35	2.54	2.71	1.48
	CC-150	Spring 2008	2.22	1082.14	1398.21	450.00	1714.29	3.16	1.53	1.14
	CC-150	Fall 2008	0.66	450.00	428.13	493.75	406.25	9.92	1.12	1.12
	CC-350	Fall 2006	1.48	1707.32	1768.29	1585.37	1829.27	1.43	3.07	1.46
	CC-350	Spring 2007	3.02	1736.36	2368.18	472.73	3000.00	1.39	1.93	1.39
	CC-350	Fall 2007	0.75	2538.46	2750.00	2115.38	2961.54	8.91	0.79	0.97
	CC-350	Spring 2008	3.13	2303.37	3061.80	786.52	3820.22	3.39	1.06	1.05
	CC-350	Fall 2008	0.61	538.46	496.15	623.08	453.85	4.44	1.23	0.73
Geometric Means - background Crow Creek				**1239.10**	**1409.00**	**796.07**	**1561.55**	**3.91**	**1.35**	**1.13**
Deer Creek	DC-600	Fall 2006	1.36	1538.46	1230.77	2153.85	923.08	7.88	0.79	0.89
	DC-600	Spring 2007	6.84	2946.67	3953.33	933.33	4960.00	0.93	1.24	0.87
	DC-600	Fall 2007	2.90	1384.42	1426.63	1300.00	1468.84	3.02	0.87	0.71
	DC-600	Spring 2008	7.93	3457.14	4835.71	700.00	6214.29	1.09	0.92	1.54
	DC-600	Fall 2008	1.58	382.35	433.82	279.41	485.29	4.25	1.23	1.35
Geometric Means - background Deer Creek				**1526.77**	**1708.62**	**874.39**	**1825.65**	**2.52**	**0.99**	**1.03**
Hoopes Spring	HS-3	Fall 2006	6.55	733.70	720.11	760.87	706.52	1.90	1.75	1.20
	HS-3	Spring 2007	11.42	505.56	586.11	344.44	666.67	1.00	1.63	1.26
	HS-3	Fall 2007	6.33	425.47	405.28	465.84	385.09	2.43	1.73	0.85
	HS-3	Spring 2008	25.86	588.46	842.31	80.77	1096.15	1.10	0.84	0.91
	HS-3	Fall 2008	22.59	430.67	538.00	216.00	645.33	1.09	0.96	1.20
Sage Creek	LSV-2C	Fall 2006	2.80	387.10	333.33	494.62	279.57	8.08	0.77	0.97
	LSV-2C	Spring 2007	7.73	466.30	532.78	333.33	599.26	1.07	1.38	1.30
	LSV-2C	Fall 2007	17.19	835.66	1064.69	377.62	1293.71	1.85	0.59	0.90
	LSV-2C	Spring 2008	10.55	450.35	636.52	78.01	822.70	2.84	0.87	0.70
	LSV-2C	Fall 2008	4.51	215.38	201.28	243.59	187.18	5.30	0.85	0.95
	LSV-4	Fall 2006	7.01	788.24	939.71	485.29	1091.18	1.43	2.00	1.08
	LSV-4	Spring 2007	10.92	772.28	965.35	386.14	1158.42	0.83	2.01	1.11
Geometric Means - Hoopes Spring and Sage Creek				**515.99**	**587.86**	**297.06**	**647.32**	**1.85**	**1.18**	**1.02**
Crow Creek	CC-1A	Fall 2006	3.46	1007.41	1177.78	666.67	1348.15	1.02	2.82	1.56
	CC-1A	Spring 2007	3.16	1870.83	2347.92	916.67	2825.00	4.08	0.72	0.84
	CC-1A	Fall 2007	2.95	879.55	1167.05	304.55	1454.55	4.15	0.64	0.99
	CC-1A	Spring 2008	6.51	1431.03	1939.66	413.79	2448.28	2.38	1.11	1.03
	CC-1A	Fall 2008	5.44	564.18	719.40	253.73	874.63	2.13	1.07	1.17
	CC-3A	Fall 2006	2.94	784.48	926.72	500.00	1068.97	1.87	2.64	1.05
	CC-3A	Spring 2007	1.78	939.29	1144.64	528.57	1350.00	3.05	2.15	1.08
	CC-3A	Fall 2007	3.50	1286.11	1698.61	461.11	2111.11	2.65	0.98	1.23
	CC-3A	Spring 2008	13.48	2992.31	4361.54	253.85	5730.77	1.32	0.74	0.99
	CC-3A	Fall 2008	1.63	256.03	271.98	224.14	287.93	6.86	1.16	1.63
Geometric Means - Crow Creek downstream Sage Creek				**997.13**	**1254.13**	**397.46**	**1372.58**	**2.56**	**1.22**	**1.13**
Total Geometric Mean			**3.12**	**939.14**	**1092.38**	**518.77**	**1226.19**	**2.71**	**1.22**	**1.09**

Notes:

$C_{particulate}$ invertebrates = ($C_{particulate}$ sediment*0.1)+($C_{particulate}$ periphyton*0.9).

Mean Periphyton and Sediment K_d = ($K_d$sediment + $K_d$periphyton)/2.

K_d (75:25 Periphyton: Sediment) = Sum ($K_d$sediment *0.25) + ($K_d$periphyton *0.75).

K_d sediment = $C_{particulate}$ sediment/Dissolved Aqueous Selenium.

K_d periphyton = $C_{particulate}$ periphyton/ Dissolved Aqueous Selenium.

$TTF_{invertebrate}$ = $C_{invertebrate}$/$C_{particulate}$invertebrates.

$TTF_{sculpin}$ = $C_{sculpin}$/$C_{invertebrate.}$

TTF_{trout} = C_{trout}/($C_{sculpin}$*0.5) + ($C_{invertebrate}$*0.5).

Table 6-4
Relationship Between Dietary and Egg Selenium in Laboratory Studies With Trout

Reference	Species	Lifestage Exposed	Dietary Selenium (µg/g dry wt)	Ovary Selenium (µg/g dry wt)	Egg Selenium (µg/g dry wt)	$TTF_{trout\ eggs}$
Hardy 2005; Hardy et al. 2009	Cutthroat trout	LC	1.2	-	1.64	1.37
Hardy 2005; Hardy et al. 2009	Cutthroat trout	LC	3.2	-	7.82	2.06
Hardy 2005; Hardy et al. 2009	Cutthroat trout	LC	5.2	-	6.61	1.03
Hardy 2005; Hardy et al. 2009	Cutthroat trout	LC	7.2	-	5.05	0.56
Hardy 2005; Hardy et al. 2009	Cutthroat trout	LC	9.2	-	5.18	0.45
Hardy 2005; Hardy et al. 2009	Cutthroat trout	LC	11.2	-	16.04	1.34
		Geometric Mean of Trout Egg to Diet TTF				**0.997**

LC = Life Cycle

Table 6-5
Variables Used in the Derivation of an Aqueous Trigger Value

Data Integration Approach	Variables										Predicted Aqueous Selenium Concentration (mg/L) based on Target Egg Threshold (20.8 mg/kg) and K_d Value Indicated		
	$C_{trout\ tissue}$ (mg/kg dw)	TTF trout eggs	$C_{particulate\ inverts}$	Average K_d	75:25 periphyton K_d	K_d sed	K_d periphyton	TTF invertebrate	TTF sculpin	TTF trout	Average K_d	75:25 periphyton K_d	K_d periphyton
Geometric mean for downstream zone data	20.8	0.997	6.07	696.13	829.57	344.06	949.90	2.14	1.20	1.07	0.0180	0.0151	0.0132
Geometric Seasonal Mean for downstream zone data	20.8	0.997	5.03	585.65	659.47	387.89	721.87	2.53	1.22	1.11	0.0190	0.0169	0.0154
Geometric Seasonal Mean downstream zone and Background data	20.8	0.997	2.34	786.05	851.59	596.98	903.10	3.36	1.25	1.01	0.0115	0.0106	0.0100

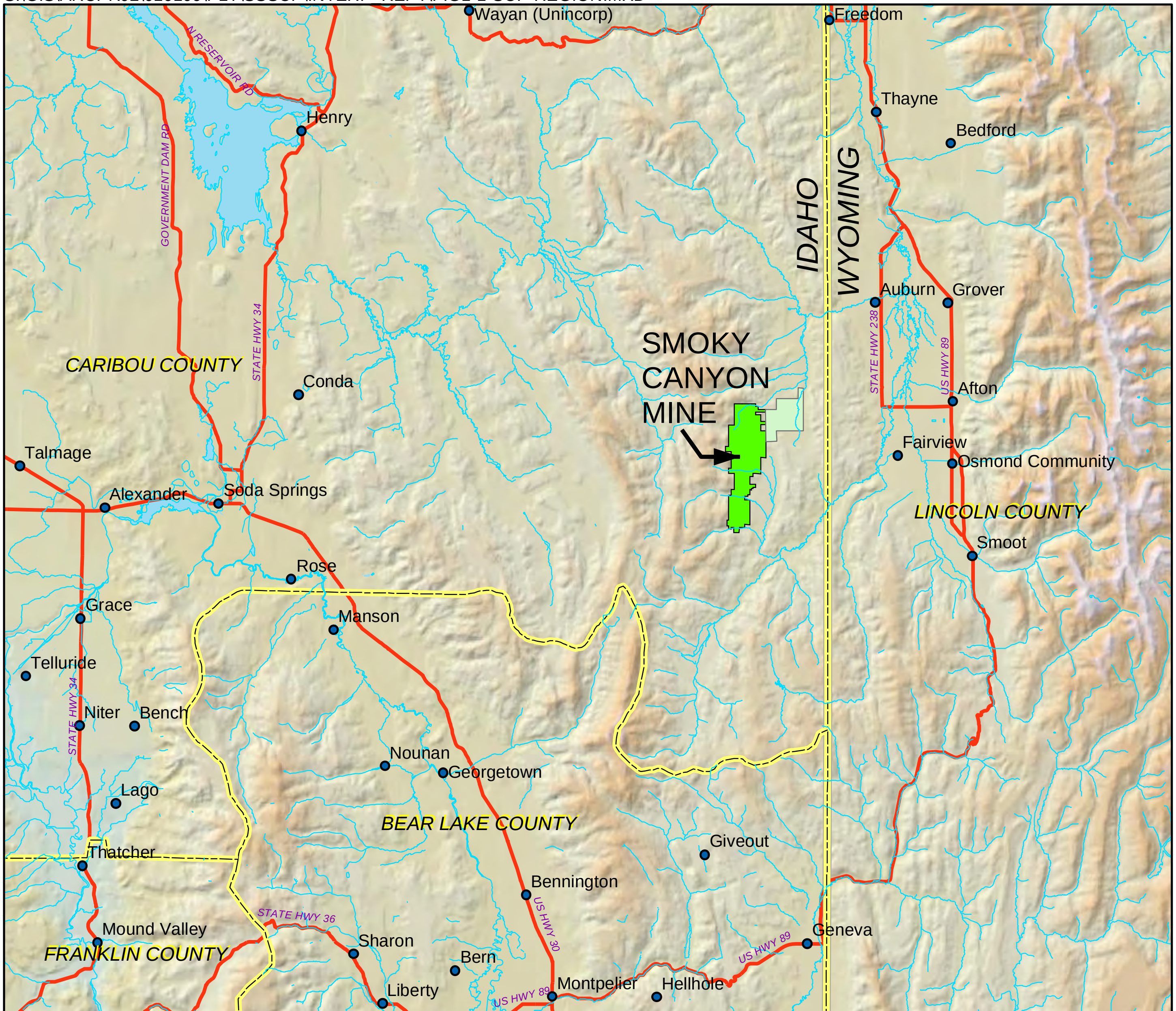
Equation:

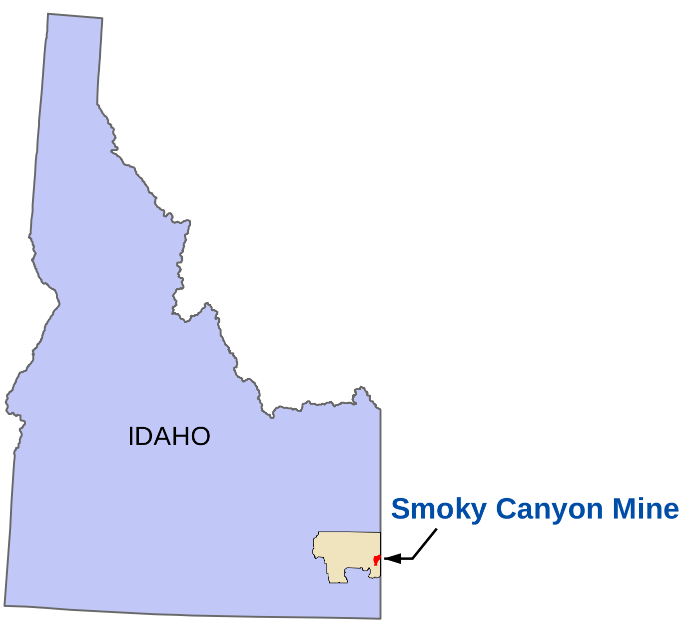
$$C_{water} = \frac{C_{trout\ tissue}}{K_d \times [(TTF_{sculpin}\ \ x\ 0.5) + (\ TTF_{invertebrate}\ \ x\ 0.5)] \times TTF_{trout\ eggs}}$$

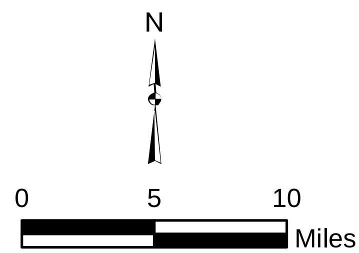
Notes:
Downstream Zone Data – Hoopes Spring (HS-3), Sage Creek (LSV-2C and LSV-4), Crow Creek d/s Sage (CC-1A and CC-3A).
Background Data – (CC-75, CC-150, CC-350, and DC-600).
Seasonal Mean – Fall Data only
Data from the location HS and SFTC-1 are excluded from all calculations.
Average K_d = mean of K_d sediment and K_d periphyton.
75:25 periphyton K_d = 75% periphyton K_d and 25% sediment $K_{d.}$
K_d periphyton = 100% periphyton $K_{d.}$
$C_{particulate\ inverts}$ = 90% invertebrate conc + 10% sediment conc.

FIGURES

S:\GIS\ARCPRJ2\010109\PLT\SSSCP\INTERP_REPT\FIG1-1-SCP-REGION.MXD







J.R. SIMPLOT COMPANY

SMOKY CANYON MINE

FIGURE 1-1

LOCATION OF THE SMOKY CANYON MINE

PRJ: 009-004.70	DATE: JULY 1, 2010	
REV: 0	BY: RCR	CHK: SMC

FORMATION ENVIRONMENTAL

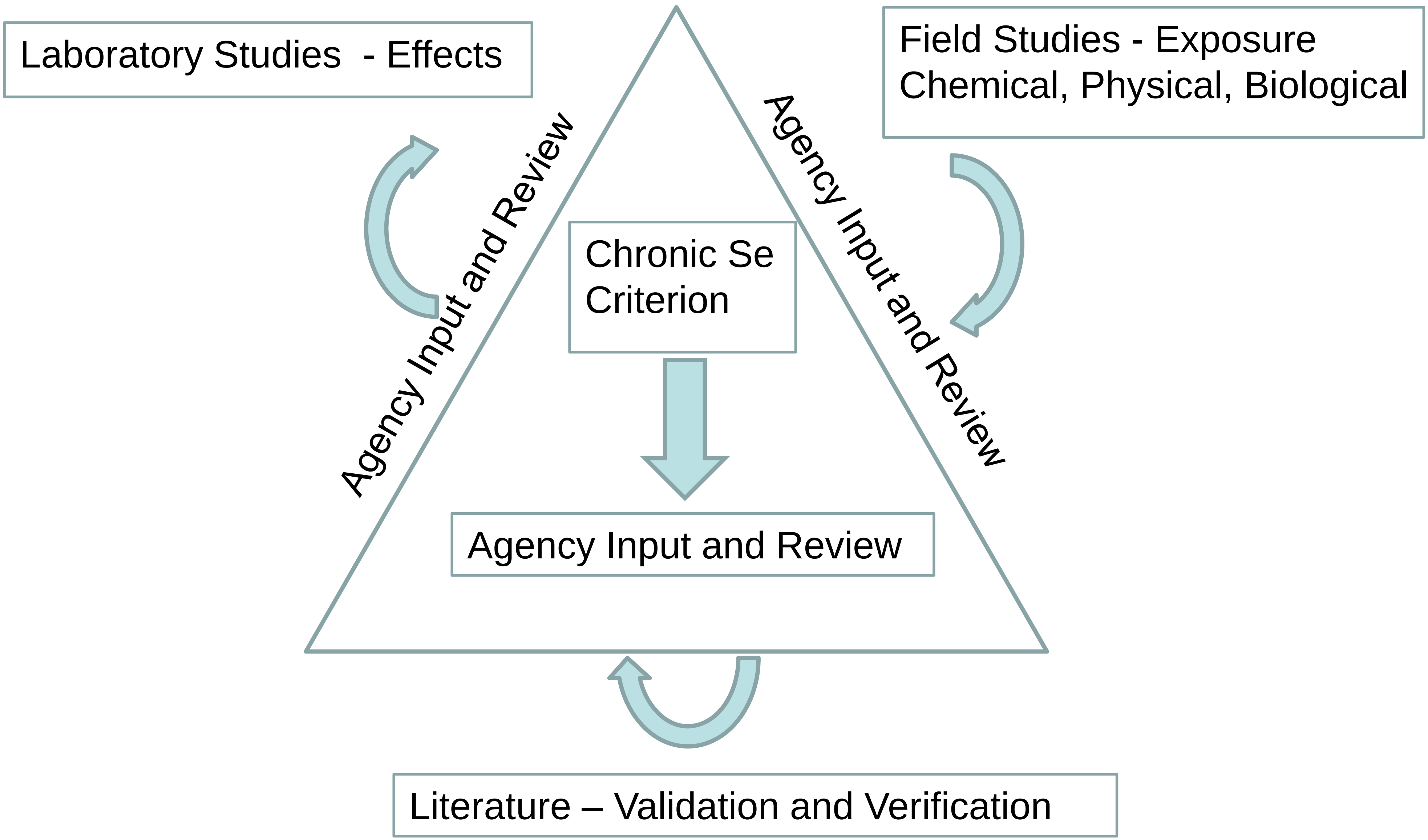
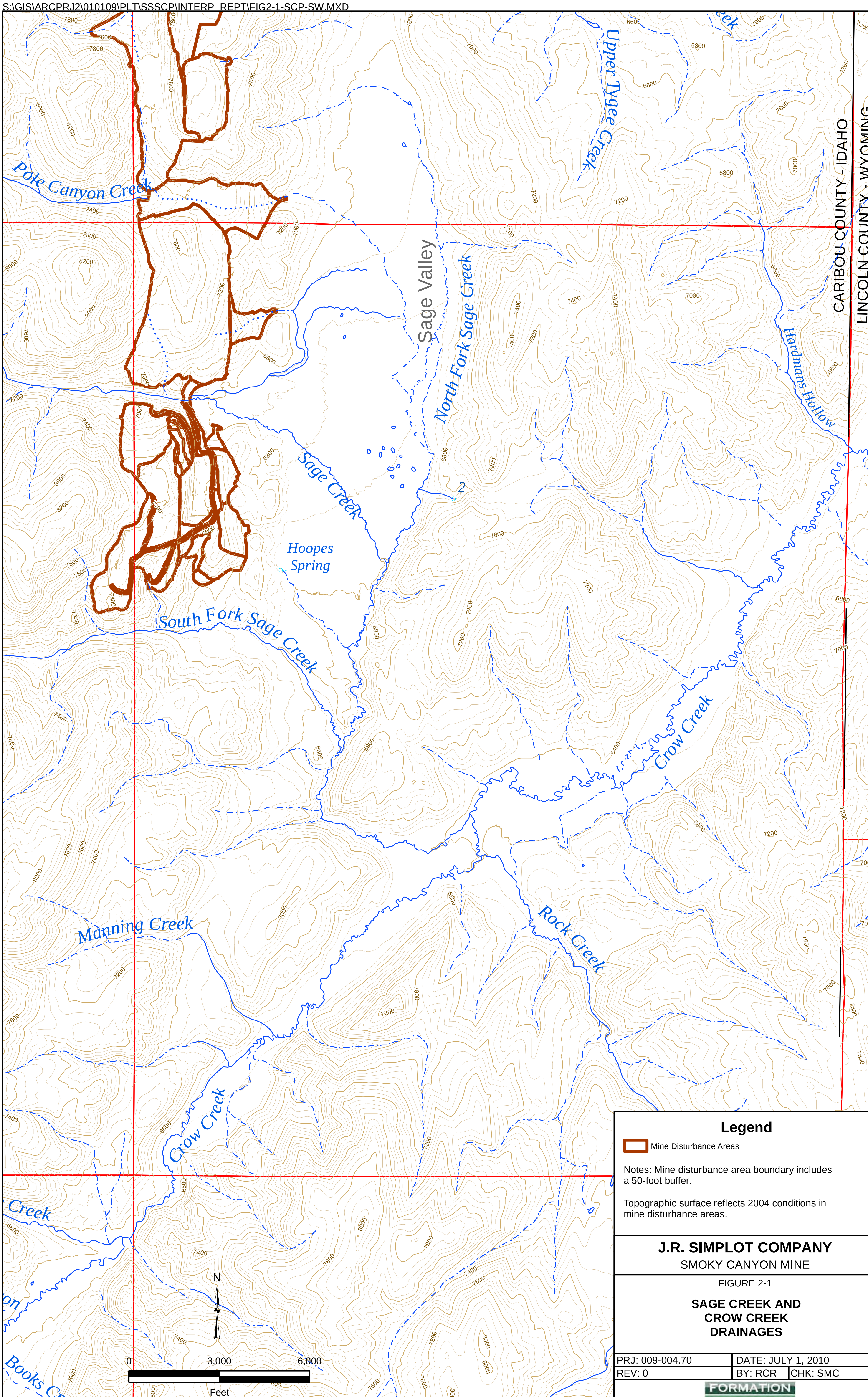


Figure 1-2
Relationship of Lines of Evidence to Developing a Site-Specific Selenium Criterion

J.R. Simplot Company Site-Specific Selenium Criterion	
PRJ: 0442-004-900.70	DATE: January 2012
REV: 1	BY: SMC / CHK: SMC

FORMATION ENVIRONMENTAL

S:\GIS\ARCPRJ2\010109\PLT\SSSCP\INTERP_REPT\FIG2-1-SCP-SW.MXD



Legend

Mine Disturbance Areas

Notes: Mine disturbance area boundary includes a 50-foot buffer.

Topographic surface reflects 2004 conditions in mine disturbance areas.

J.R. SIMPLOT COMPANY

SMOKY CANYON MINE

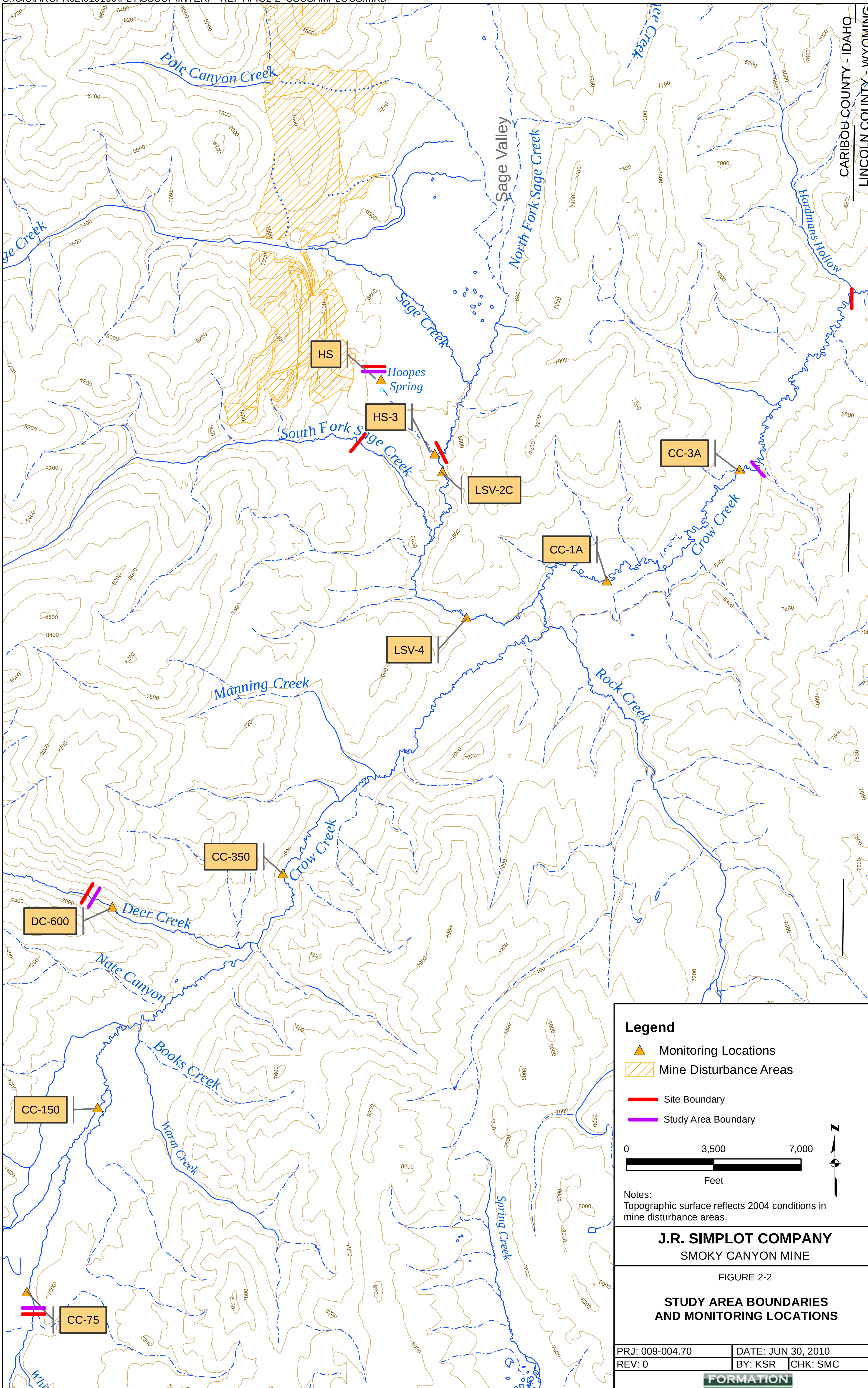
FIGURE 2-1

SAGE CREEK AND CROW CREEK DRAINAGES

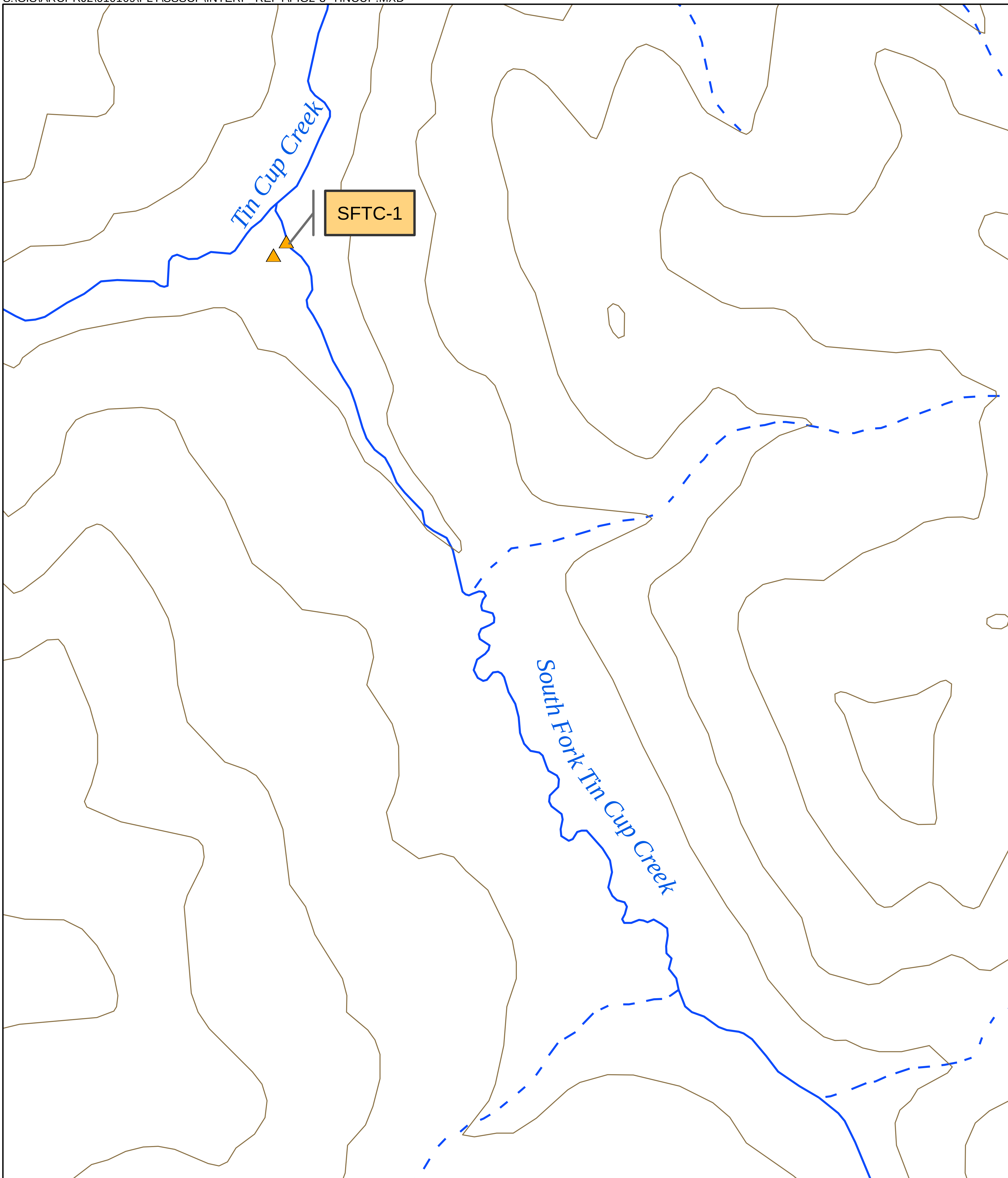
PRJ: 009-004.70	DATE: JULY 1, 2010	
REV: 0	BY: RCR	CHK: SMC

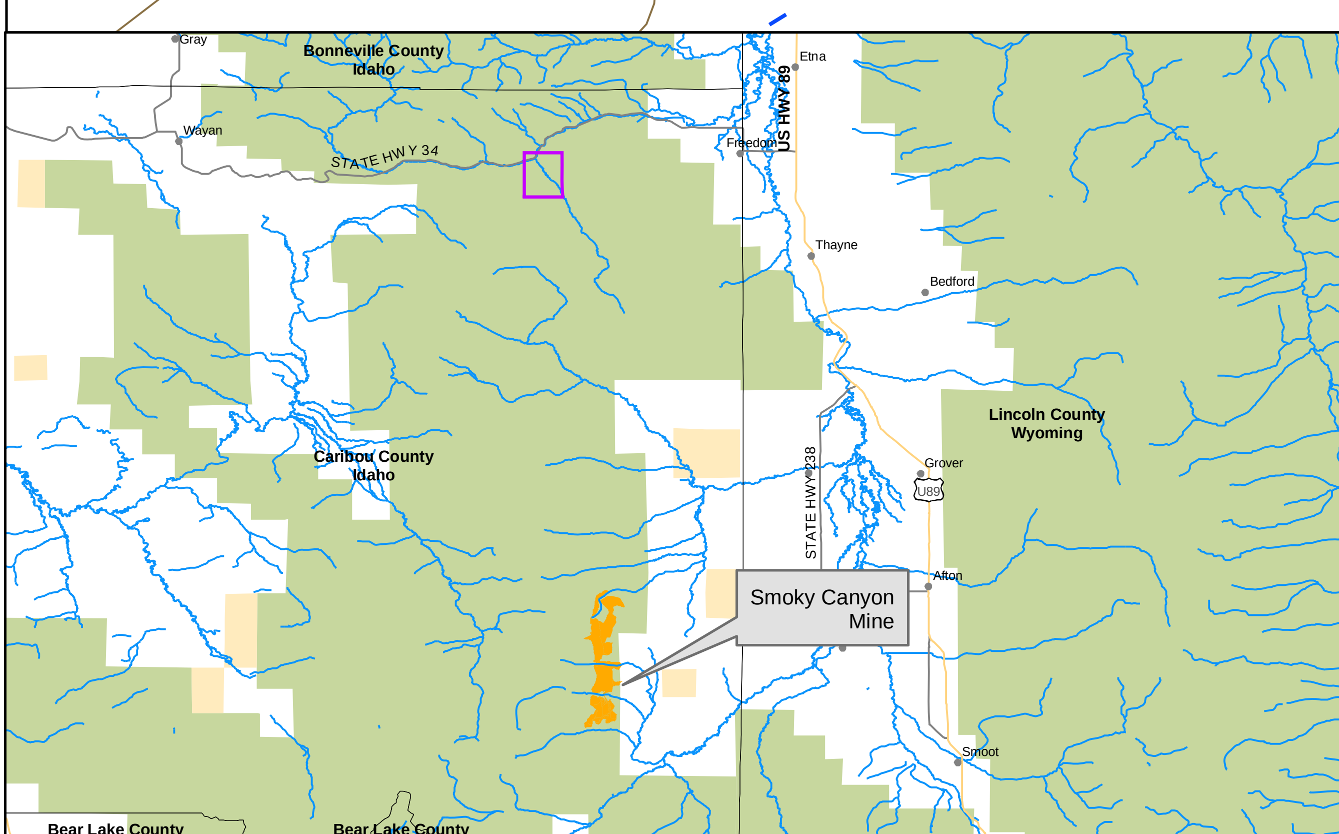
FORMATION ENVIRONMENTAL

S:\GIS\ARCPRJ2\010109\PLT\SSSCP\INTERP_REPT\FIG2-2_SSCSAMPLOCS.MXD



S:\GIS\ARCPRJ2\010109\PLT\SSSCP\INTERP_REPT\FIG2-3_TINCUP.MXD





Legend

△ Monitoring Location

Feet

0 600 1,200

N

J.R. SIMPLOT COMPANY

SMOKY CANYON MINE

FIGURE 2-3

SOUTH FORK TINCUP CREEK MONITORING LOCATION

PRJ: 009-004.70	JULY 1, 2010	
REV: 0	BY: KSR	CHECKED: SMC

FORMATION ENVIRONMENTAL

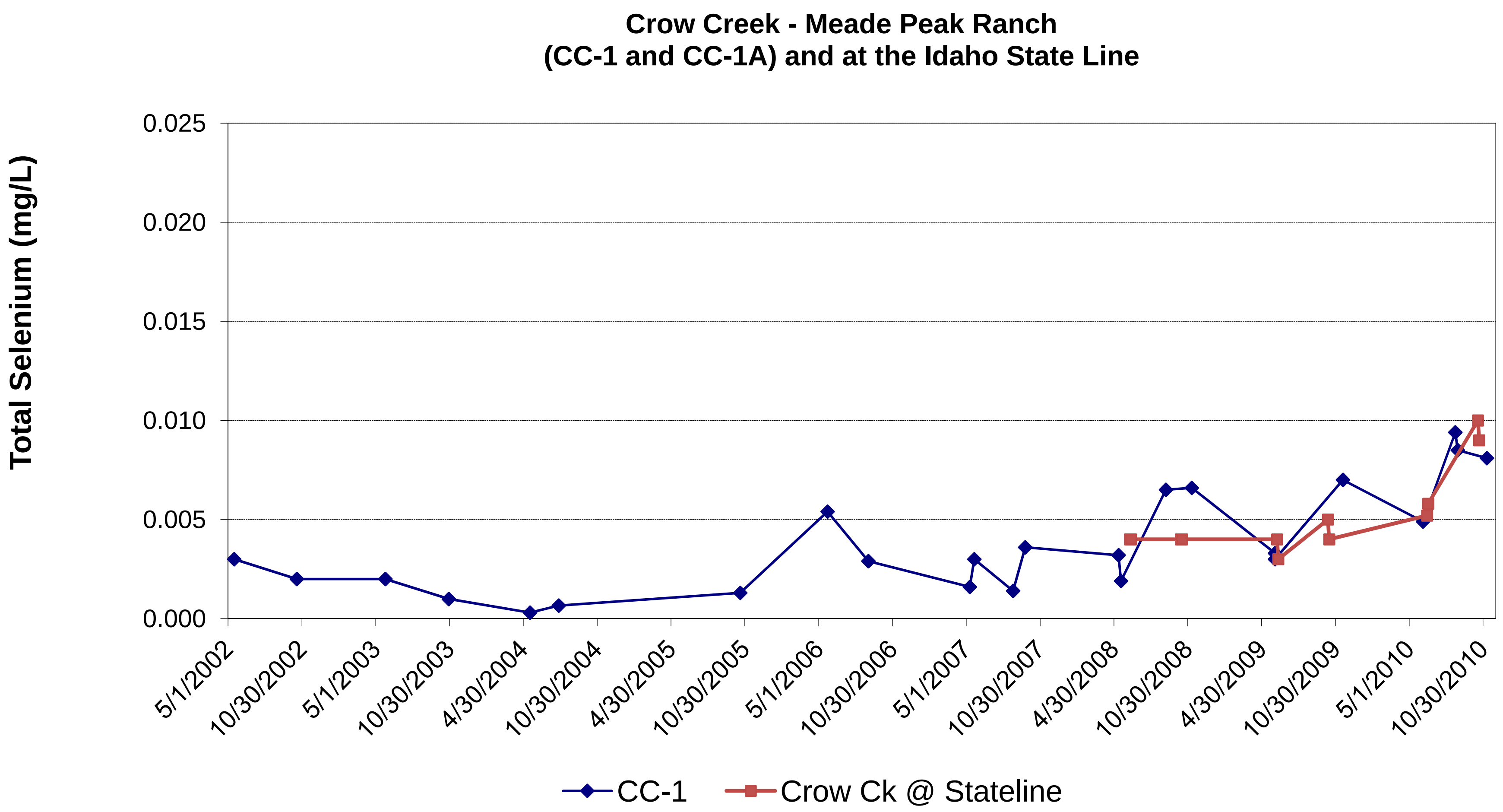
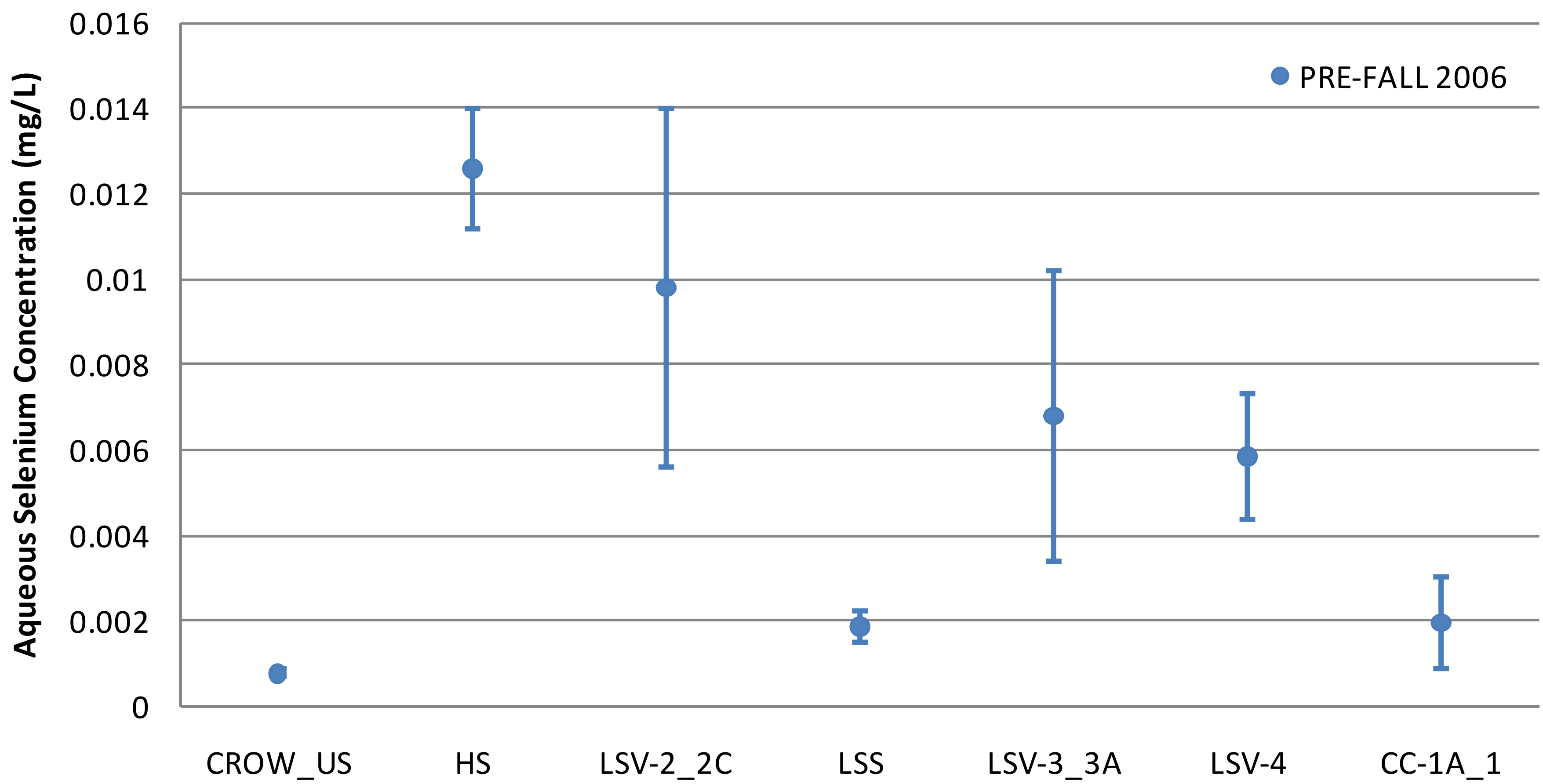


Figure 2-4
Total Selenium in Surface Water Measured at Crow Creek-Meade Peak Ranch (2002-2010)

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 0	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL



Error bars denote 95 CI.

CROW_US includes Crow Creek locations upstream of Sage Creek (CC-7, SW-CC-100, SW-CC-300, SW-CC-50, AWI012-29, CC-300, CC-350, CC-75, and CC-150); all locations are upstream of SSSC location CC-350.

