Appendix F -
Yellowstone Cutthroat Trout
Early Life Stage Laboratory Studies

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

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1.0 INTRODUCTION

A chronic site-specific selenium criterion (SSSC) is being developed 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). In accordance with the Administrative Order on Consent (AOC) entered into by Simplot, Idaho Department of Environmental Quality (IDEQ), the US Forest Service (USFS), and the US Environmental Protection Agency (USEPA), a Site Investigation (SI) was conducted at the mine site in 2003 and 2004. Investigations to date 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. Selenium released from overburden disposal areas (ODAs) at the mine has the potential to migrate vertically downward into the Wells Formation aquifer. Groundwater from the Wells Formation aquifer discharges at Hoopes Spring and South Fork Sage Creek Springs.

Laboratory studies have been conducted as part of developing the data necessary to define effects to trout species found at the Site. The first study examined the reproductive success of maternal brown trout (*Salmo trutta)* from ambient exposure to selenium in their diet and aqueous environment. Results of the brown trout study are reported in the June 2011 *Final Brown Trout Report Laboratory Reproduction Studies Conducted in Support of Development of a Site-Specific Selenium Criterion* (Formation 2011a). A similar study was conducted for Yellowstone cutthroat trout (YCT) (*Oncorhynchus clarki*) and these results are reported in the Draft *Yellowstone Cutthroat Trout Adult Reproduction Studies for Developing a Site-Specific Selenium Criterion* (Formation 2011b).

A third study was identified in the Laboratory Studies Work Plan (NewFields 2008) that was designed to assess potential effects of selenium accumulated in tissues of naïve YCT eggs. This third study using YCT early life stages (ELS) examines the effects of aqueous and dietary selenium concentration effects on the egg through post-hatch life stage of YCT that were not previously exposed via maternal transfer. The ELS laboratory study was developed to complement information available from previous laboratory studies for the Site, literature, and the extensive field monitoring program for the study area.

1.1 Background

Studies conducted prior to this ELS study evaluated the success of young fish spawned from parents (in particular maternal parent) with previous exposure to different ranges of selenium in the environment. The field studies conducted prior to these laboratory studies indicated that

Sage Creek downstream of Hoopes Spring regularly exceeds the water quality standard for selenium and that the frequency and magnitude of the exceedences decline downstream with tributary inflows. Only infrequent exceedences have been observed in Crow Creek immediately downstream of Sage Creek. Both studies found some level of effect on survival and deformities in young fish, although brown trout have been found through comparison to be more sensitive to selenium effects than YCT.

Early planning for the ELS study using YCT was designed initially to utilize eggs from pre-exposed maternal females, reared in varying aqueous selenium treatments. At swim up, alevins would be fed varying levels of bioaccumulated selenium in live *Lumbriculus*. Following the YCT adult studies, this approach was abandoned due to the difficulty of obtaining adequate numbers of wild-collected females from which to extract eggs. Instead, the approach used eggs from the Henry's Lake Fish Hatchery run of YCT. Prior maternal exposure was thus eliminated using the later approach and reproductive success was eliminated as an endpoint in this study. Despite the loss of the reproductive element of this study, using eyed eggs as a starting point for exposure still encompasses a sensitive life stage where survival, growth, and development can be evaluated.

1.2 Objectives

The ELS toxicity testing studies evaluated the concentrations of selenium in diet and aqueous exposures that may adversely affect young developing trout. The reproduction studies identified above were terminated at 15 days post-swim-up to evaluate the transition from endogenous to exogenous feeding. The ELS studies extend into the post swim-up stage to 30 days post hatch. The objective of this testing approach is to evaluate the combined effects of dietary and aqueous exposure concentrations of selenium that affect survival, growth, and deformities of young trout with no maternal exposure. Additional objectives of the testing presented herein are as follows:

- Document the range of aqueous selenium concentrations that may affect young developing fish;
- Document the range of selenium concentrations in dietary media that may affect young developing fish; and
- Assess if differences exist in similar endpoints of survival, growth and deformities between maternally exposed young and those that had no maternal exposure.

2.0 METHODS

The methods for testing YCT ELSs, including the study design plan and analysis details for the assessment of selenium exposure, were presented in a SSSC Workgroup–reviewed *Revised Draft Work Plan – Laboratory Toxicity Tests for Developing a Site-Specific Selenium Threshold for Trout* (May 2008) (NewFields 2008).

Laboratory portions of this testing were carried out at ENSR|AECOM's environmental toxicology laboratory in Ft. Collins, Colorado by Dr. Rami Naddy. The deformities assessment was performed by Dr. Kevin Bestgen at Colorado State University's Larval Fish Laboratory. Columbia Analytical Services (CAS) (Kelso, Washington) conducted the analytical chemistry for selenium concentrations in tissues. Details of the exposure system are described in Appendix A of this document, and are briefly described below.

2.1 Test Organisms

Gametes were obtained from Henry's Lake Fish Hatchery, Henry's Lake, ID (courtesy of Damon Keen, Idaho Fish and Game). 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 setup near the lake outlet to the river and pre-spawn trout are captured as a hatchery source from this location for other areas. Eyed eggs, which were obtained late in the spawning season, were shipped via overnight courier and delivered at ENSR|AECOM's laboratory. on 14 May 2008. Eggs from Henry's Lake fish, with limited pre-parental exposure, were used for controls and treatments.

2.2 Test Setup and Treatments

Viable eyed eggs were impartially distributed among the 35 egg cups (5 replicates and 6 treatments plus control) by adding no more than five eggs at a time into each egg cup and repeating the process until each cup contained the required number. Egg cups were then placed in their randomly assigned test chambers. Twenty eyed eggs were placed in replicates A through D for each treatment, while 34 eggs were placed in each E replicate to maximize the available organisms for whole body selenium analysis.

2.2.1 Aqueous Exposure

Six treatments of aqueous and dietary selenium exposure were used. Aqueous selenium exposures targeted nominal concentrations ranging from 40, 20, 15, 10, 5, 2.5 and control for water (µg/L). Selenium was introduced to the aqueous system as sodium selenate, which

resulted in a predominantly selenate exposure, the form which is predominant in Site streams. Aqueous exposures began at hatch and continued through the end of the test.

2.2.2 Dietary Exposure

Dietary exposure to young trout was from bioaccumulated selenium in invertebrate (*Lumbriculus variegatus*) feedstocks. *Lumbriculus* were fed selenized yeast similar to the methods developed by Besser et al. (2006) and McIntyre et al. (2008). Aqueous selenium exposure for *Lumbriculus* was in the form of sodium selenate, similar to the aqueous exposure for YCT. Concentrations of selenium introduced to the feedstock were similar to the nominal concentrations described above for the aqueous exposure treatments. These concentrations bracket the selenium residues in periphyton and benthic invertebrate tissues measured to date from field monitoring at various locations. Exposed feedstock was then fed live to young trout. See Appendix A for exposure details related to *Lumbriculus* bioaccumulation.

At swim-up, the period when young fish begin to feed, dietary exposures were slated to begin. The original plan was to start feeding the larval YCT the live, dosed *Lumbriculus* diet at swim-up (Day 22); however, after several feeding attempts it was determined that the fish would not eat the *Lumbriculus*, presumably because the worms were too large. The start of the dietary study was delayed to allow the larval fish to grow so they could consume the *Lumbriculus*. In the interim (Days 16-17), two different food types (freshly hatched brine shrimp nauplii and salmon starter #1) were offered to the larval fish with the goal of enticing the young fish to eat live food. The diet of choice was the brine shrimp nauplii.

The brine shrimp diet was offered to the swim-up fry once each day starting on Day 18 for two days and increasing to three times a day (i.e., morning, noon, evening) until initiation of the dietary study. The volume typically consisted of either 0.75 ml or 1.0 ml three times per day, which amounted to 55.2 – 73.5 mg per chamber per day. Given the estimated weight of 0.119 g, based on a subsample of the test organisms from the E reps in all treatments (measured at thinning on June 10, 2008), this translated to a 3.4 to 4.6 percent food ration per body weight (wet) of the fish until fish were fed the live *Lumbriculus* diet. The brine shrimp was analyzed for contaminants by lot; the results of the contaminant analysis are on file at ENSR.

The weight of an adult *Lumbriculus* was estimated at 5-12 mg each (ASTM 2006). Given that the plan was to feed fish at a 4 percent body weight ration, (i.e., 4.8 mg per fish), the initial feeding rate was calculated at one worm per every two fish (wet weight of fish = 0.119 mg). By Day 38, trial studies indicated that most fish were large enough to eat the *Lumbriculus* and; therefore, the dietary study was initiated (June 21, 2008). The dietary study used bioaccumulated selenium in *Lumbriculus* for approximately 32 days.

2.3 Test Monitoring

After the hatching phase, alevins (recently hatched young with yolk sacs) were monitored daily for mortality. This daily monitoring for mortality continued throughout the study. Dead organisms were removed and placed in Davidson's solution. As alevins approached swim-up, food was offered to the organisms to determine if they were actively feeding. The swim-up date was set based on absorption of the yolk sac and active feeding by at least 70 percent of the alevins. After the swim-up stage and before the dietary study had begun, a subset of fish (16) from the E replicates were sacrificed for measurement of whole-body selenium concentrations, dry weight (via freeze drying), and percent solids at Columbia Analytical Laboratories (CAS; Kelso, WA). One additional fish from each replicate was also sacrificed for wet weights to estimate feeding rates.