Figure 2-5
Average Aqueous Selenium Concentrations Prior to Fall 2006

J.R. Simplot Company Site-Specific Selenium Criterion	
PRJ: 0442-004-900.70	DATE: January 2012
REV: 1	BY: SMC / CHK: SMC

FORMATION ENVIRONMENTAL

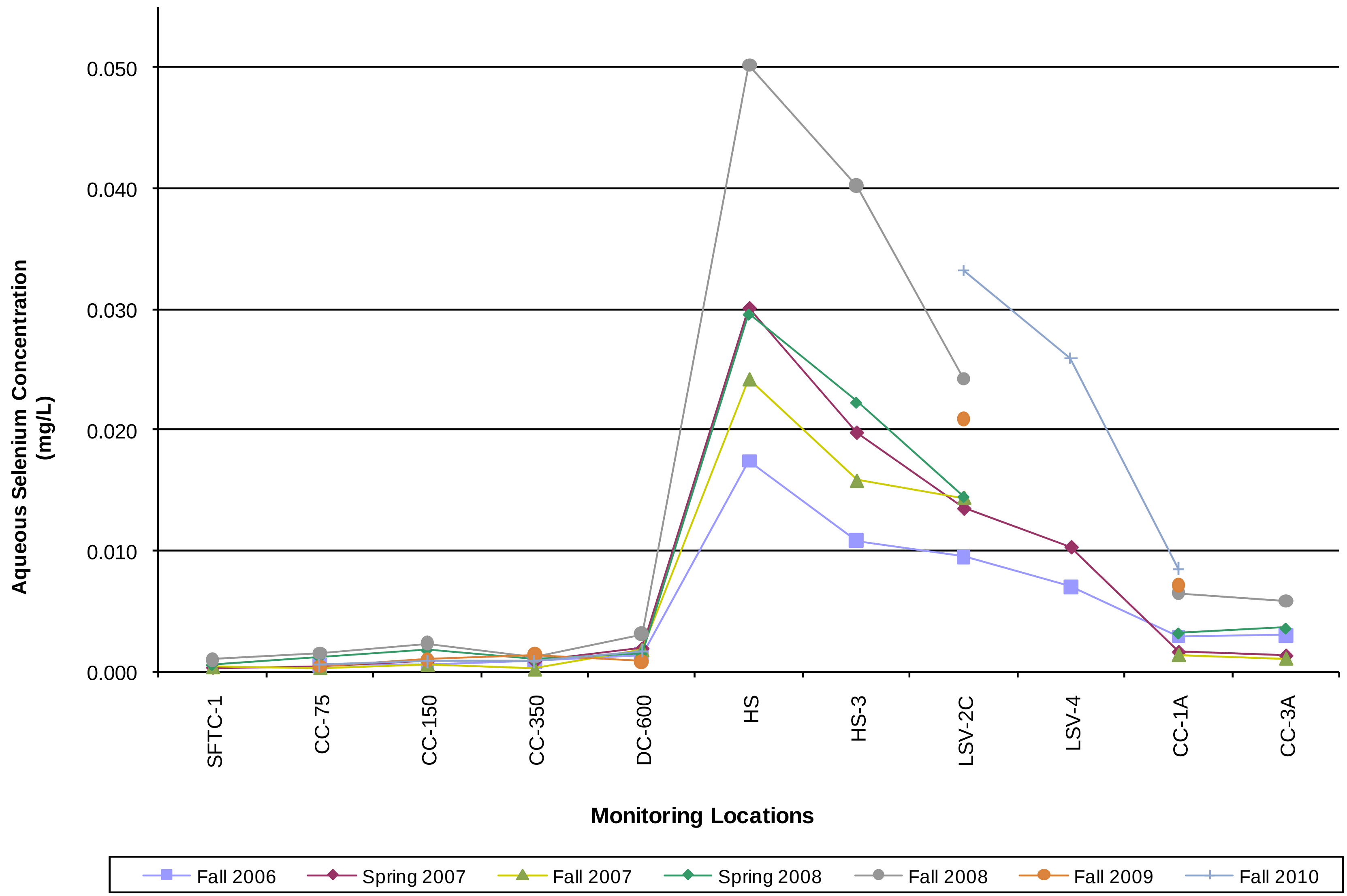


Figure 4-1
Aqueous Selenium Concentrations from 11 Locations During 2006 - 2010

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012
REV: 1	BY: SMC CHK: SMC

FORMATION ENVIRONMENTAL

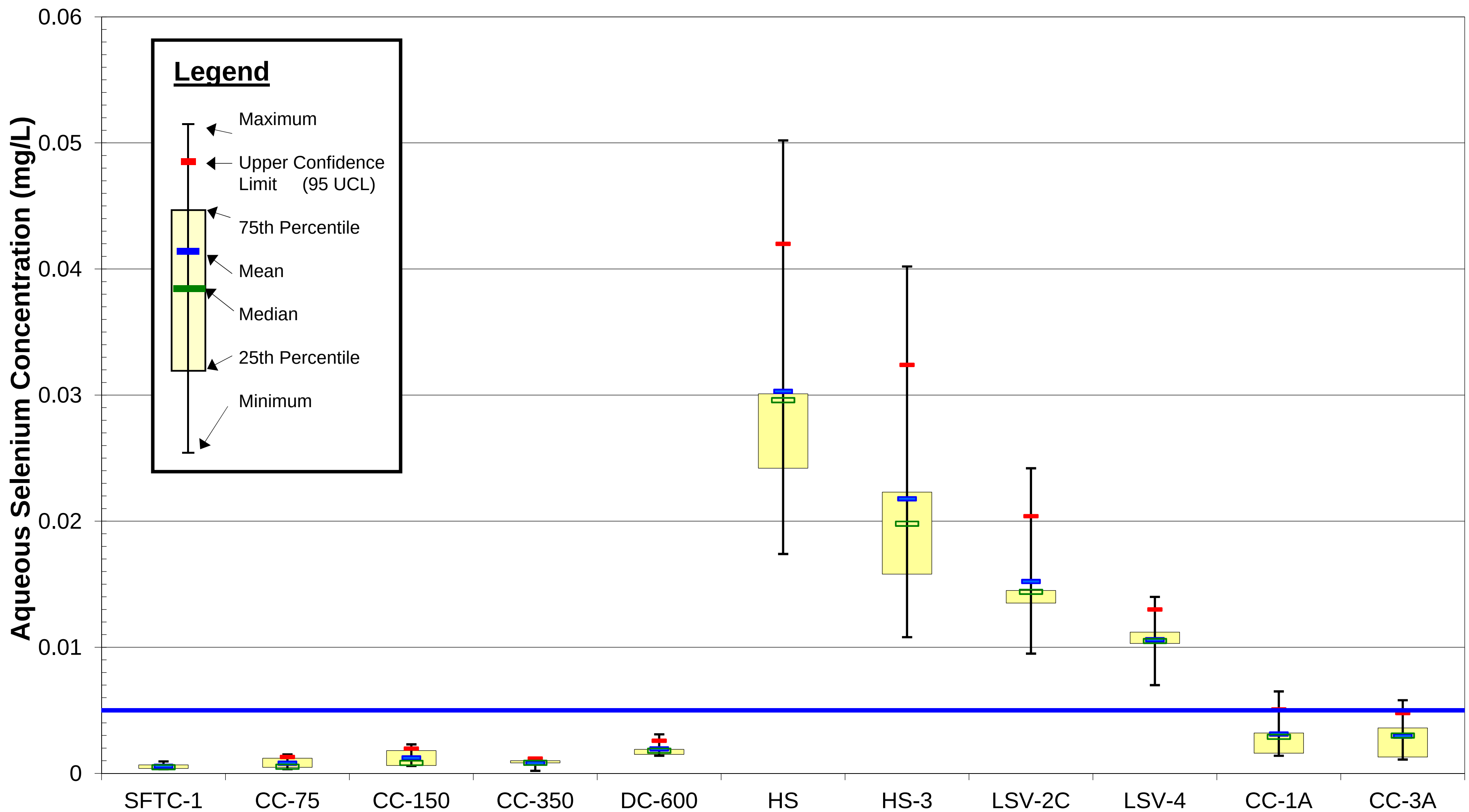


Figure 4-2
Summary Statistics for Aqueous Selenium Concentrations During 2006, 2007 and 2008

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

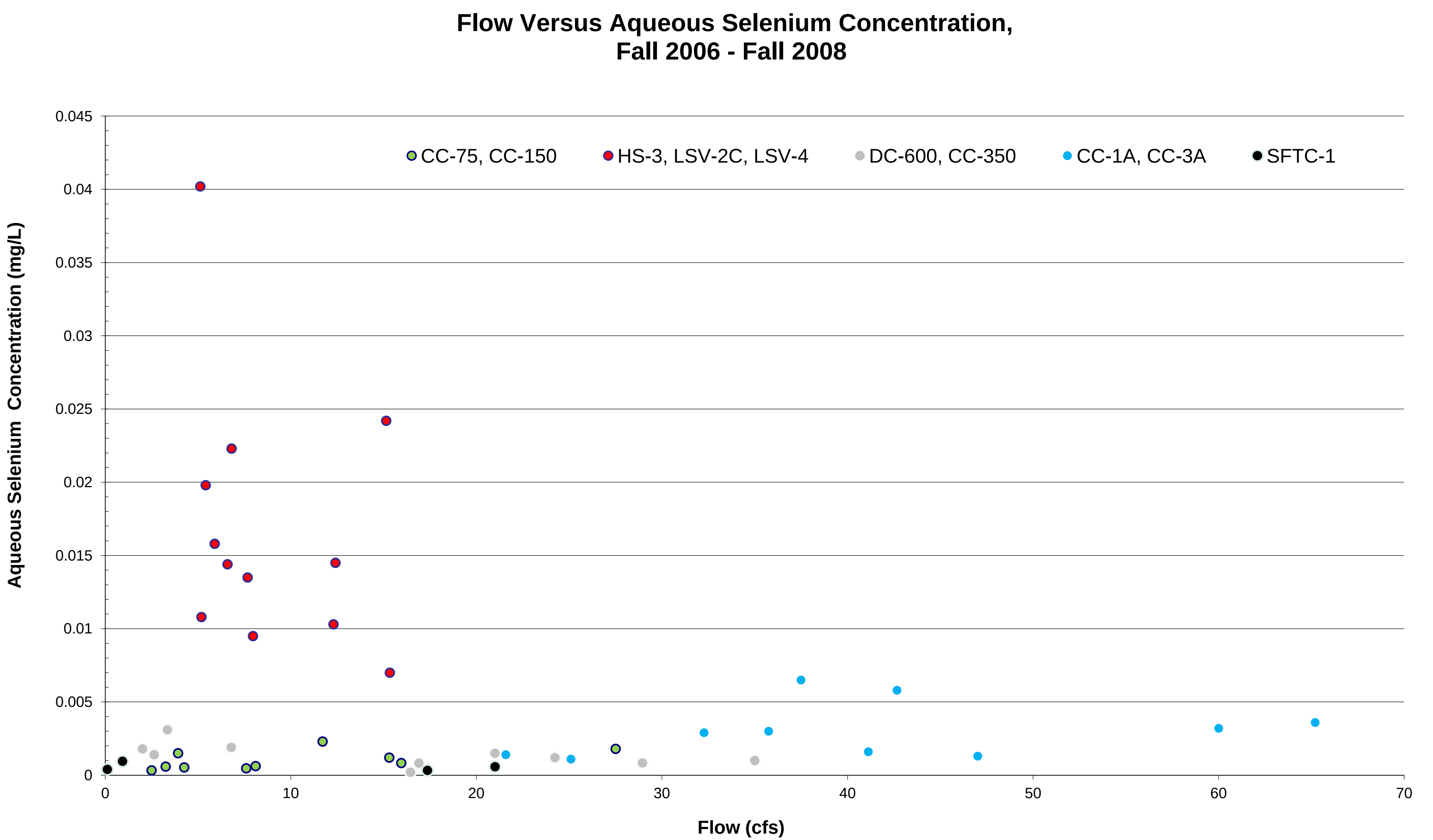
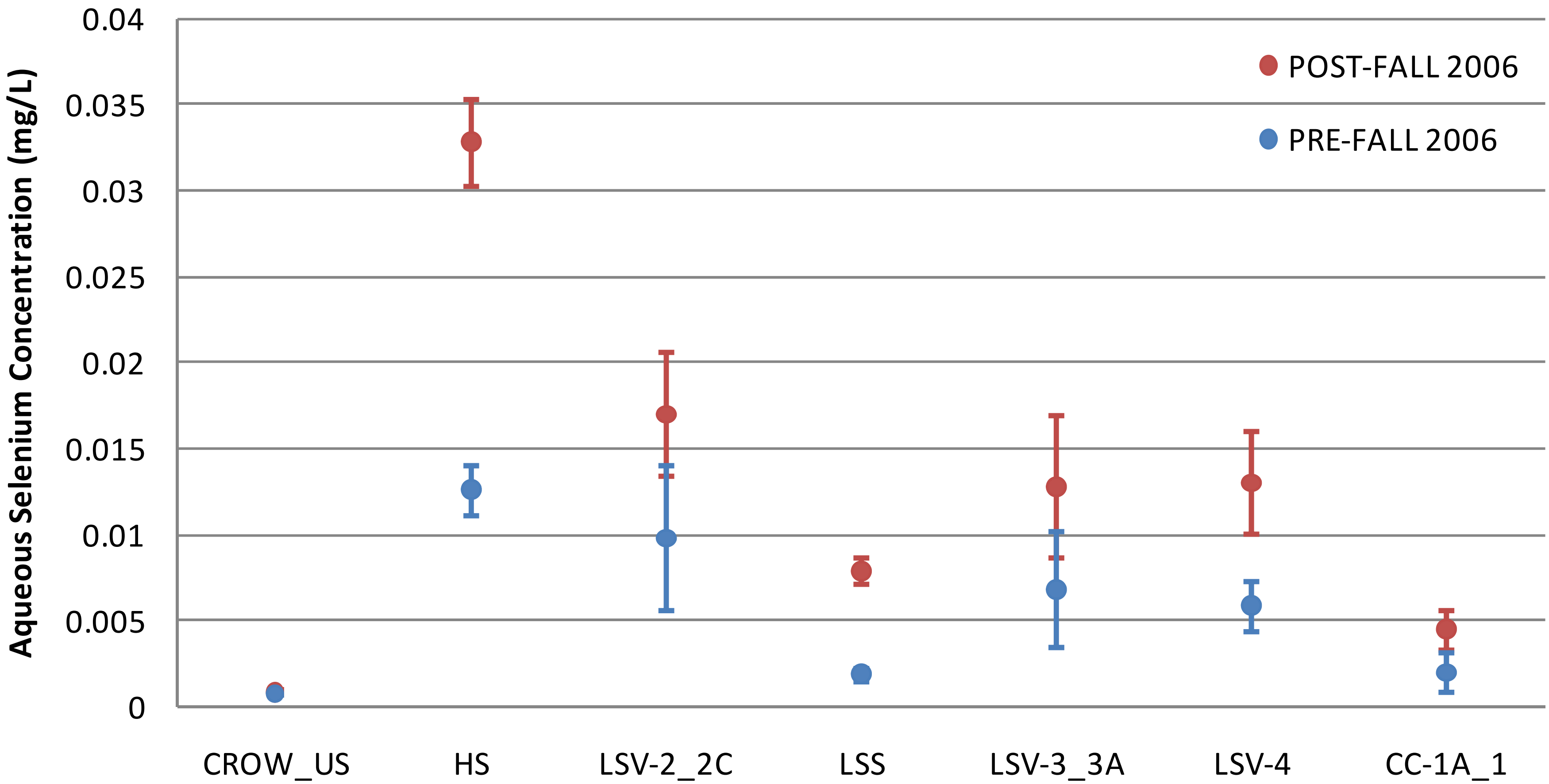


Figure 4-3
Flow Versus Aqueous Selenium Concentrations
Fall 2006-Fall 2008

J.R. Simplot Company Site-Specific Selenium Criterion	
PRJ: 0442-004-900.70	DATE: January 2012
REV: 1	BY: SMC CHK: SMC

FORMATION ENVIRONMENTAL



Error bars denote 95 CI.

CROW_US includes Crow Creek locations upstream of Sage Creek (CC-7, SW-CC-100, SW-CC-300, SW-CC-50, AWI012-29, CC-300, CC-350, CC-75, and CC-150); all locations are upstream of SSSC location CC-350.

Figure 4-4
Average Aqueous Selenium Concentrations Prior to SSSC Study (2000 to 2006) and During/Following the SSSC Study (2006 to 2010)

J.R. Simplot Company Site-Specific Selenium Criterion	
PRJ: 0442-004-900.70	DATE: January 2012
REV: 0	BY: SMC / CHK: SMC

FORMATION ENVIRONMENTAL

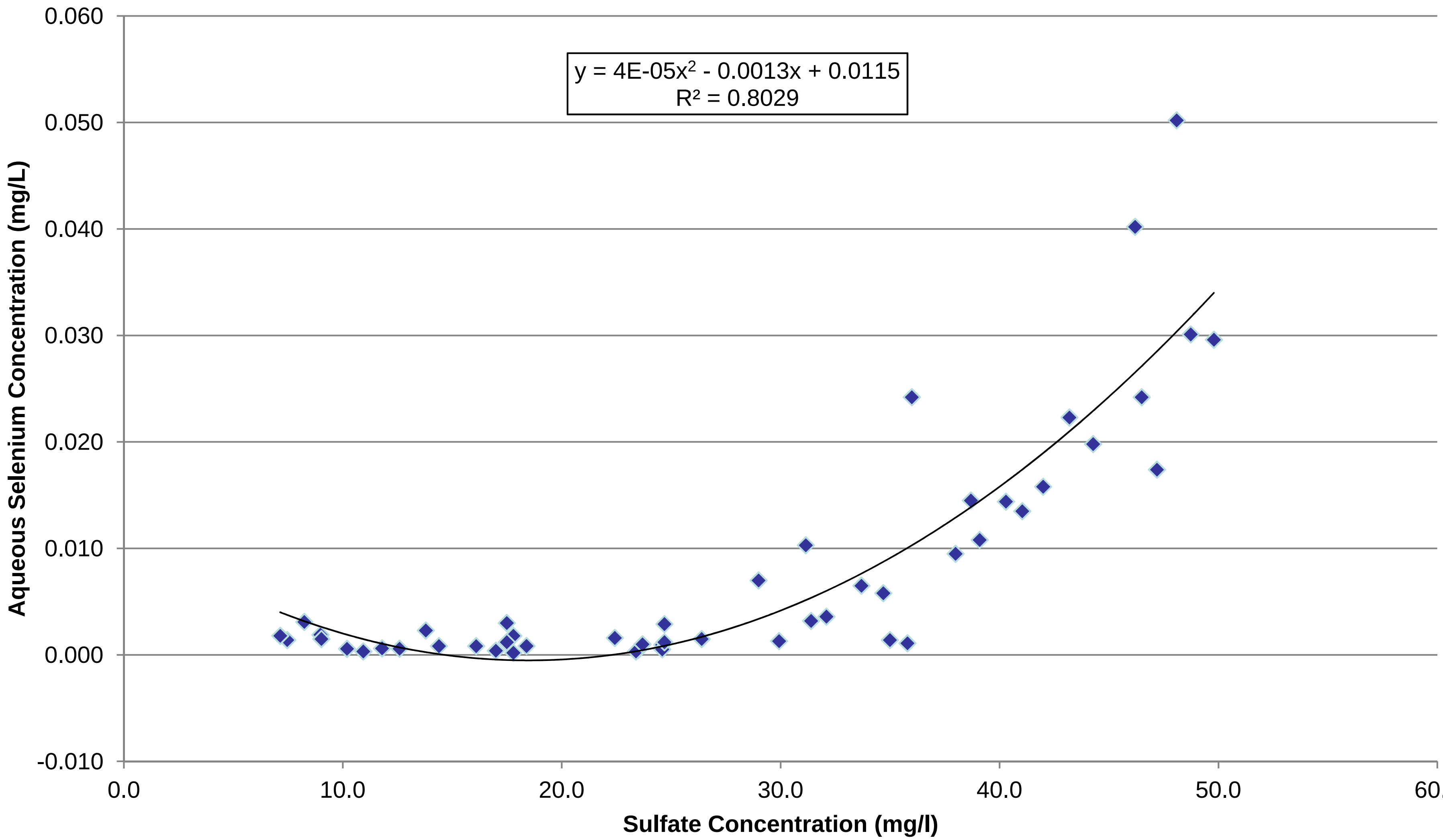


Figure 4-5
Relationship of Aqueous Selenium Concentrations to Sulfate Concentrations in Study Area Surface Waters, 2006 to 2008

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

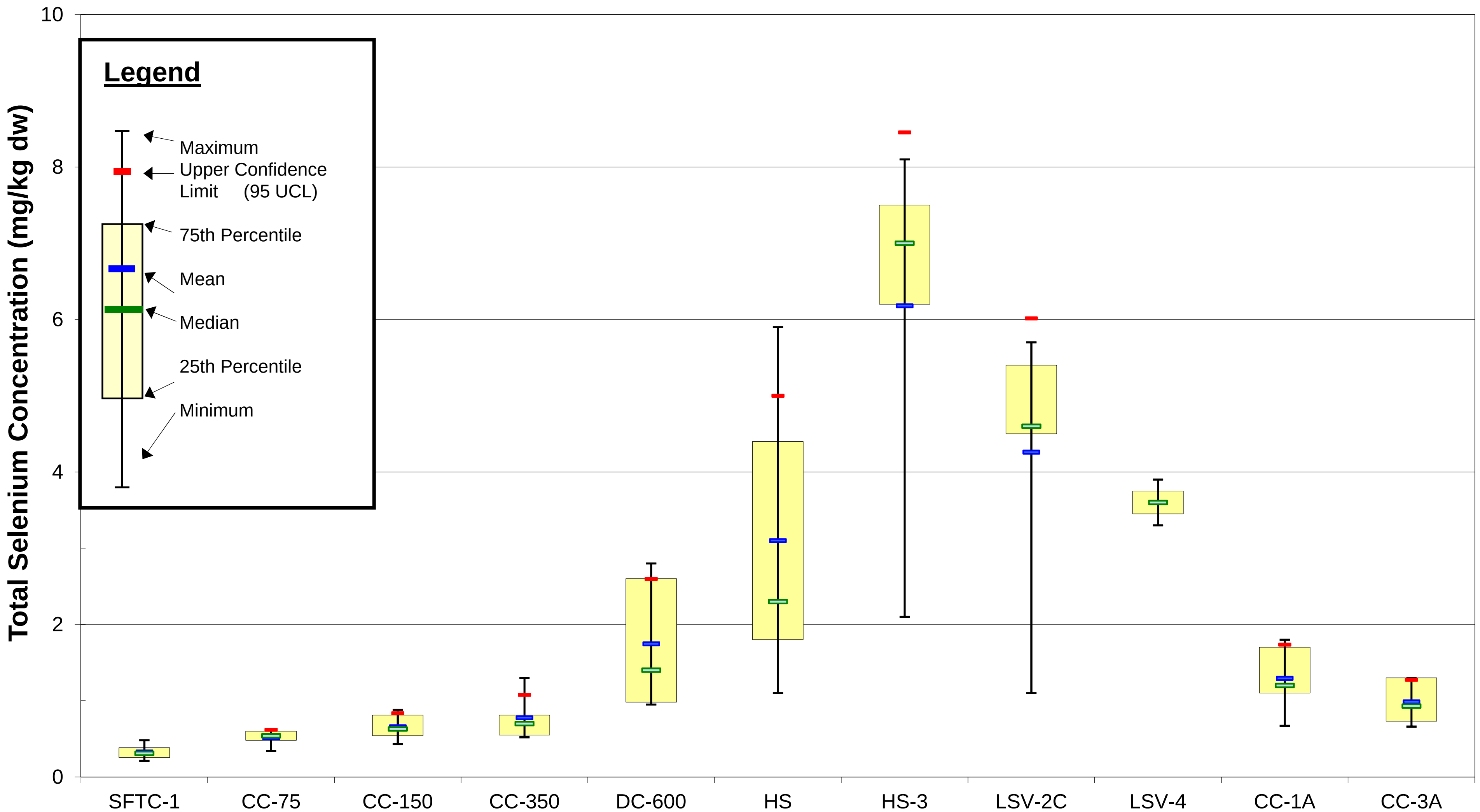


Figure 4-6
Summary Statistics for Total Selenium in Sediment During 2006, 2007, and 2008

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012
REV: 1	BY: SMC / CHK: SMC

FORMATION ENVIRONMENTAL

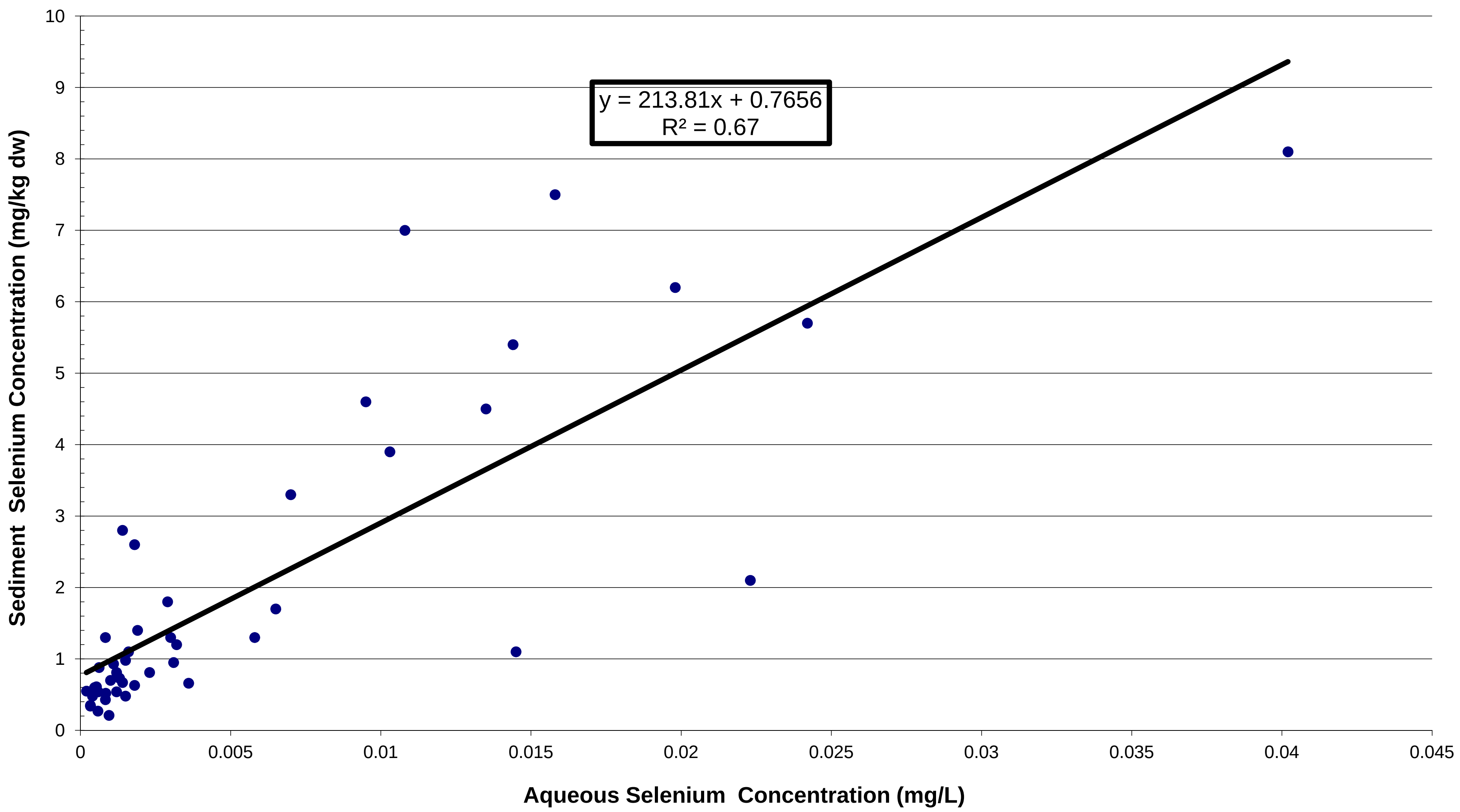


Figure 4-7
Relationship of Aqueous Selenium Concentrations to Sediment Selenium Concentrations, Fall 2006 – Fall 2008

J.R. Simplot Company Site-Specific Selenium Criterion	
PRJ: 0442-004-900.70	DATE: January 2012
REV: 0	BY: SMC / CHK: SMC

FORMATION ENVIRONMENTAL

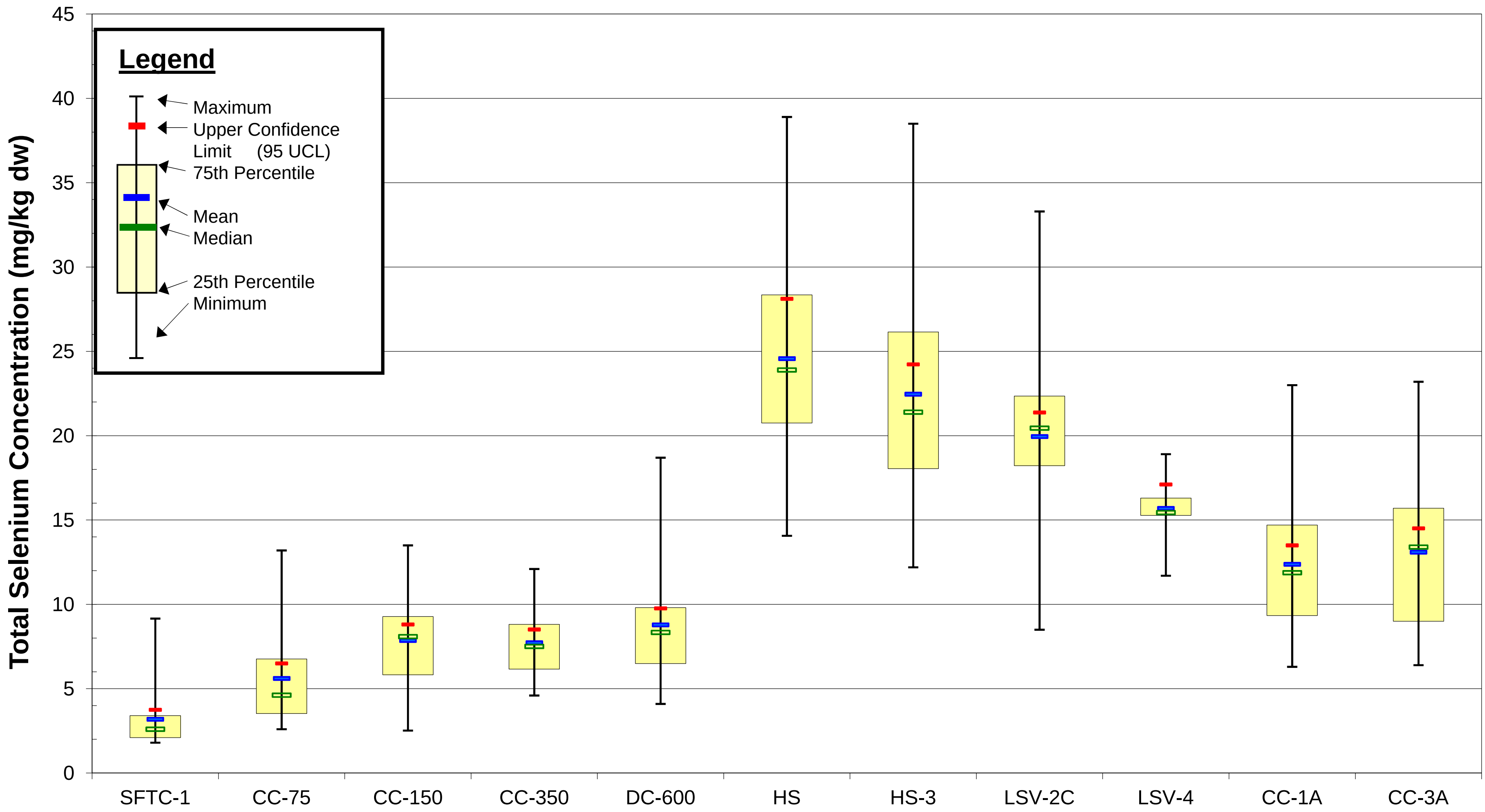
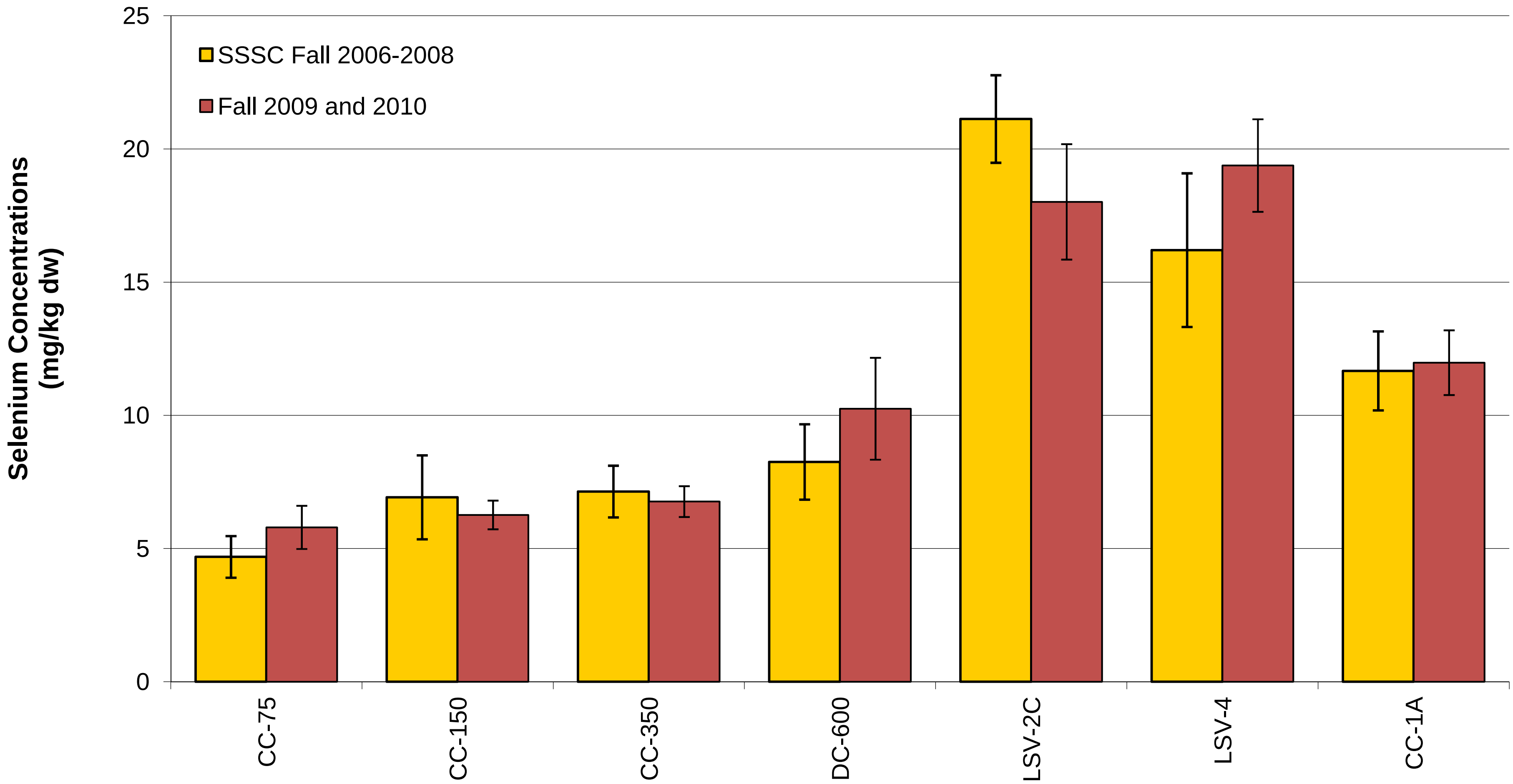


Figure 4-8
Summary Statistics for Total Selenium in Trout Tissue During 2006, 2007, and 2008

J.R. Simplot Company Site-Specific Selenium Criterion		
PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL



*error bars are 95 CI

Figure 4-9
Mean Total Selenium in Trout Tissues, Comparison of Fall 2009 and 2010 to Previous Fall SSSC Data (2006-2008)

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

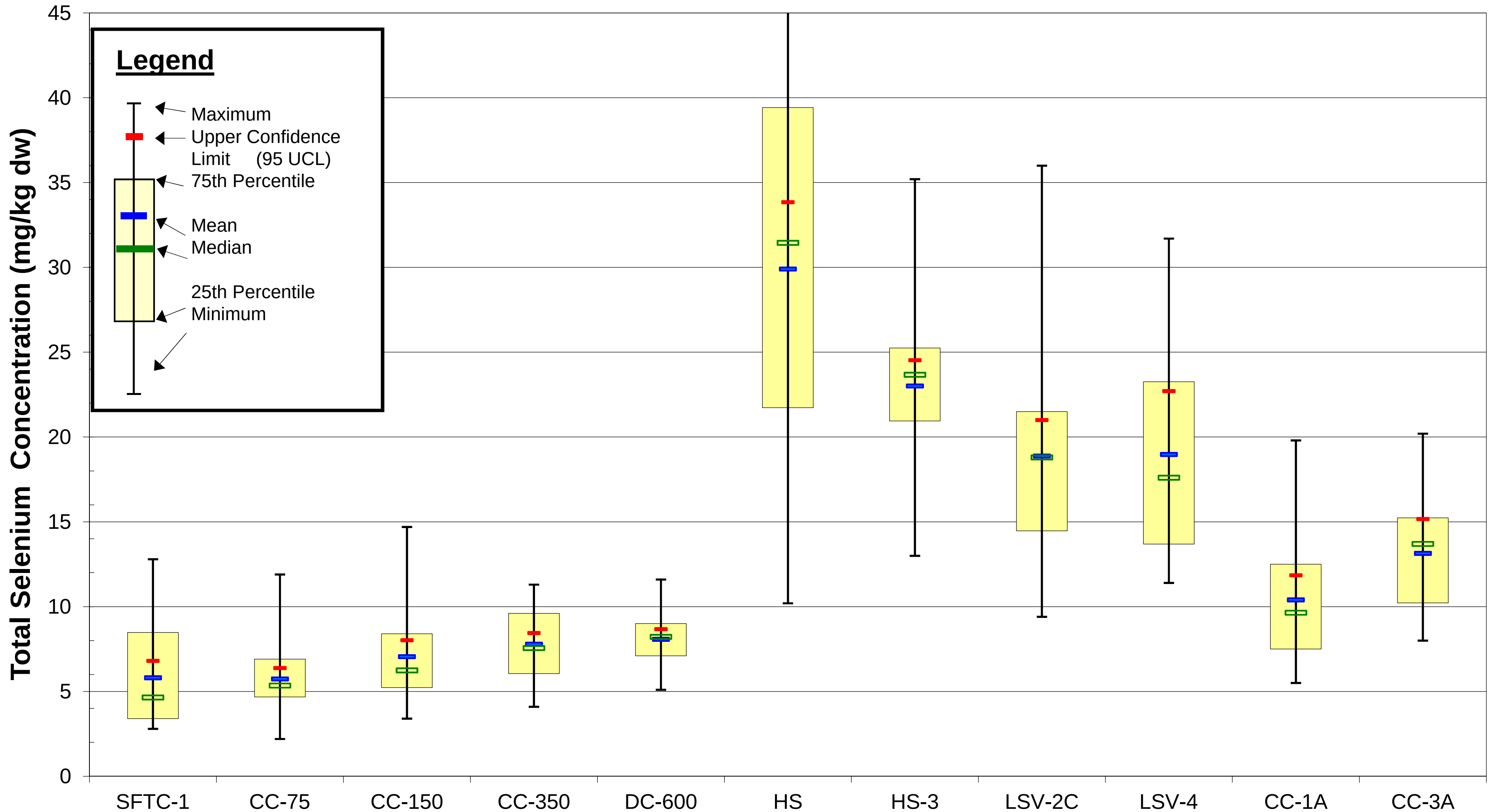
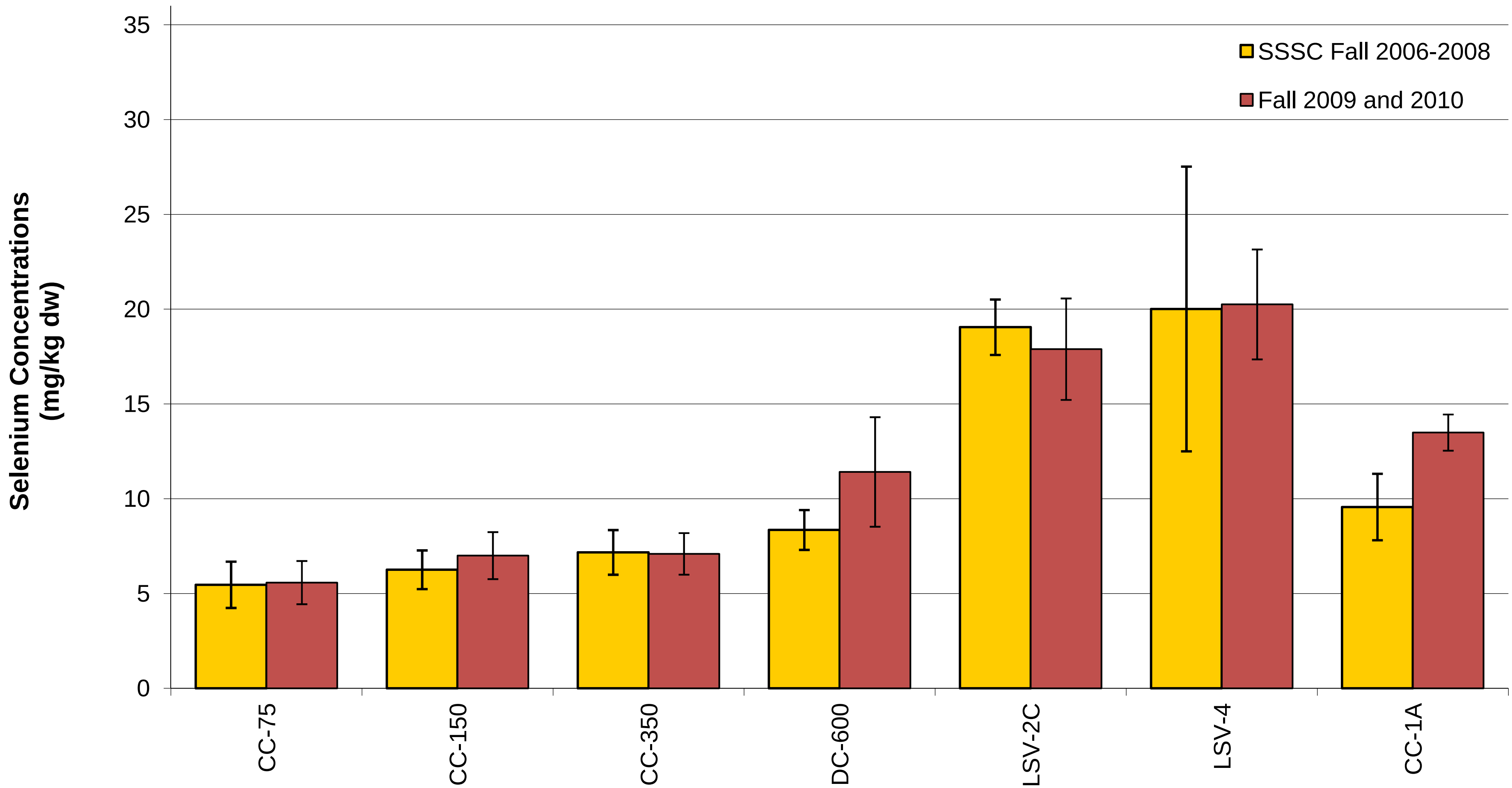


Figure 4-10
Summary Statistics for Total Selenium in Sculpin Tissue During 2006, 2007, and 2008

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL



*error bars are 95 CI

Figure 4-11
Mean Total Selenium in Sculpin Tissues, Comparison of Fall 2009 and 2010 to Previous Fall SSSC Data (2006-2008)

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

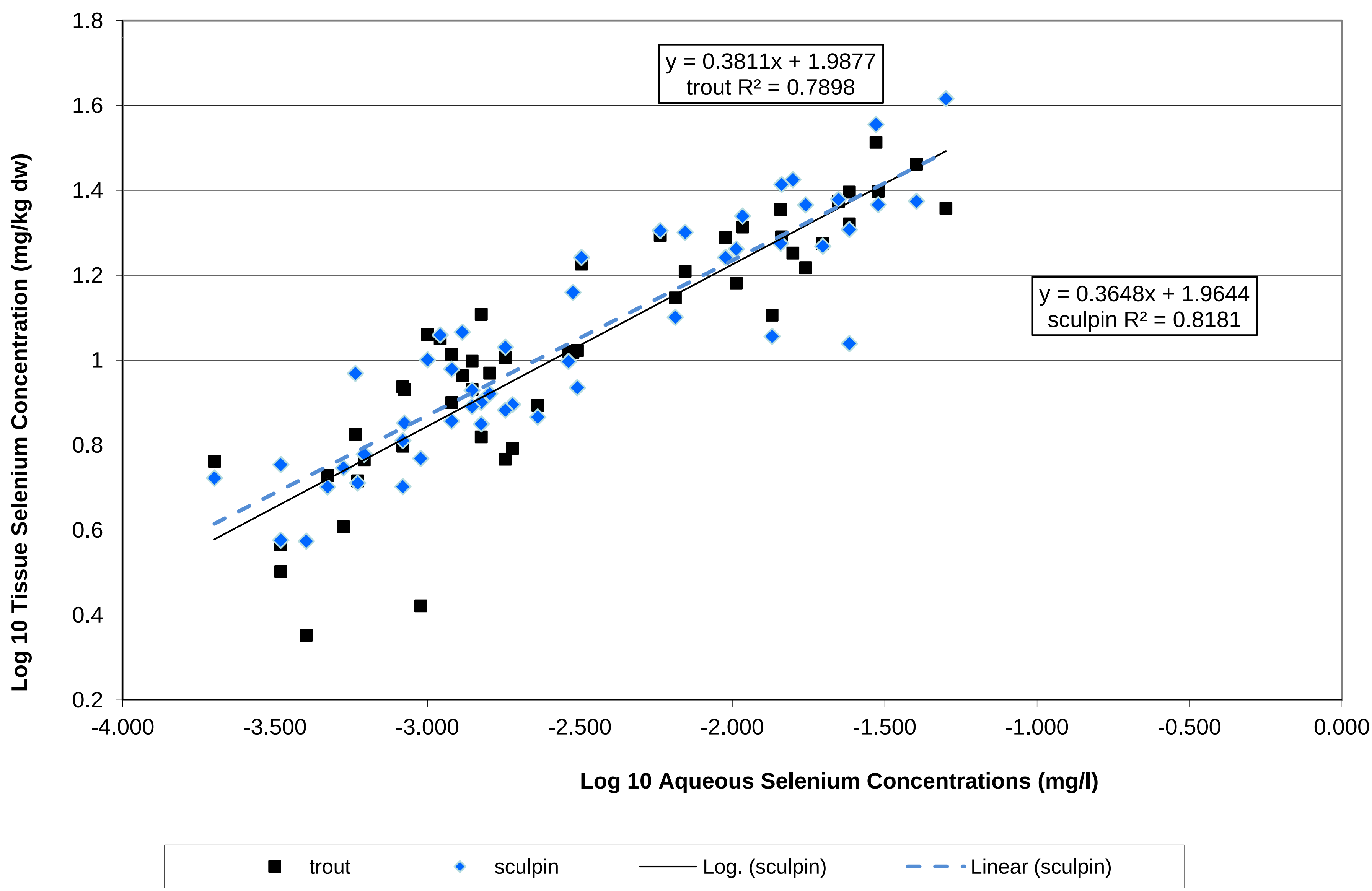


Figure 4-12
Relationship of Aqueous Selenium to Mean Trout and Sculpin Tissue Selenium Concentrations Collected from the Same Locations - Fall 2006-Fall 2008

J.R. Simplot Company Site-Specific Selenium Criterion		
PRJ: 0442-004-900.70	DATE: January 2012	
REV: 0	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

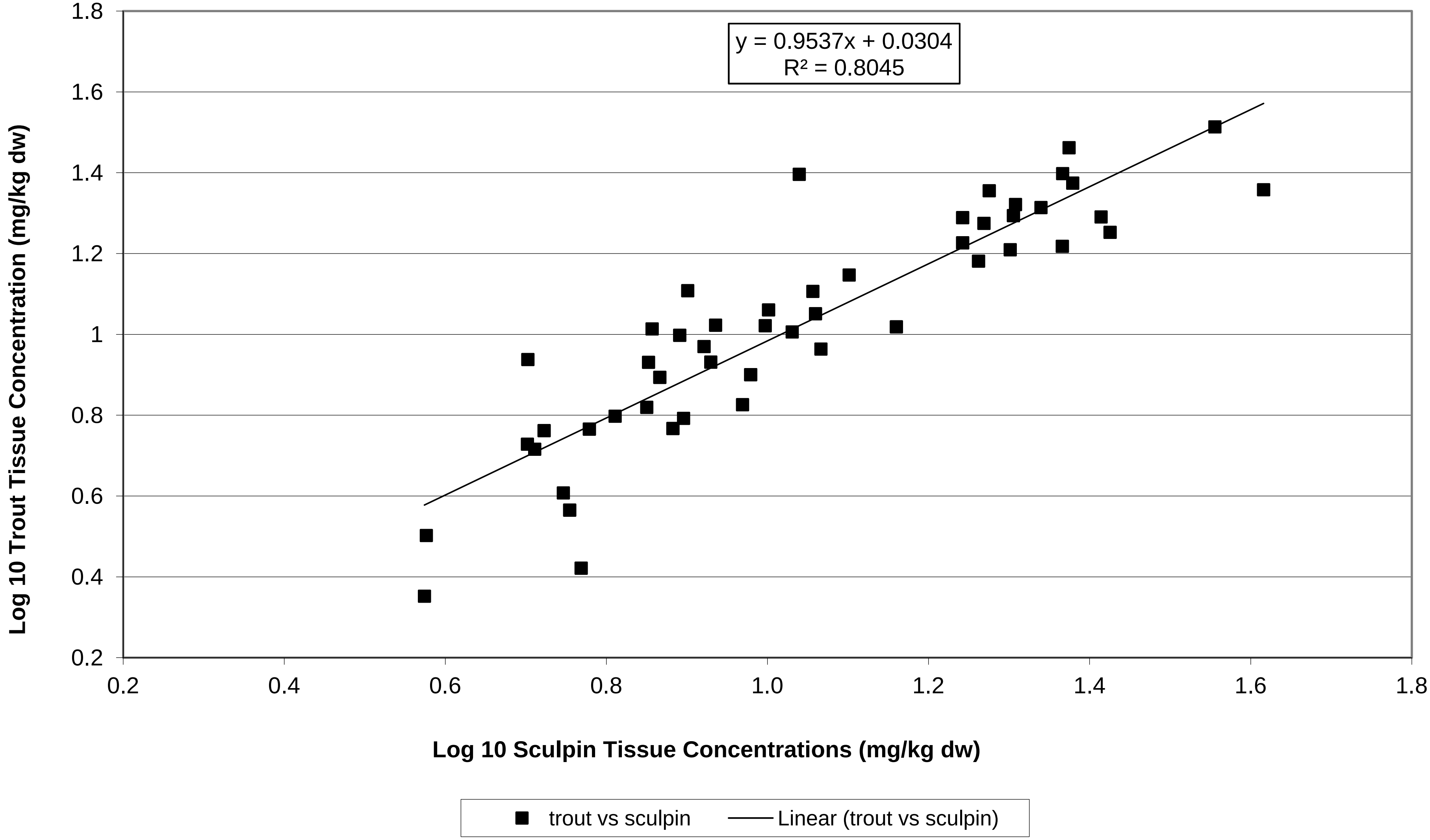


Figure 4-13
Relationship of Mean Trout Tissue Selenium to Mean Sculpin Tissue Selenium Concentrations, Fall 2006 – Fall 2008

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

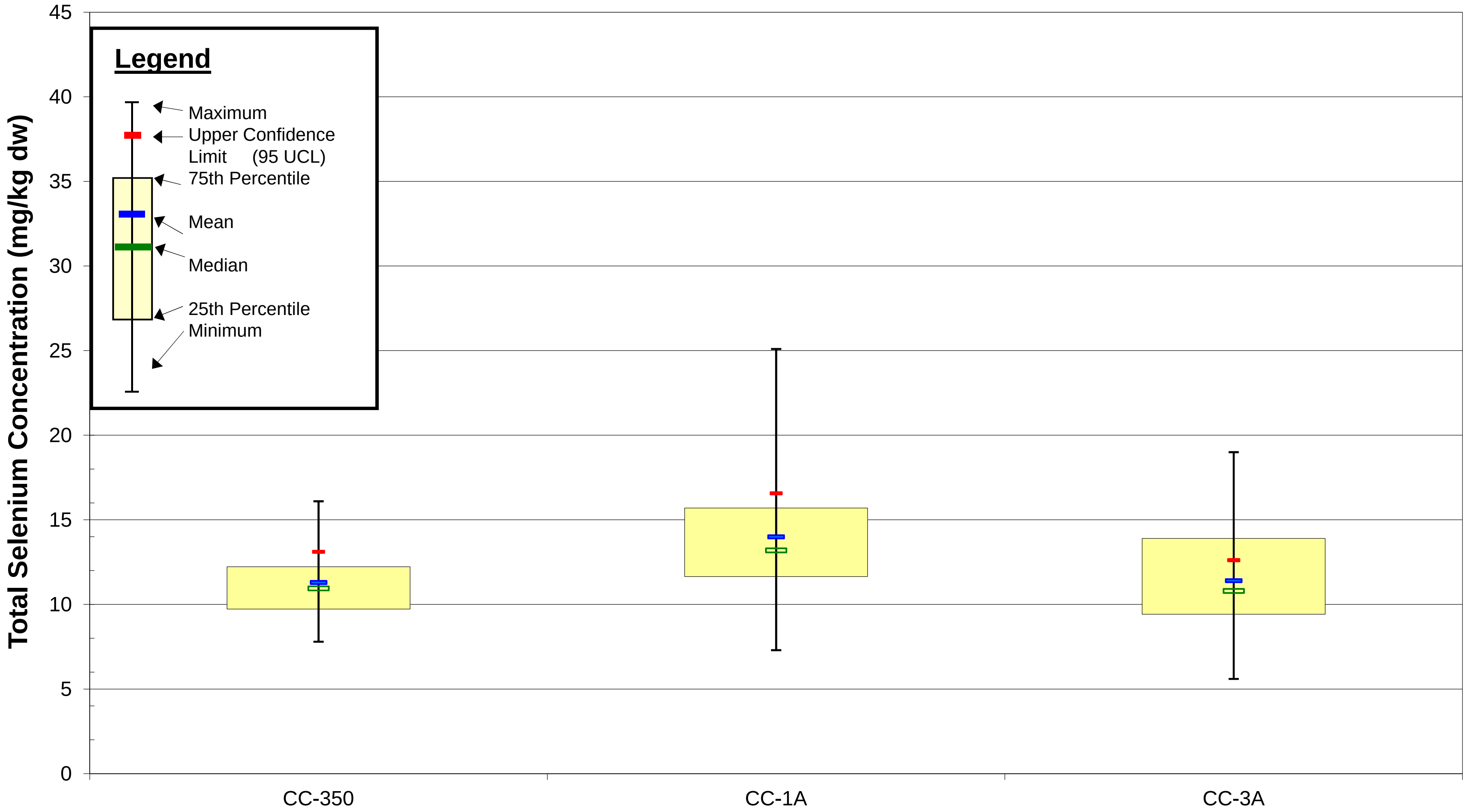


Figure 4-14
Summary Statistics for Total Selenium in Cyprinid and Catostomid Tissue During 2006, 2007, and 2008

J.R. Simplot Company Site-Specific Selenium Criterion		
PRJ: 0442-004-900.70	DATE: January 2012	
REV: 0	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

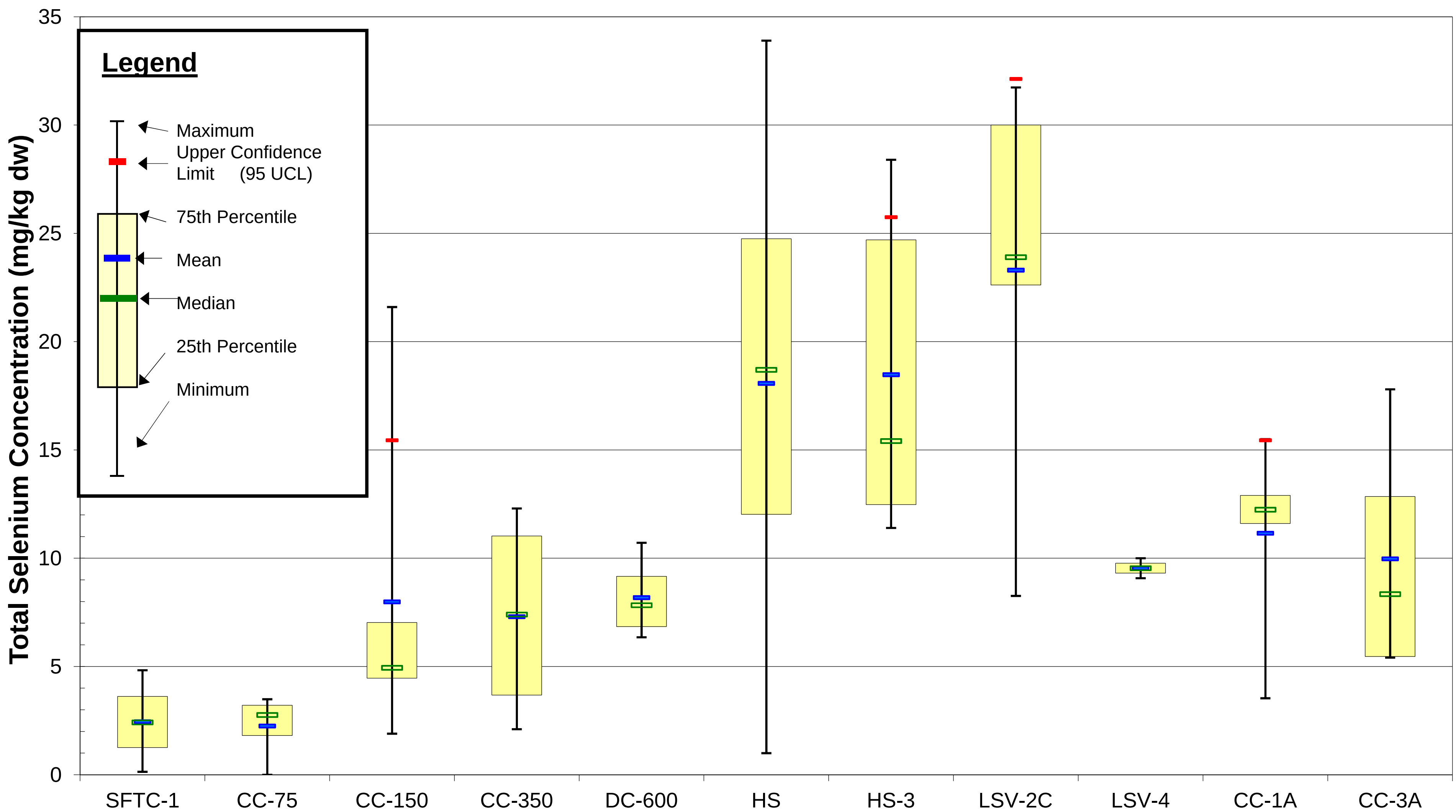


Figure 4-15
Summary Statistics for Selenium Concentrations in Invertebrate Tissues During 2006, 2007, and 2008

J.R. Simplot Company Site-Specific Selenium Criterion		
PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC
FORMATION ENVIRONMENTAL		

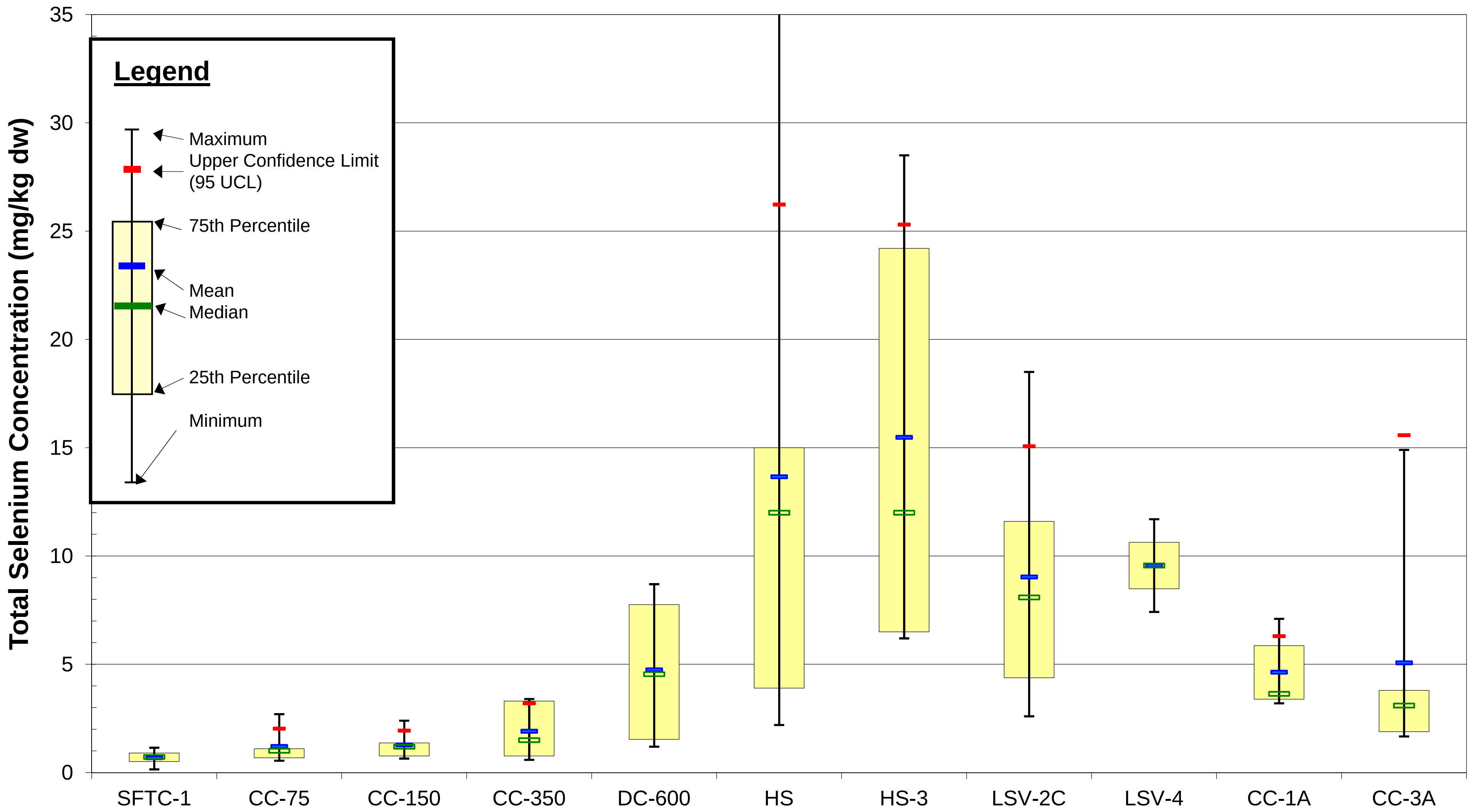


Figure 4-16
Summary Statistics for Selenium Concentrations in Periphyton Tissue During 2006, 2007, and 2008

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

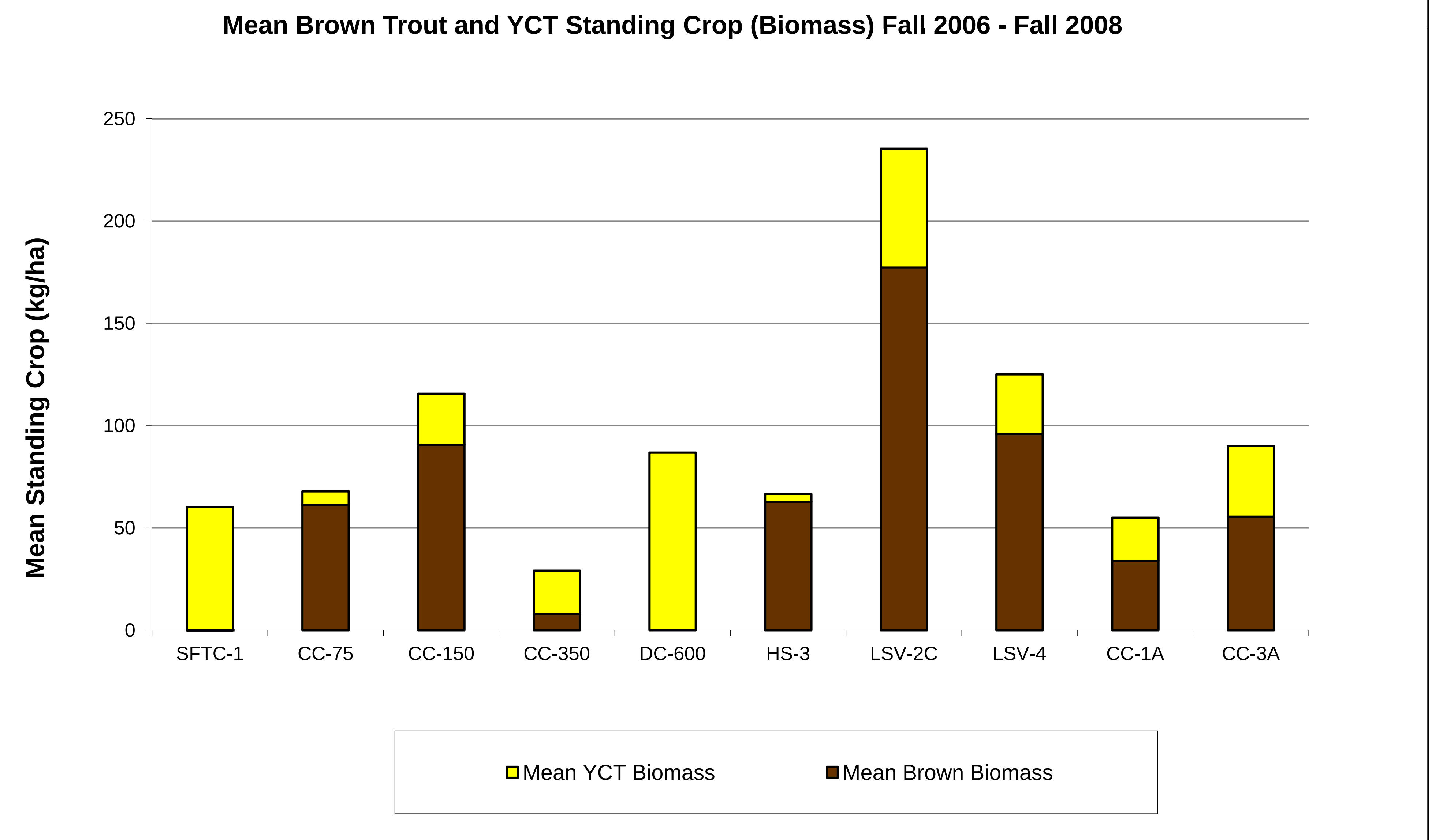


Figure 4-17
Mean Brown Trout and YCT Standing Crop (Biomass) Based on Population Estimates, Fall 2006 - Fall 2008

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012
REV: 1	BY: SMC / CHK: SMC

FORMATION ENVIRONMENTAL

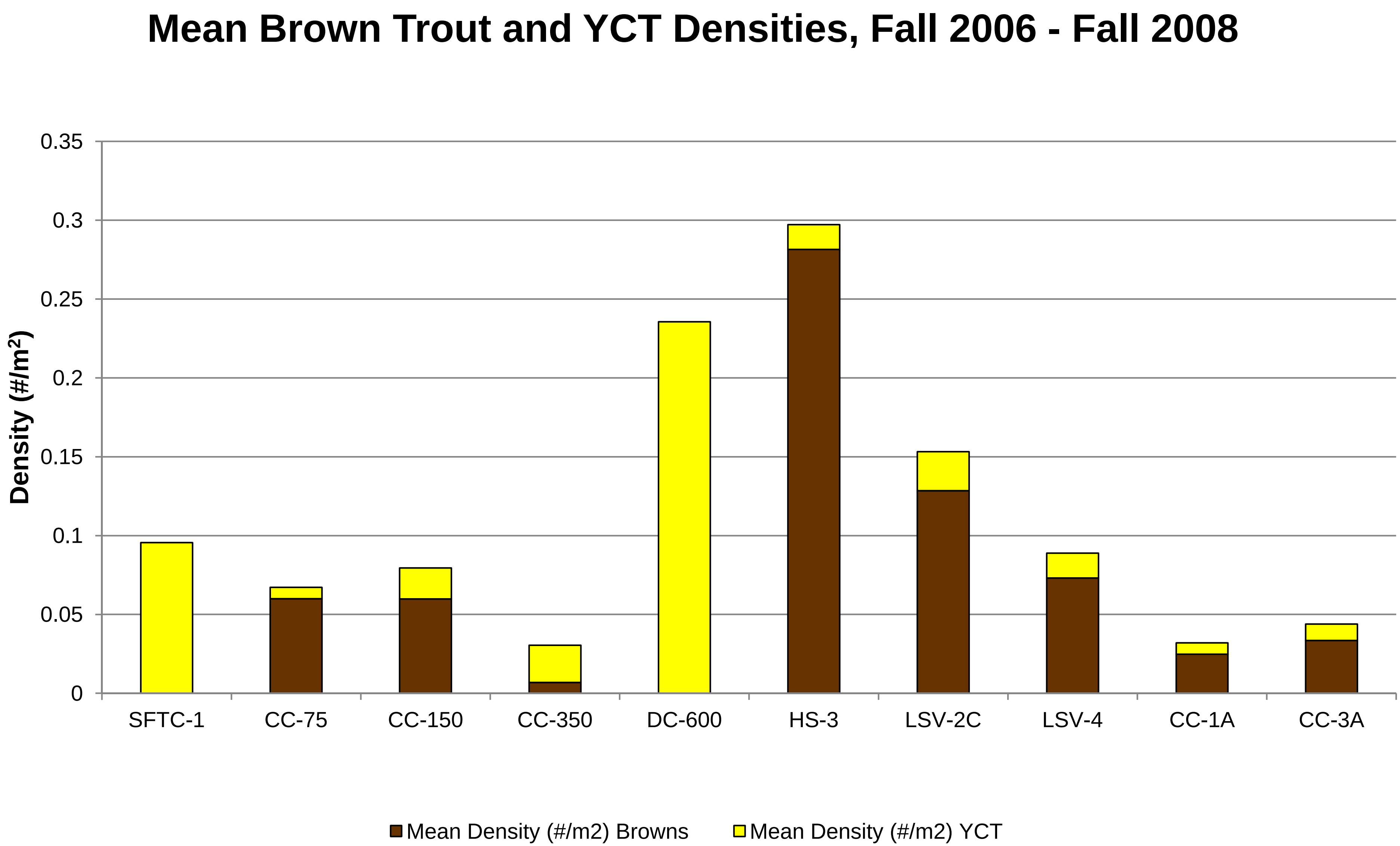


Figure 4-18
Mean Brown Trout and YCT Densities Based on Population Estimates
Fall 2006 - Fall 2008

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012
REV: 1	BY: SMC CHK: SMC

FORMATION ENVIRONMENTAL

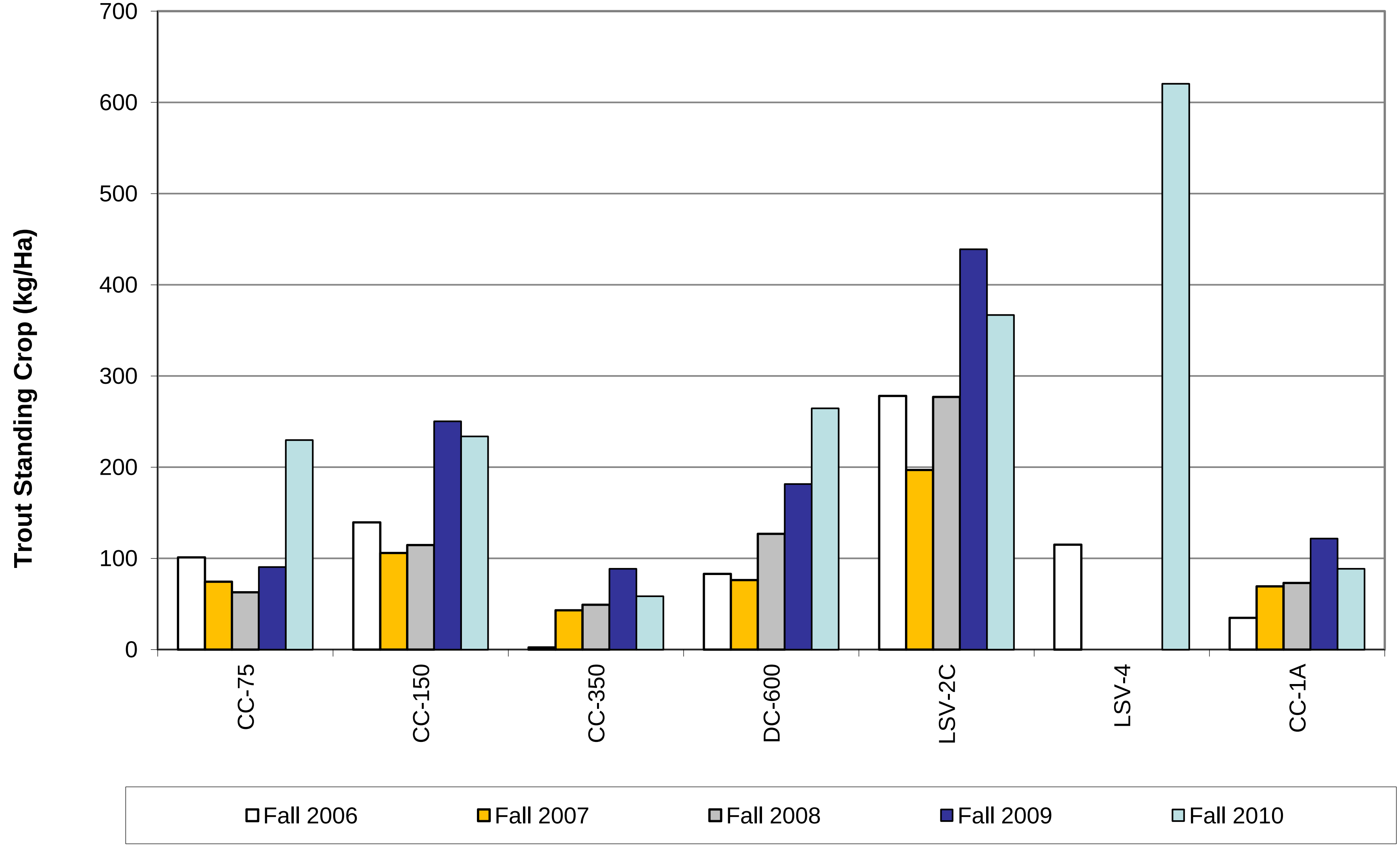
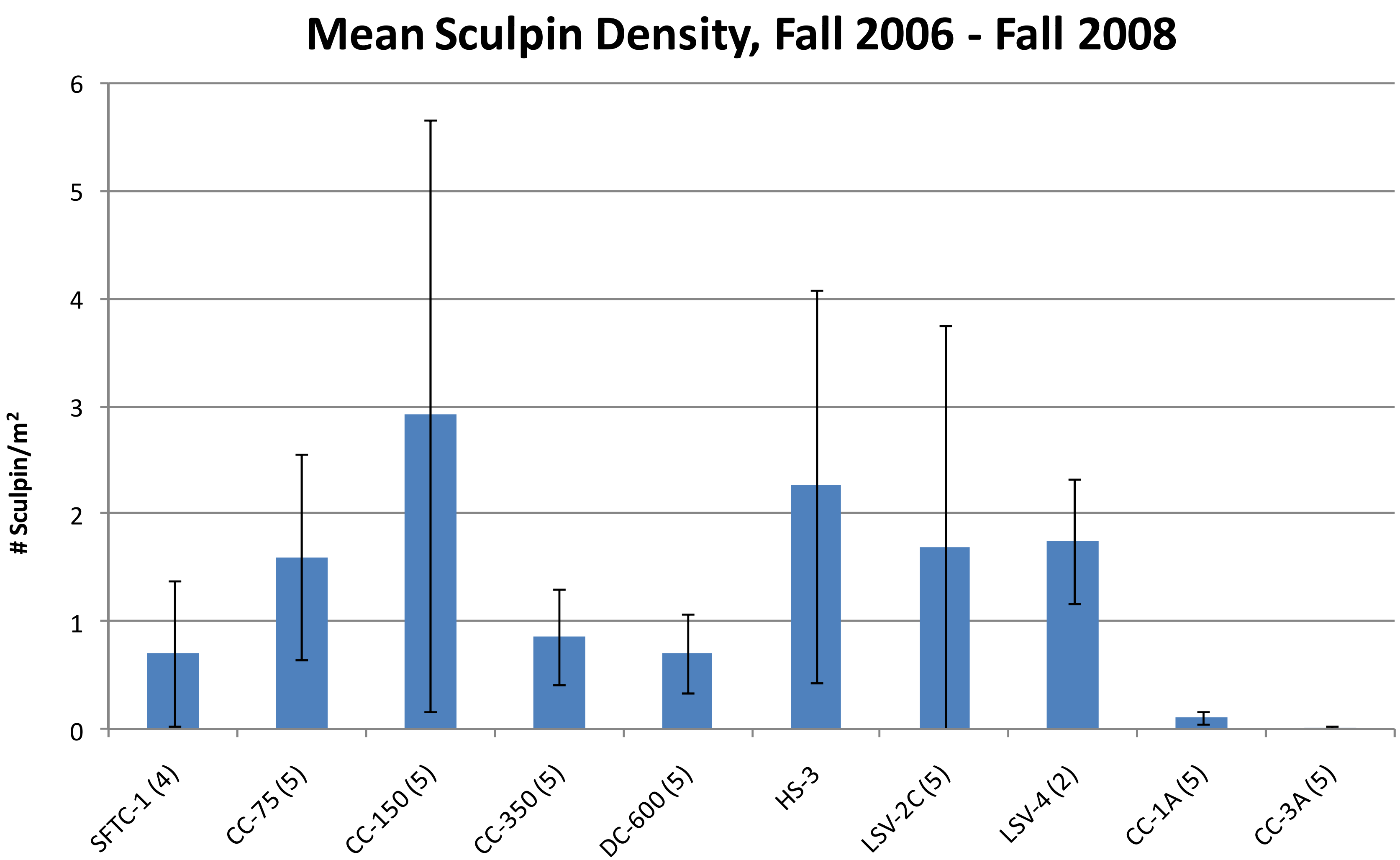


Figure 4-19
Total Trout Standing Crop Estimates, Comparison of Fall 2009 and 2010 to Previous Fall SSSC Data (2006-2008)

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL



Notes: Density values based on statistically-derived population estimates. Error bars are one standard deviation. Parentheses indicate number of sampling seasons.

Figure 4-20
Mean Aerial Sculpin Density Based on Population Estimates
Fall 2006 - Fall 2008

J.R. Simplot Company Site-Specific Selenium Criterion		
PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

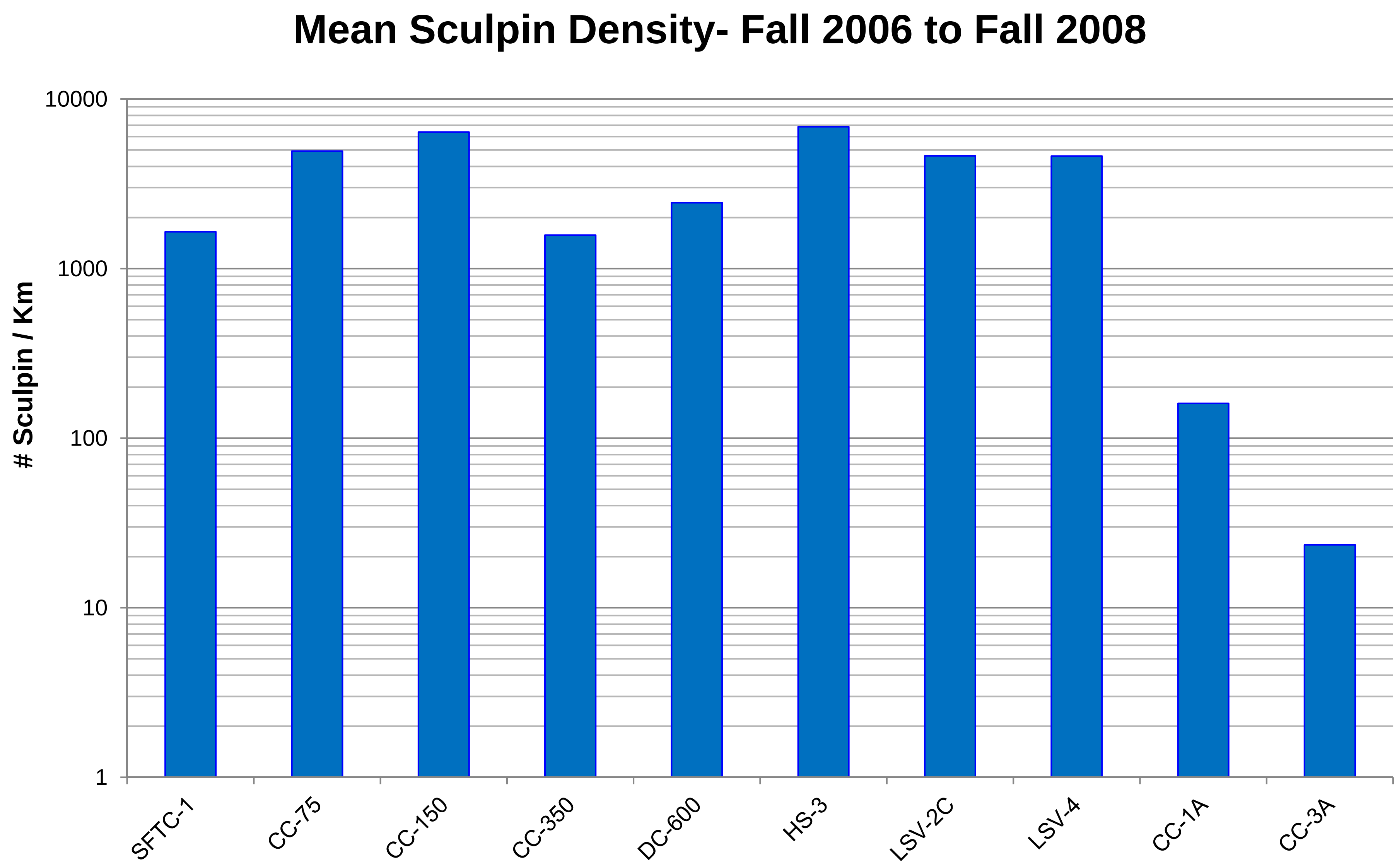


Figure 4-21
Mean Linear Sculpin Density Based on Population Estimates
Fall 2007 - Fall 2008

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012
REV: 1	BY: SMC / CHK: SMC

FORMATION ENVIRONMENTAL

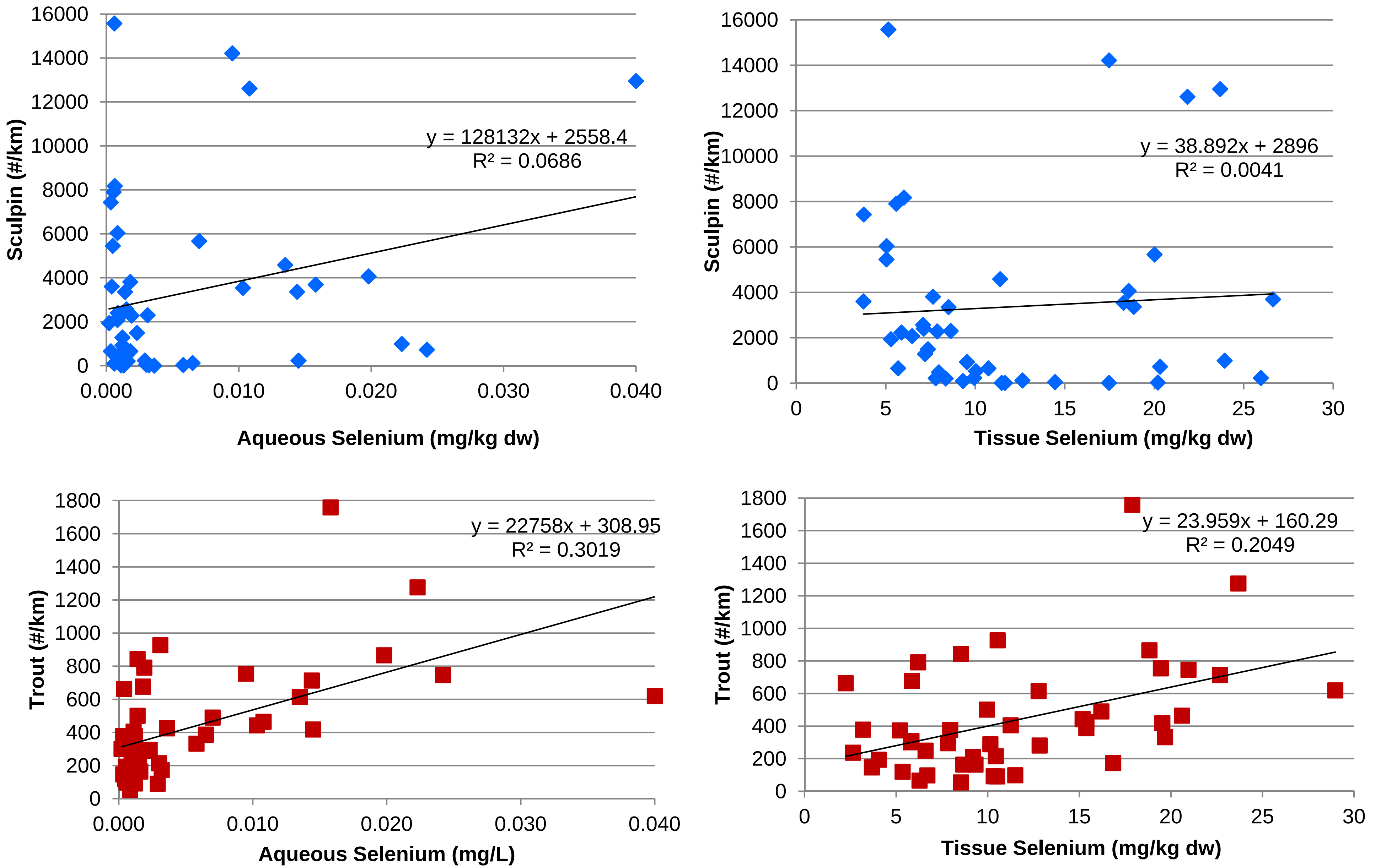


Figure 4-22
Sculpin and Trout Density Versus Aqueous and Tissue Selenium
Fall 2006 - Fall 2008