At the end of the dietary study (Day 71), five randomly selected organisms from each treatment were obtained for whole-body analysis of selenium (analysis was performed on individual fish). Fish were sacrificed using isopropyl alcohol, rinsed with deionized water, blotted dry, and then measured for standard lengths (tip of snout to caudal peduncle) and wet weights. These organisms were then frozen and sent via overnight courier to CAS for measurement of whole body selenium concentration, dry weight, and percent solids.

Remaining fish were sacrificed via immersion in isopropyl alcohol, rinsed with deionized water, blotted dry and measured for standard length. Wet weights were taken on a subset of organisms. Organisms were then preserved in Davidson's solution and saved for deformities assessment by CSU. After deformities assessment was performed on all these organisms, they were returned to the ENSR|AECOM's environmental toxicology laboratory and then measured for dry weight. For dry weight analysis, each fish was transferred to a tared weight boat and dried at 100 °C for at least 48 hours. After removal from the drying oven, the weight boats were placed in a dessicator to prevent absorption of moisture from the air, until weighed (dry weight) to the nearest 0.01 mg.

2.4 Deformity Assessment

Dr. Kevin Bestgen at Colorado State University's Larval Fish Laboratory was contracted to conduct the deformity assessment. Dr. Bestgen developed a process for assessing deformities which gave specific measurements to each ranking, thereby allowing for some measure of repeatability and accuracy. He received samples essentially as blinds because he did not know what the sample locations were or their locations relative to selenium concentrations.

The 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. The original publication showed pictures of some deformities but

others, particularly the intermediate categories, were not illustrated or were poorly described. More specific definitions for each of the assessment categories were developed to give better repeatability and consistency across studies, and to aid others in learning the range of deformities possible.

Deformities in each of the categories described above were given a score from 0-3, with 0 being a normal condition and 3 being the most deformed. Some range finding was conducted over the first several samples to find background and severe levels of deformities in each category. Initial samples were rescored as necessary to bring them into compliance with the standards that were used throughout the assessment.

The protocol for assessing damage was to place several fish, head to the left, in a Petri dish and examine them under a dissecting microscope and 10X magnification. The lateral side was examined for spinal deformities (lordosis, kyphosis), appearance of the eye, head and snout shape, edema, and fin deformities. The fish was turned ventrally to look for mouth deformities and further spinal deformities (scoliosis), turned laterally again for the same criteria as the other side, and then dorsally for issues associated with eyes, head size, spinal deformities.

Craniofacial deformities included shortening of the jaw, snout, and missing or poorly developed eye or eyes, and head shape abnormalities. A slightly shortened lower jaw (<= 1 lip width) received a 1, a shortened jaw = 2 lip widths or a slightly shortened and slightly disfigured jaw = 2, and a flat lower jaw or much disfigured (non-functional) jaw = 3. An assessment of fish independent of this study revealed that other brown trout of the same size and developmental state did not have the slight deformity that was assessed as CF =1 for the jaw (J). Thus, the CF = 1 score where the J was concerned were deemed real. A slightly blunted snout (about 50 percent eye diameter, usually is > than that) = 1, very blunt or flat = 2, deformed or bulbous = 3. Eye deformities were scored as one eye blind or poorly pigmented or poorly developed =1, both poorly developed = 2, both blind = 3. Skulls that were slightly bulbous (1/3 > normal) = 1, moderately bulbous (2/3 > normal) = 2, and bulbous (1x or > than normal) = 3.

Skeletal deformities included any deformity of the vertebrae or spines. A slight bend of less than 45 degrees (but > than body width off of straight) or a minor body constriction (e.g., a tight rubber band about the body effect) was given a score of 1, 2 slight bends or constrictions anywhere, or bend of > 45-90 degrees was scored a 2, and multi-directional bends > 90 degrees were given a 3.

Fin deformities included variation in fin or finfold morphology and a slightly smaller or missing fin (in thin fish, the adipose fin was often absent, indicating fat absorption, not uncommon and scored 1) or one with a bend or incomplete ray development (in older fish) was given a 1, 2 fins damaged or malformed = 2, and > 2 fins malformed or if fins were missing (except adipose) was = 3.

Edema was detected by an obvious swelling and fluid buildup, usually abdominally, and ventrally, which often displaced the gut, and was usually clear fluid that was slightly soft when touched with a blunt probe. Slight edema = 1 was for a fish with up to 1X swelling of the normal body width or depth, up to 2x = 2, and > 2x = 3.

2.4.1 Data Reduction of Deformity Rankings

Individual files, representing scoring sheets, were received for each sample evaluated. All files were combined in Excel to form a master file. Data were summarized using the Pivot table function in Excel to produce counts and percentages of normal fish, deformed fish, and total number of fish evaluated. Similar to the method of Holm et al. (2005), a Graduated Severity Index (GSI) was derived based on the deformity rankings and counts for progeny from each parent. A total score was computed as follows:

[(# fish for CF=1) x (1)] + [(# fish for CF=2) x (2)] + [(# fish for CF=3) x (3)].

This method differs slightly from Holm et al. (2005) as it weights each ranking with more weight given to more severe deformities. Fish scored as 0 (normal) observations did not enter into this calculation of total score. The final GSI score was computed as the sum score/total # fish including those ranked as "0". The total scores were summed and divided by the number of categories of deformities assessed (usually 4) to derive a mean GSI score.

Because the USEPA's Toxicity Relationship Analysis Program (TRAP) version 1.2 (Erickson 2008) logistic functions were designed to derive an inverse sigmoidal curve, commonly used to illustrate the dose-response curve of increasing exposure concentration and declining biological observation (e.g., survival, growth, etc.), deformities were evaluated as the sum fraction of normal fish (sum of normal fish/ total number of fish) for each deformity. This approach did not take into account severity of deformity, simply the frequency of normal fish relative to the total number of fish which is consistent with USEPA's (2004) approach to analysis of similar data. A percentage of normal fish (based on the total number) will be low if high numbers of fish present with some level of deformity, and conversely high if low numbers of fish present with some level of deformity.

2.5 Statistical Analysis

Multiple test-effects endpoints were measured at different times during the test including: hatching success, survival (different times during the study), tissue concentrations (whole body), and feeding success as measured by growth endpoints.

Scatter plots and best-fit ordinary least squares (OLS) regressions were used as an exploratory tool to evaluate the potential for meaningful relationships. Ordinary least squares regression

analysis was 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 measured in the study. If a relationship was found to exist between an exposure and effects measure that was meaningful (i.e., was logical with respect to either increase or decrease in effects relative to change in exposure), then the potential dose-response relationships for exposure and effects endpoints were evaluated further using USEPA regression-analysis software (TRAP version 1.2; Erickson 2008). USEPA's TRAP software provides a number of statistical analysis tools, including logistic, piece-wise linear and threshold sigmoidal regression analyses to evaluate the best model fit to the data distribution. Data transformations were also utilized to achieve the best model fit.

3.0 RESULTS

3.1 Aqueous Selenium Exposure

Samples were collected for total and dissolved selenium analyses on Days 1, 8, 15, 23, 29, 36, 43, 50, 57, and 64. Time weighted average concentrations for total recoverable and dissolved selenium concentrations are presented in Table 1. Measured total recoverable selenium concentrations were 101 to 112 percent of nominal concentrations, while measured dissolved values were 115 to 119 percent of nominal values (Table 1). This indicates that measured values were very similar to target aqueous concentrations. Dissolved values ranged from 107 to 116 percent of total recoverable selenium values, indicating strong agreement between dissolved and total recoverable values.

3.2 Dietary Selenium Exposure

Selenium concentrations were measured in yeast treatments and in *Lumbriculus.* The average (±SD) selenium concentration in yeast and *Lumbriculus* are presented in Table 2. Figure 2 shows the relationship of selenium concentrations in yeast and uptake in *Lumbriculus*.

Measured selenium concentrations were lower than target values in most of the yeast diets but very similar to target nominal values in *Lumbriculus*. Except for the highest treatment, the mean selenium concentration in *Lumbriculus* was within 2.5 mg/kg dw of the target nominal concentration. In the highest treatment (i.e., 40), the mean *Lumbriculus* concentration was 5.2 mg/kg dw higher than the target nominal concentration. A summary of the analytical measurements for these samples are included in Appendix A, including specific concentrations in *Lumbriculus* from small and large tanks. Nutritional content for *Lumbriculus* from each treatment is presented in Appendix A.

3.2.1 ELS Whole Body Selenium Concentrations

Whole body selenium concentrations in Henry's Lake YCT were measured in a subset of organisms on Day 27 (at thinning, prior to introduction of selenium diet), and at test termination. The results of these analyses are presented in Table 3. Selenium exposure measured at Day 27 resulted in a very low and flat response across all aqueous selenium treatments. Whole-body selenium in these treatments ranged from 1.47 to 1.84 µg/g dw. For fish measured at test termination, whole-body selenium concentrations in the control were similar to measurements at Day 27, but increased to an average of 34.48 mg/kg dw in the highest selenium treatment. From these results, it is clear that the body burden of selenium increased in YCT due to dietary selenium exposure from *Lumbriculus* (Figure 3).

3.3 Endpoints for Testing

3.3.1 Survival

Survival was determined based on the number of surviving fish at different periods in the test compared to the number of eggs at test initiation, including at hatch on Day 6, at swim up on Day 22, at thinning on Day 27, at commencement of the selenium diet on Day 38, and at test termination on Day 71 (Table 4).

Total survival (i.e., including hatch) of YCT at different stages of the study is presented in Table 4. Survival at hatch was lower than expected, ranging from 72.1 to 84.9 percent, with survival in all treatments being higher than survival in the controls. Survival at hatch may have been lower than expected because the eyed eggs were received late in the spawning season. Survival from hatch was fairly consistent through thinning (±5.6%) at Day 27 for all treatments. At this point, survival averaged 74.7 percent and ranged from 67.9 to 79.3 percent. Survival at the start of the initiation of the selenium *Lumbriculus* diet (Day 38) averaged 68.5 percent and ranged from 60.9 to 73.6 percent in treatments with control survival being 67.1 percent. By test termination, survival ranged from 28.5 to 45.4 percent in treatments and was 50.6 in controls.

Survival was also measured from hatch to test termination, which removed initial egg mortality from the survival assessment. While eggs were received as eyed eggs, indicating successful fertilization, a number of other variable including shipping, handling, and fungus could affect early egg survival. Control survival from hatch to test termination averaged 74 percent. Excluding hatching success from the survival term for all treatments, the range for survival was 52 percent at 2.5 µg/L to 59 percent at 10 µg/L. At the highest aqueous and dietary dose (40), survival was 48 percent, whereas the lowest survival occurred at the 20 µg/L treatment (37%).

As noted in the AECOM Laboratory Report (Appendix A), once the feeding study was initiated, survival rates were affected by fish gorging on *Lumbriculus* (to the point of rupturing stomachs in some fish) and territorial behavior (i.e., larger fish guarding excess food) that kept smaller fish from being able to feed freely.

Despite the reduction in total survival, as measured from the beginning of the test to test termination in controls, survival from hatch to test end at 74 percent is within acceptable test limits[1] (ASTM 2006). Table 5 illustrates both survival endpoints (total and from hatch to test termination).

[1] 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.

3.3.2 Growth

Dry weights were measured for all fish at test termination, either at CAS or ENSR. Dry weight determinations were collected on a "per surviving" and on a "per original" weight basis (Tables 6 and 7), respectively. Dry weights on a "per surviving" weight basis exclude any potential survival effect, whereas dry weight measurements on a "per original" basis take into account any potential survival and / or growth effects. Dry weights on a "per surviving" basis ranged from 72.8 to 87.4 mg per fish, with the highest weights observed in the highest selenium treatment. Mean dry weights on a "per original" basis ranged from 24.0 to 45.27 mg per fish, with the highest weights observed in the control treatment. A summary of dry weight measurements on a "per surviving" and "per original" basis is included in Appendix A.

Length data were also collected for YCT at test termination (Table 8). Among each treatment, the length data were consistent based on the means (± SD) of measurement data from replicates.

3.3.3 Deformities

Appendix A provides a summary of the counts of deformity rankings for each sample and a series of graphics illustrating sample deformities as a percentage of each sample. Observations made during scoring that resulted in defining a level of severity for a fish being examined are reported below:

- **Cranio-Facial Deformities** - Usually factors occurred together so a combination of two "1" conditions = 2, three "1" conditions = 3, or a 1 and a 2 = 3, and so on. For example, a deformed jaw and a blind eye = 2, two blind eyes = 2, but a badly deformed jaw (= 2 alone) plus a blind eye (= 1 alone), = 3.

- **Skeletal Deformities** - Bends caused by skeletal deformities were usually detectable from normal bending of the body during preservation (these fish were usually well preserved, very straight) by presence of a slight or greater bump below the surface of the epidermis on the outside of the bend. However, some fish with SD = 1 had just a very slight bend in the range the deformity described but could be due to preservation or the poor condition of the fish. This was sometimes especially true in larger fish, which may be more muscular and undergo stronger contraction during preservation and thus, bend slightly. A score "SD = 1" was a slight deformity, if at all. The scores of SD = 1 involving kyphosis or lordosis were deemed real because that is an unusual preservation deformity.

- **Fin and Finfold Deformities -** Often fins were malformed associated with vertebral deformities that did not permit proper development. Folded finfolds as a result of preservation were not counted.

- **Edema** - Edema was not originally scheduled for assessment because it was thought sometimes not a teratogenic effect and may be transitory as fish develop. However, it was assessed because it was common in one early sample and not others, and because it was thought a condition that could affect emergence, mobility, and other factors that may limit survival of fish in the wild. The yolk, which was present in some quantity in some study specimens, also created some swelling but was typically yellowish, opaque, and small, and hard to the touch in preservation.

The results of the cranio-facial (CF), skeletal (SK), finfold (FD), and edematous tissue (ED) deformity frequency are depicted separately in Figures 4 through 7. Each of these figures illustrate the percent normal trout for the deformity assessed.

Results of the deformity assessment are included in Appendix A. This assessment included categorization of individual fish into one or more types of deformities (i.e., cranio-facial, skeletal, fin or finfold, or edema) if present and ranking the severity of deformity. Specific ranking methods are presented together with a summary of the scoring for each fish in each treatment. Finally, a series of summary graphics showing the percentage of fish in each treatment for the deformity type are shown by deformity severity. Generally, very few fish showed any type of deformity, and those that did, were ranked as slightly deformed. Table 9 summarizes the percentage of deformities and normal fish for each treatment.

The percentage of normal fish evaluated for cranio-facial deformities across all treatments and controls ranged from 89.2 to 100 percent. The 2.5 and 10 nominal treatments both had about 10 percent of the total rated as slight cranio-facial deformities. The two highest treatments (e.g., 20 and 40) had zero and 6.9 percent, respectively, of the total individuals ranked as having slight cranio facial deformities.

For skeletal deformities, the percentage of normal fish ranged from 96.6 to 100 percent. Only a small percentage of slight ranked skeletal abnormalities were identified in the 10 and 40 nominal treatment.

For finfold deformities, the percentage of normal fish ranged from 56.5 to 89.7 percent. Of the finfold deformities, almost all were categorized as level 1 (slight or few) and less than 3% were categorized as level 2 (moderate or several). Finally, edema deformities ranged from 52.2 to 100 percent normal. Again, for edema all fish considered not normal were ranked as level 1.

The mean fraction of normal fish was derived for each treatment (# normal fish/ total # assessed per treatment) and ranged from 79 to 94 percent normal (Figure 8). The highest percentage of normal fish was found at the 10 µg/L treatment, while the lowest percentage of normal fish was found at the 20 µg/L treatment. At 40 µg/L, the mean fraction normal was 90 percent.

3.4 Graduated Severity Index (GSI)

The GSI summed and weighted deformities based on their severity for each sample from each location. A total GSI score was derived by summing the individual GSI scores for each deformity for a sample and the higher the GSI score the higher the number and severity of the deformities. Figure 9 shows the GSI scores for each treatment. Discernable trends of increasing GSI score with increasing egg selenium concentrations are not evident.

4.0 ANALYSIS OF RESULTS

The following section presents analysis of the YCT ELS study data. The combination of aqueous and dietary treatments yielded significant selenium bioaccumulation in fry by test termination. Table 3 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. While an exact time to bioaccumulation in YCT cannot be determined, the time frame of ~33 days can be used as a reasonable estimate of the time frame for accumulation once the selenium diet was initiated in the exposure system.

4.1 ELS Study Endpoint Analysis

4.1.1 Survival

Survival was measured at multiple time periods during the test including at hatch, swim up, at thinning stage, at commencement of the dietary exposure, and at test termination (Table 4). 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 = 72.1 percent) but within acceptable limits (ASTM 2006). 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 10).

- 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 10).

- 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.

- Survival data (hatch to test end) were plotted and evaluated using the TRAP software logistic and piecewise linear functions on transformed and untransformed data and no dose response models could be derived given the variability of the data within each replicate at each treatment.

- Despite the difference, no dose response is observed for these data as there is no consistent decrease in survival with increasing selenium in diet or water.

4.1.2 Growth

Growth was measured based on dry weight and length (Tables 6 through 8). 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 11). Growth measured based on the total test population takes into account growth and survival, whereas growth based on the 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 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 8).

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

- Growth data, regardless of how it was measured provides no dose response relative to selenium treatments.

4.1.3 Deformities

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 9). Of the four deformities evaluated (craniofacial, skeletal, finfold, and edema), only finfold deformities resulted in fish scored as moderate or several, albeit the percentage was low. All other deformities evaluated resulted in high percentages of normal fish or fish with only slight or few deformities. Figures 4 through 8 present scatter plots of each deformity assessed relative to the actual dietary concentrations rather than the nominal treatment levels to evaluate if any relationships might be viable. For each individual deformity, there is no apparent relationship of decreasing percentages of normal fish with increasing dietary selenium.

When considering all deformity measures (or lack thereof) using a mean fraction normal, the range across all treatments was 79 to 91 percent normal, with controls having 93 percent normal (Table 9). One-way ANOVA indicates that the means for fraction normal fish in treatments are not significantly different from one another or controls ($p = 0.21$). Figure 8 shows the mean fraction normal versus the dietary treatment levels and illustrates no relationship is present.

5.0 DISCUSSION

The YCT ELS studies provide additional important information relative to pathway of exposure and life stage effects. Both dietary and aqueous exposures were used as treatments to eyed eggs through hatching and swim up that received no prior maternal transfer. Fish at Day 71 clearly showed increased bioaccumulation at increasing dietary selenium concentrations. Aqueous exposure alone showed no apparent negative effects on survival through the range of concentrations tested. Addition of the selenium-bioaccumulated diet at Day 38 through Day 71 resulted in a significant survival effect observed at the 20 mg/kg nominal treatment level. However, at the next highest exposure level, no significant differences from control were noted. It is unclear if the response observed at 20 µg/L treatment was due to some of the issues identified with feeding or an effect due to selenium exposure.