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012
REV: 1	BY: SMC CHK: SMC

FORMATION ENVIRONMENTAL

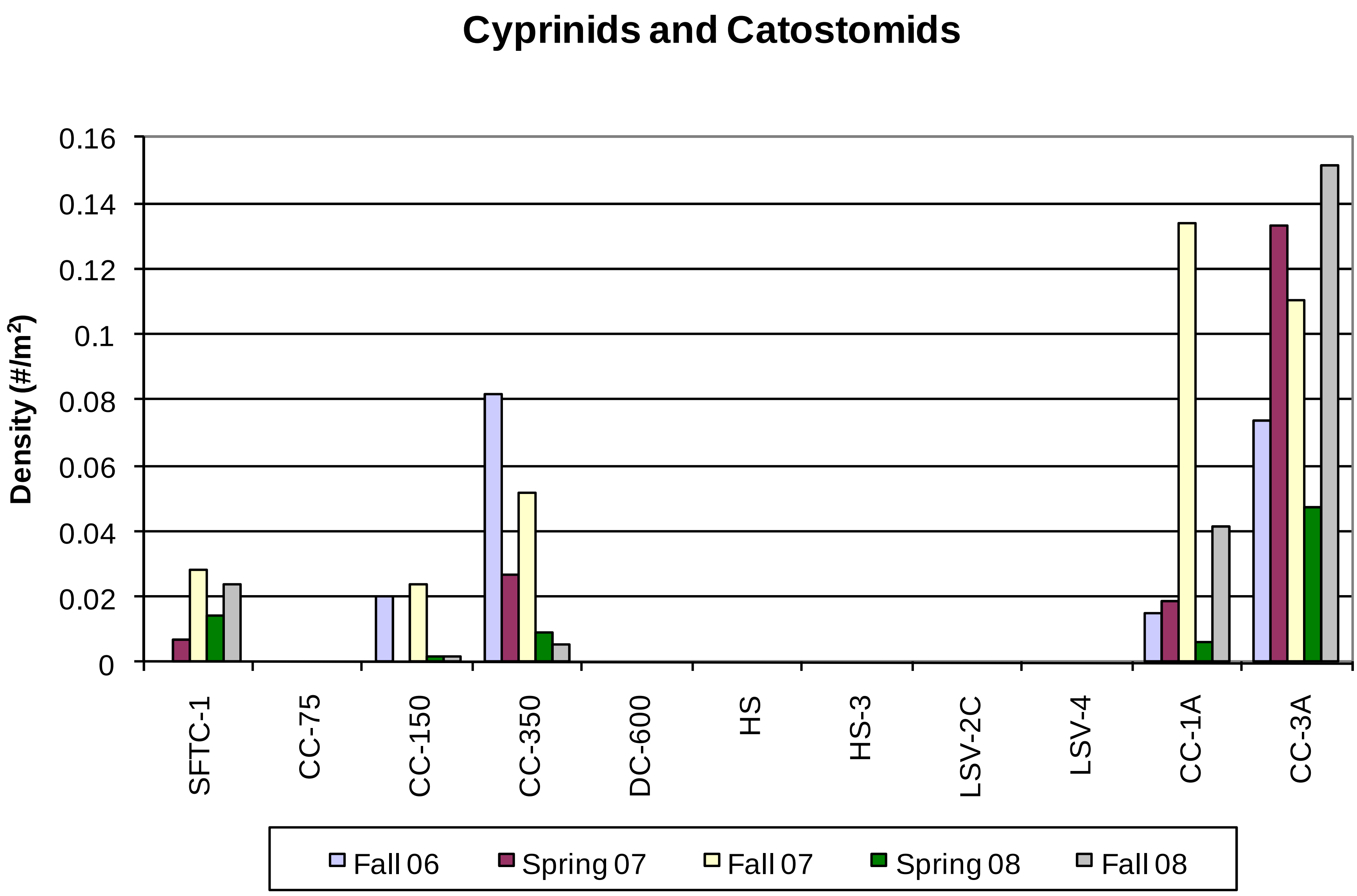


Figure 4-23
Cyprinid and Catostomid Density
Fall 2006 - Fall 2008

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

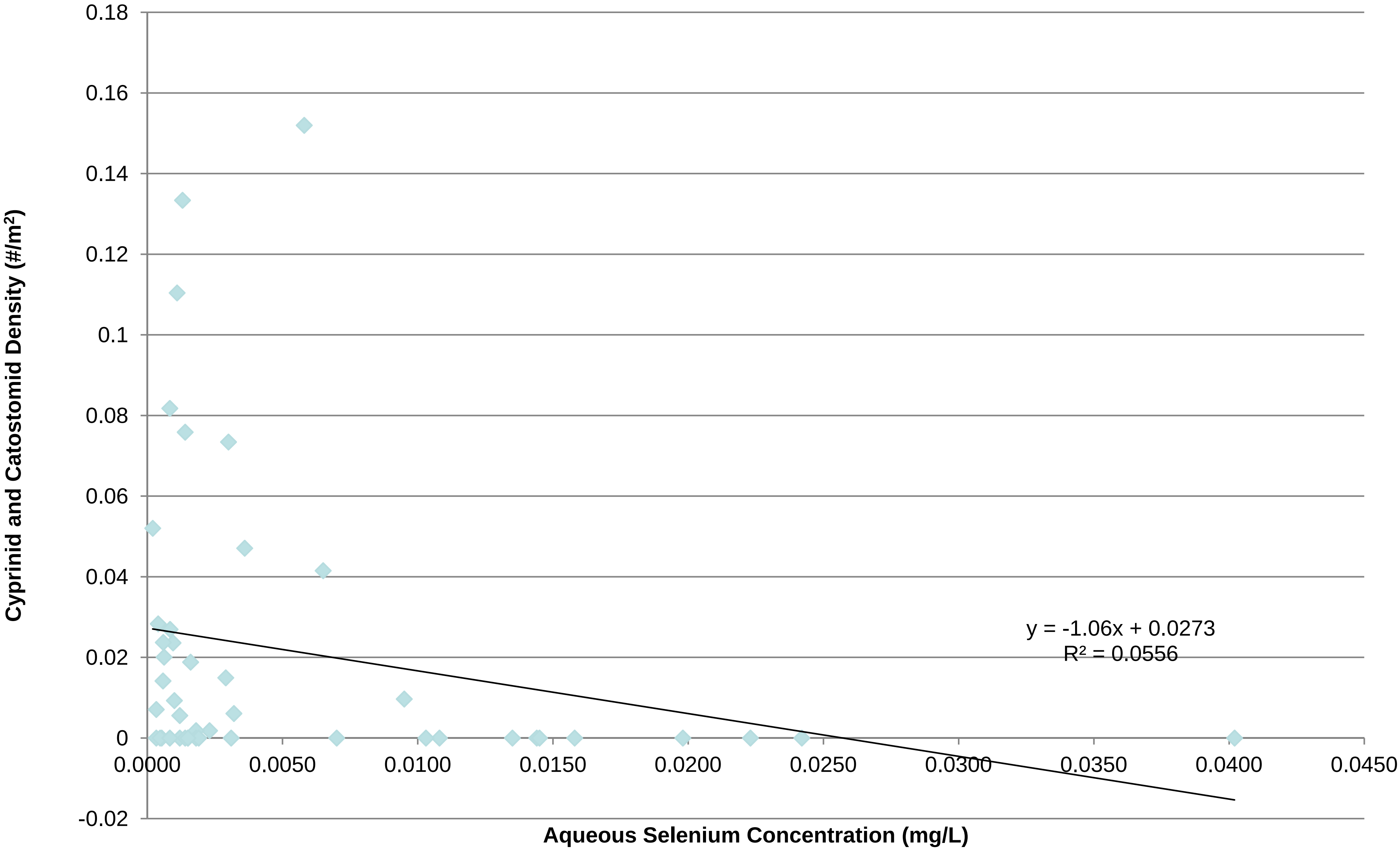


Figure 4-24
Aqueous Selenium Concentrations Versus Cyprinid and Catostomid Density

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

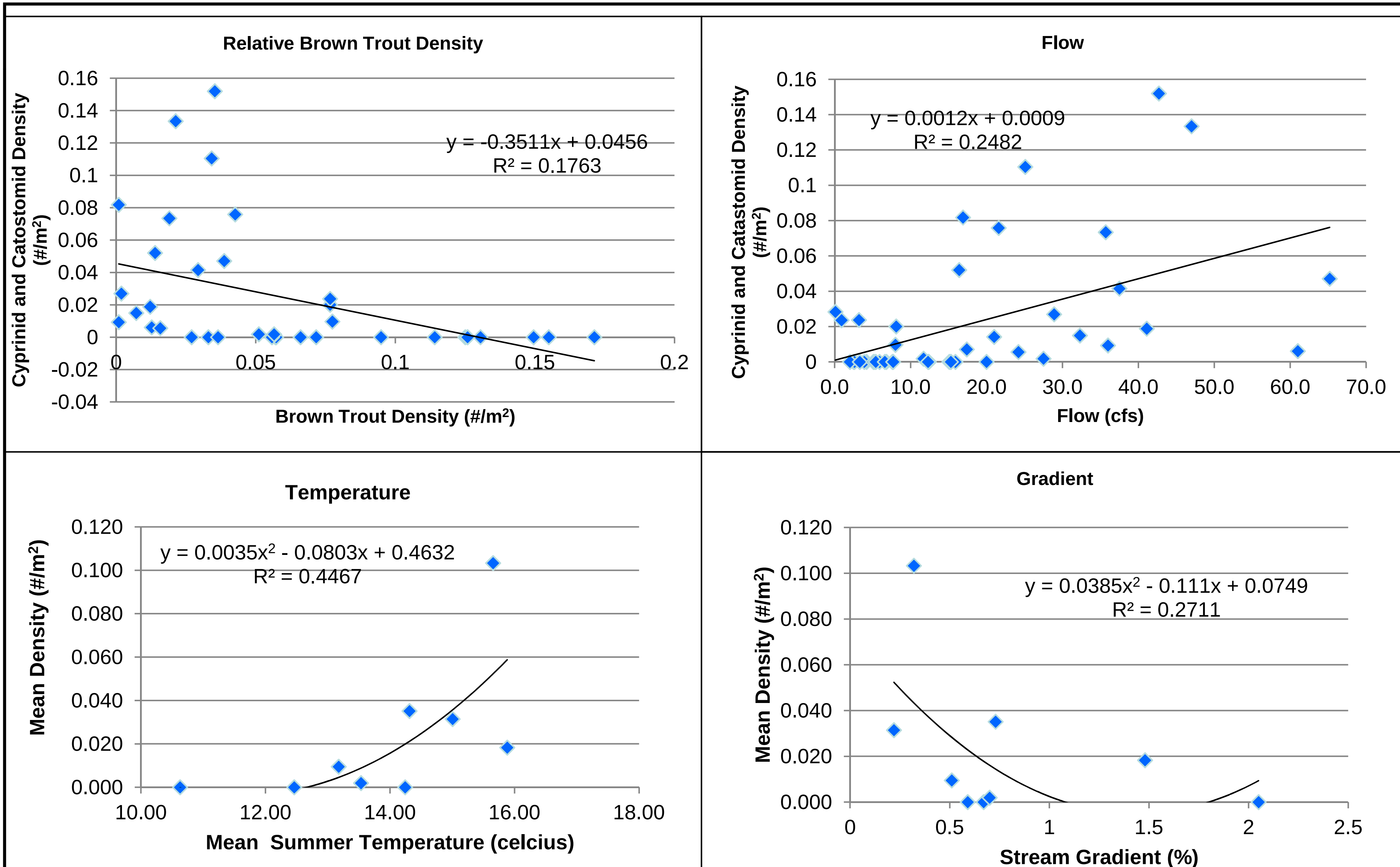


Figure 4-25 a-d
Relative Brown Trout Density, Flow, Mean Summer Temperature, and Stream Gradient Versus Relative Cyprinid and Catostomid Density

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

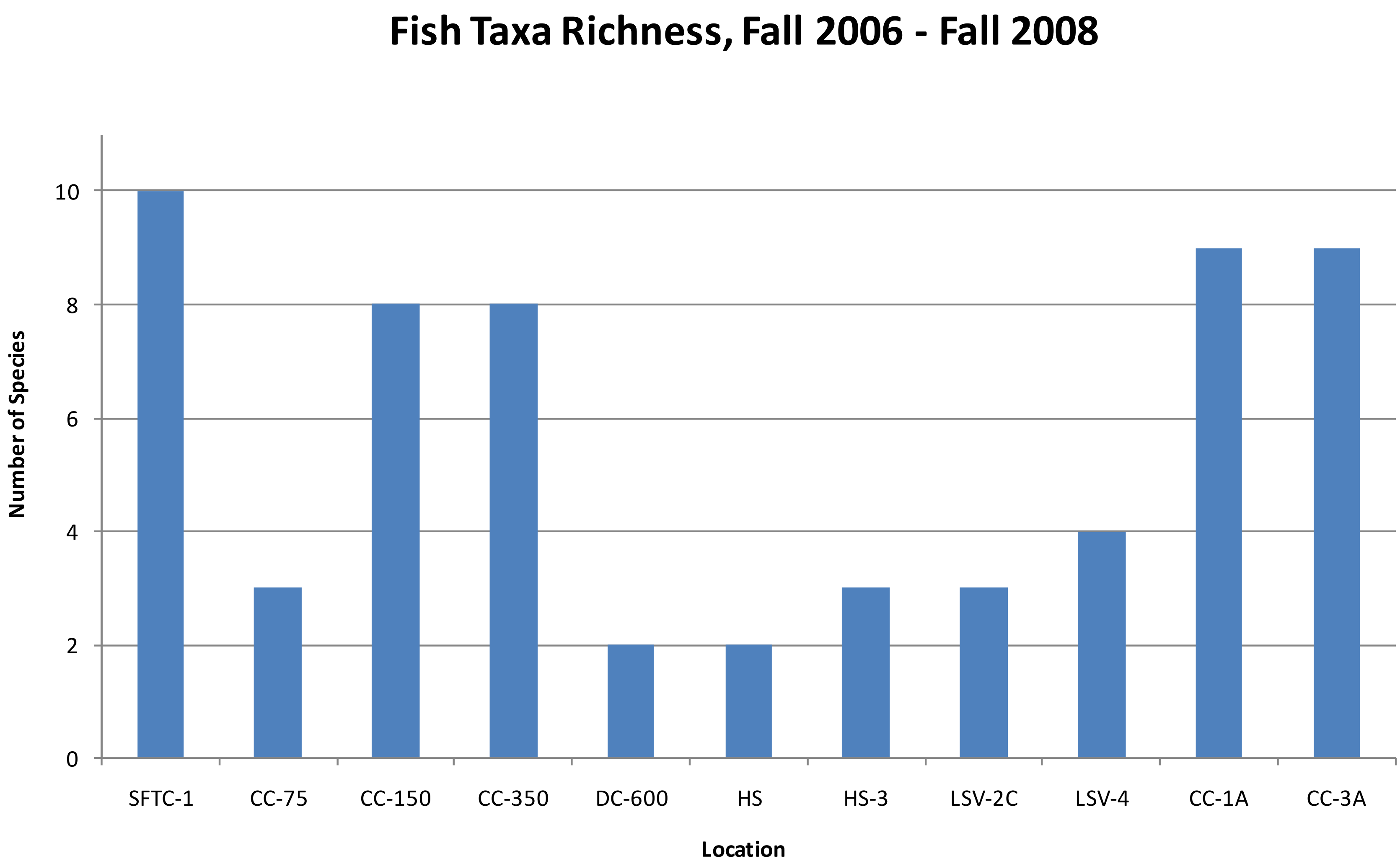


Figure 4-26
Fish Taxa Richness
Fall 2006 - Fall 2008

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

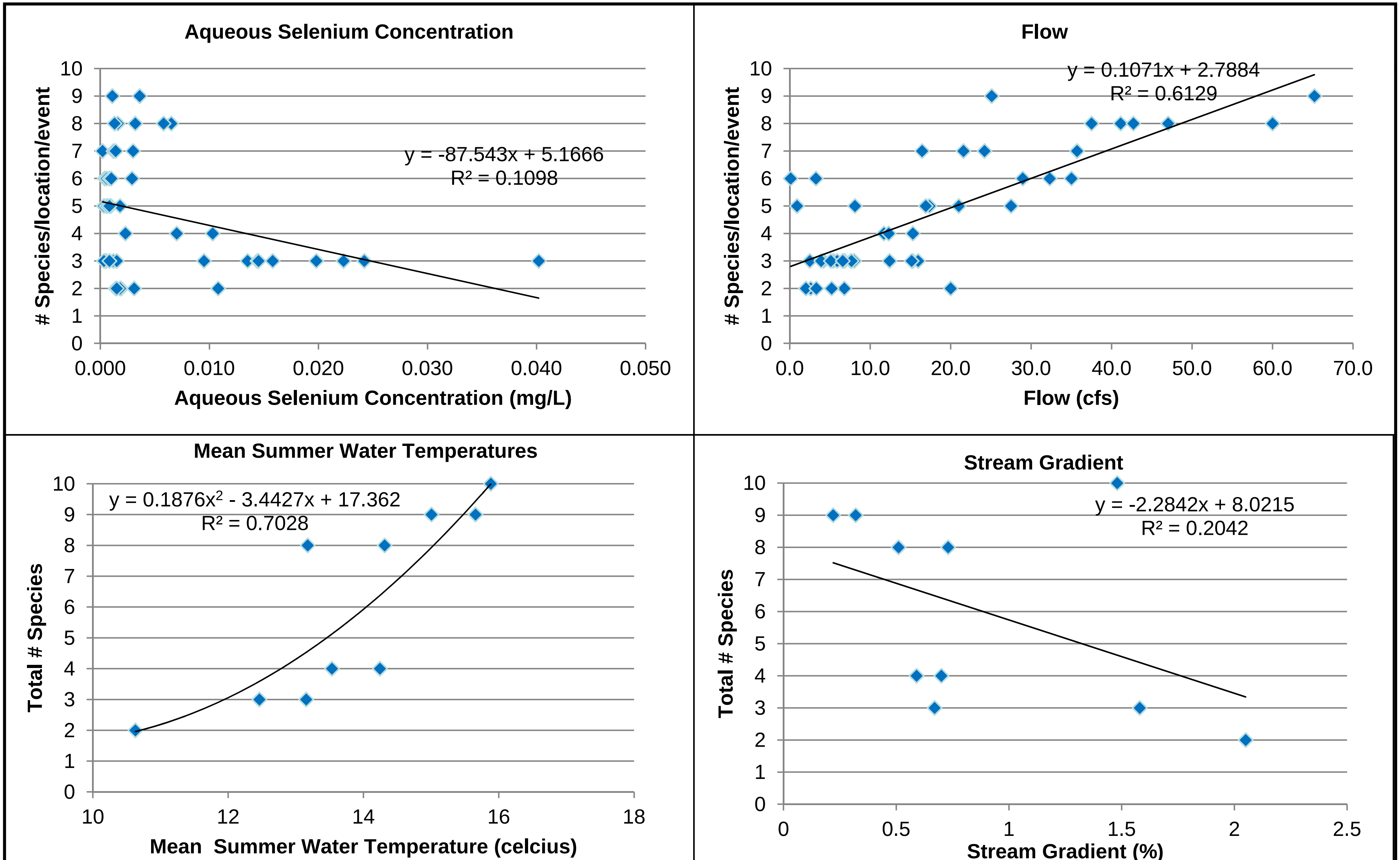


Figure 4-27 a-d
Number of Fish Species (per event and period) and Total Number of Species Versus Aqueous Selenium Concentration, Flow, Mean Summer Water Temperature, and Stream Gradient

J.R. Simplot Company Site-Specific Selenium Criterion	
PRJ: 0442-004-900.70	DATE: January 2012
REV: 1	BY: SMC / CHK: SMC

FORMATION ENVIRONMENTAL

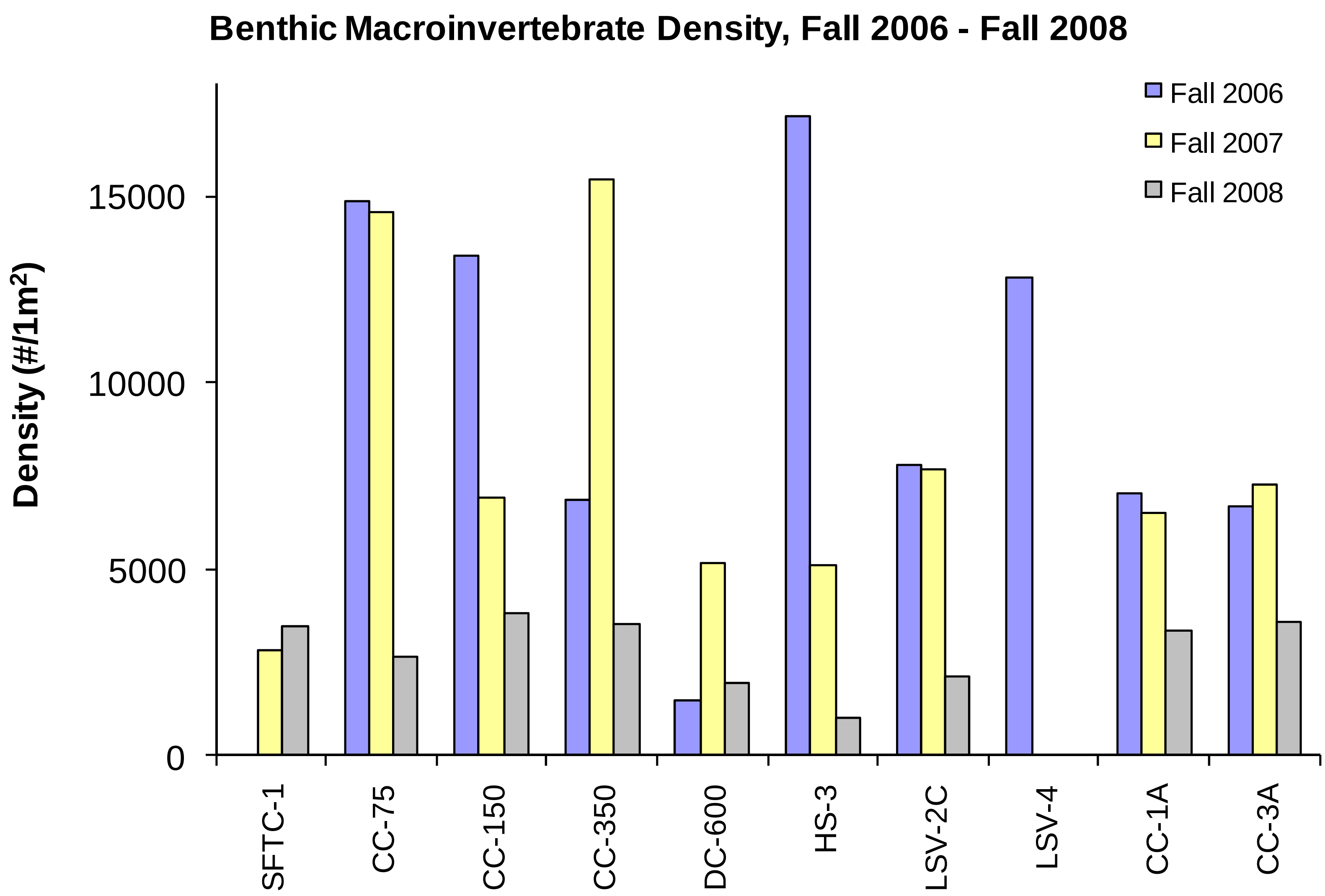


Figure 4-28
Benthic Macroinvertebrate Density
Fall 2006 - Fall 2008

J.R. Simplot Company Site-Specific Selenium Criterion	
PRJ: 0442-004-900.70	DATE: January 2012
REV: 1	BY: SMC CHK: SMC

FORMATION ENVIRONMENTAL

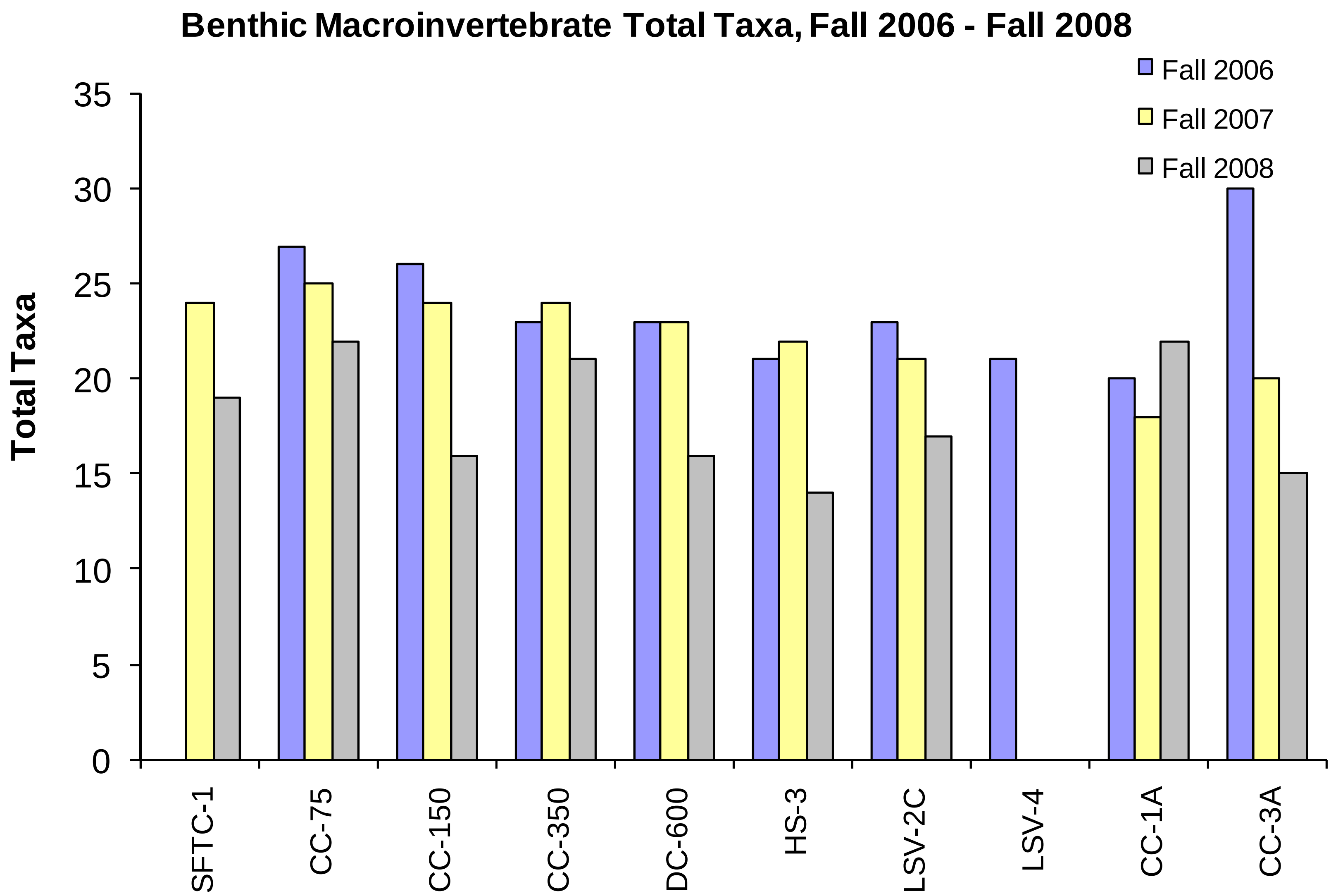


Figure 4-29
Benthic Macroinvertebrate Total Taxa
Fall 2006 - Fall 2008

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012
REV: 1	BY: SMC · CHK: SMC

FORMATION ENVIRONMENTAL

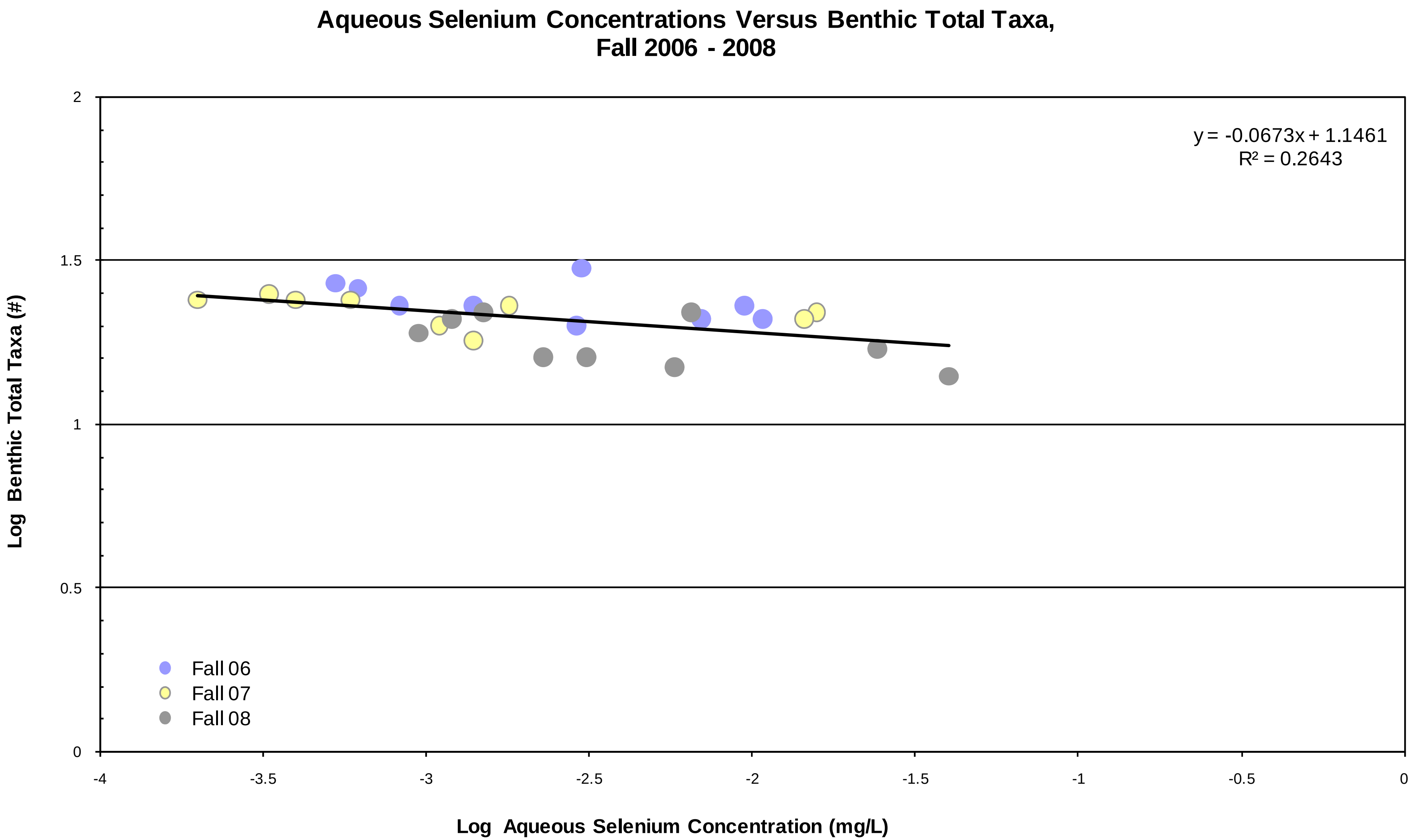


Figure 4-30
Aqueous Selenium Concentrations Versus Benthic Total Taxa
Fall 2006 - Fall 2008

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012
REV: 1	BY: SMC / CHK: SMC

FORMATION ENVIRONMENTAL

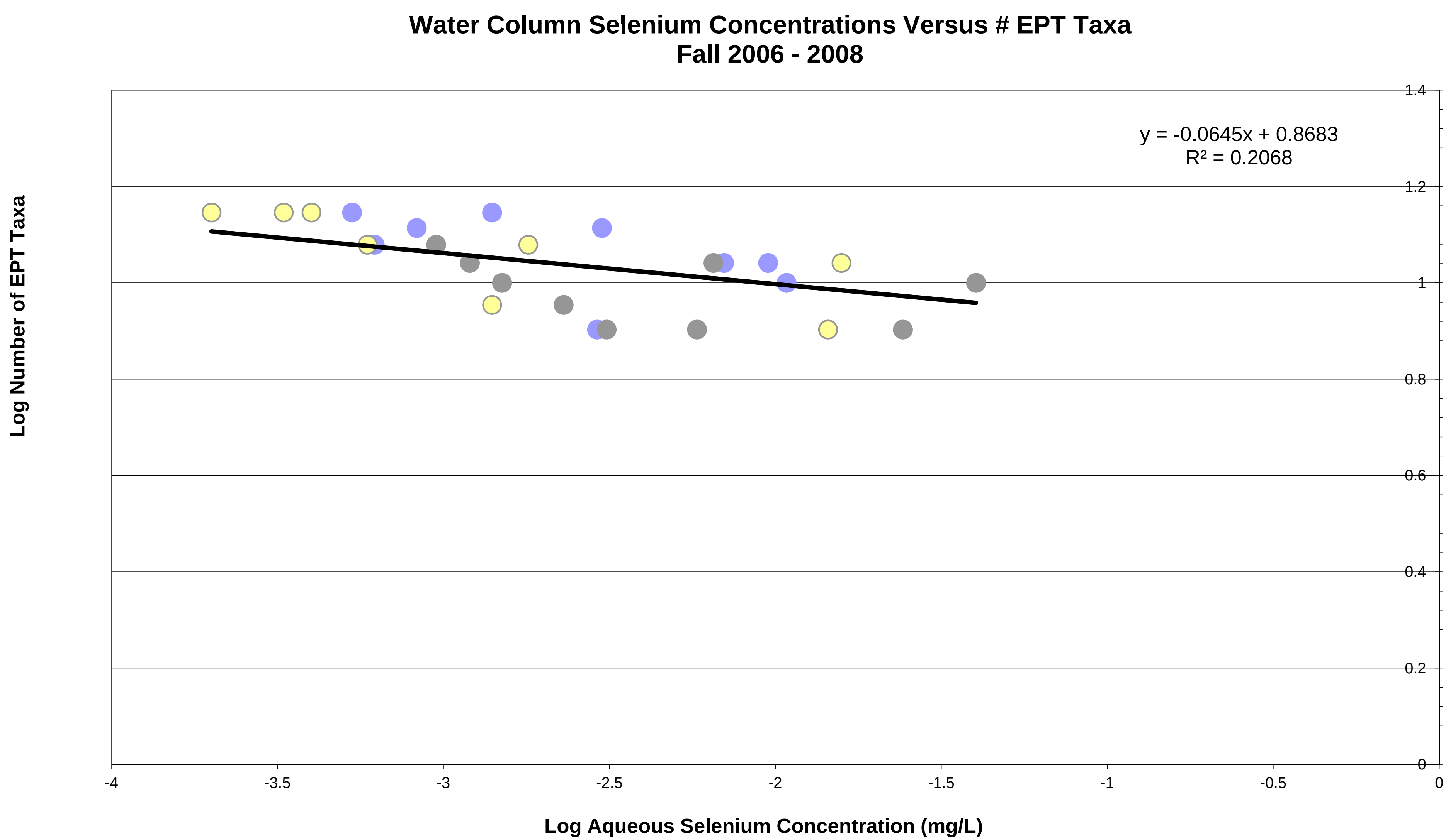


Figure 4-31
Aqueous Selenium Concentrations Versus the Number of EPT Taxa
Fall 2006 - Fall 2008

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012
REV: 1	BY: SMC / CHK: SMC

FORMATION ENVIRONMENTAL

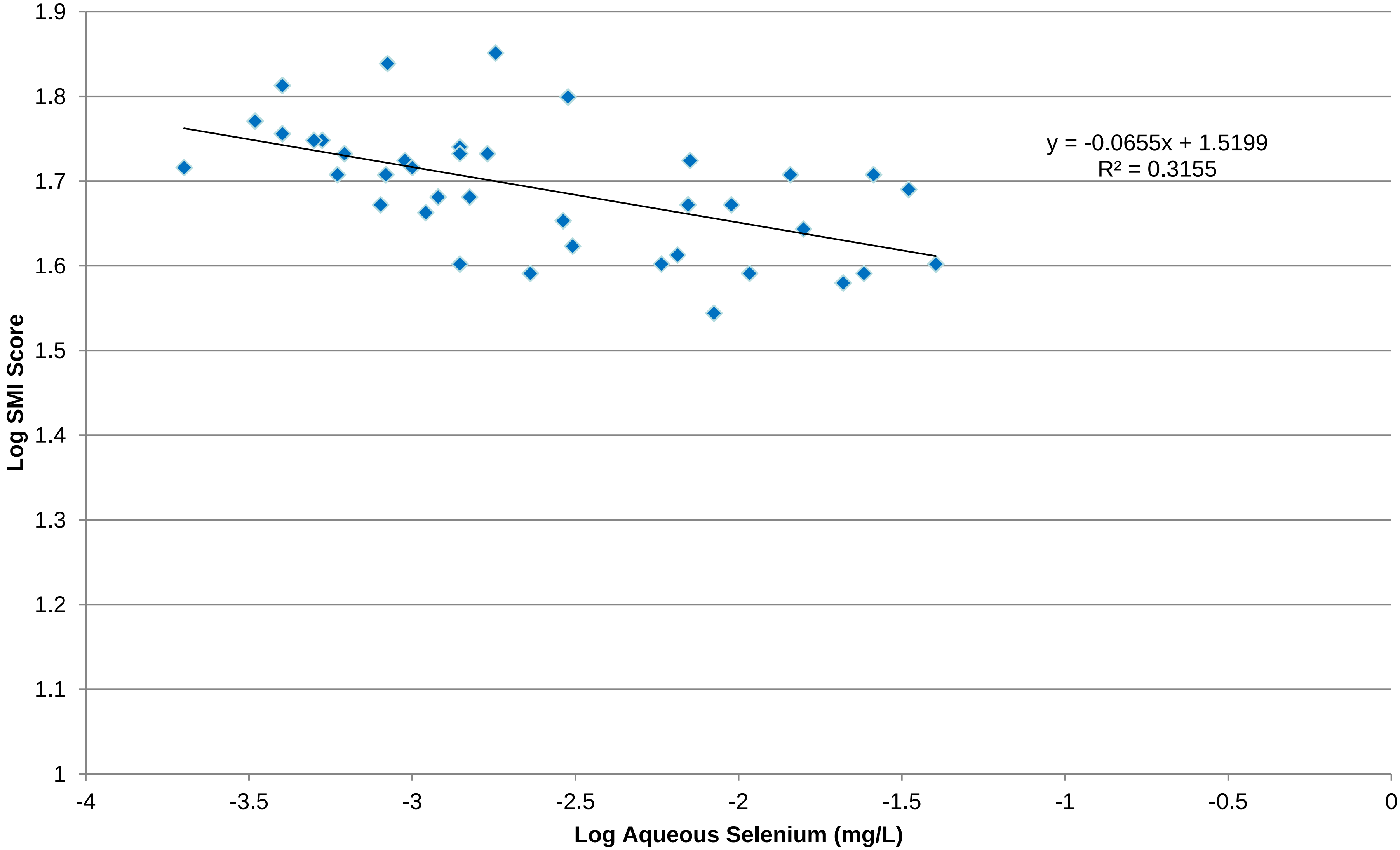


Figure 4-32
Aqueous Selenium Concentrations Versus SMI Scores
Fall 2006 - Fall 2010

J.R. Simplot Company Site-Specific Selenium Criterion	
PRJ: 0442-004-900.70	DATE: January 2012
REV: 1	BY: SMC CHK: SMC

FORMATION ENVIRONMENTAL

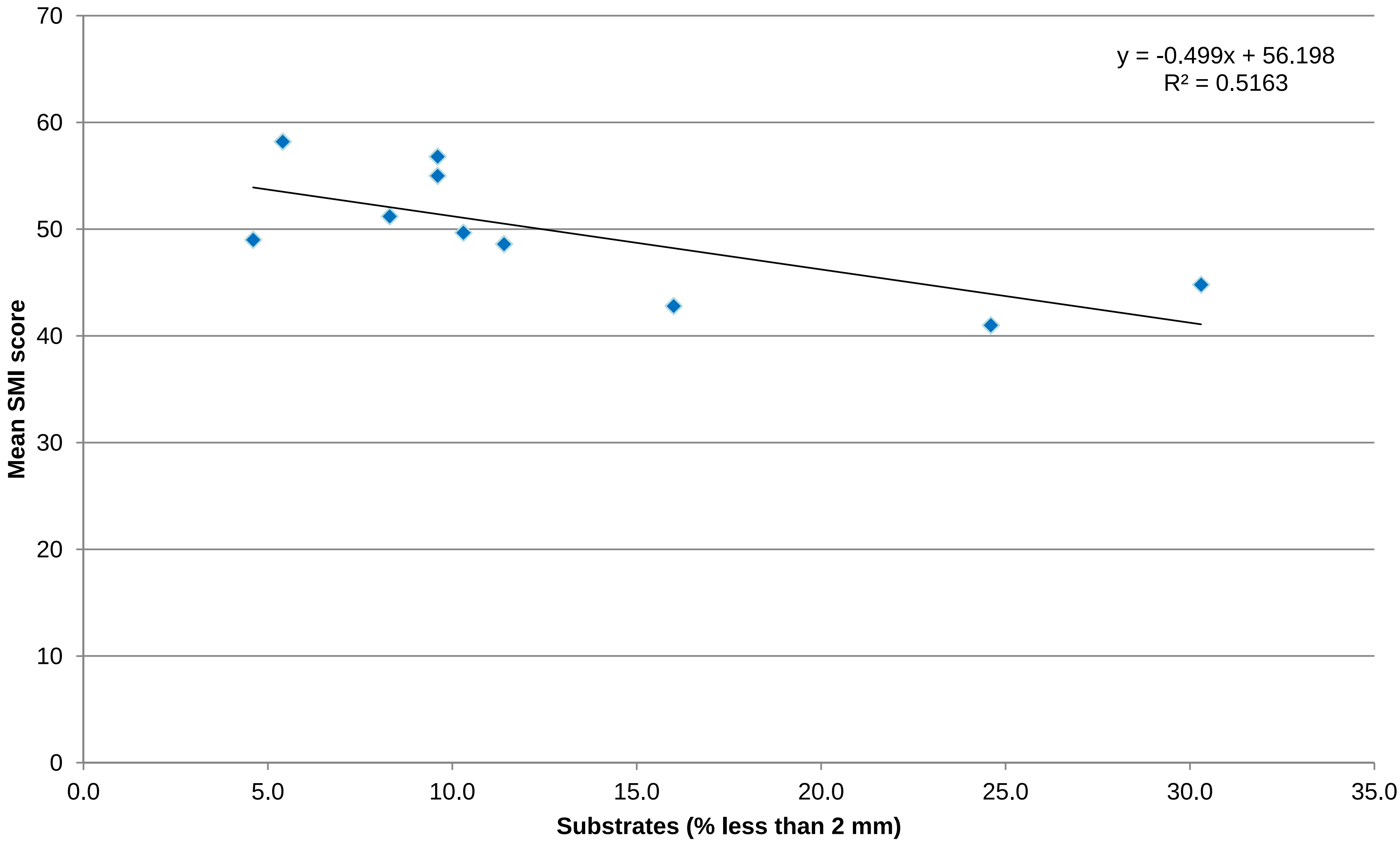
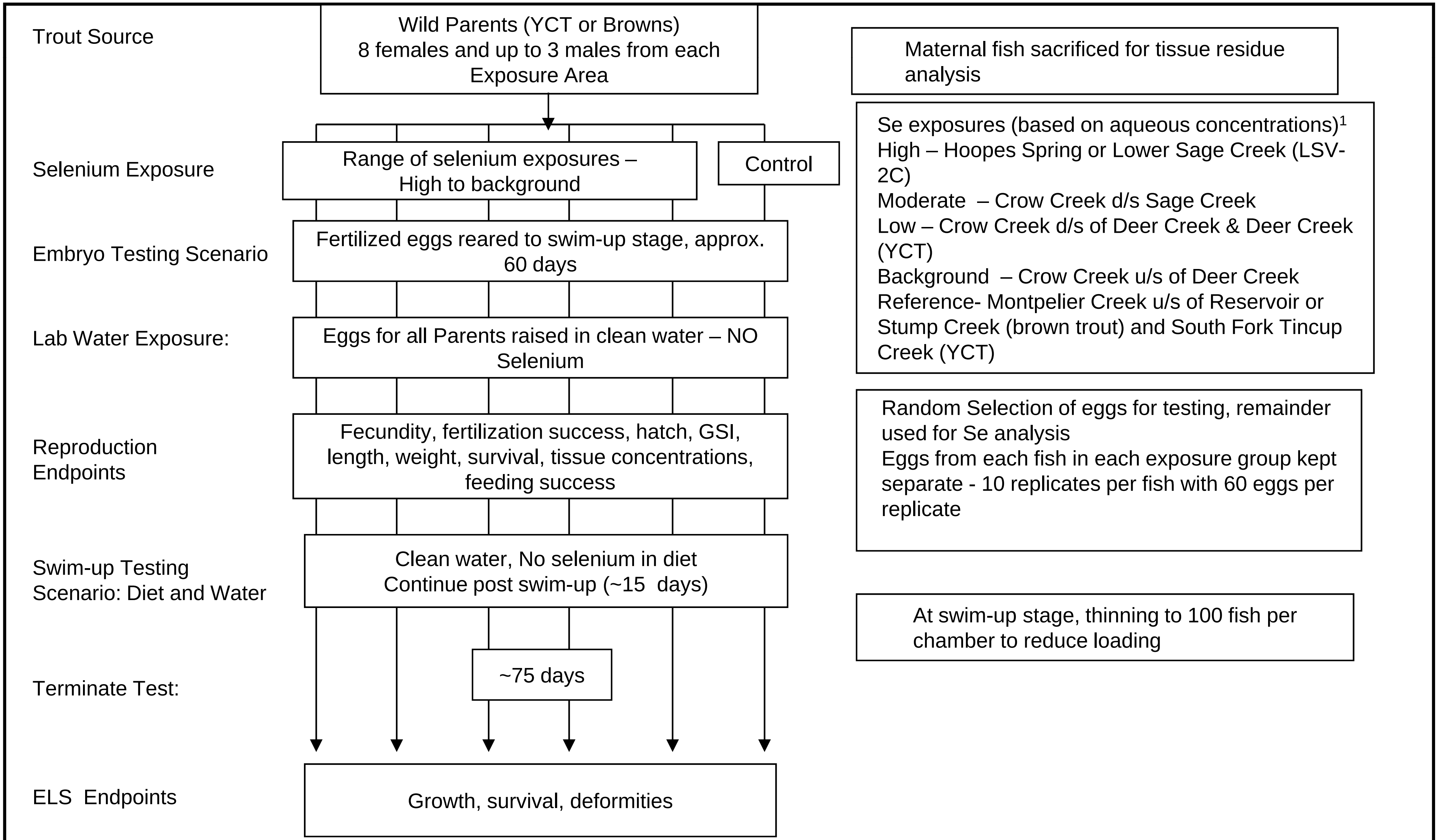


Figure 4-33
Substrate Percent Fines (<2mm) Versus Mean SMI Scores
Fall 2006 - Fall 2010

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012
REV: 1	BY: SMC CHK: SMC

FORMATION ENVIRONMENTAL



[1] Objective is to capture trout from as many of the exposure areas as possible to provide a representative sample of different exposures. Fish are not grouped by exposure area.

Figure 5-1
Flow Diagram of Laboratory Testing Methods to Assess Reproduction of Wild-Collected Parents Exposed to a Range of Selenium Concentrations

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

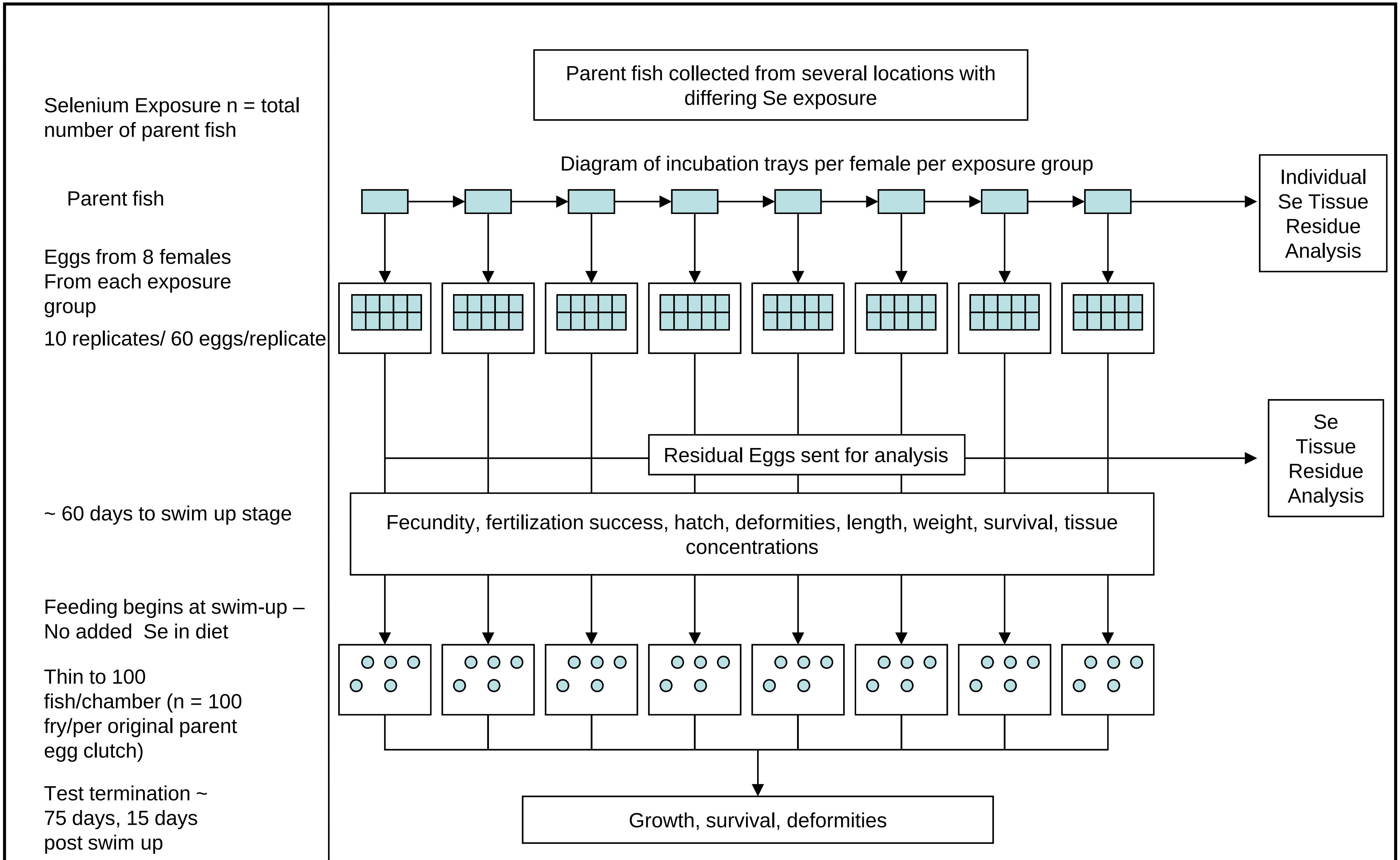


Figure 5-2
Example Diagram of Laboratory Testing Regime per Exposure Group For Adult Reproduction

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012
REV: 1	BY: SMC CHK: SMC

FORMATION ENVIRONMENTAL

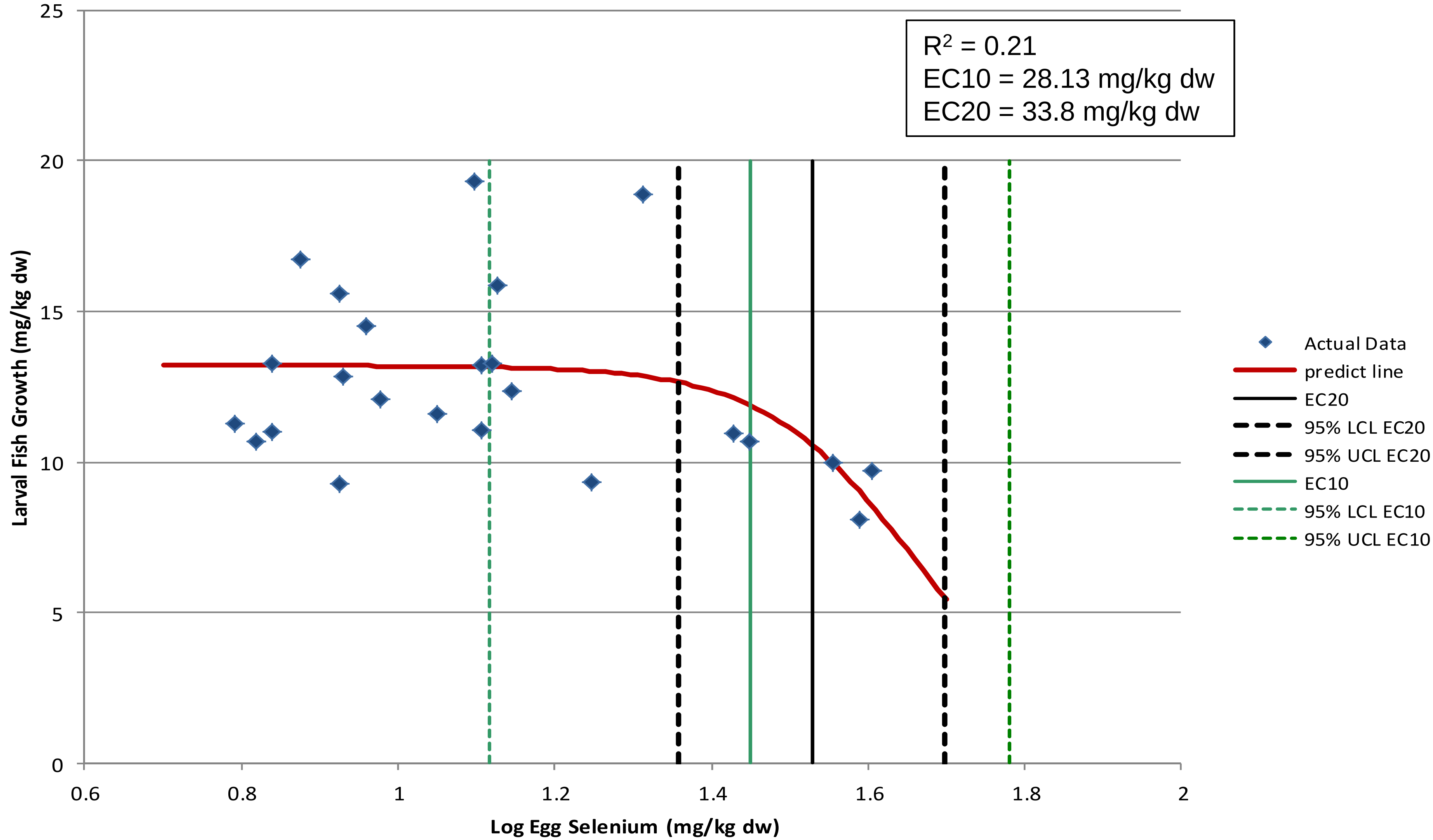


Figure 5-3
Logistic Regression of Brown Trout Egg Selenium Concentrations Versus Larval Fish Growth from the 15-Day Post-Swim-Up Feeding Trial

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

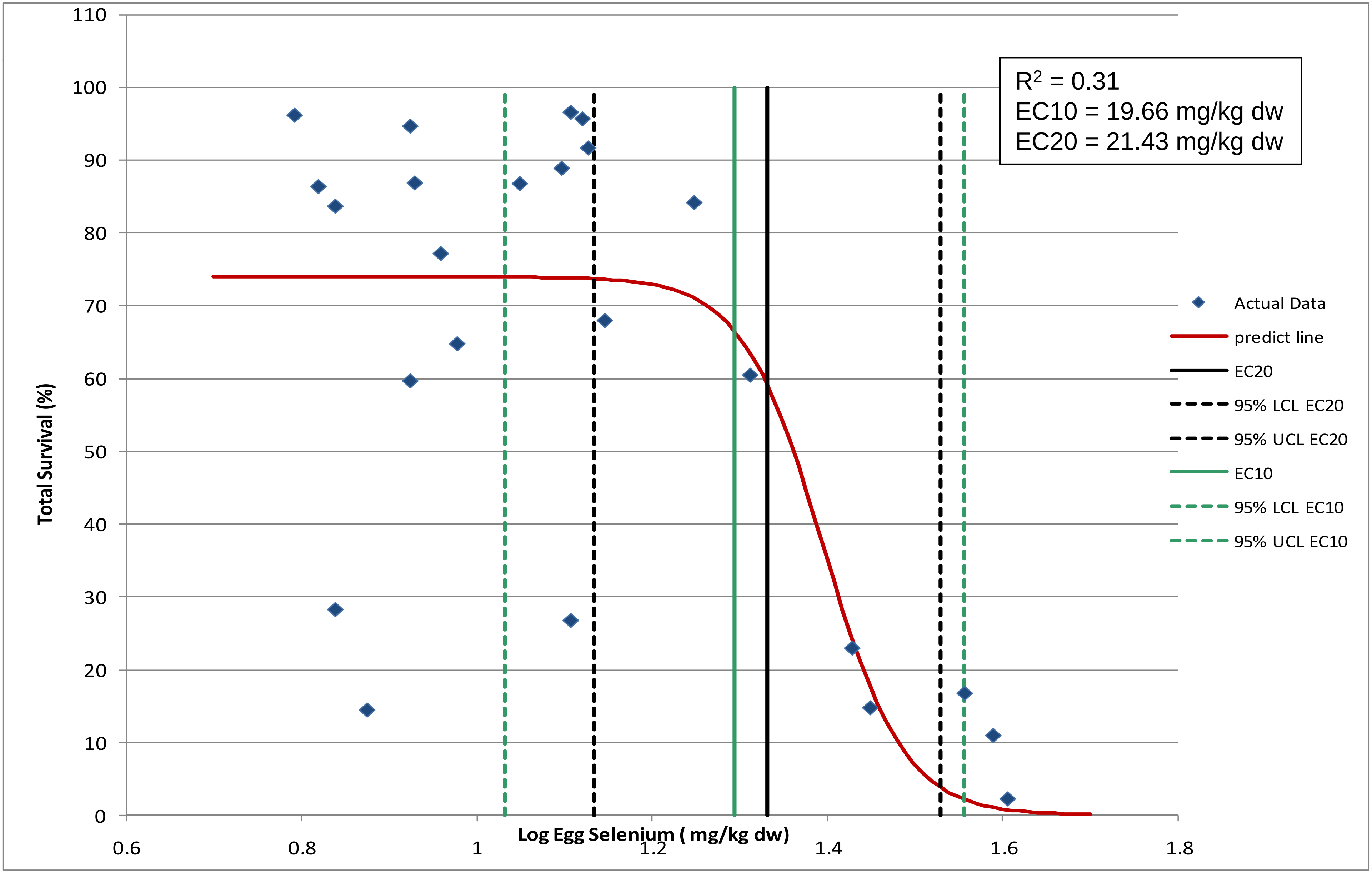


Figure 5-4
Logistic Regression of Brown Trout Egg Selenium Concentrations Versus Total Survival Percentage

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

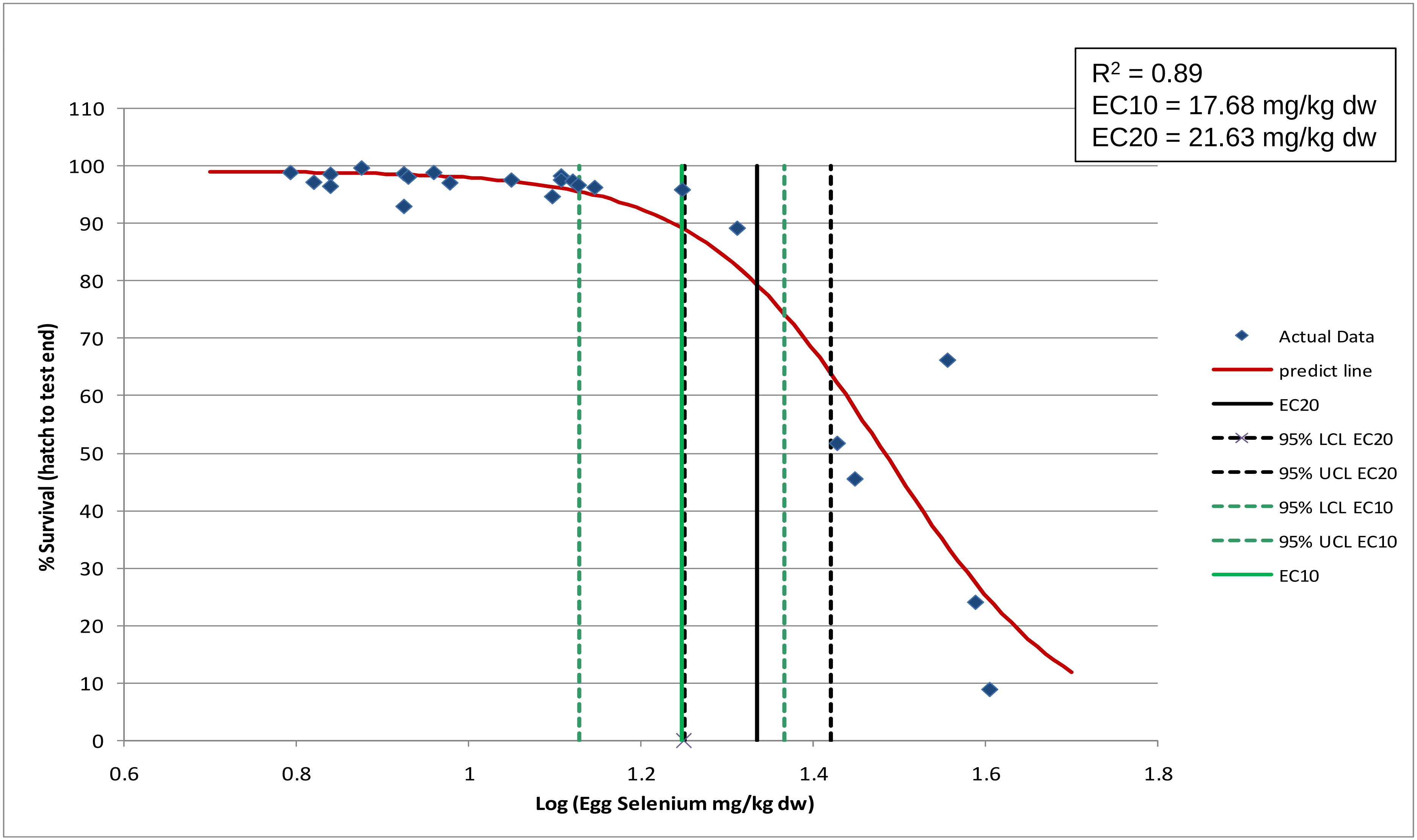


Figure 5-5
Logistic Regression of Brown Trout Egg Selenium Concentrations Versus Survival Percentage (Hatch to Test End)

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

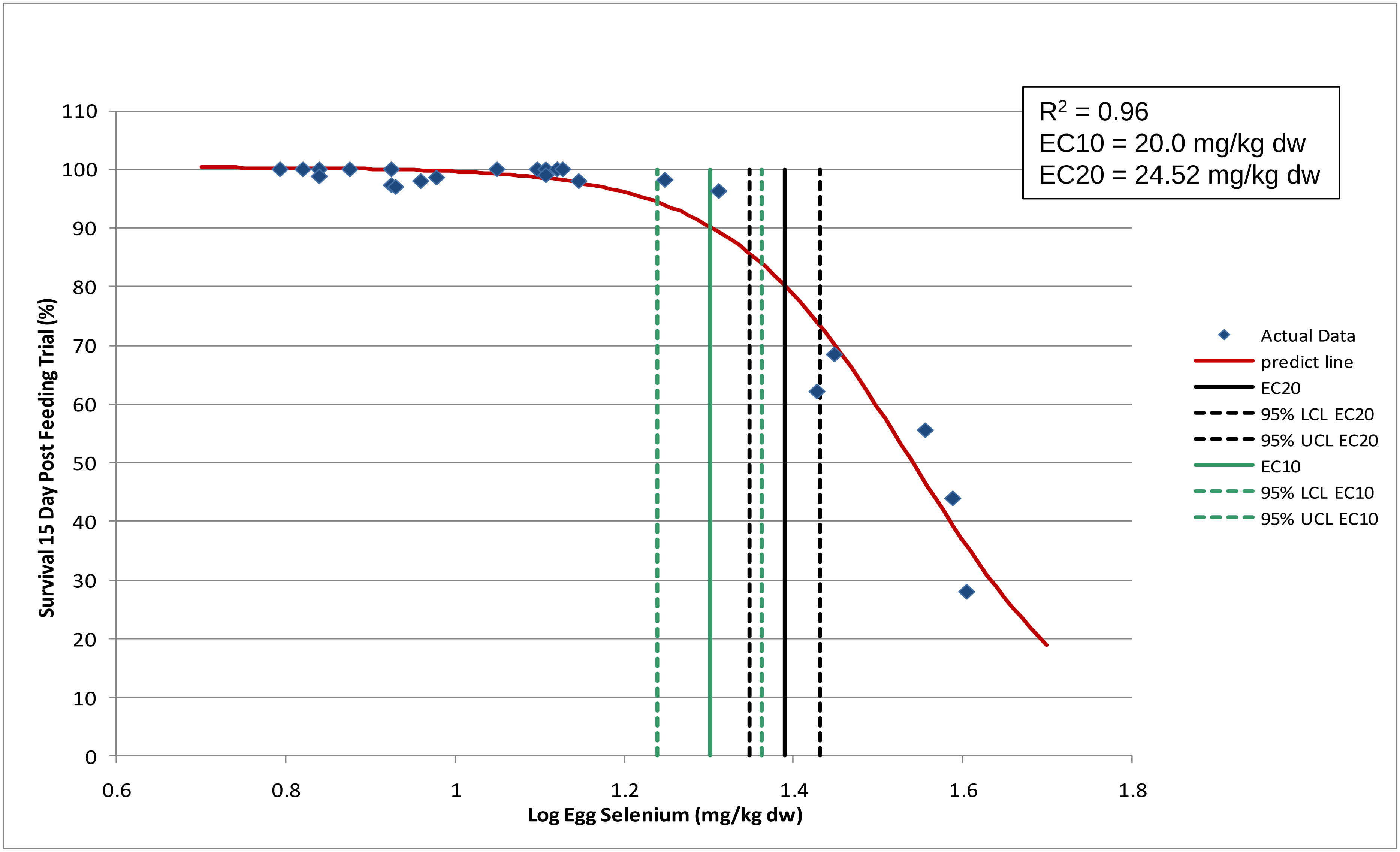


Figure 5-6
Logistic Regression of Brown Trout Egg Selenium Concentrations Versus Survival Percentage (15-Day Post-Feeding Trial)

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

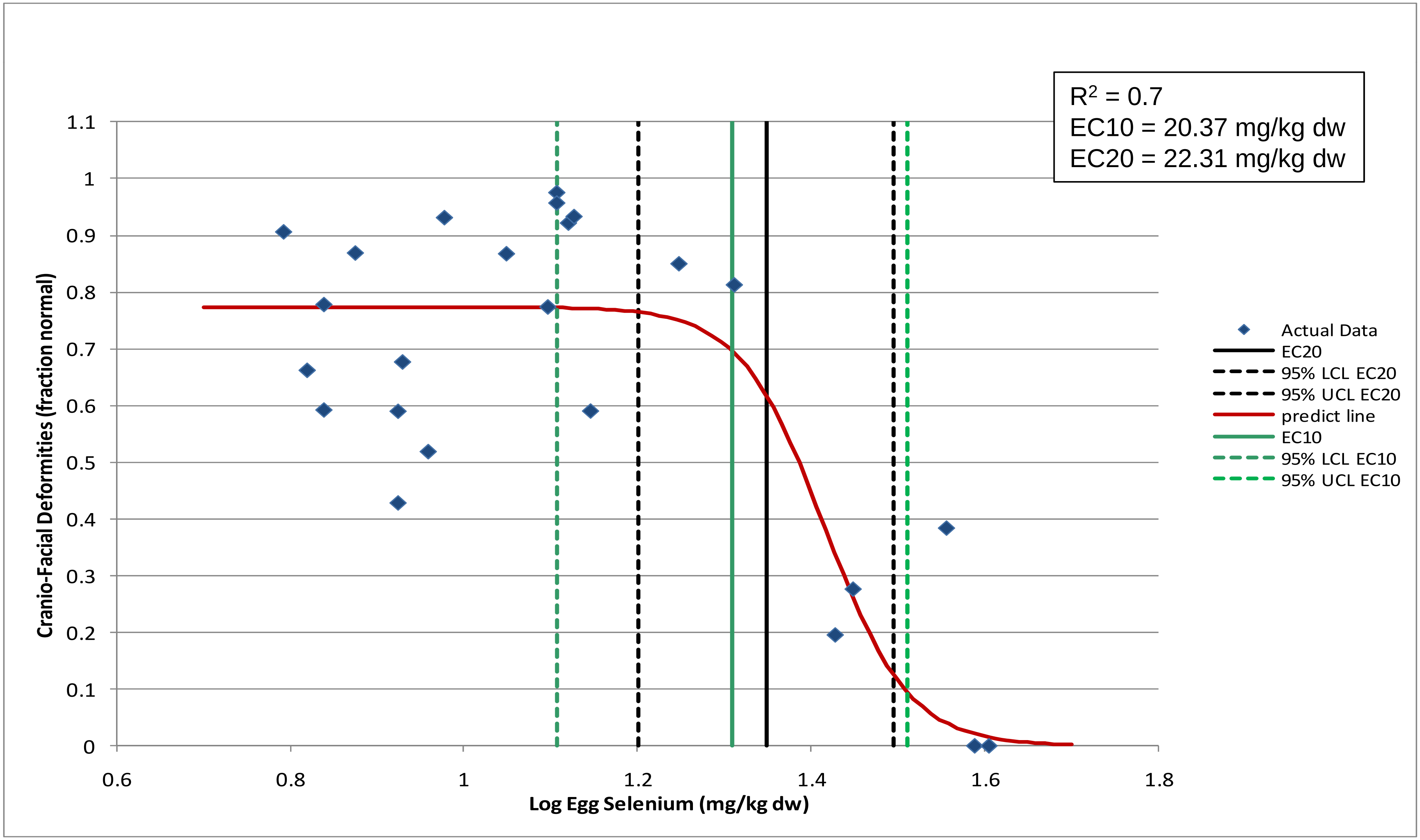


Figure 5-7
Logistic Regression of Brown Trout Egg Selenium Concentrations Versus Cranio-Facial Deformities (fraction normal)

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

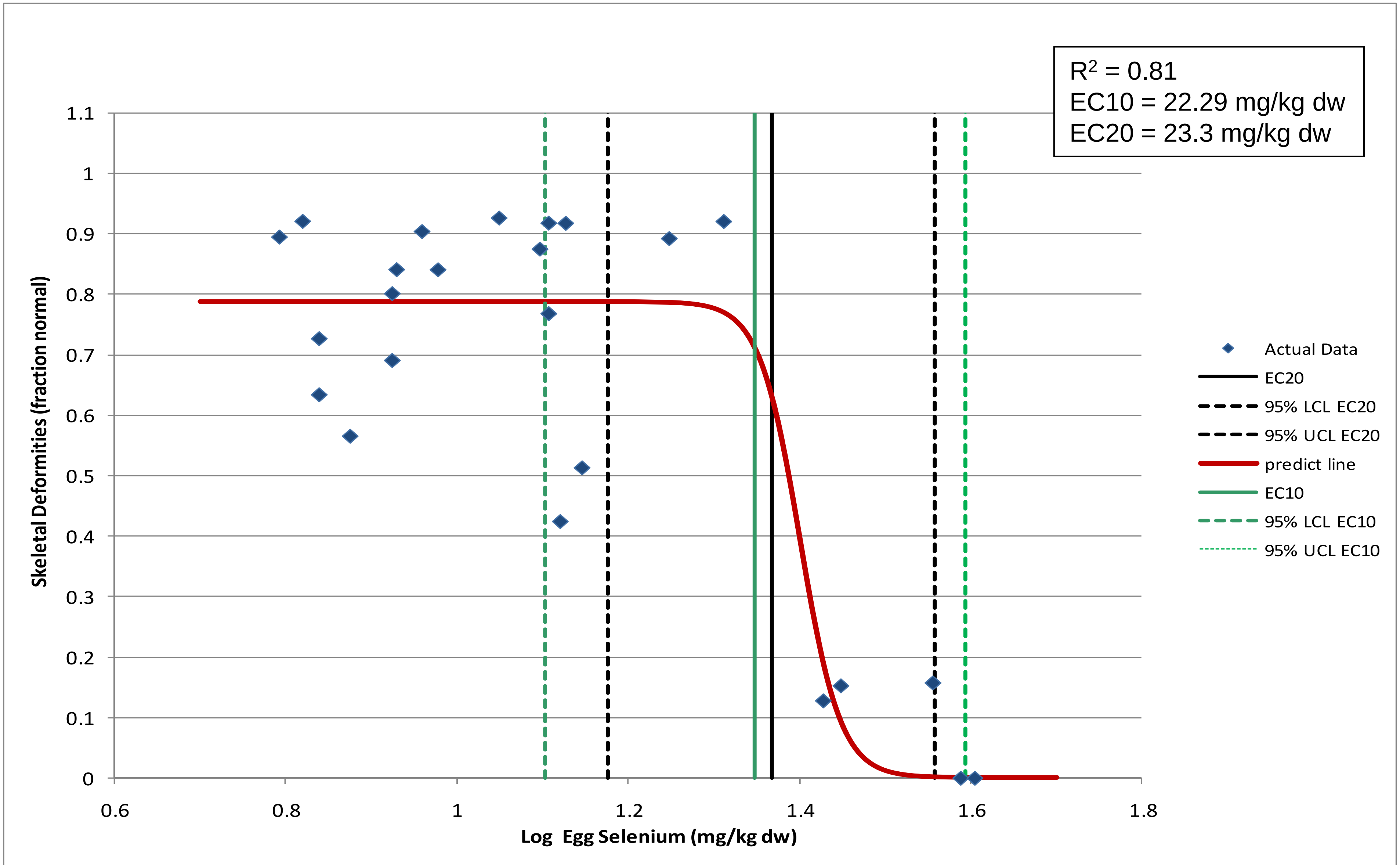


Figure 5-8
Logistic Regression of Brown Trout Egg Selenium Concentrations Versus Skeletal Deformities (fraction normal)

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012
REV: 1	BY: SMC CHK: SMC

FORMATION ENVIRONMENTAL

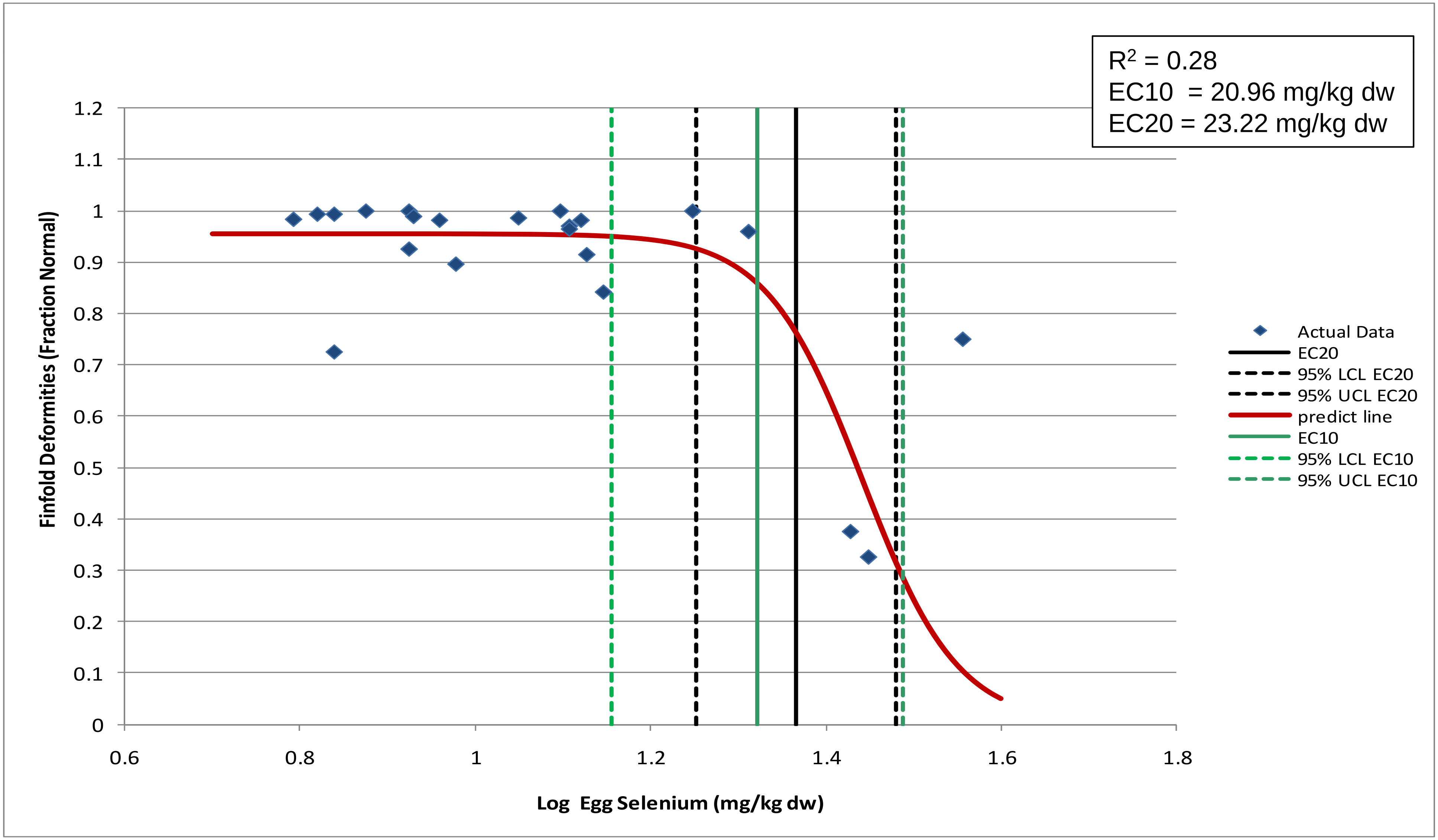


Figure 5-9
Logistic Regression of Brown Trout Egg Selenium Concentrations Versus Finfold Deformities (fraction normal)

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

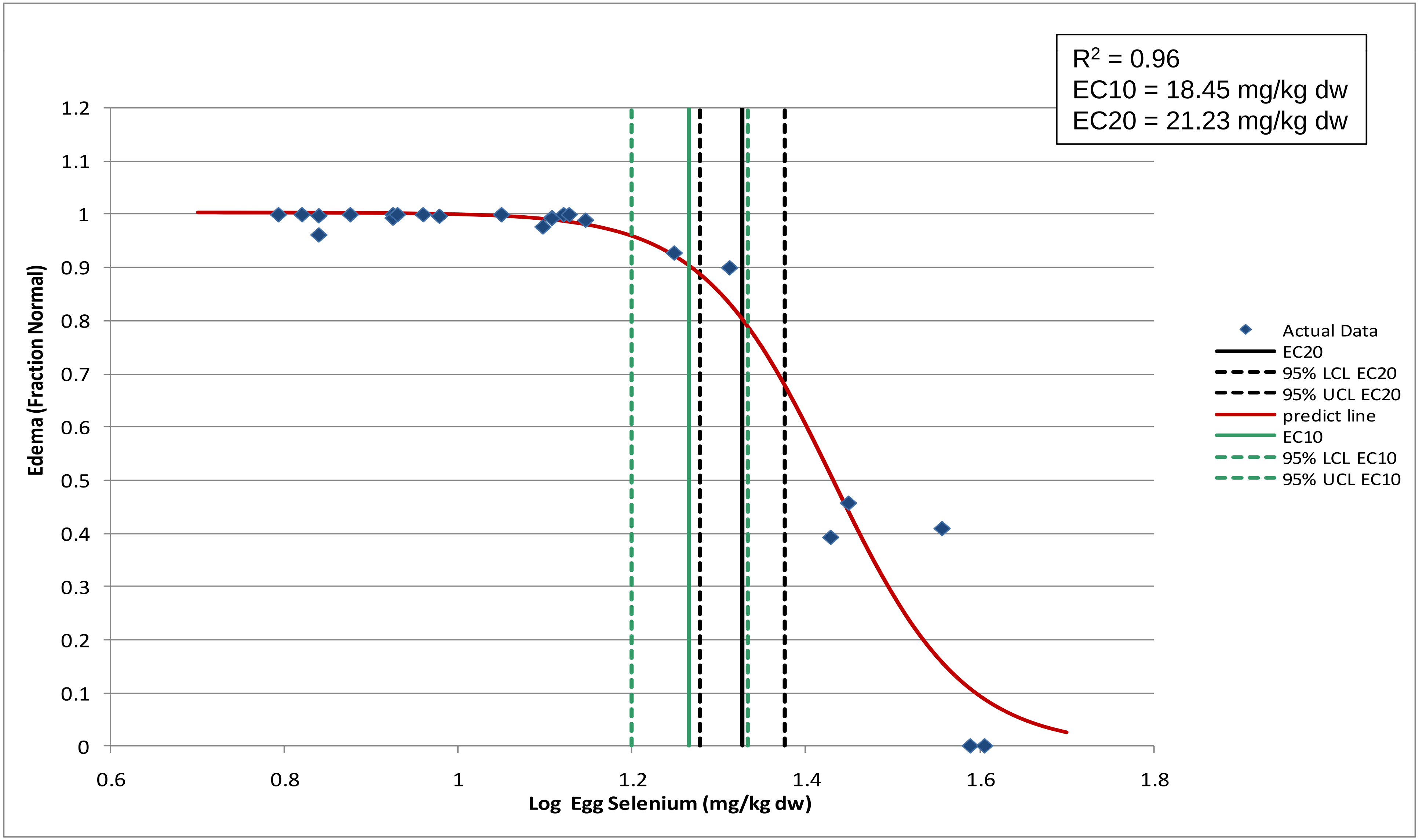


Figure 5-10
Logistic Regression of Brown Trout Egg Selenium Concentrations Versus Edema (fraction normal)

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

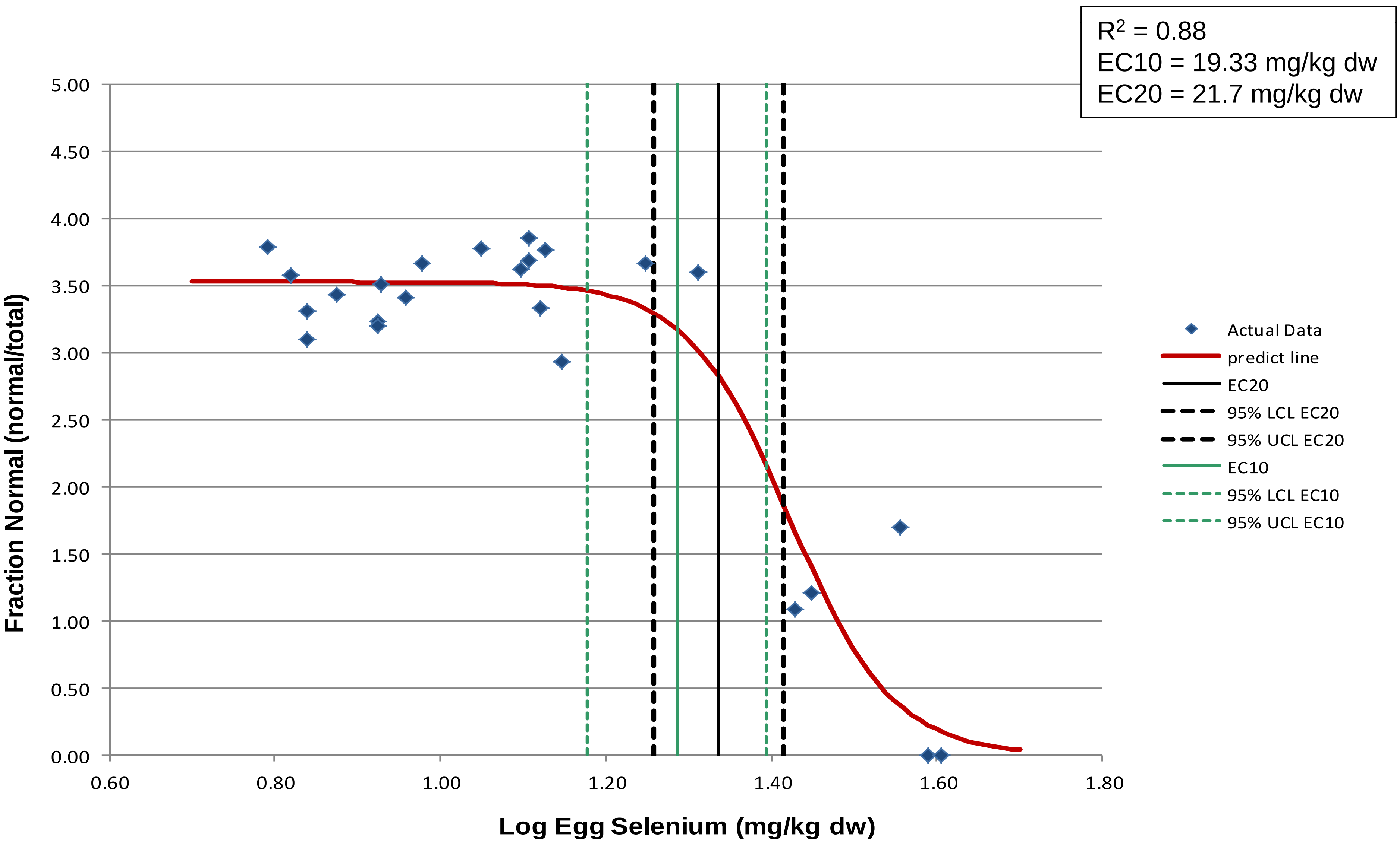


Figure 5-11
Logistic Regression of Brown Trout Egg Selenium Concentrations Versus Normal Fish Larvae (fraction normal/total)

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

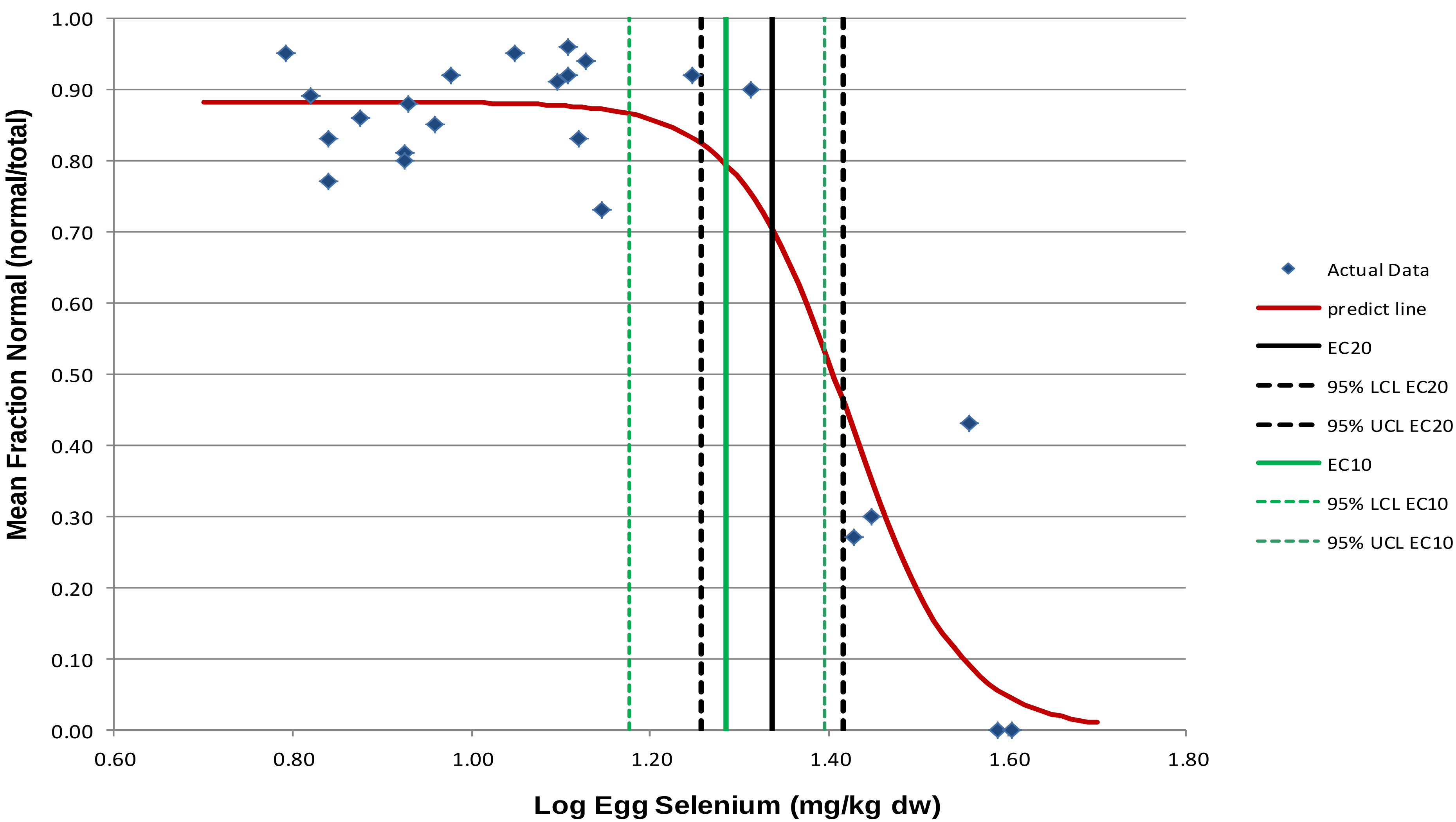


Figure 5-12
Logistic Regression of Brown Trout Egg Selenium Concentrations Versus Fish Larvae (mean fraction normal)