Based on these data and the data generated as part of the adult reproduction studies, maternal exposure is a key determinant of effects for young developing fish. Conclusive results for effects to survival, growth, or frequency or severity of deformities to ELS YCT despite nominal exposures up to 40 µg/L in water and 40 mg/kg in the diet were not observed. For survival, while there was an inverse linear response of survival relative to increasing selenium body burden in fish and selenium body burden in the *Lumbriculus* diet, there is no dose-response relationship indicating decreasing survival with increasing selenium in whole body tissue or dietary media. The survival response is highly variable both within treatment concentrations and across treatments. No relationships were observed between dietary selenium concentrations and growth as measured by dry weight.

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. Results of the YCT ELS studies conducted appear to support this conclusion.

Studies by Vidal et al. (2005) and Hamilton et al. (1990) that used juvenile 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.

6.0 SUMMARY AND CONCLUSIONS

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

- Survival rate at test termination, growth, and deformities in treatments were not significantly different from controls.

- Despite selenium tissue residues in young fish with no pre-parental exposure up to 34.5 mg/kg dw 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.

The hypothesis for this ELS 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 that may apply to resident Crow Creek trout that travel to Sage Creek to spawn.

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TABLES

Table 1
Measured Aqueous Selenium Concentration in Trout Dietary Study

Nominal Selenium Treatment (µg/L)	Time Weighted Average Total Recoverable Selenium	% of Nominal Concentrations	Time Weighted Average Dissolved Selenium	% of Nominal Concentrations	Dissolved/ Total Recoverable (%)
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 2
Selenium Concentrations 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 Worms (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 3
Whole Body Selenium Concentration in Trout Prior to Dietary Study and at Test Termination

Nominal Se Treatment (µg/L)	Whole Body Fish	
	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 4
Percent Survival of Trout at Different Stages during the Aqueous-Dietary Study

Nominal Se 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
Percent Survival of Trout from Hatch to Test Termination during the Aqueous-Dietary Study

Nominal Selenium Treatment (µg/L)	% Survival (hatch to test termination)						
	Rep A	Rep B	Rep C	Rep D	Rep E	Overall Mean	Std. Dev.
Control	69	69	90	69	75	74.4	9.1
2.5	65	41	33	82	40	52.2	20.58
5	40	65	53	61	62	56.2	10.08
10	50	33	67	44	100	58.8	26.11
15	40	53	64	63	40	52	11.77
20	31	19	41	65	27	36.6	17.74
40	42	71	44	41	43	48.2	12.79

Table 6
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 7
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 8
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 9
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.

FIGURES

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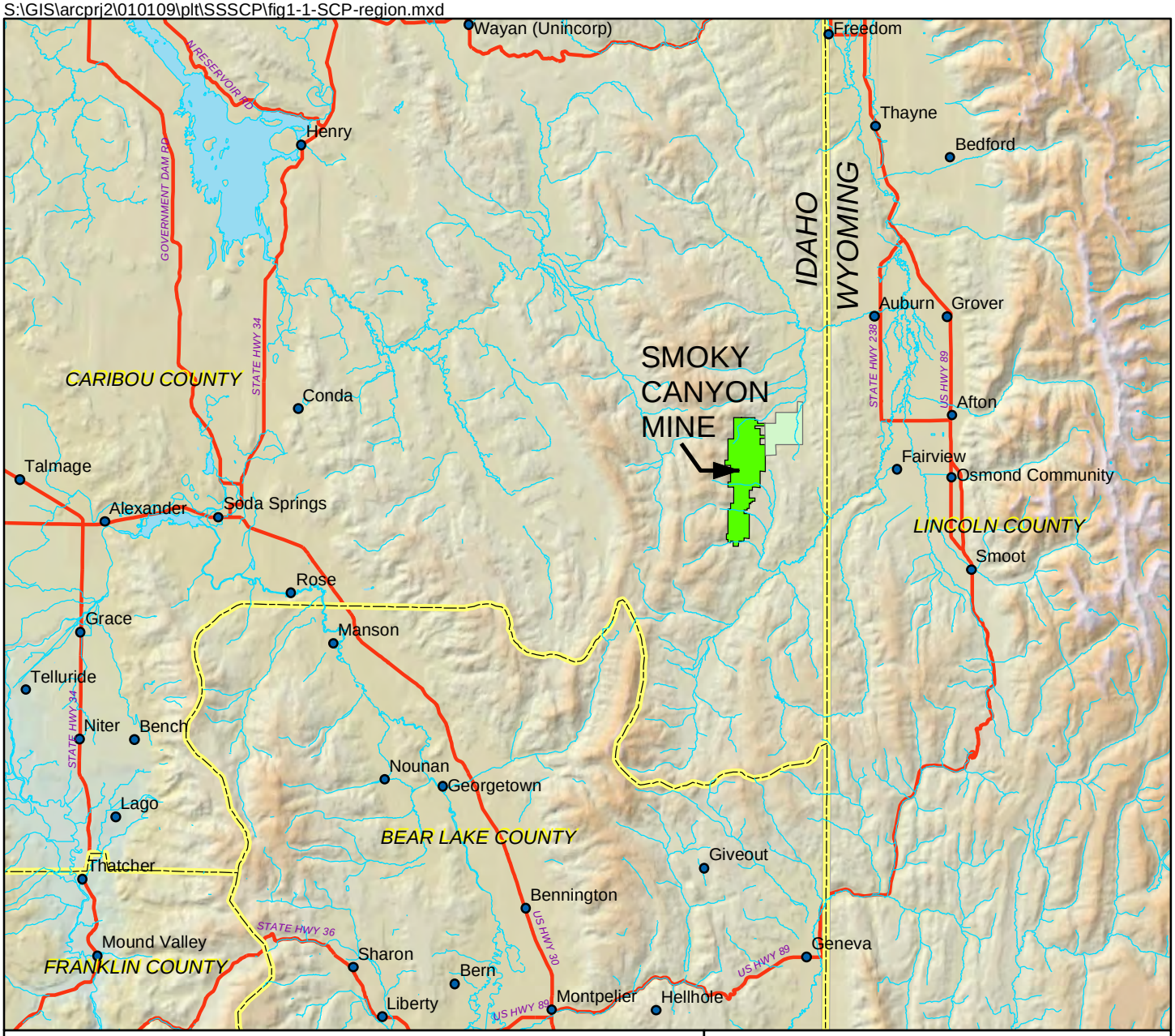

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SMOKY CANYON MINE

FIGURE 1

LOCATION OF THE SMOKY CANYON MINE

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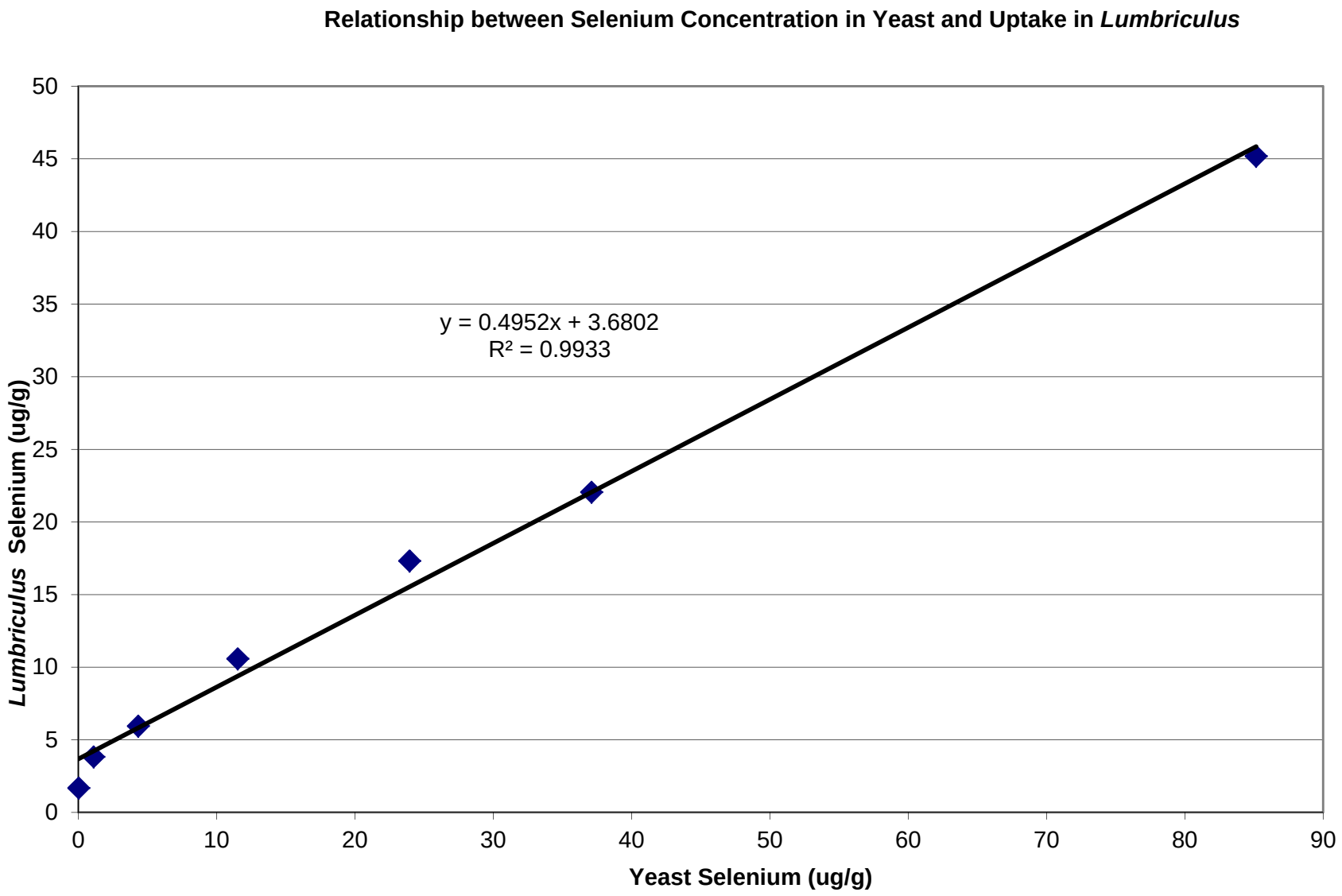