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 0	BY: SMC	CHK: SMC

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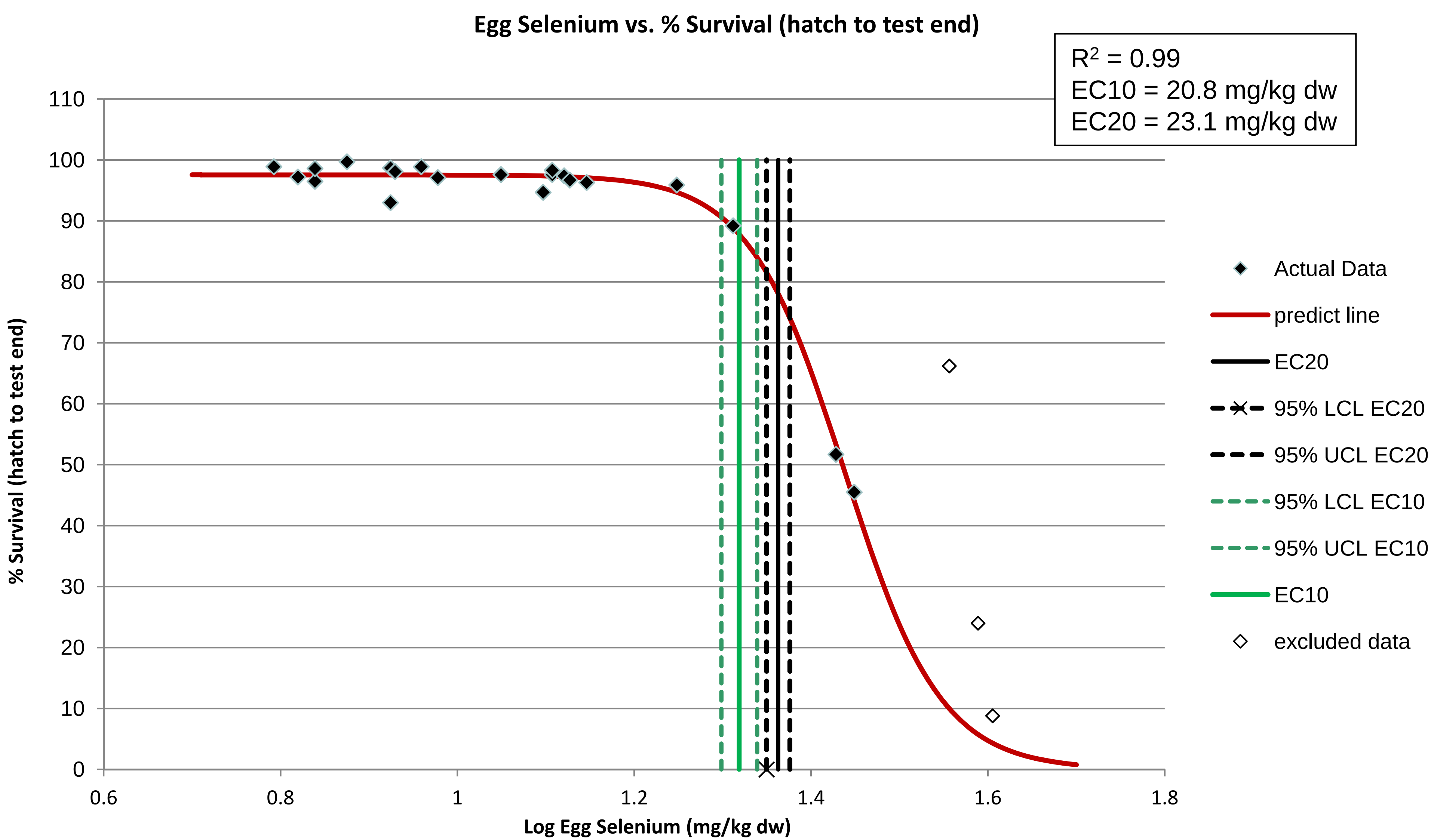

Figure 5-13
Logistic Regression of Brown Trout Egg Selenium Concentrations Versus Survival Percentage (Hatch to Test End) Focused on Effect Region for Derivation of EC_x Values

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012
REV: 1	BY: SMC / CHK: SMC

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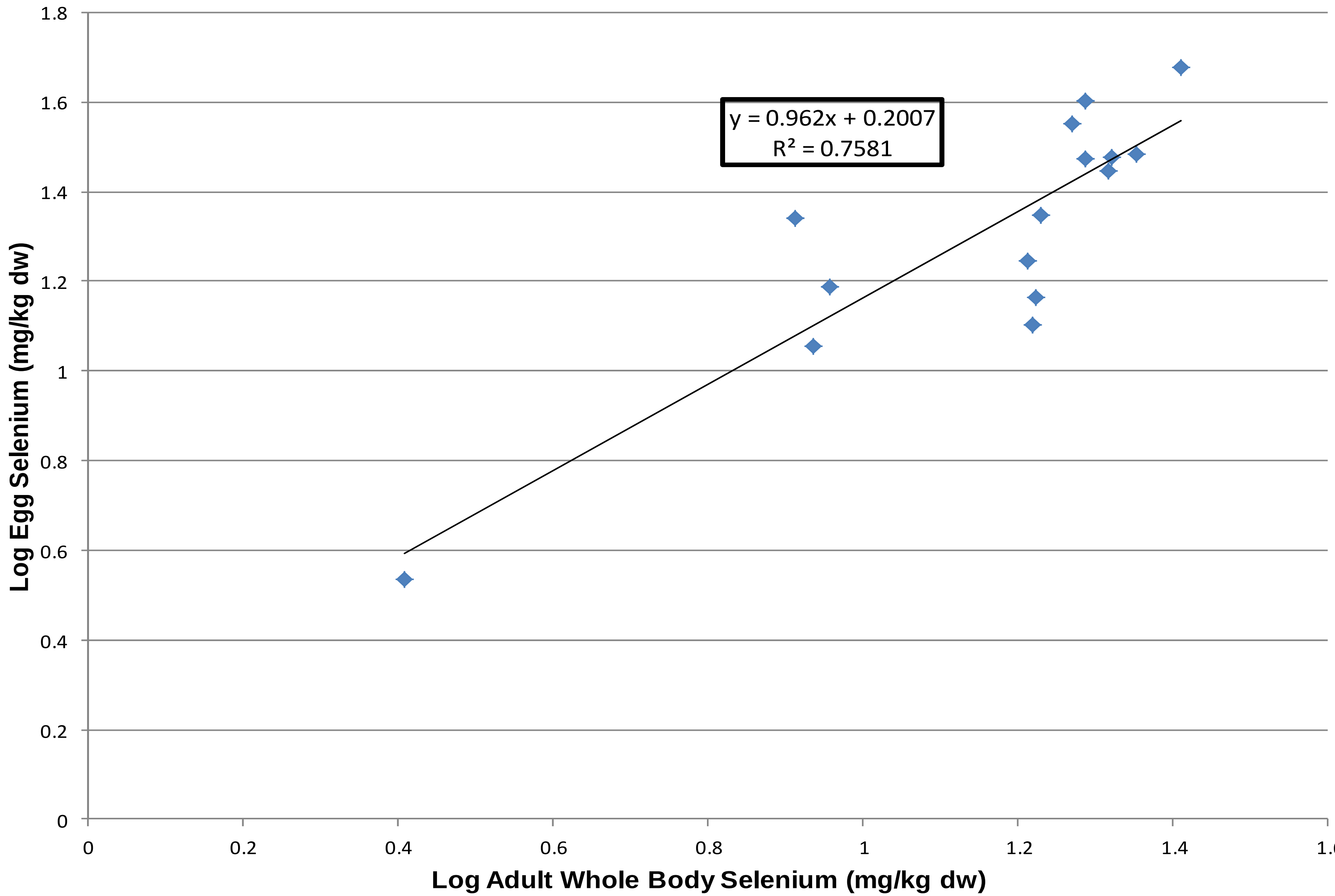

Figure 5-14
YCT Adult Maternal Whole Body Selenium Concentrations Versus Egg Selenium Concentrations

J.R. Simplot Company Site-Specific Selenium Criterion		
PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

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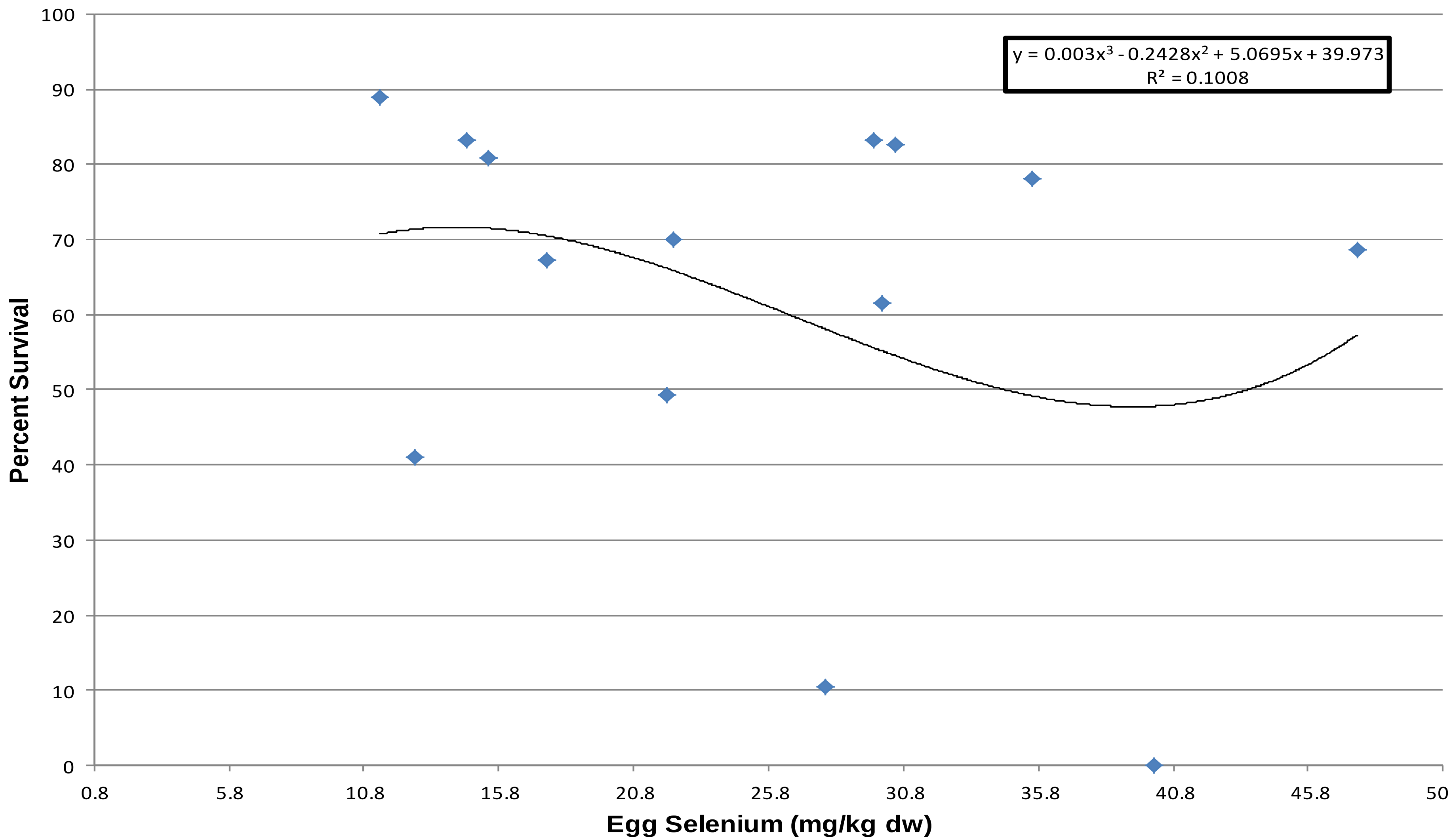

Figure 5-15
YCT Egg Selenium Concentrations Versus Total Survival Percentage

J.R. Simplot Company Site-Specific Selenium Criterion		
PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC
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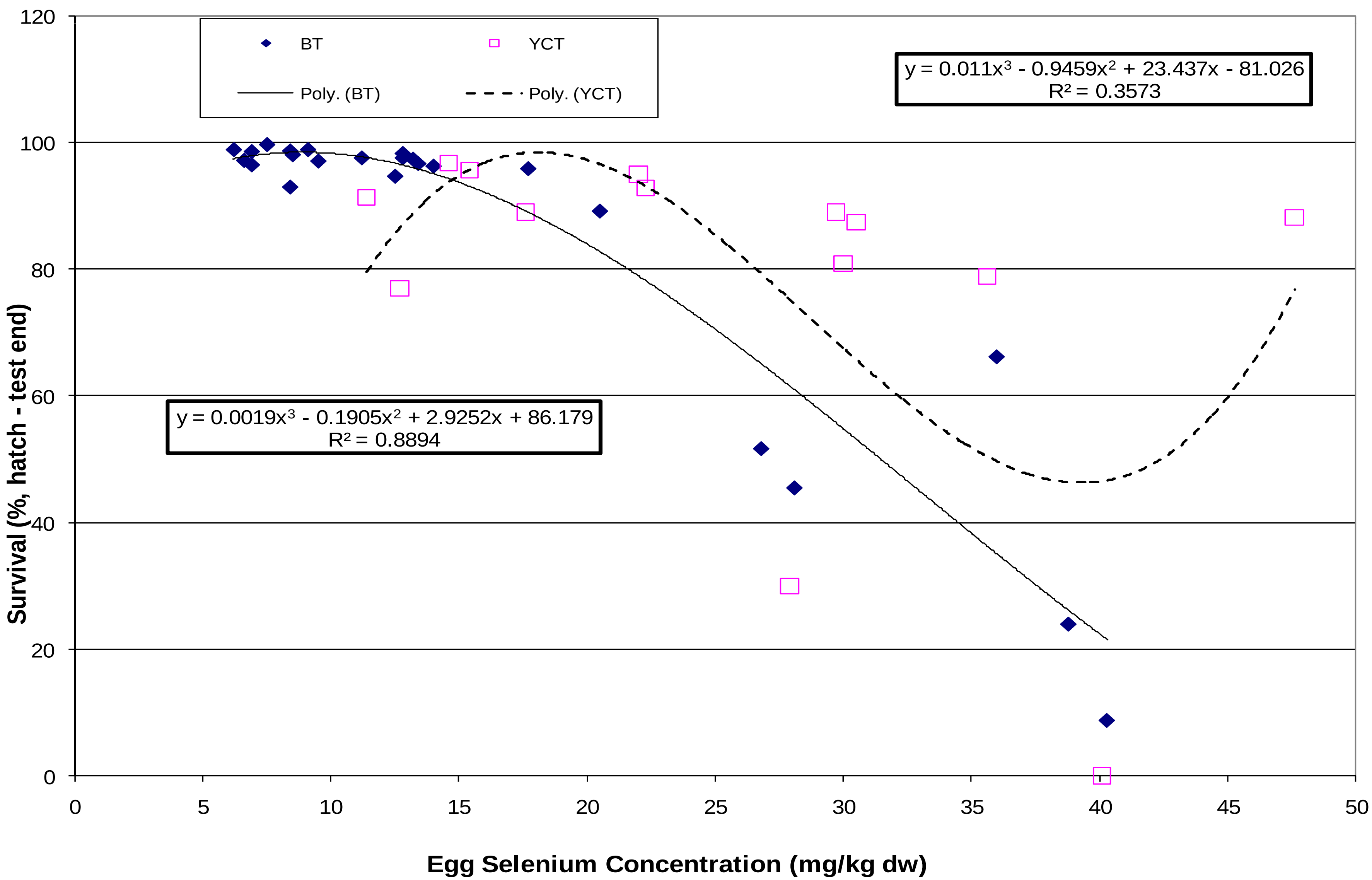

Figure 5-16
Brown Trout and Yellowstone Cutthroat Trout Egg Selenium Concentrations Versus Percent Survival (Hatch to Test End) – All Data

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 0	BY: SMC	CHK: SMC

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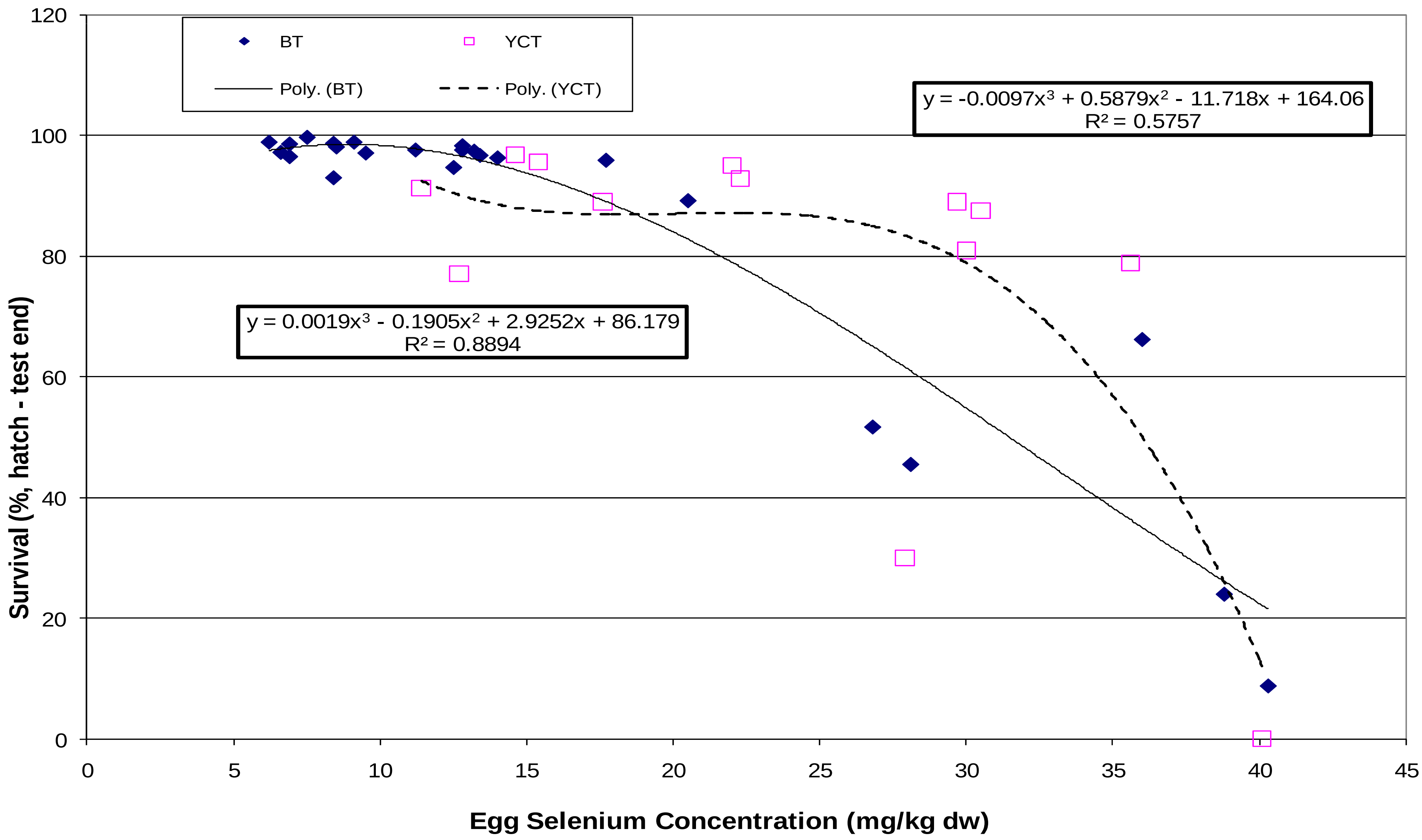

Figure 5-17
Brown Trout and Yellowstone Cutthroat Trout Egg Selenium Concentrations Versus Percent Survival (Hatch to Test End) – High Egg Selenium Concentration and High Survival Concentration Excluded

J.R. Simplot Company Site-Specific Selenium Criterion	
PRJ: 0442-004-900.70	DATE: January 2012
REV: 0	BY: SMC / CHK: SMC
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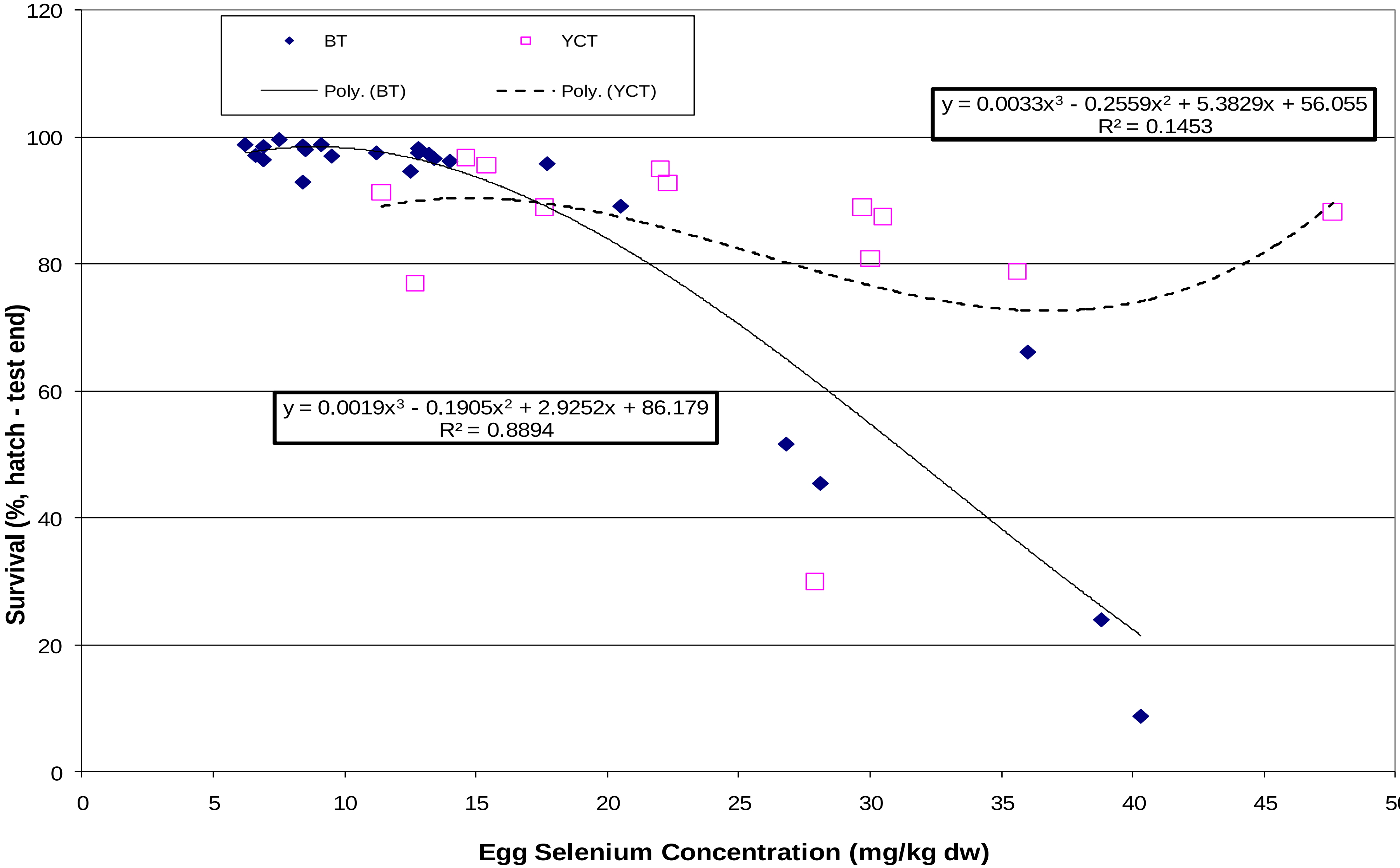

Figure 5-18
Brown Trout and Yellowstone Cutthroat Trout Egg Selenium Concentrations Versus Percent Survival (Hatch to Test End) – High Egg Selenium Concentration and Low Survival Concentration Excluded

J.R. Simplot Company Site-Specific Selenium Criterion	
PRJ: 0442-004-900.70	DATE: January 2012
REV: 0	BY: SMC / CHK: SMC

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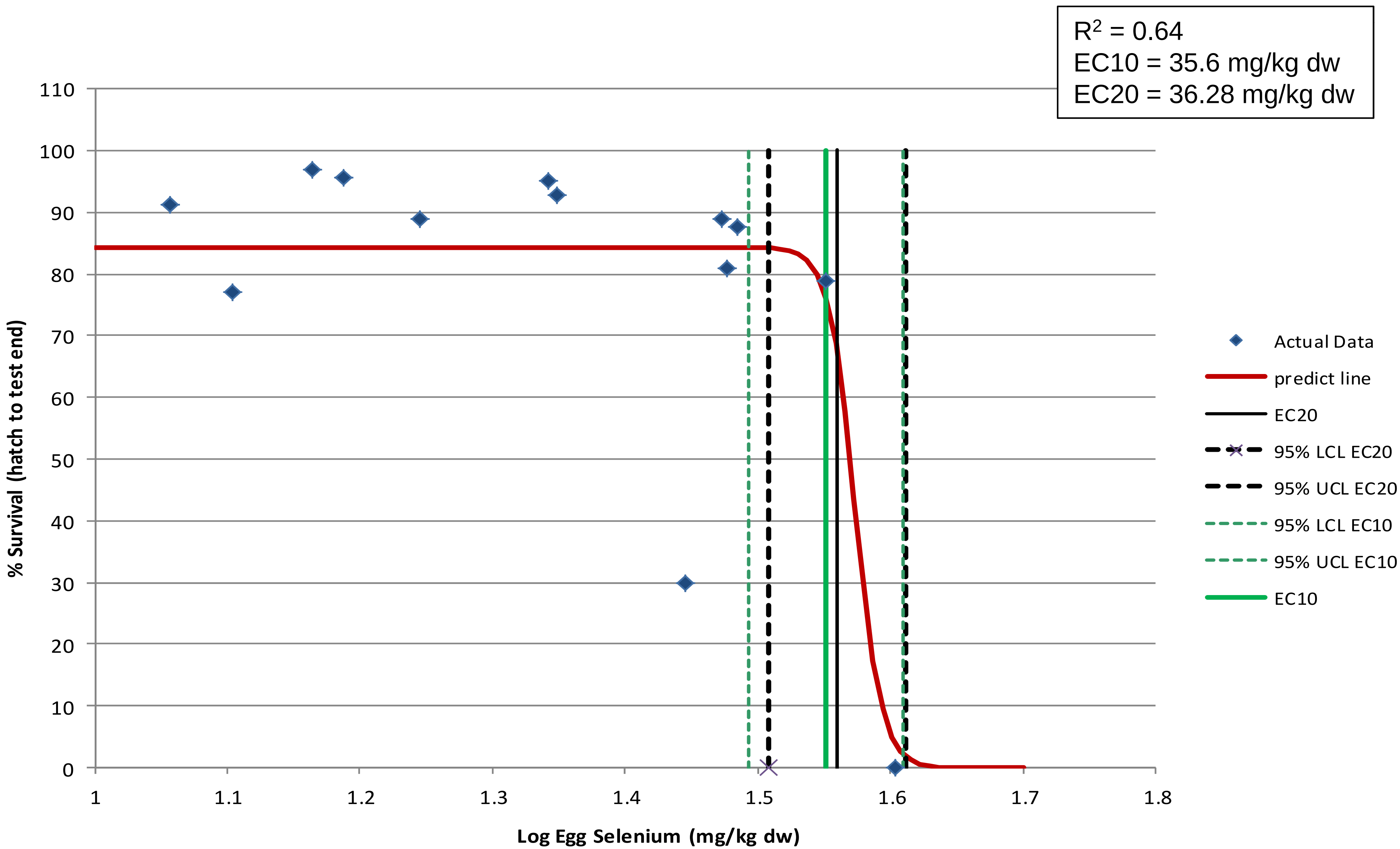

Figure 5-19
Logistic Regression for Yellowstone Cutthroat Trout Egg Selenium Concentrations Versus Survival Percentage (Hatch to Test End)

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012
REV: 1	BY: SMC CHK: SMC

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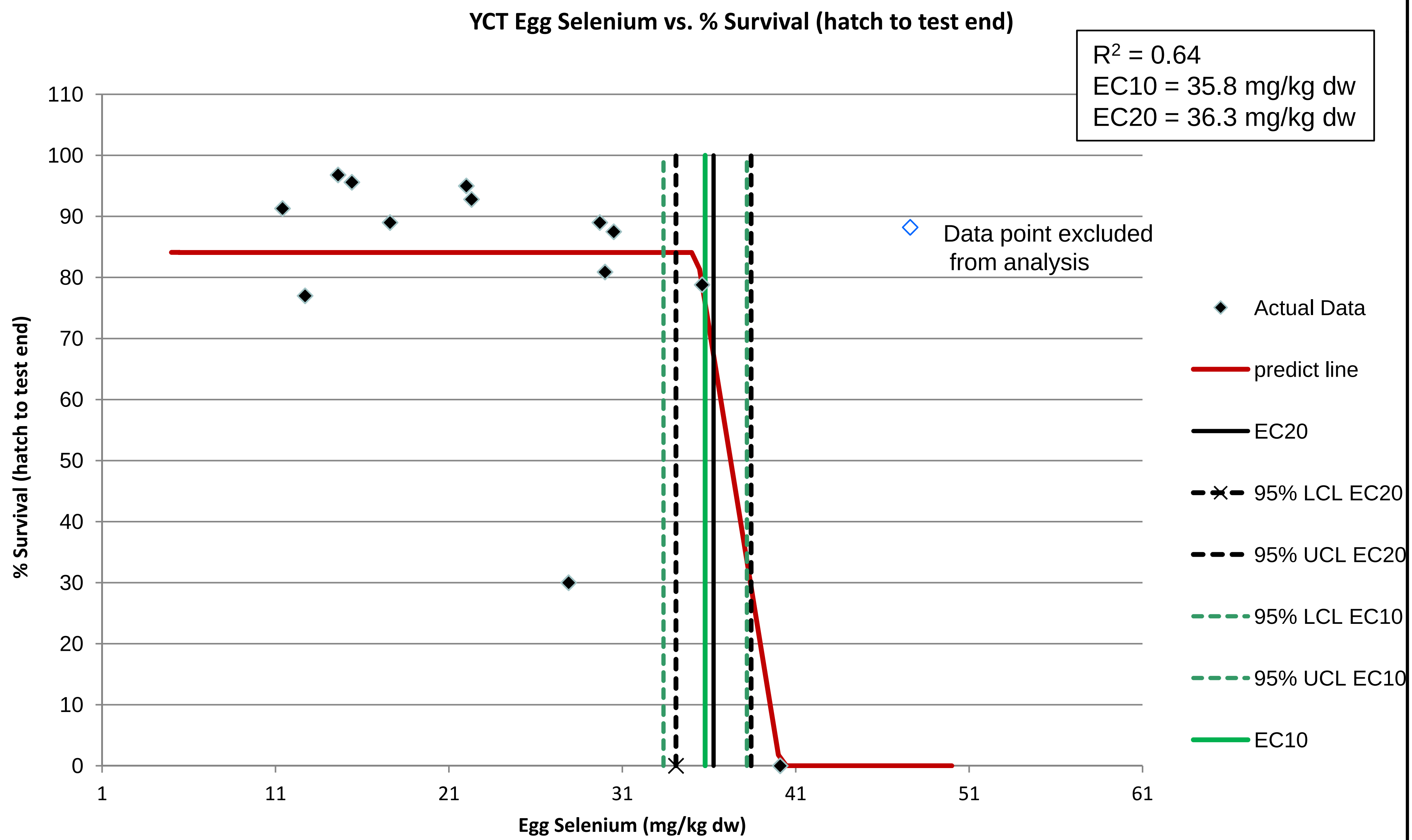

Figure 5-20
Piecewise Linear Regression for Yellowstone Cutthroat Trout Egg Selenium Concentrations Versus Survival Percentage (Hatch to Test End)

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012
REV: 1	BY: SMC / CHK: SMC

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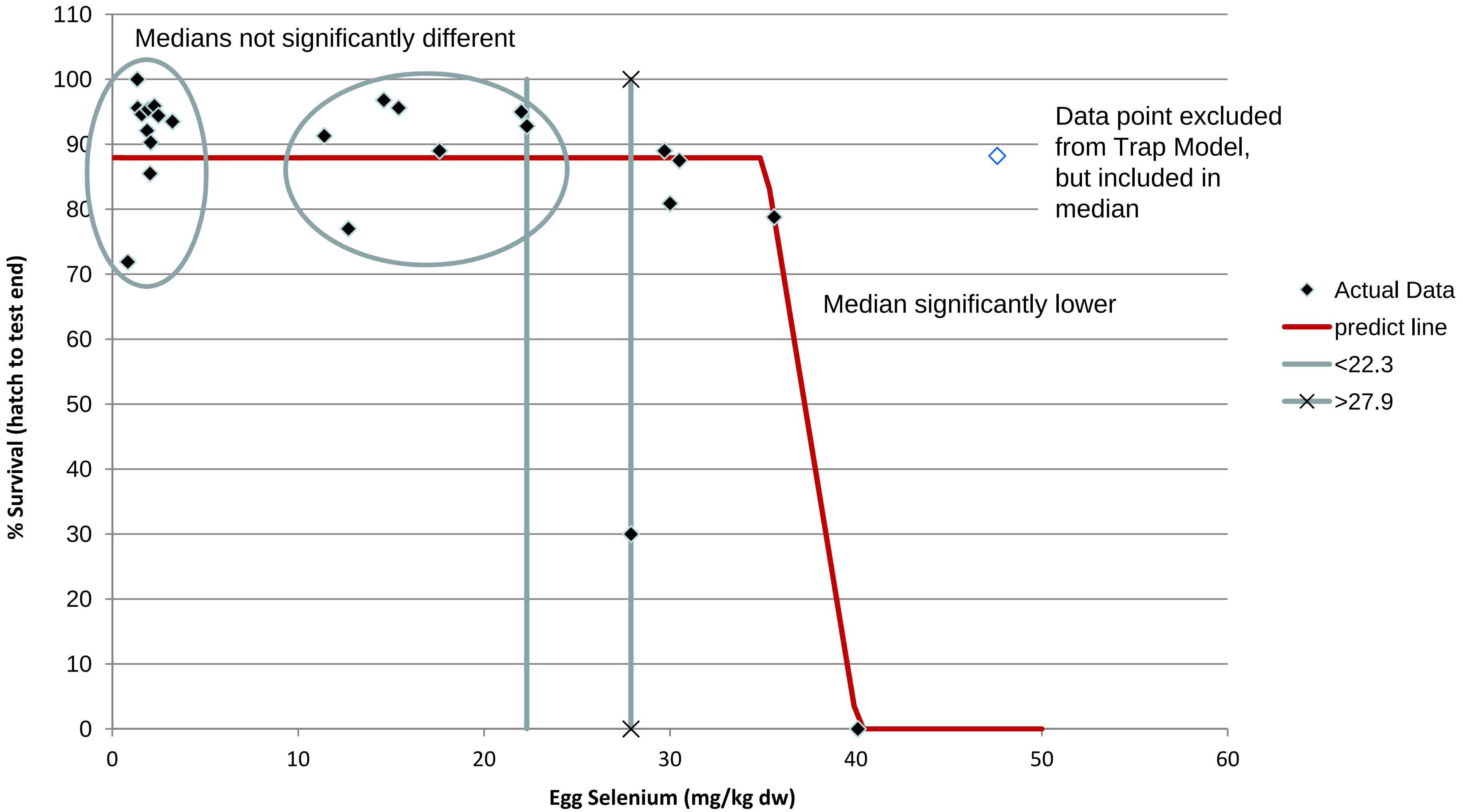

Figure 5-21
YCT Egg Selenium Concentrations Versus Survival Percentage (Hatch to Test End) Including Henry's Lake Survival Data

J.R. Simplot Company Site-Specific Selenium Criterion		
PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

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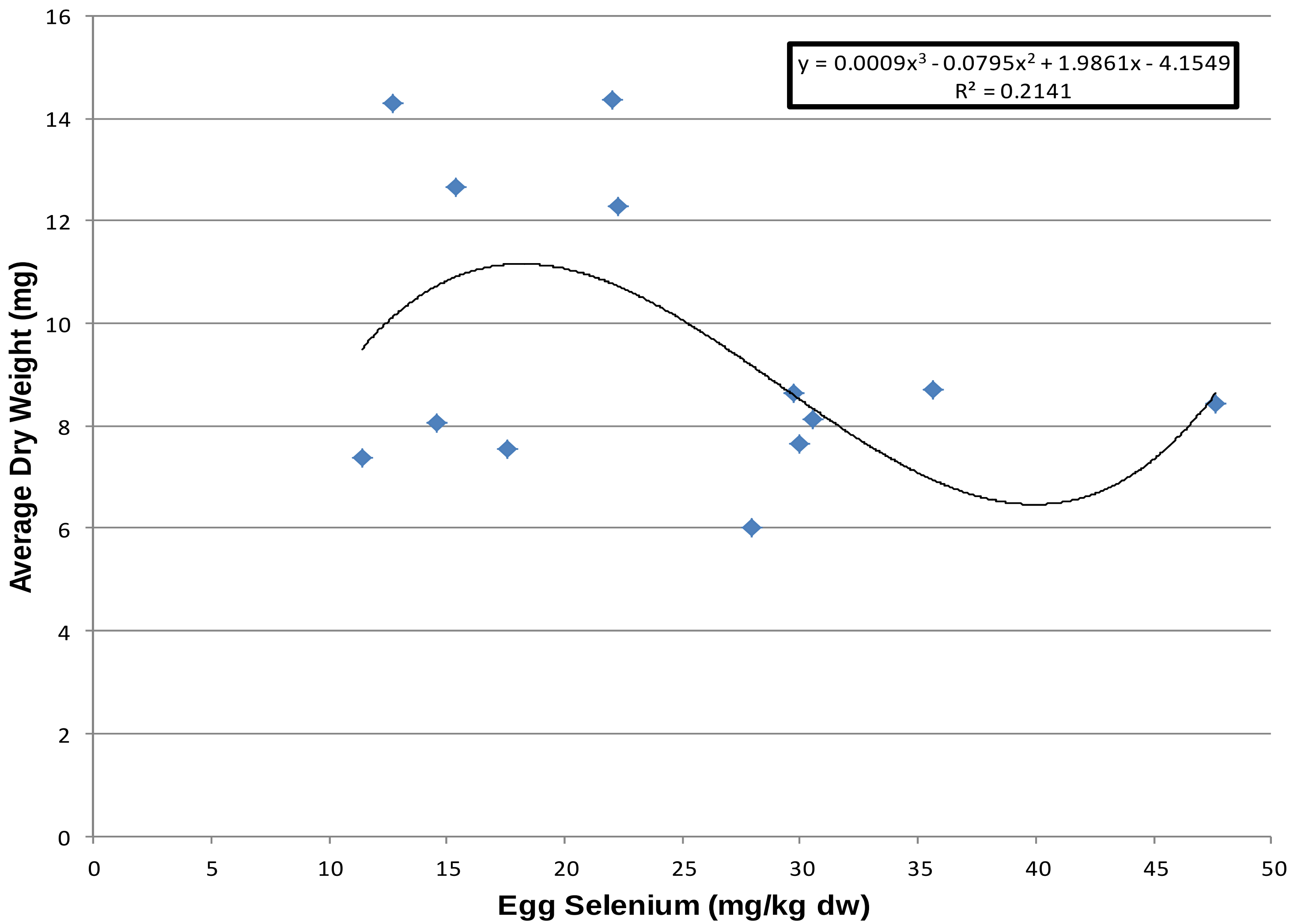

Figure 5-22
YCT Egg Selenium Concentrations Versus Growth

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

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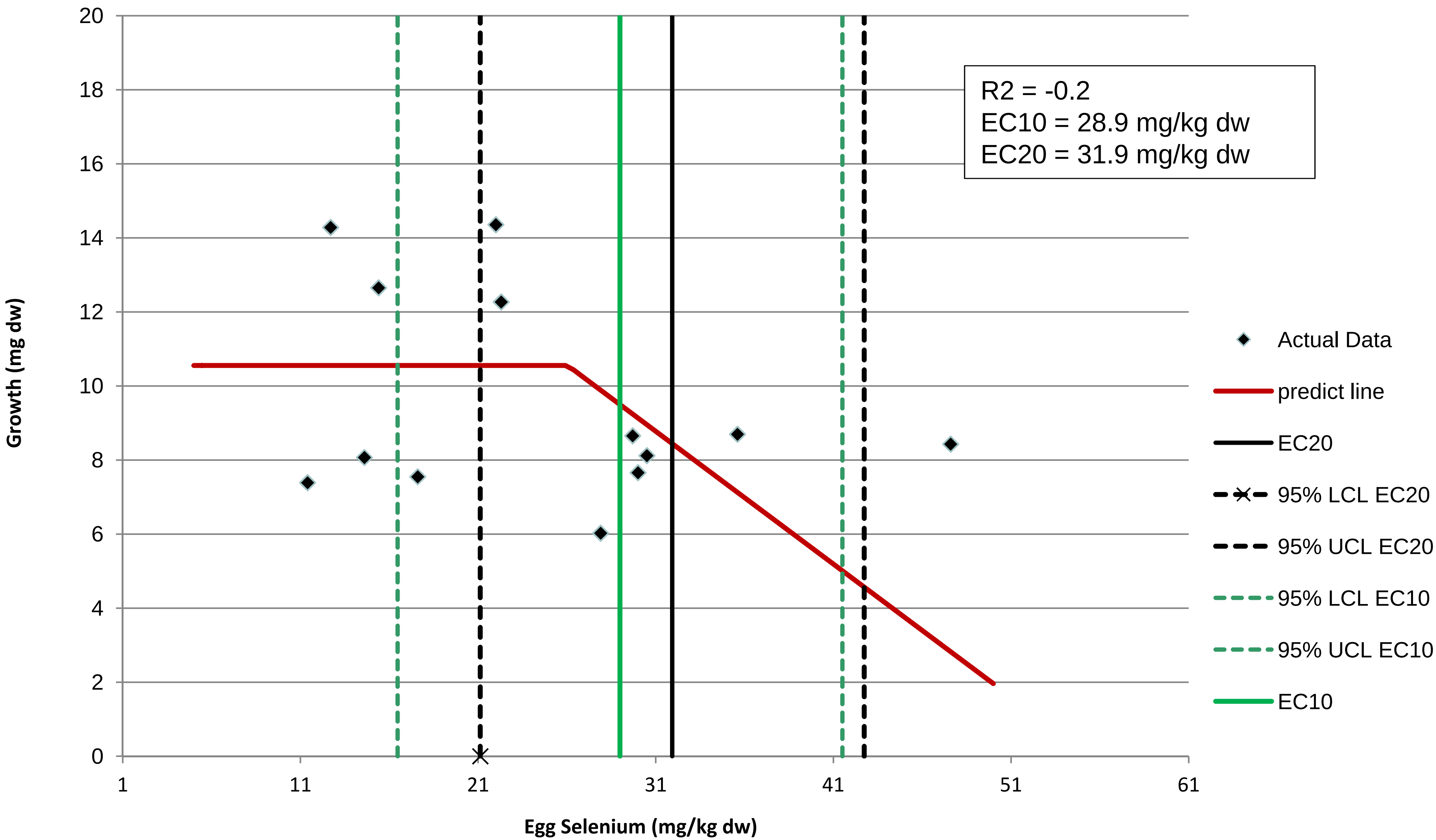

Figure 5-23
Piecewise Linear Regression for Yellowstone Cutthroat Trout Egg Selenium Concentrations Versus Growth (Hatch to 15 Days Post-Swim-Up)

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

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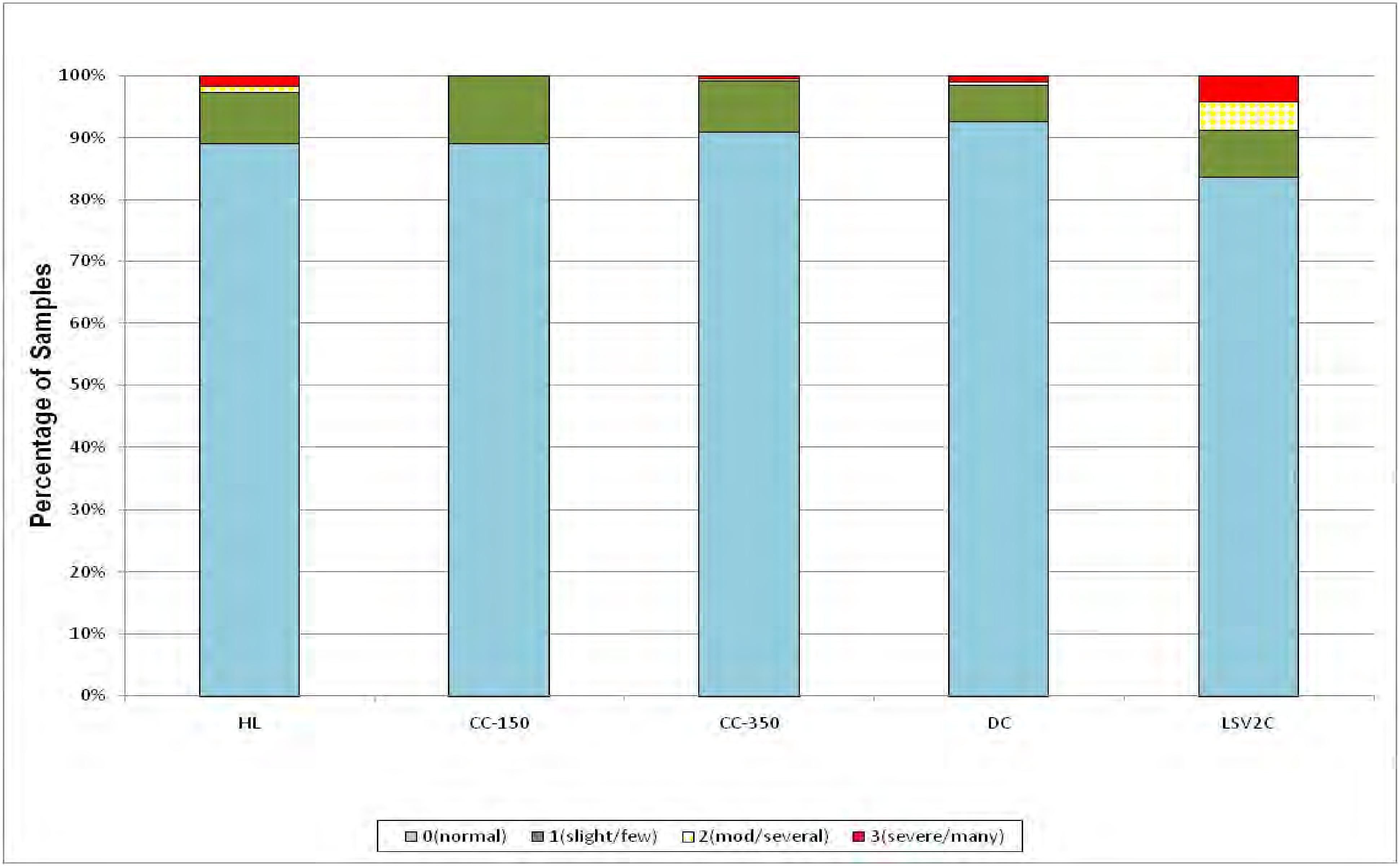

Figure 5-24
Yellowstone Cutthroat Trout – Average Percentage of Larvae with Cranio-Facial Deformities

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

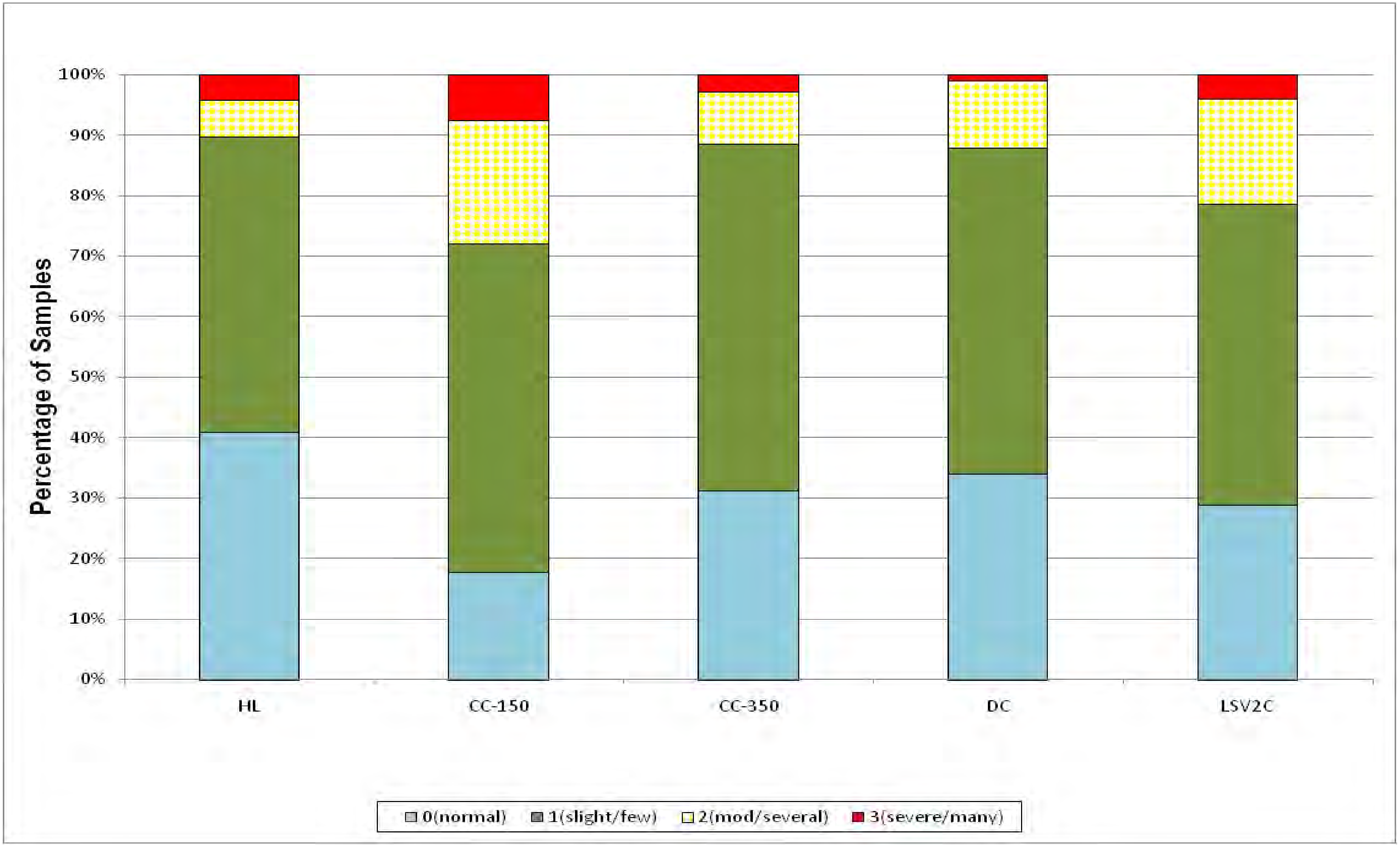

Figure 5-25
Yellowstone Cutthroat Trout – Average Percentage of Larvae with Skeletal Deformities

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

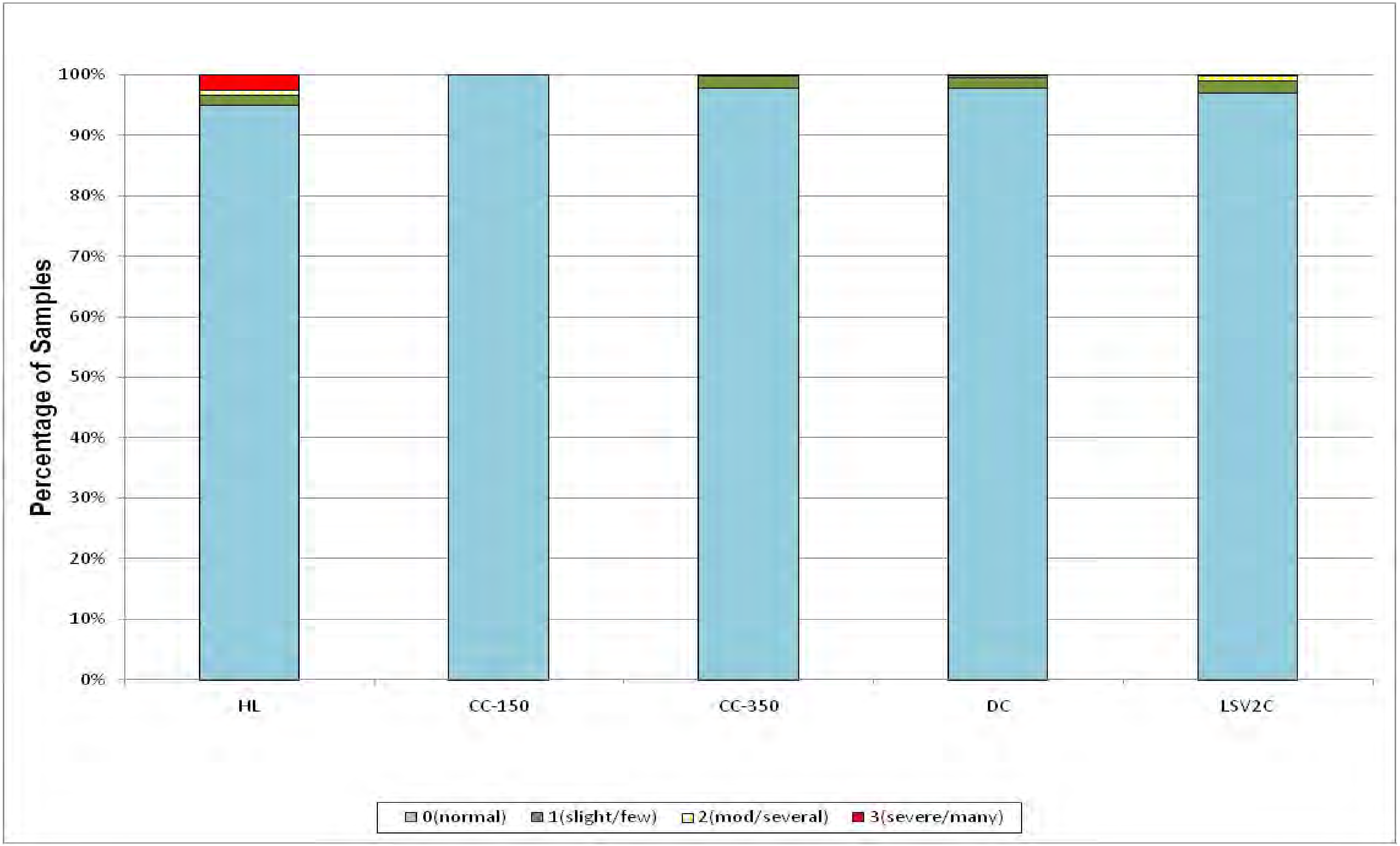

Figure 5-26
Yellowstone Cutthroat Trout – Average Percentage of Larvae with Fin or Finfold Deformities

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

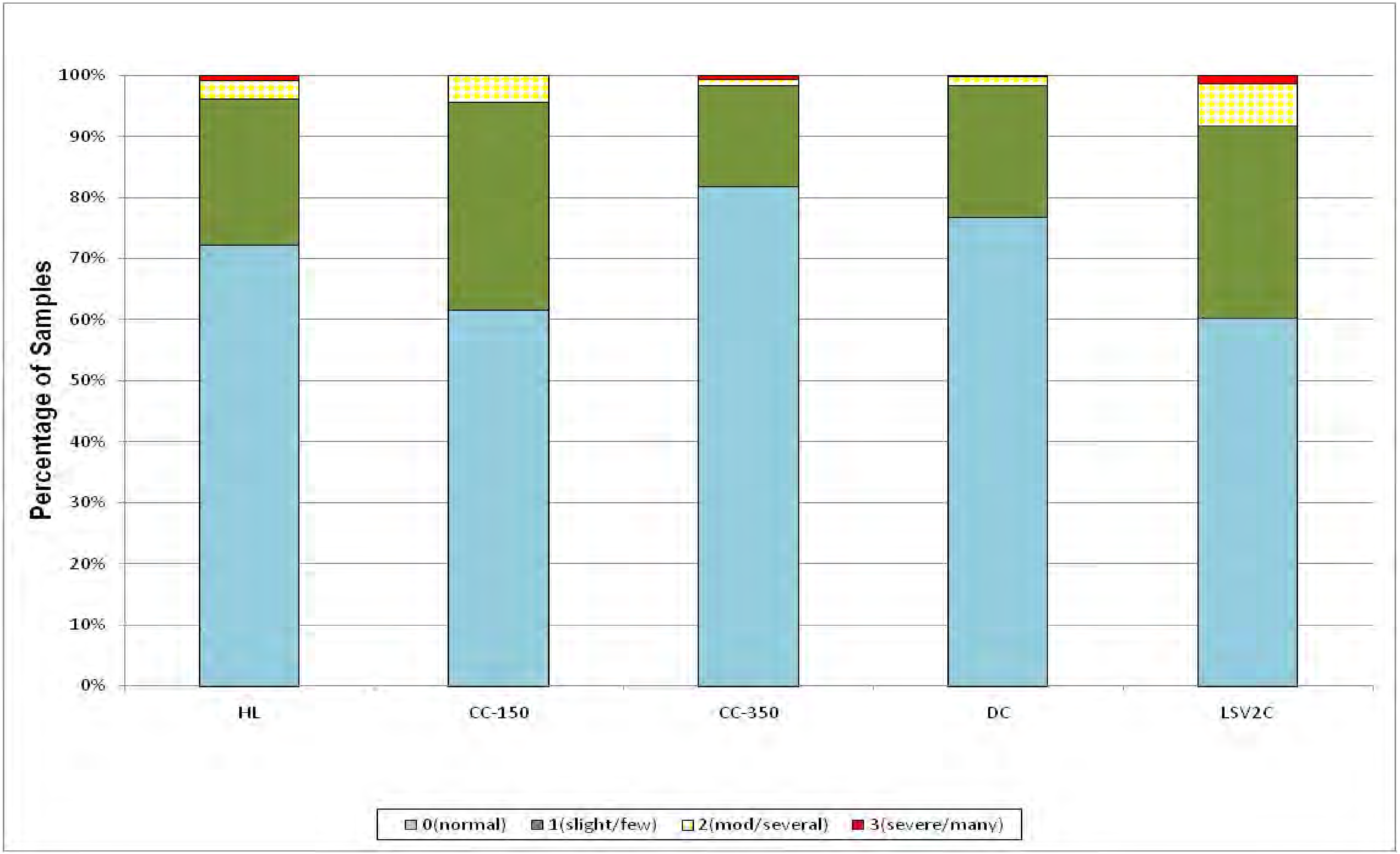

Figure 5-27
Yellowstone Cutthroat Trout – Average Percentage of Larvae with Edematous Tissue Deformities

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

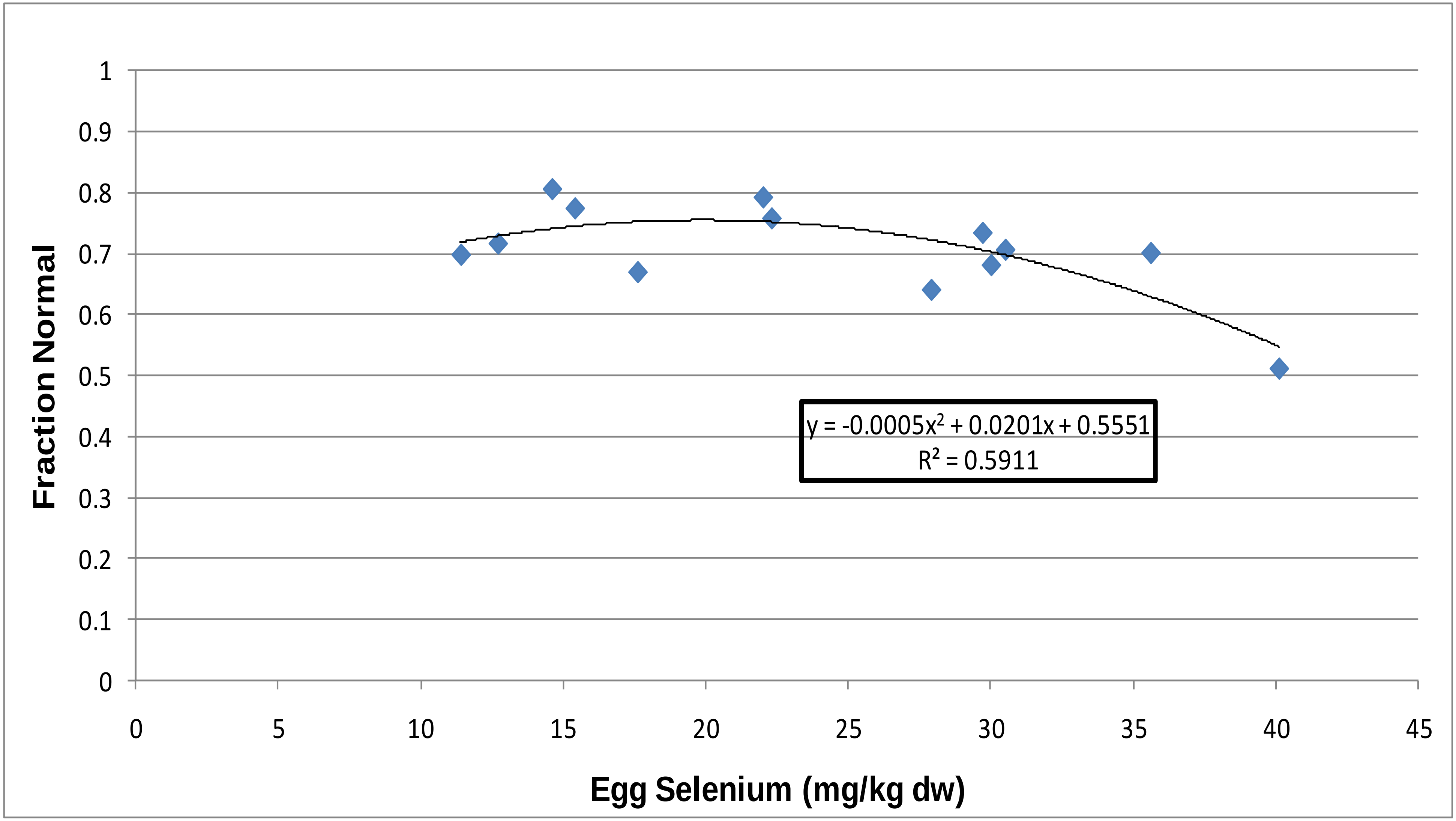

Figure 5-28
YCT Egg Selenium Concentrations Versus Mean Fraction Normal Larvae

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

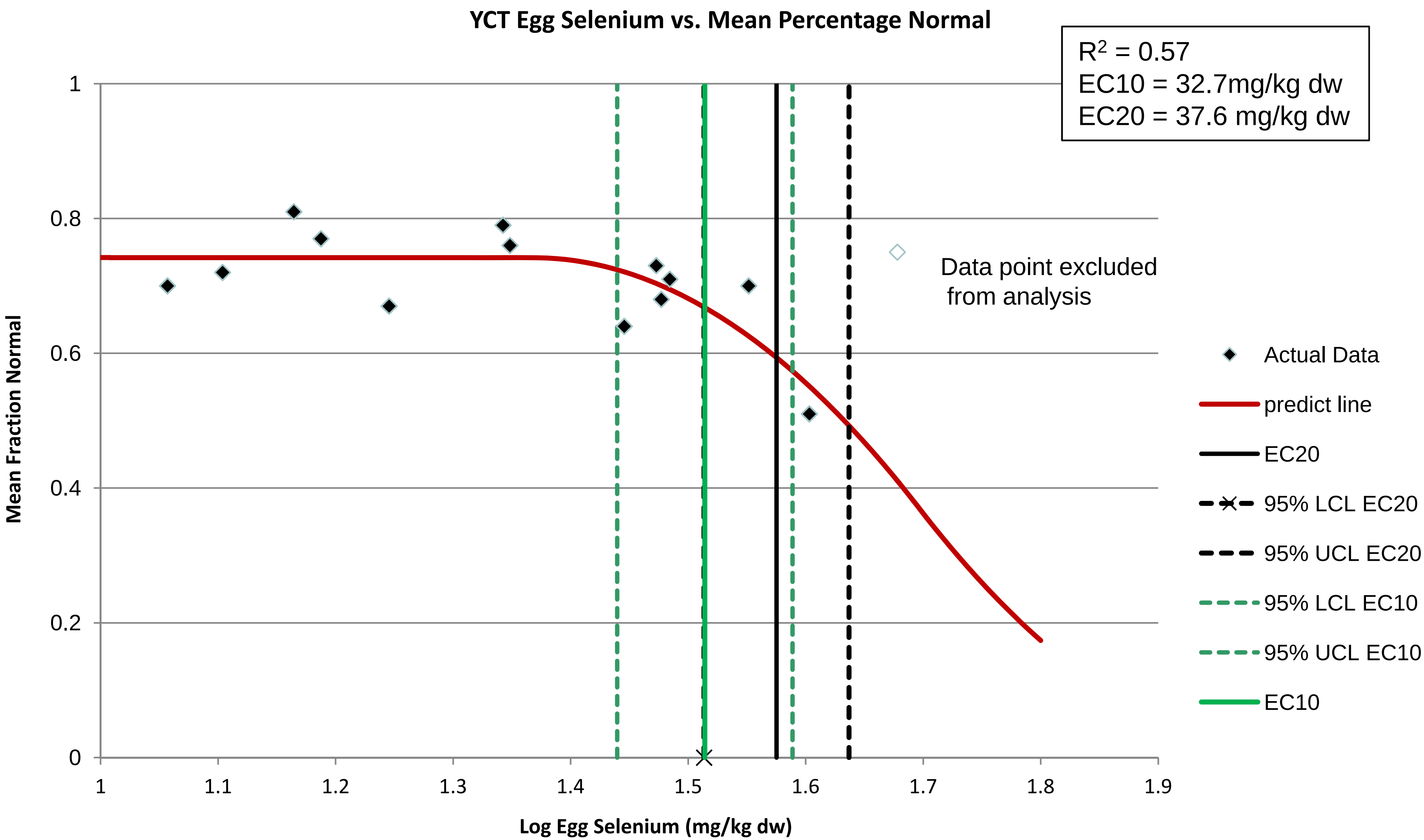

Figure 5-29
Logistic Regression for Yellowstone Cutthroat Trout Egg Selenium Concentrations Versus Mean Fraction Normal Larvae

J.R. Simplot Company Site-Specific Selenium Criterion	
PRJ: 0442-004-900.70	DATE: January 2012
REV: 1	BY: SMC CHK: SMC

FORMATION ENVIRONMENTAL

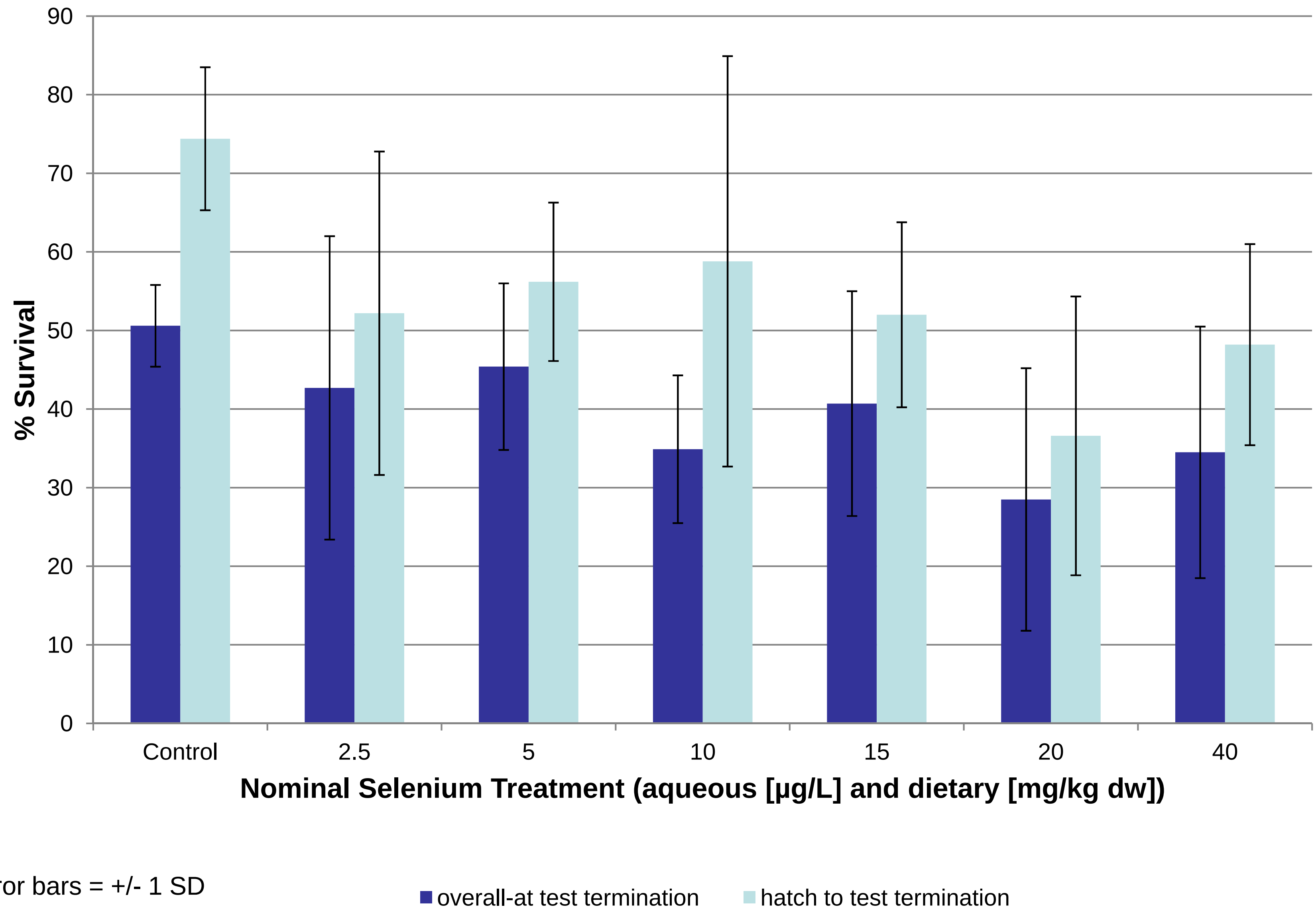

Figure 5-30
Percent Survival for Early Life Stage YCT Exposed to Different Levels of Aqueous and Dietary Selenium

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

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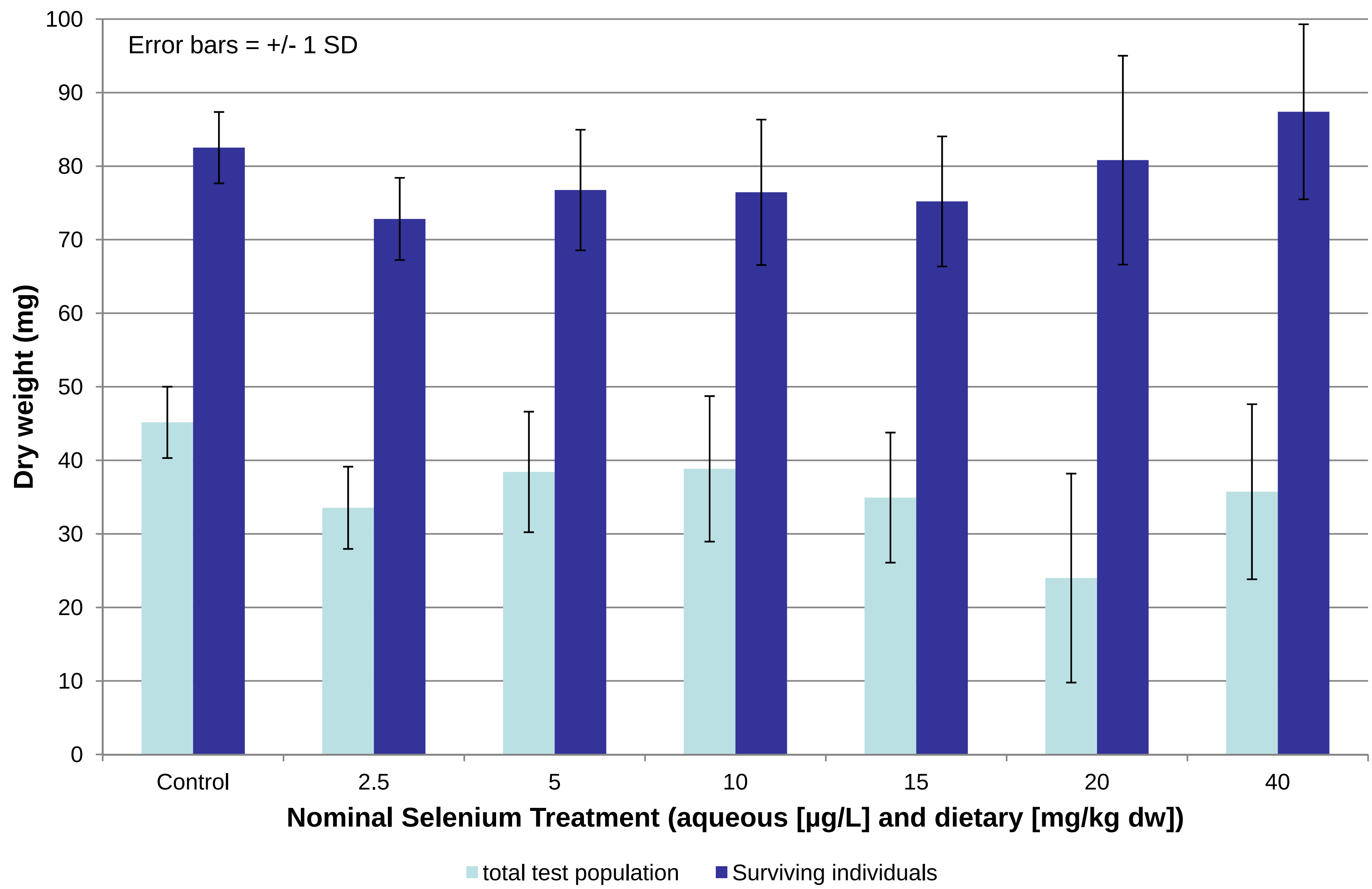

Figure 5-31
Growth as Measured by Weight for Early Life Stage YCT Exposed to Different Levels of Aqueous and Dietary Selenium

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

FORMATION ENVIRONMENTAL

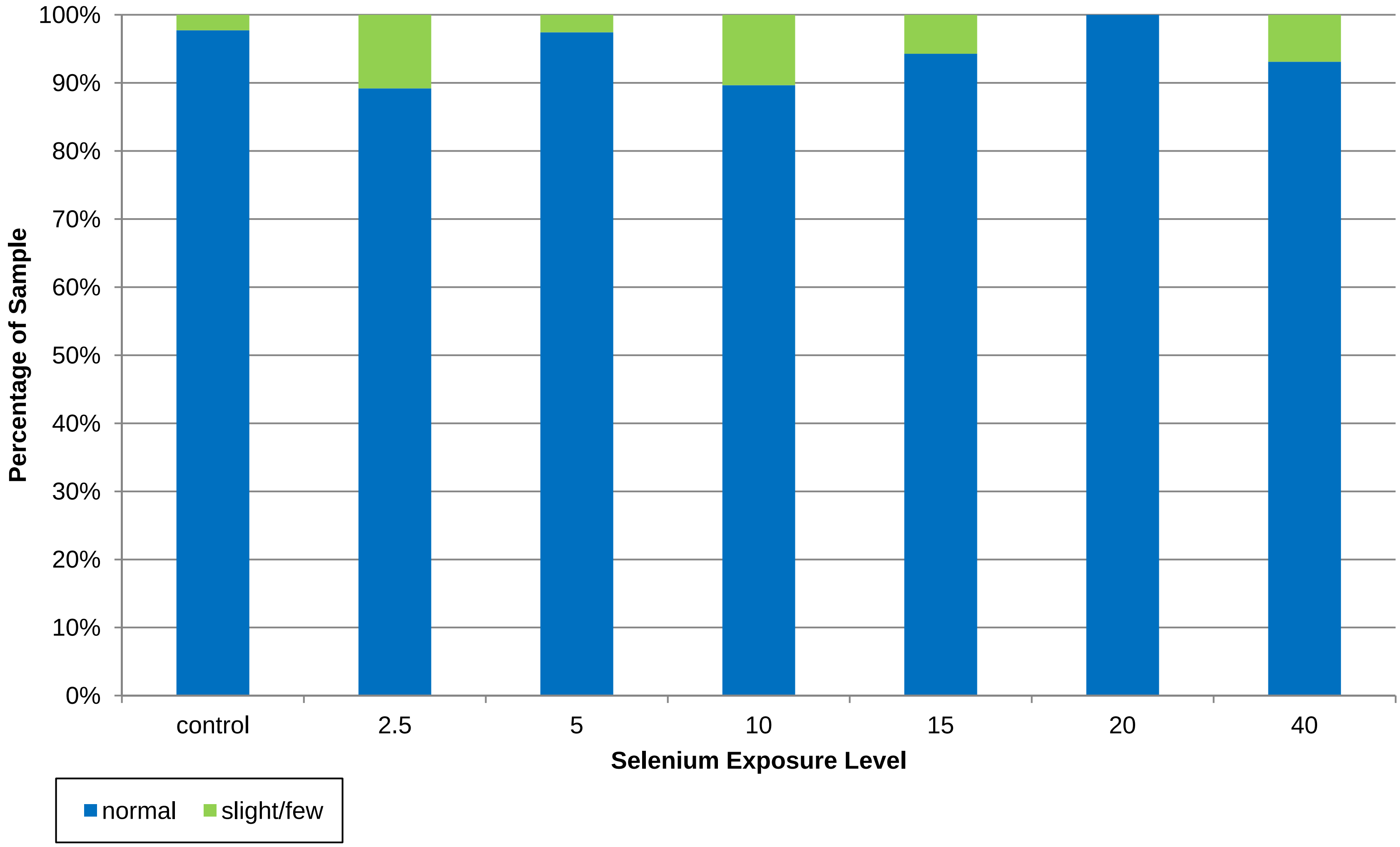

Figure 5-32
Selenium Exposure Levels vs. Average Percentage of Samples with Craniofacial Deformities in Early Life Stage Yellowstone Cutthroat Trout

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

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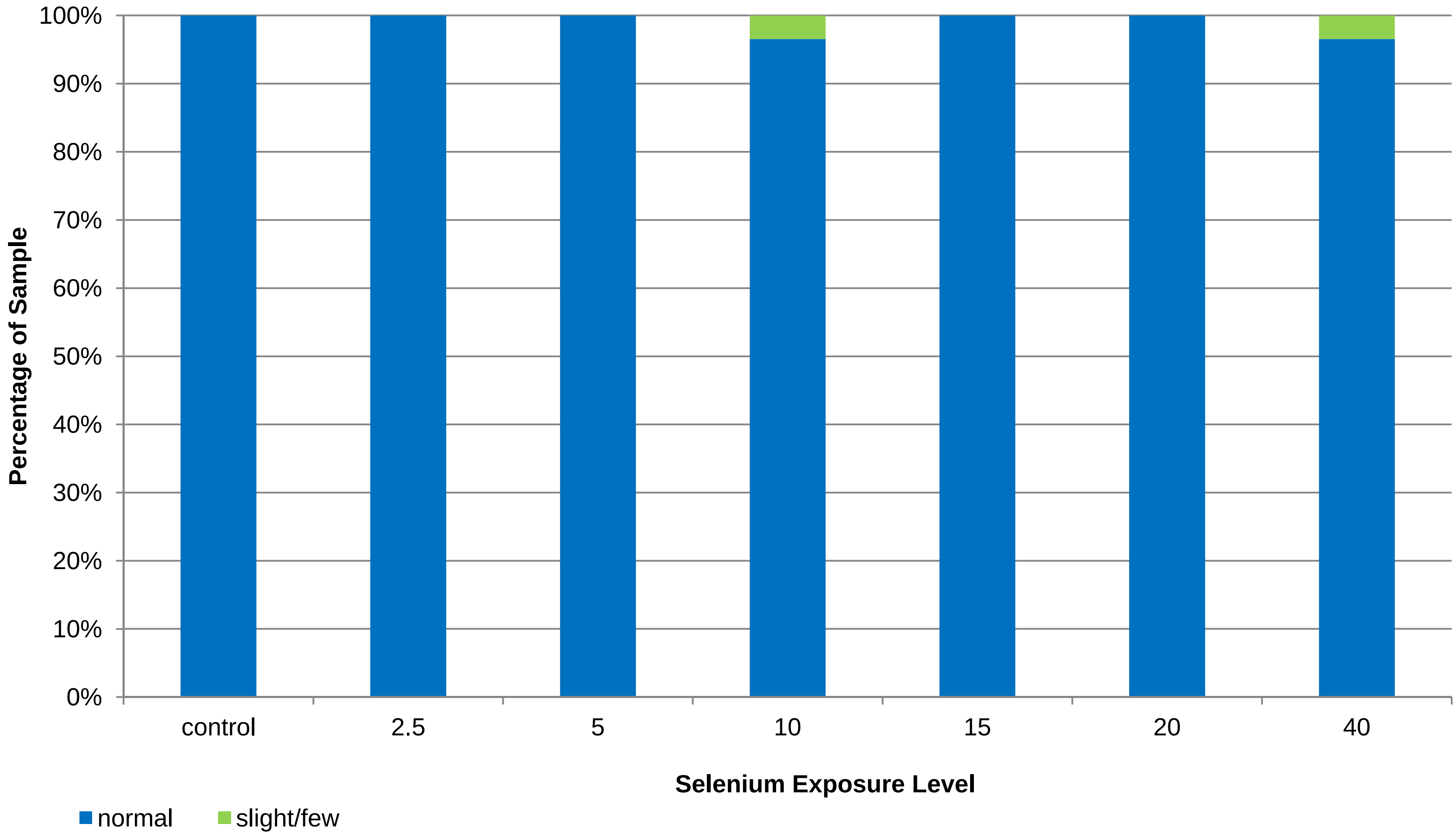

Figure 5-33
Selenium Exposure Levels vs. Percentage of Samples with Skeletal Deformities in Early Life Stage Yellowstone Cutthroat Trout

J.R. Simplot Company Site-Specific Selenium Criterion		
PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

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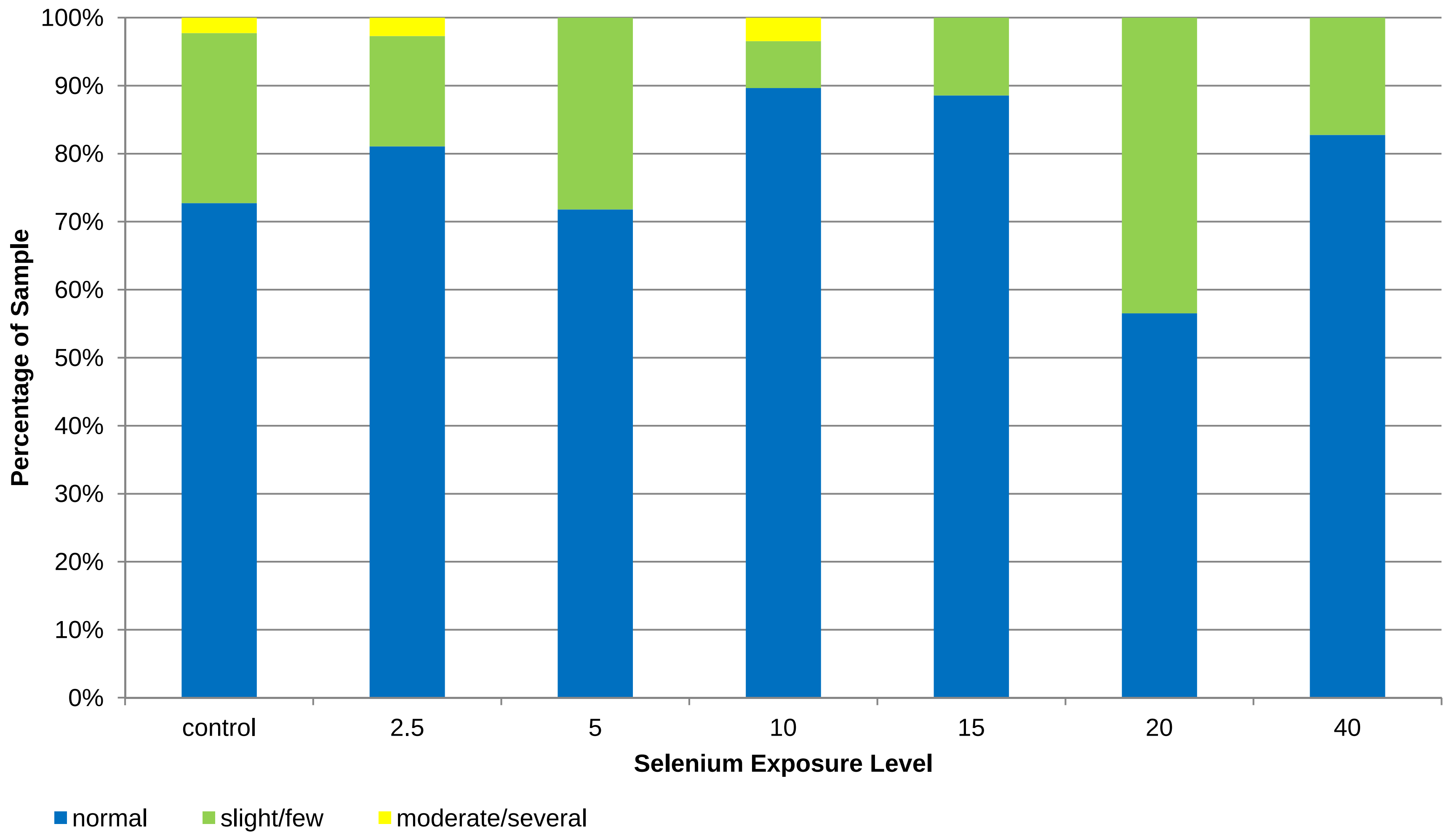

Figure 5-34
Selenium Exposure Levels vs. Percentage of Samples with Finfold Deformities in Early Life Stage Yellowstone Cutthroat Trout

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

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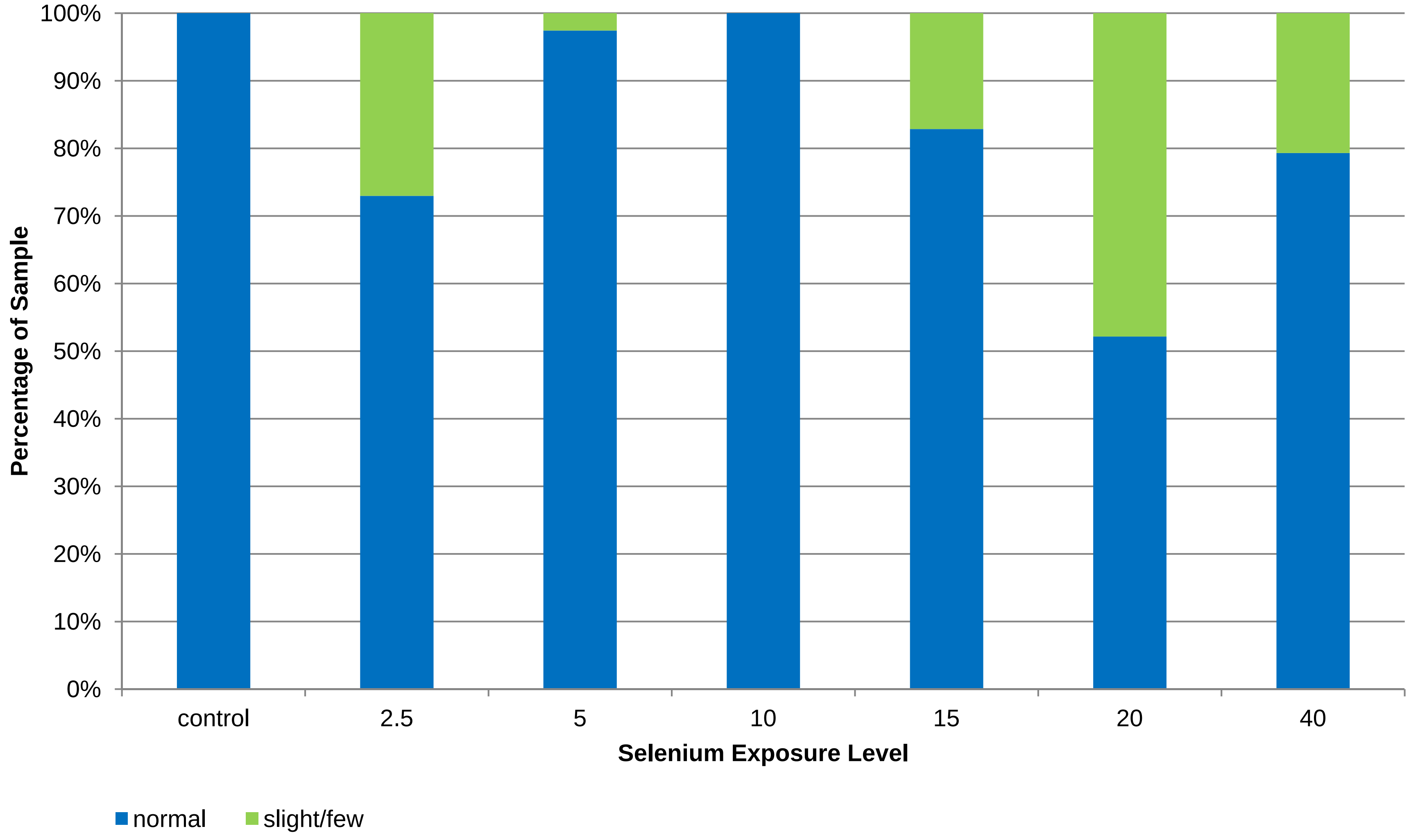