Figure 2
Relationship between Selenium Concentration in Yeast and Uptake in *Lumbriculus*

J.R. Simplot Company
Site-Specific Selenium Criterion

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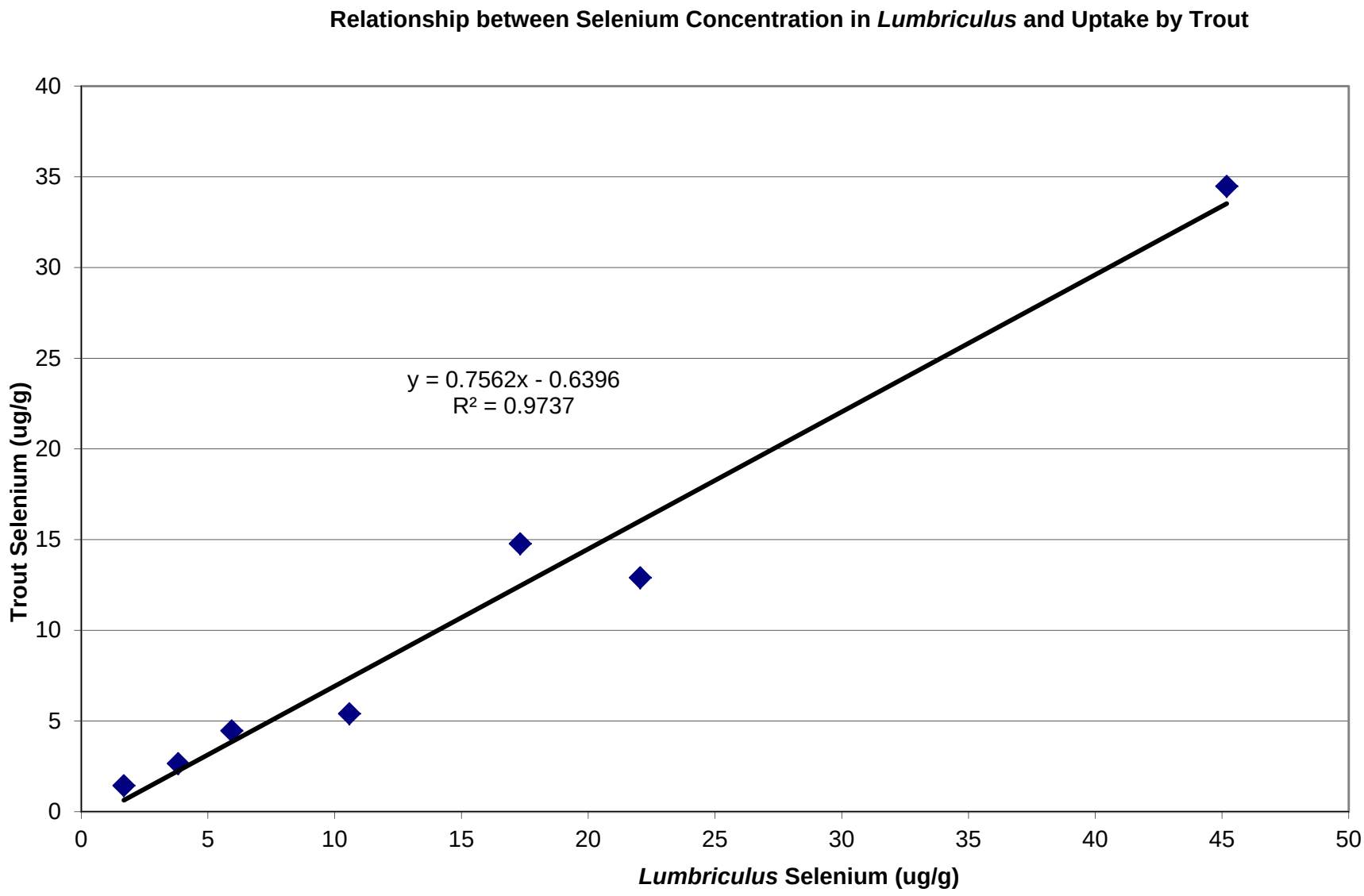

Figure 3
Relationship between Selenium Concentration in *Lumbriculus* and Uptake by Trout

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Site-Specific Selenium Criterion

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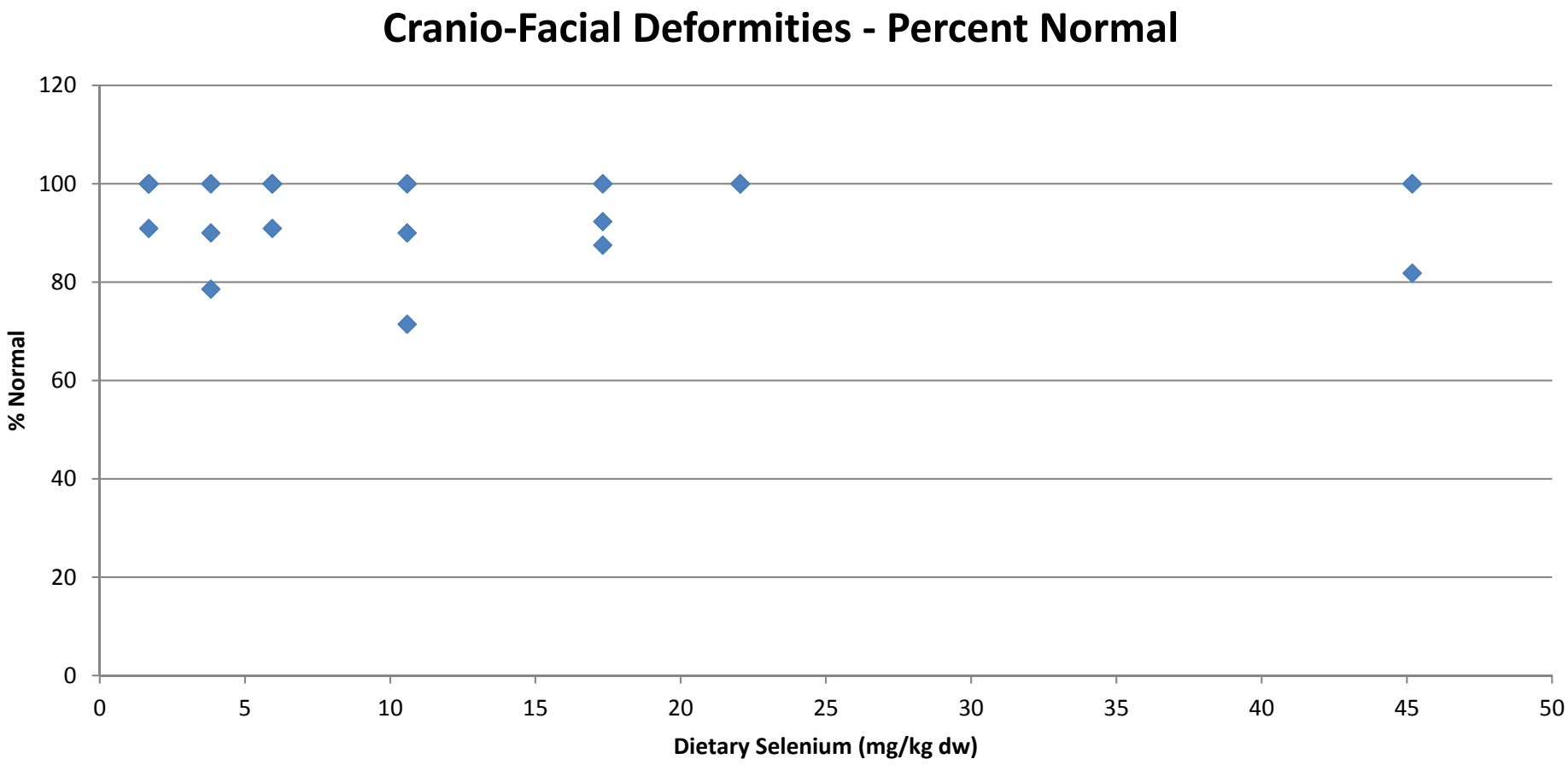

Figure 4
Percent Normal YCT Evaluated for Cranio-Facial Deformities in the Selenium Aqueous and Dietary Study