Figure 5-35
Selenium Exposure Levels vs. Percentage of Samples with Edema Deformities in Early Life Stage Yellowstone Cutthroat Trout

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

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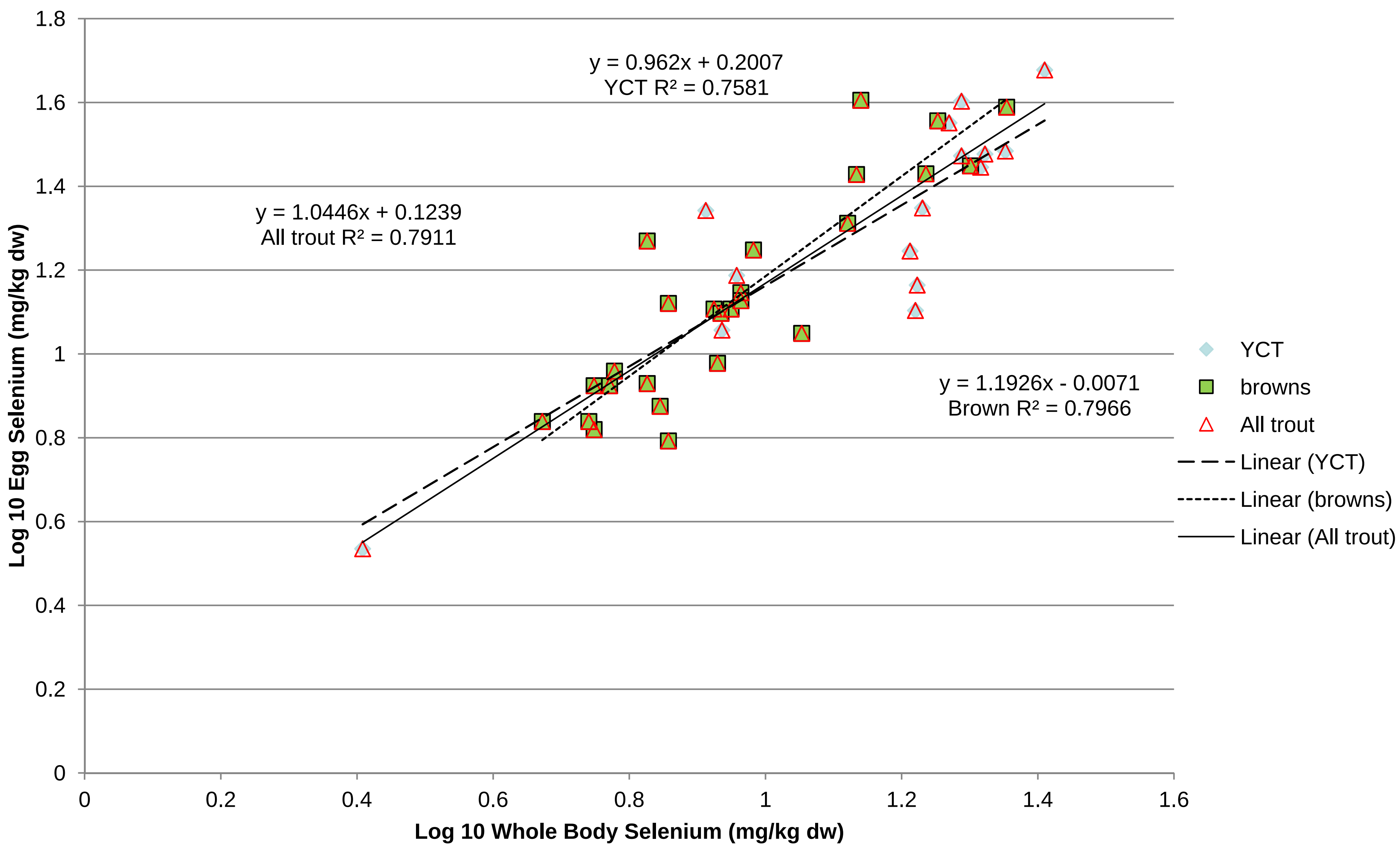

Figure 5-36
Relationship of Selenium Concentrations in Parental Whole Body Tissues of Brown Trout and YCT to Egg Selenium Concentrations

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012
REV: 1	BY: SMC / CHK: SMC

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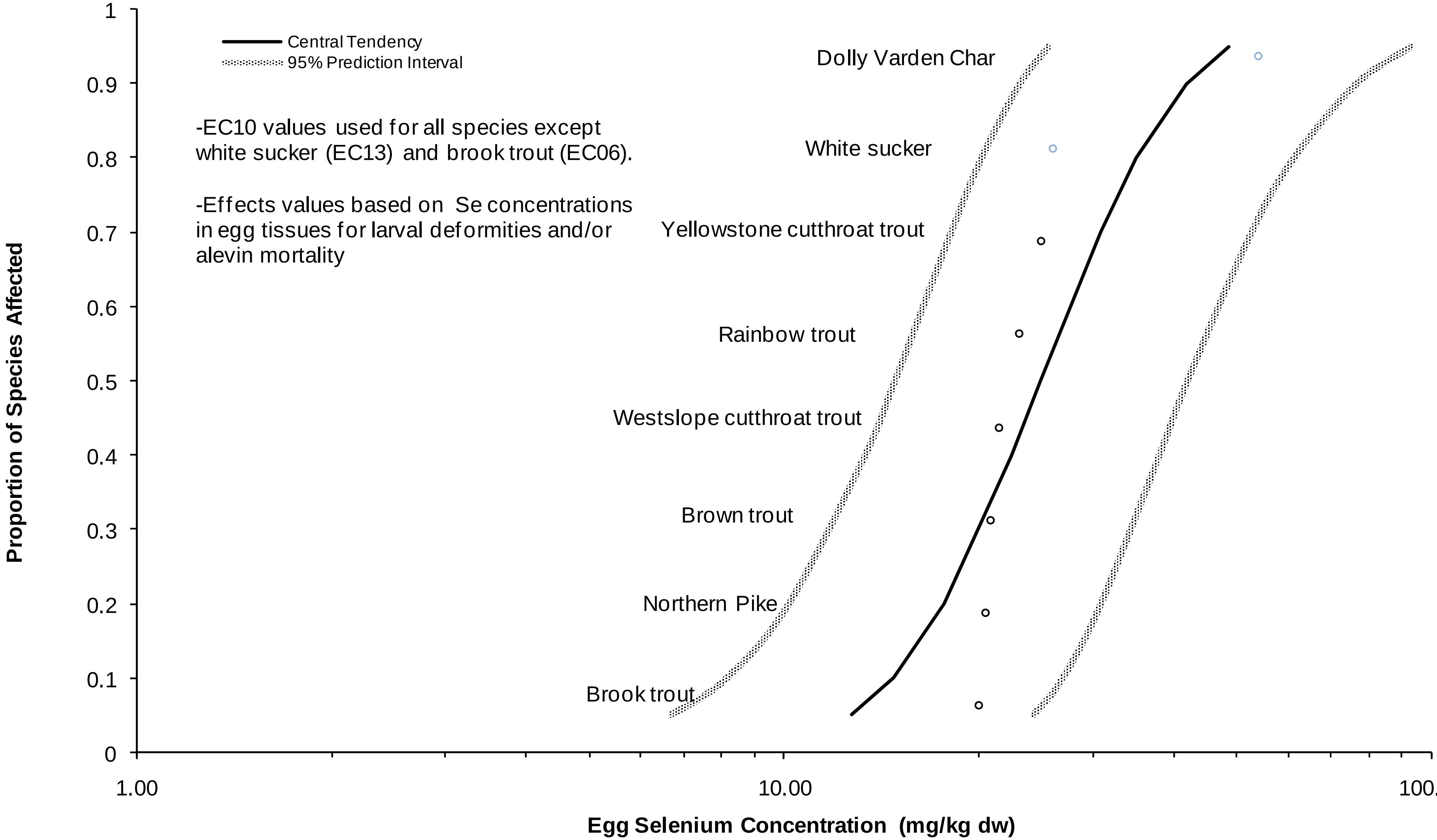

Figure 6-1
Species Sensitivity Distribution for Cold and Cool Water Species Effects Data Derived from Maternal Transfer Studies

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012
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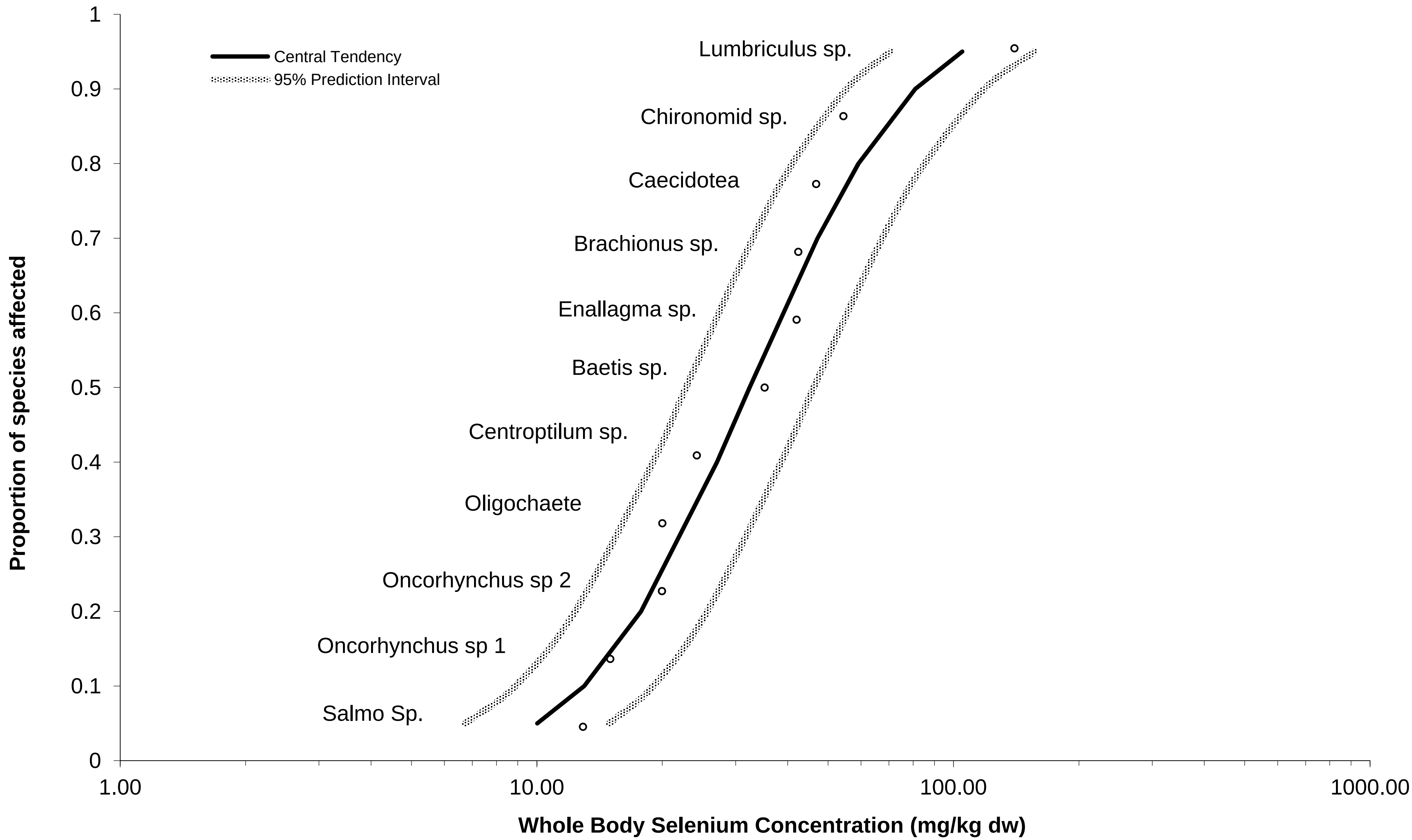

Figure 6-2
Species Sensitivity Distribution of a Representative Cold Water Aquatic Community to Selenium

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
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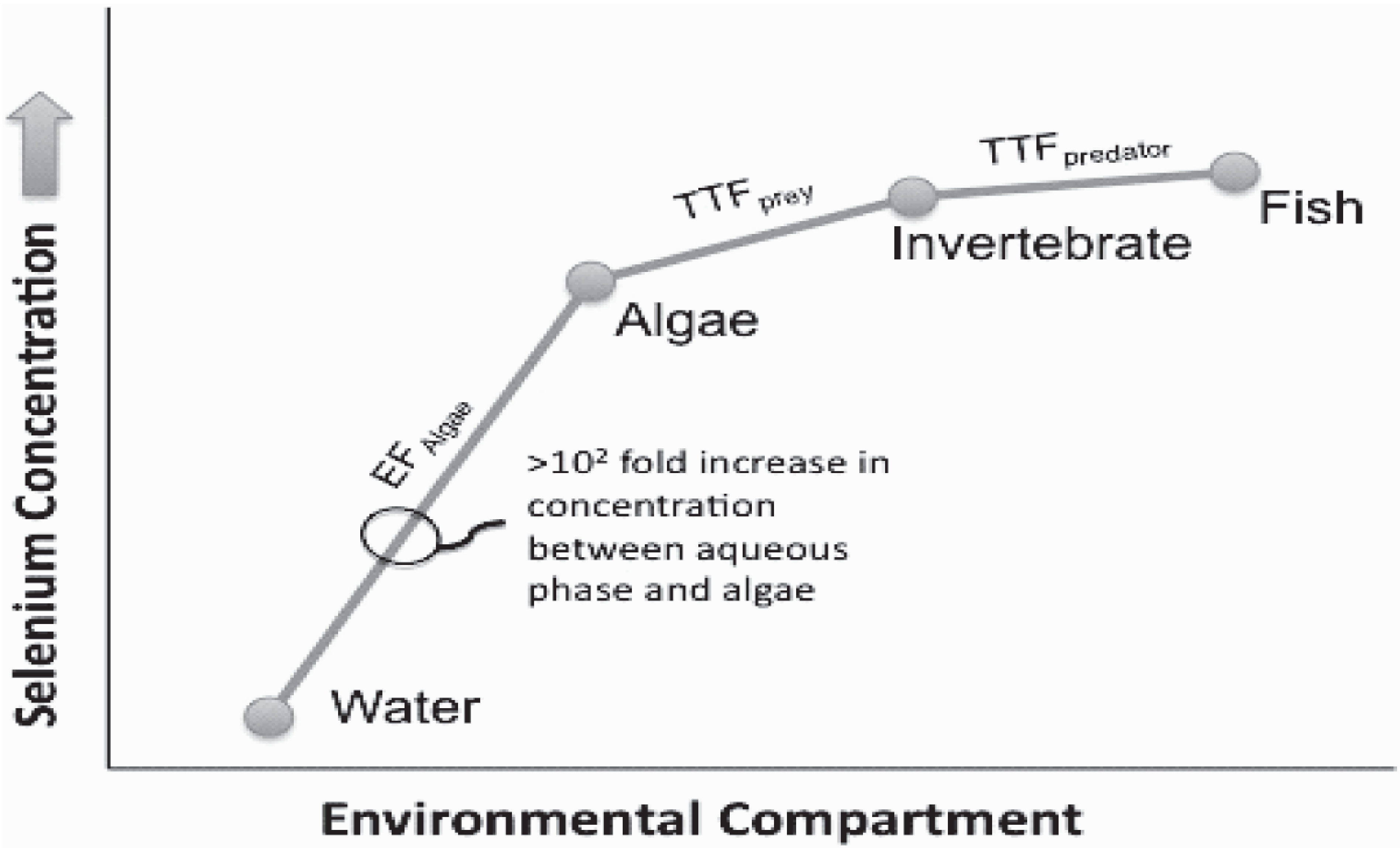

Figure 6-3
Diagram of Selenium Bioaccumulation in a General Aquatic Food Web

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
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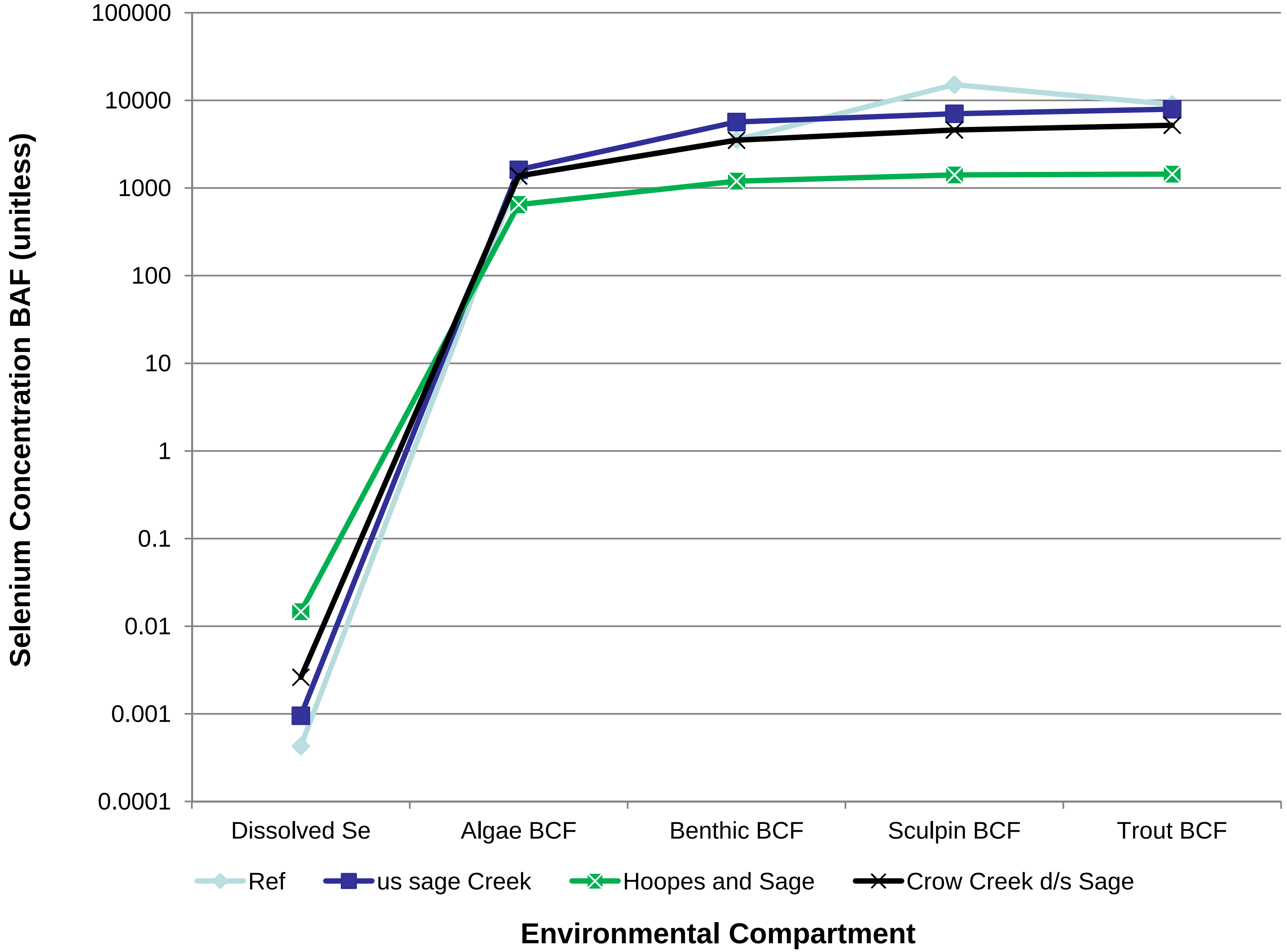

Figure 6-4
Selenium Bioaccumulation in the Crow Creek Drainage Based on Site-Specific Data

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
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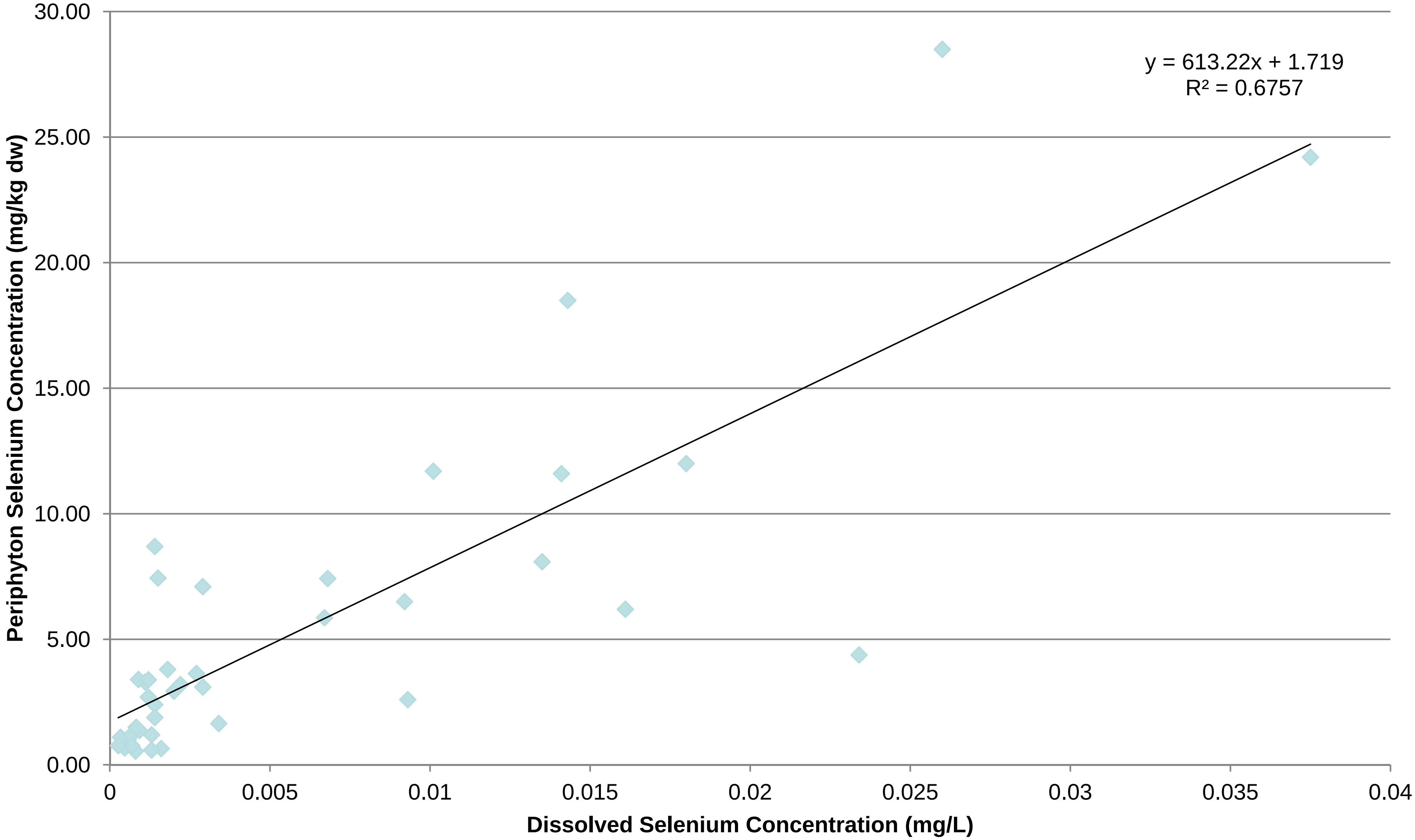

Figure 6-5
Relationship of Aqueous Selenium to Periphyton Selenium Concentrations

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

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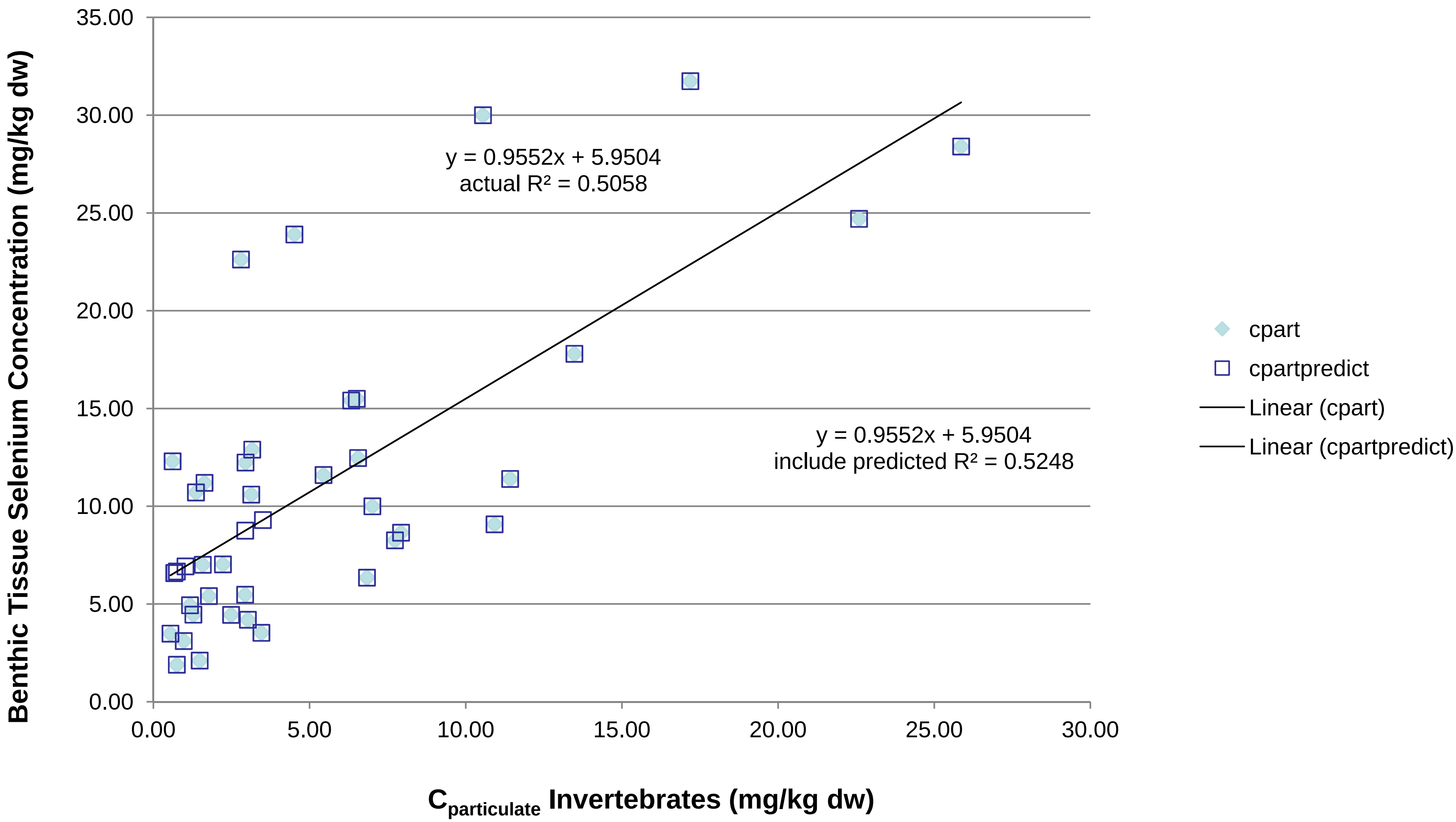

Figure 6-6
Relationship of $C_{particulate}$ Invertebrates (Periphyton and Sediment) to Benthic Invertebrate Tissue Selenium Concentrations

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012
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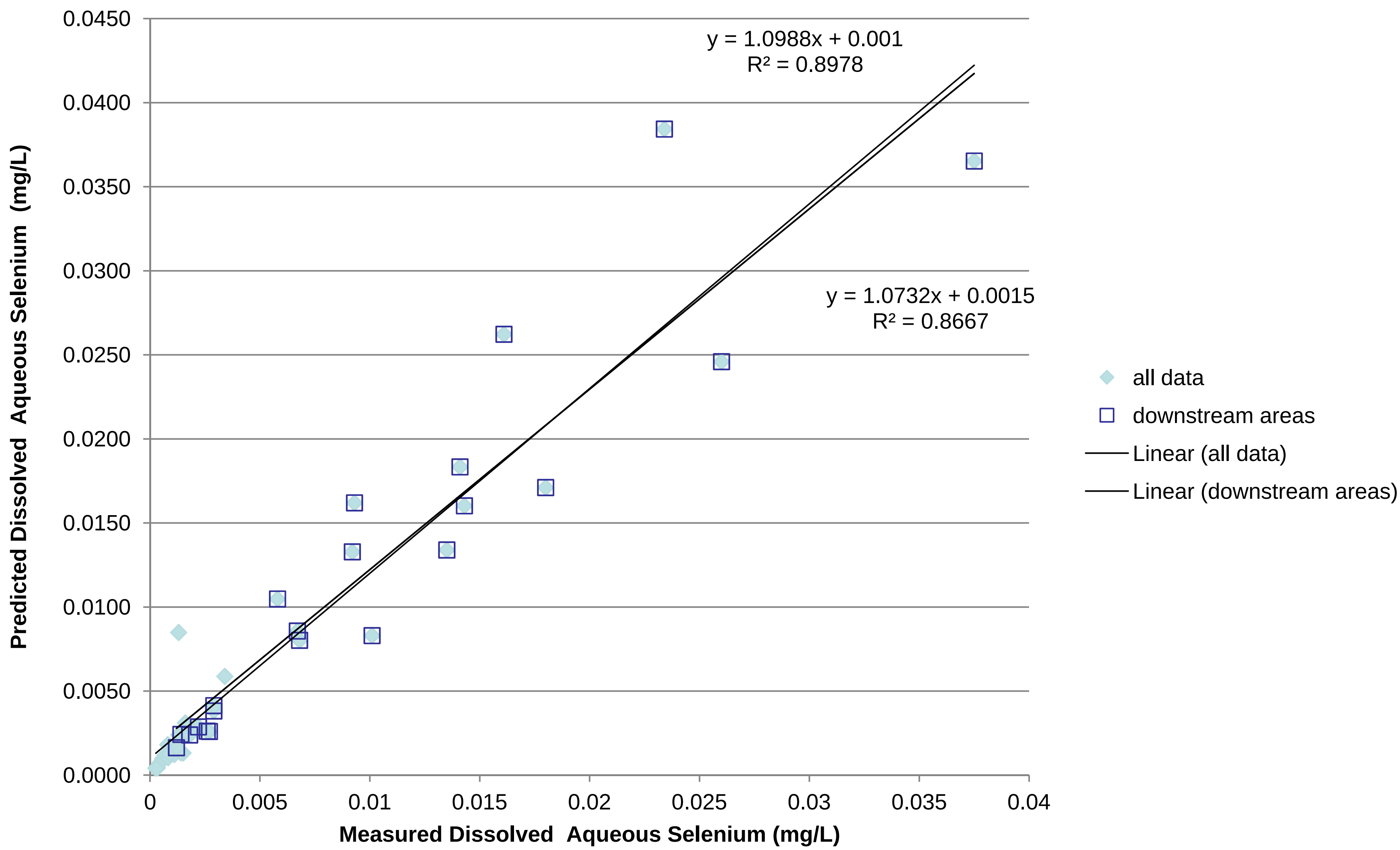

Figure 6-7
Relationship of Measured Dissolved Aqueous Selenium to Aqueous Selenium Predicted using the Presser and Luoma (2010) Trophic Model

J.R. Simplot Company Site-Specific Selenium Criterion	
PRJ: 0442-004-900.70	DATE: January 2012
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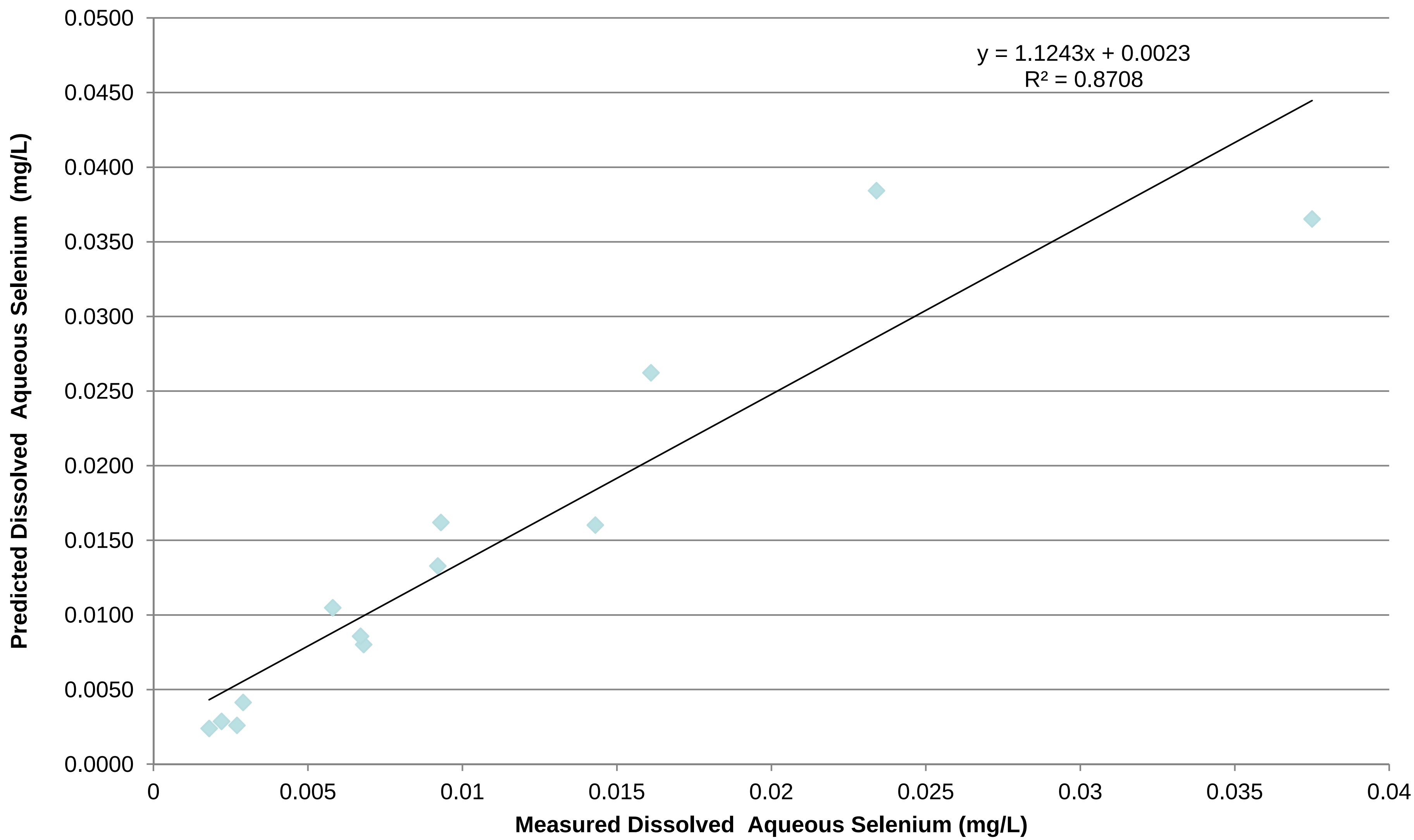

Figure 6-8
Relationship of Measured Dissolved Aqueous Selenium to Aqueous Selenium Predicted using the Presser and Luoma (2010) Trophic Model using only Fall Data from the Downstream Locations

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012
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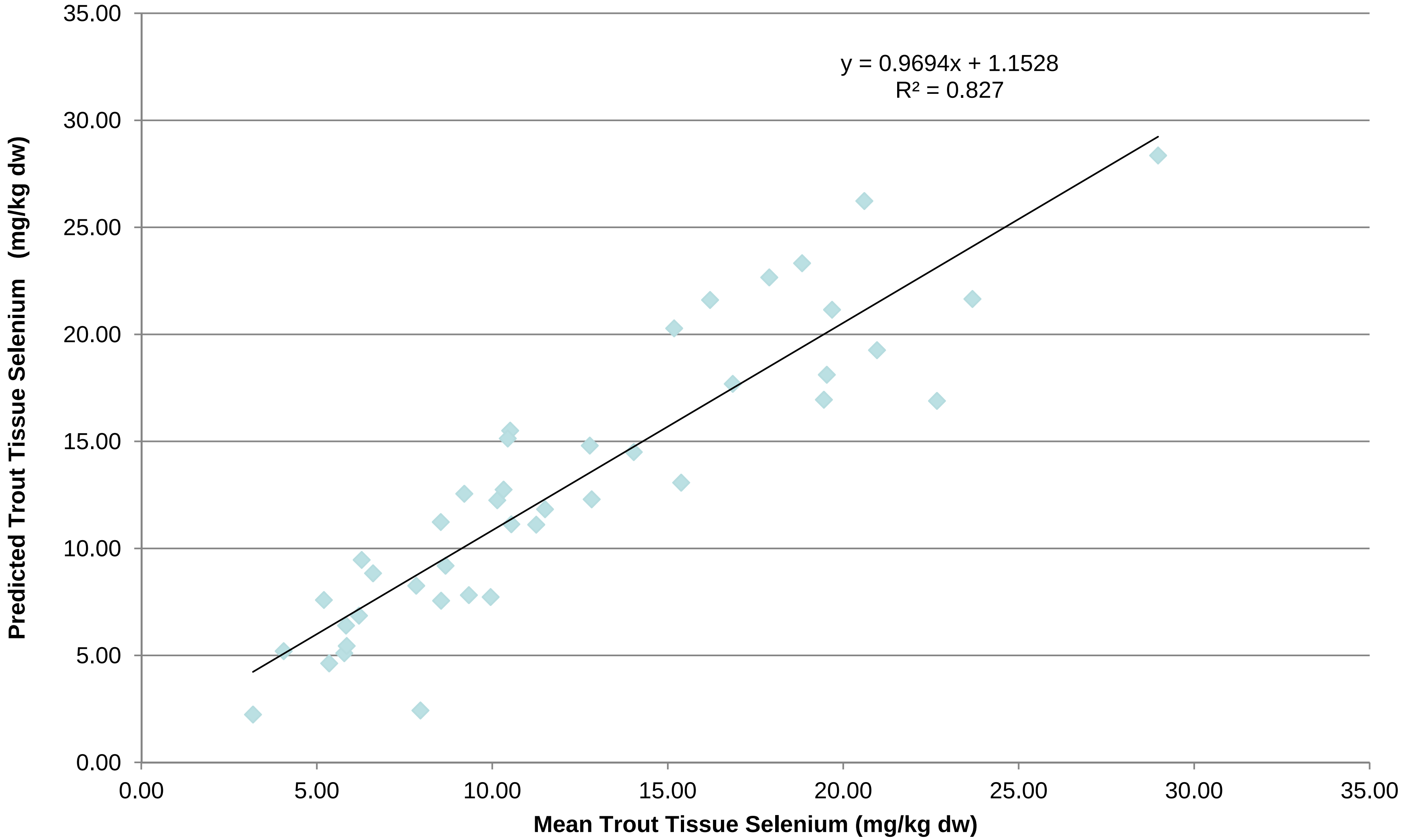

Figure 6-9
Relationship of Mean Selenium in Whole Body Trout Tissues to Selenium in Trout Tissues Predicted Using the Presser and Luoma (2010) Trophic Model

J.R. Simplot Company
Site-Specific Selenium Criterion

PRJ: 0442-004-900.70	DATE: January 2012	
REV: 1	BY: SMC	CHK: SMC

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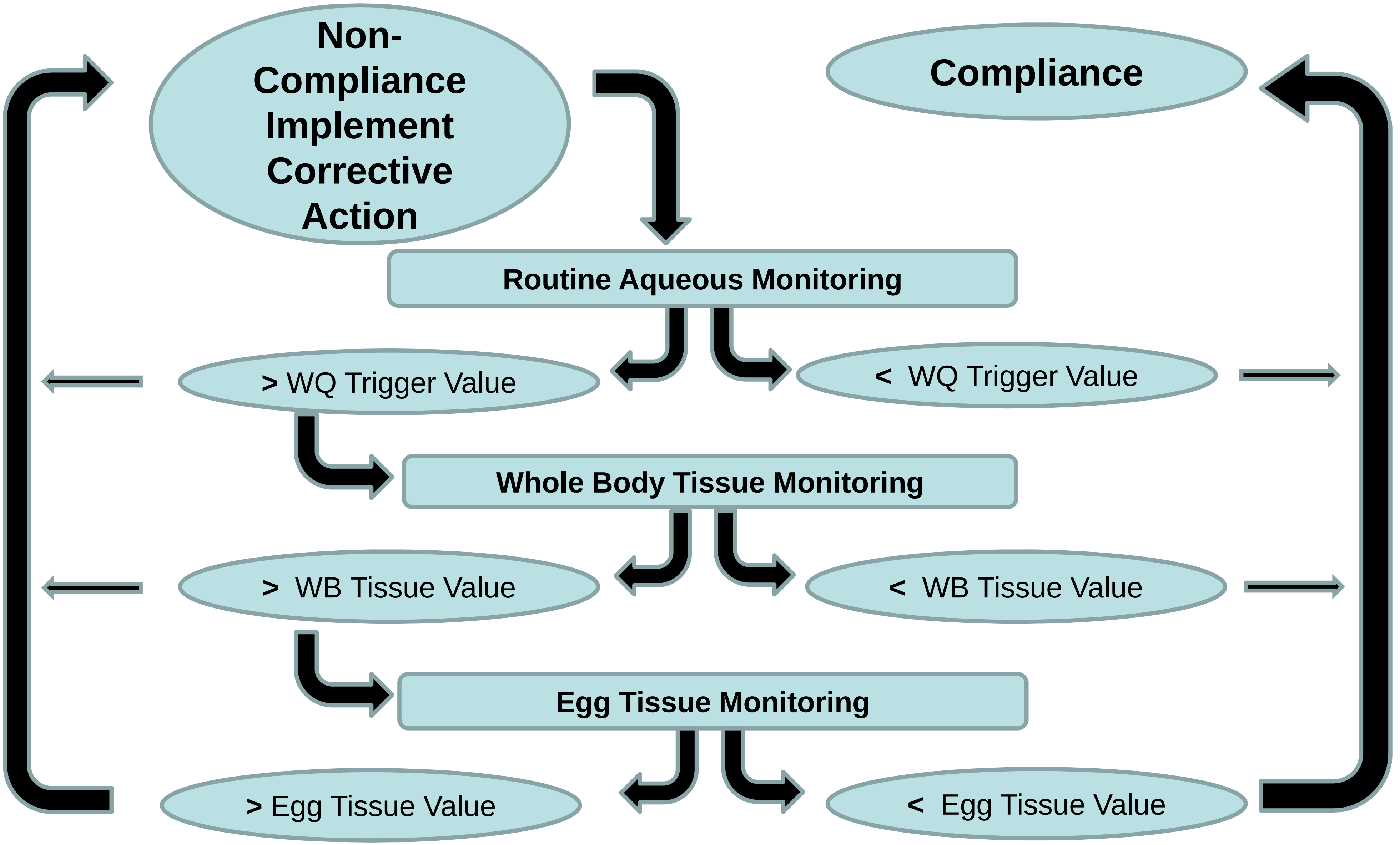

Figure 7-1
Flow Diagram for Implementing the Site-Specific Selenium Criterion for the Site

J.R. Simplot Company Site-Specific Selenium Criterion	
PRJ: 0442-004-900.70	DATE: January 2012
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FORMATION ENVIRONMENTAL	