J.R. Simplot Company
Site-Specific Selenium Criterion

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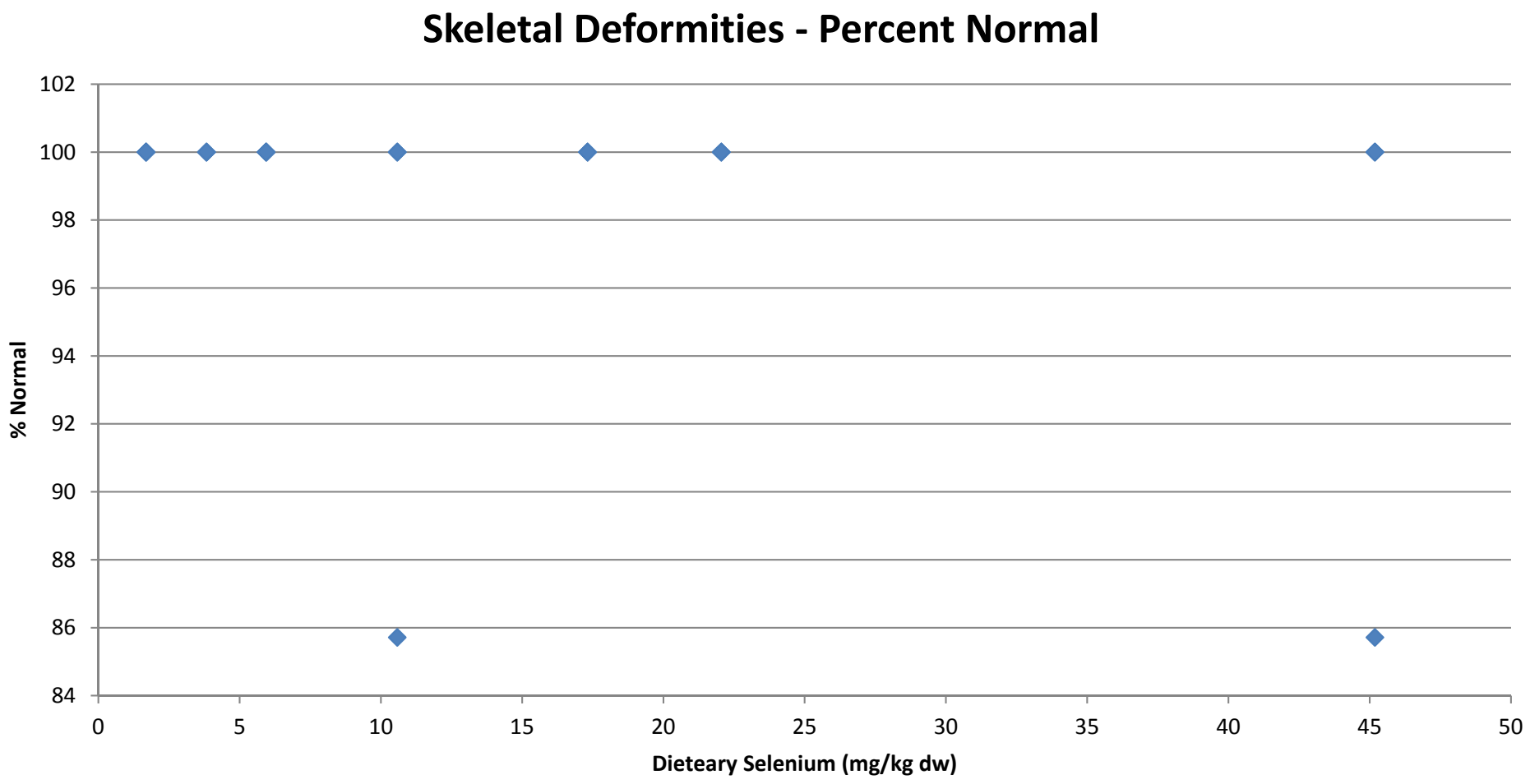

Figure 5
Percent Normal YCT Evaluated for Skeletal Deformities in the Selenium Aqueous and Dietary Study

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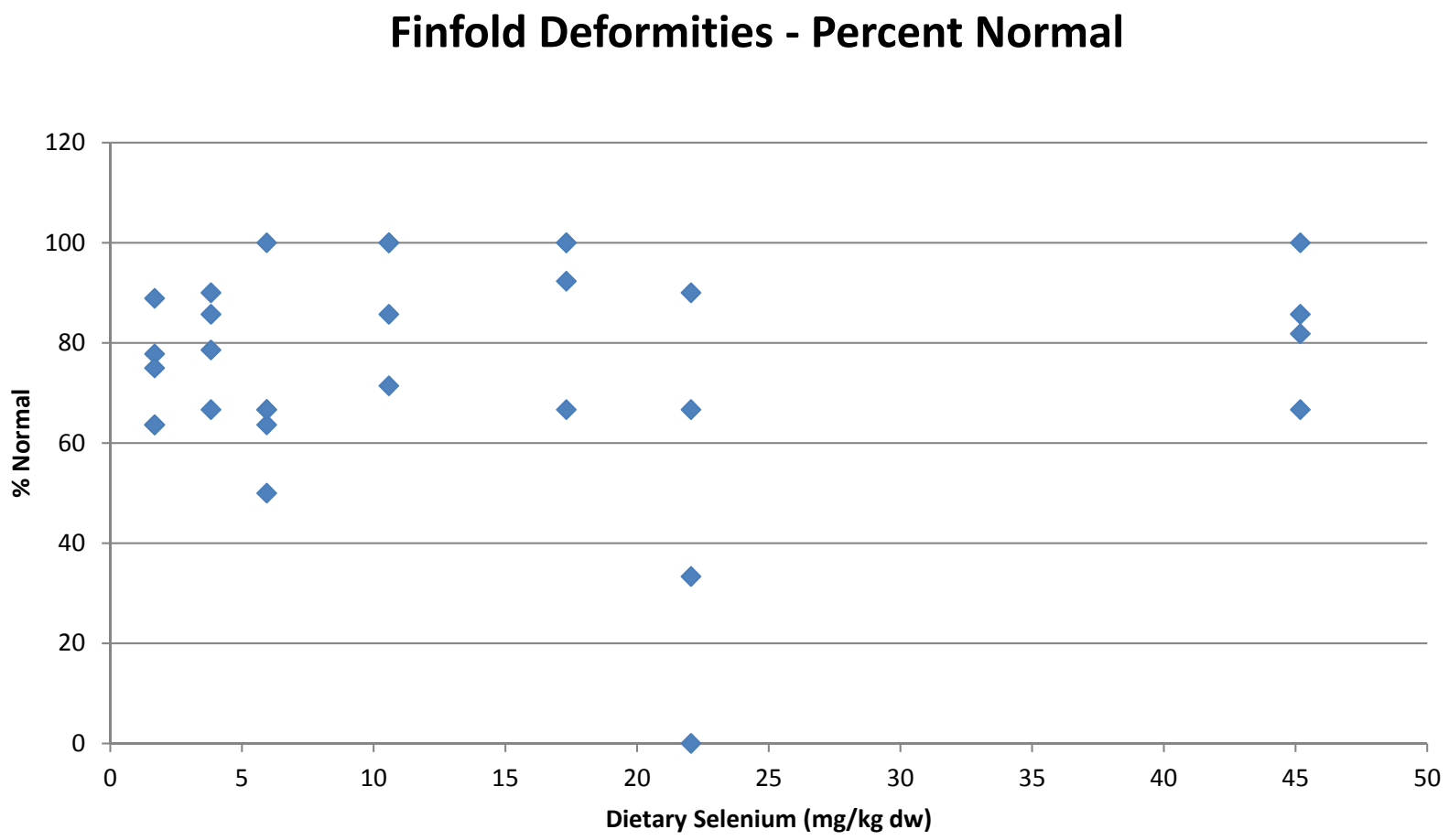

Figure 6
Percent Normal YCT Evaluated for Finfold Deformities in the Selenium Aqueous and Dietary Study

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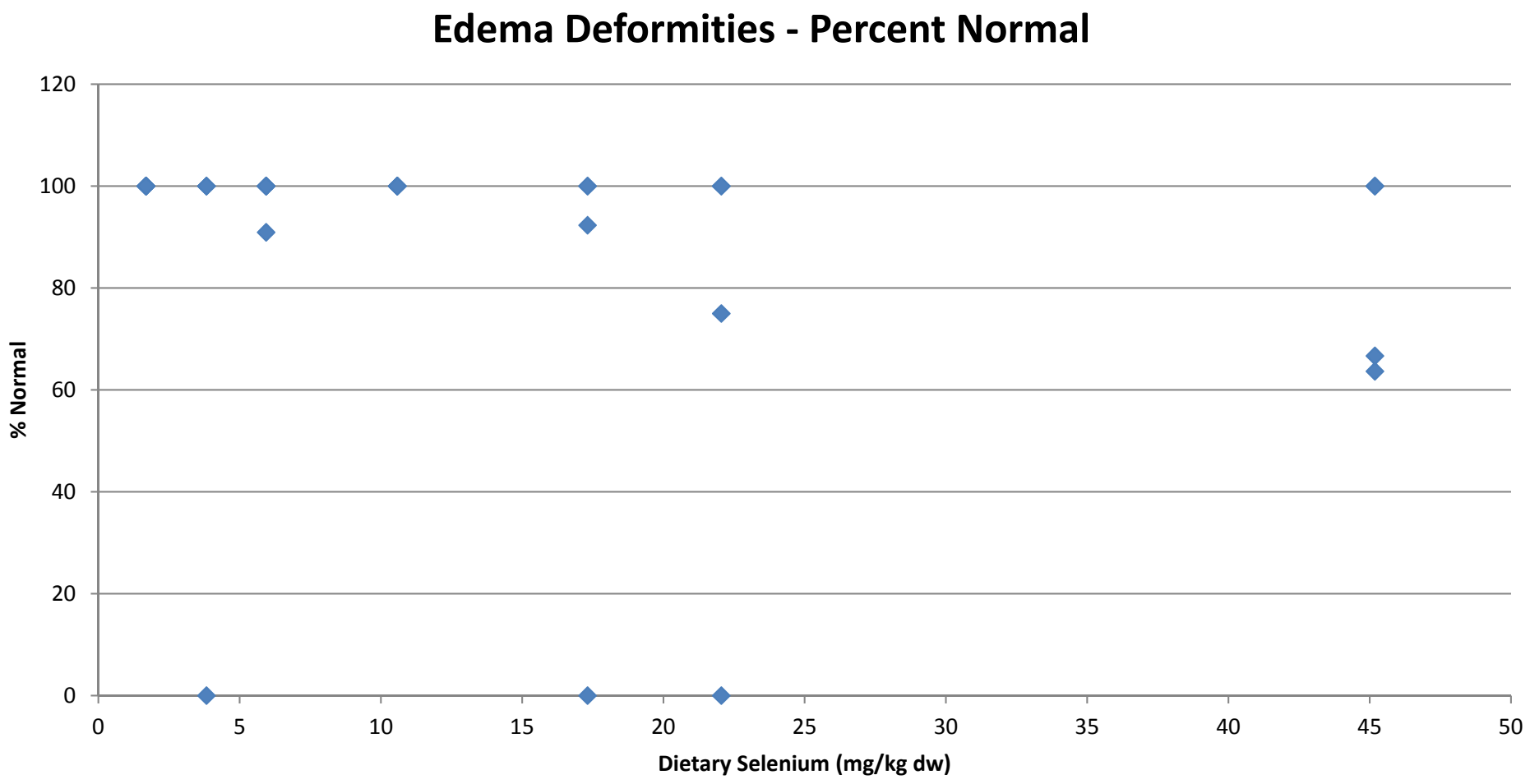

Figure 7
Percent Normal YCT Evaluated for Edema Deformities in the Selenium Aqueous and Dietary Study

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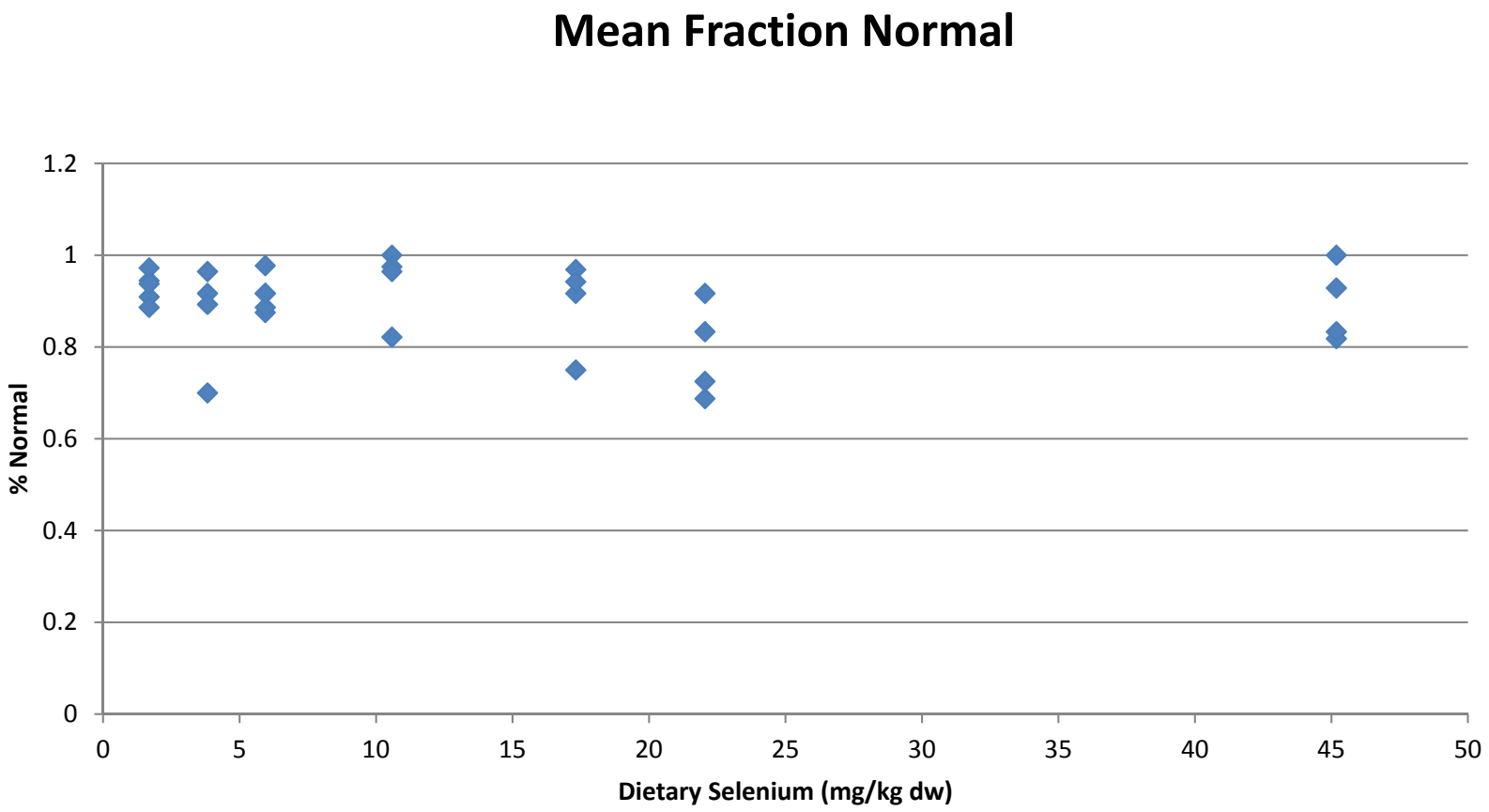

Figure 8
Mean Fraction Normal YCT Evaluated for All Deformities in the Selenium Aqueous and Dietary Study

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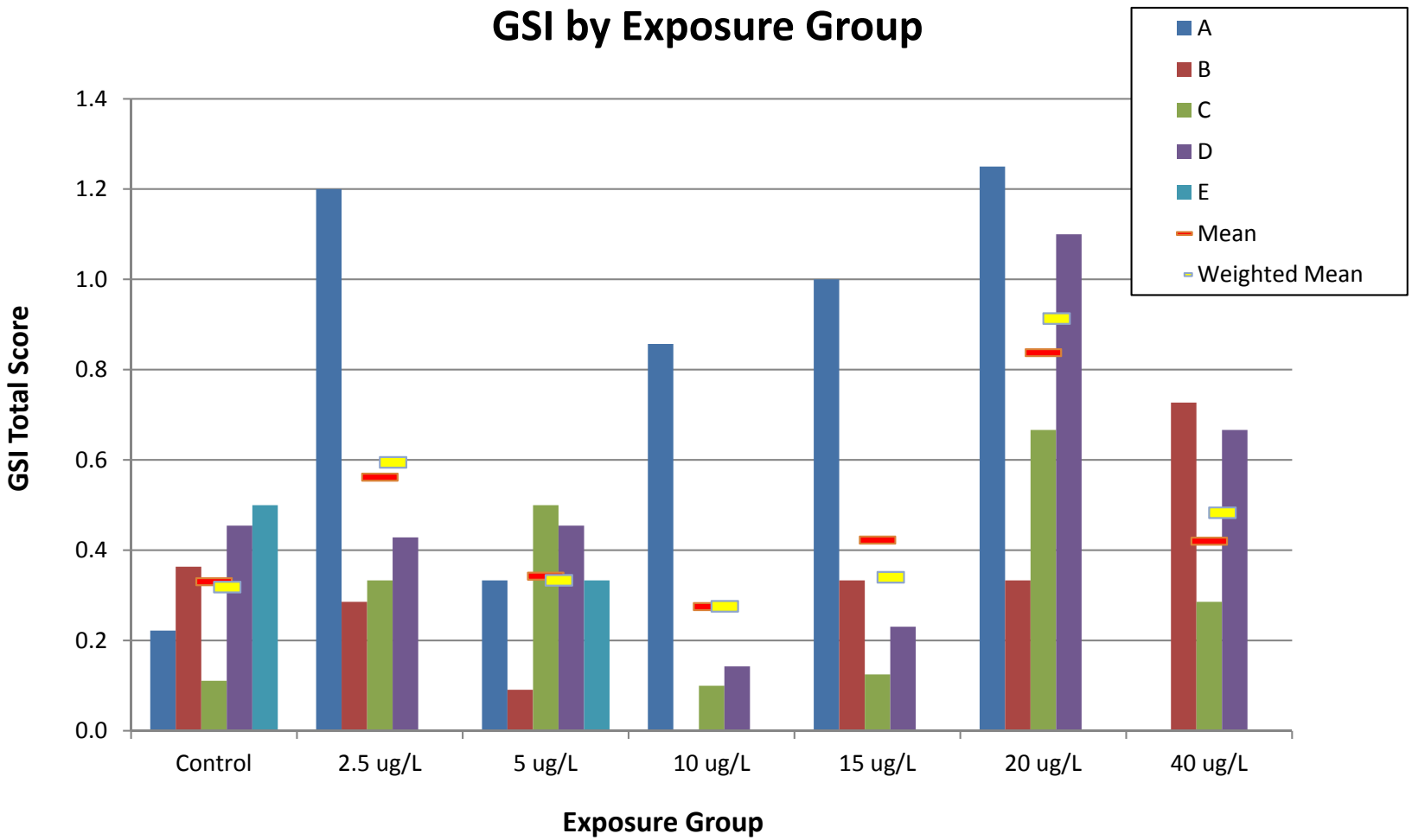

Note: "Mean" is calculated for each exposure group by averaging the GSI totals for the subgroups, with each subgroup weighted equally. "Weighted mean" normalizes the GSI scores by accounting for the number of individuals in each replicate.

Figure 9
Graduated Severity Index for Larvae Evaluated for Deformities from Each Treatment

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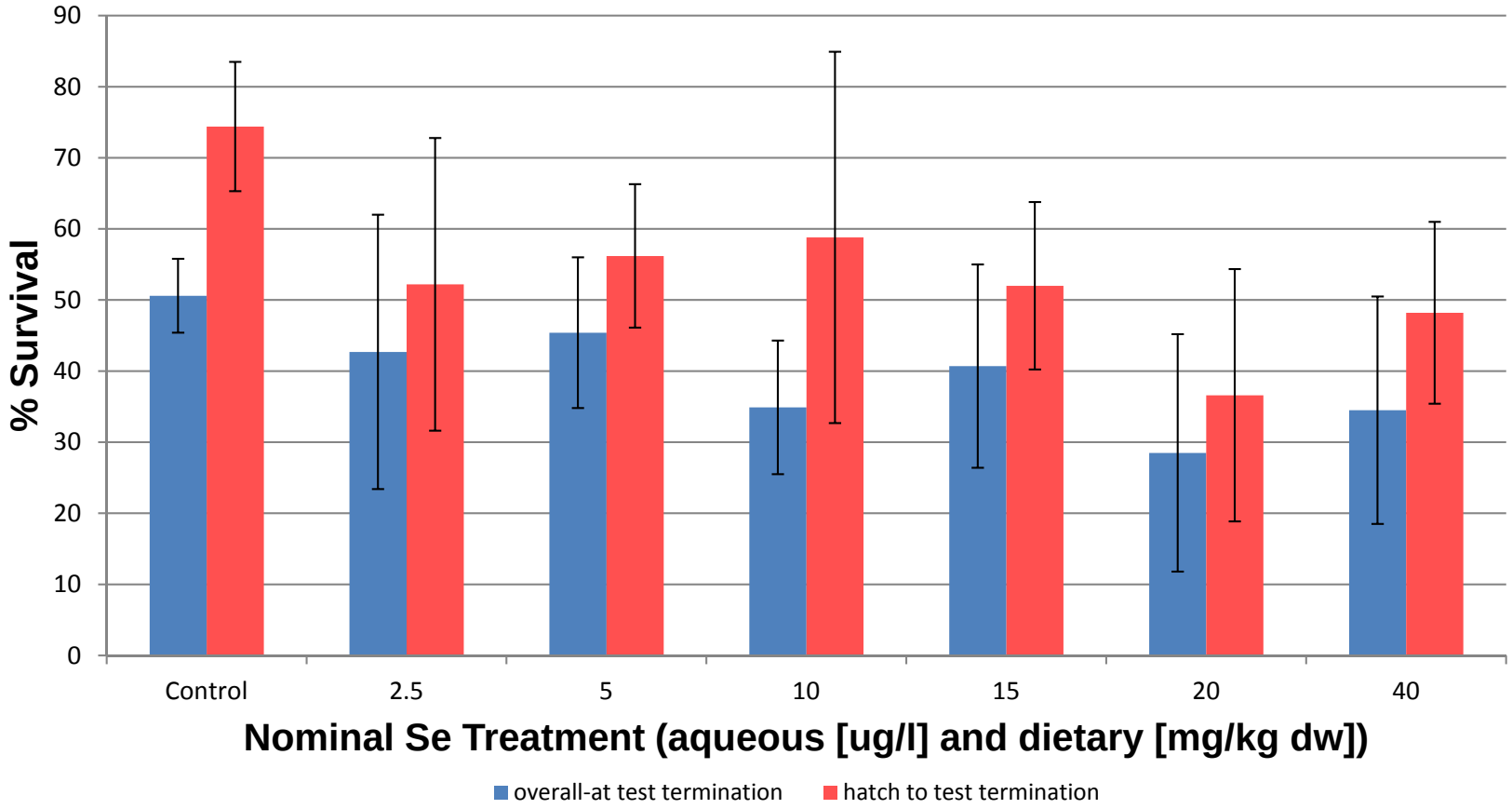

Error bars represent +/- 1 SD

Figure 10
Percent Survival for Early Life Stage YCT Exposed to Different Levels of Aqueous and Dietary Selenium

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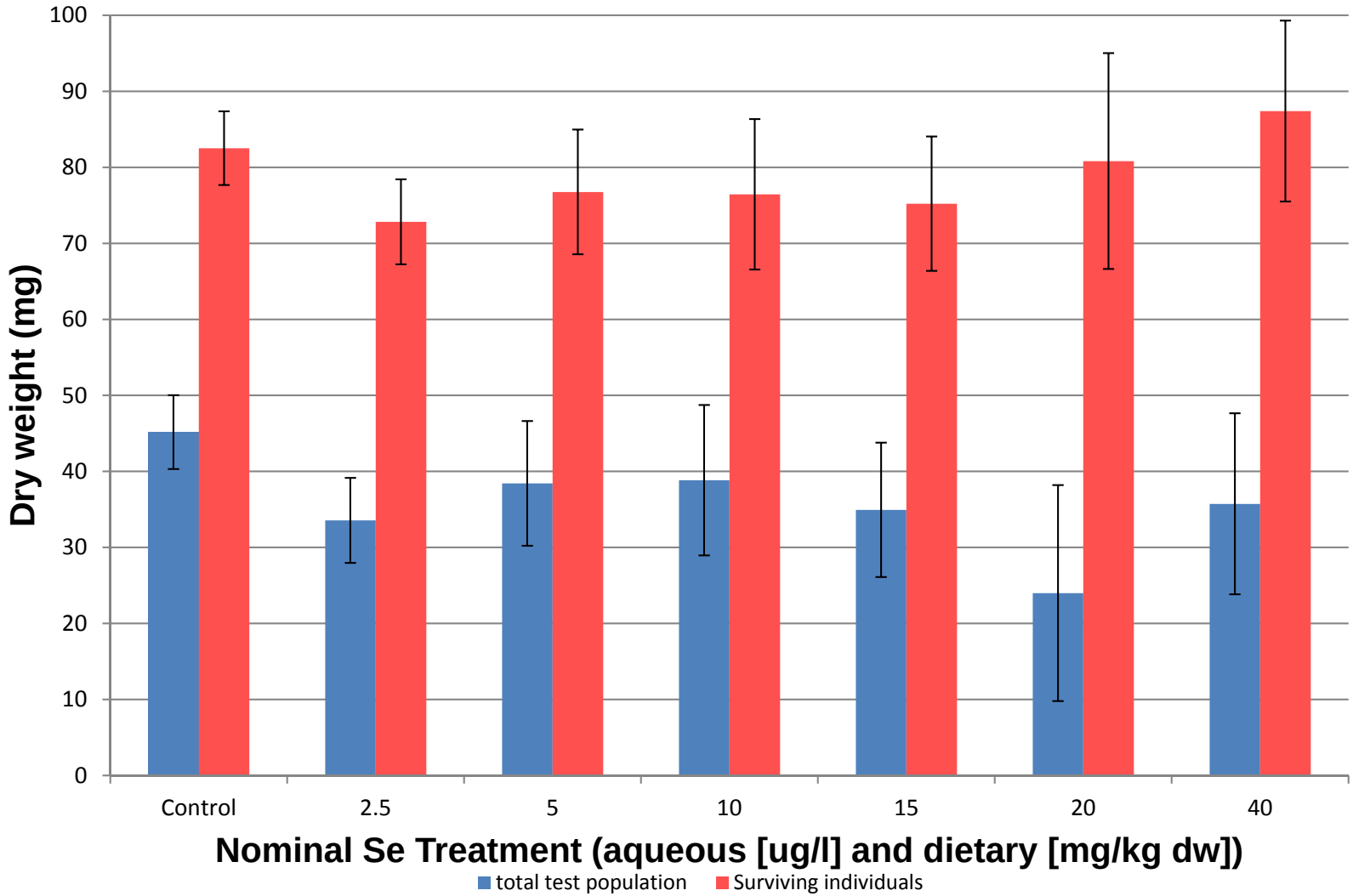

Error bars represent +/- 1 SD

Figure 11
Growth as Measured by Weight for Early Life Stage YCT Exposed to Different Levels of Aqueous and Dietary Selenium

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Site-Specific Selenium Criterion

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APPENDIX A

Prepared for:
JR Simplot Company and Newfields Company, LLC.
Pocatello, Idaho

Data Report - Draft

Early Life Stage Study with Yellowstone Cutthroat Trout (*Oncorhynchus clarki*) - Aqueous and Dietary Exposure of Selenium

October 2009

ENSR | AECOM

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1.0 Introduction

As outlined in the *Work Plan – Laboratory Toxicity Tests for Developing a Site-Specific Selenium Threshold for Trout* (Newfields 2008), a laboratory study was performed to evaluate aqueous and dietary exposure of selenium to Yellowstone cutthroat trout (*Oncorhynchus clarki*) in an early life stage study (ELS). While Study Design A, which entailed using wild caught fish for this ELS study, was the preferred method from the work plan, Study Design B (hatchery obtained fertilized eggs) had to be used because there were not enough wild caught fish obtained in the field (i.e., Se pre-exposed organisms) to initiate this study.

ENSR's (presently AECOM) Fort Collins Environmental Toxicology Laboratory (FCETL), Fort Collins, CO was retained to conduct the laboratory biological exposure portions of this study according to the study design plan outlined by Newfields Inc. (presently Formation Environmental Inc.). An assessment of larval trout deformities at the end of the feeding period was performed under the direction of Dr. Kevin Bestgen at Colorado State University's Larval Fish Laboratory. This report presents the results / data from the laboratory portion of this work.

Key dates of this project are outlined in the table below.

Stage of study	Date	Test day
Initiation	5/14/2008	0
Hatch	5/20/2008	6
Swim-up	6/5/2008	22
Thinning[1]	6/10/2008	27
Start of feeding study	6/21/2008	38
Termination	7/24/2008	71

[1]Whole-body selenium was measured in a sub-sample of larval fish at thinning

2.0 Methods

2.1 Test Substance

2.1.1 ELS Study

The test substance for the aqueous study was selenium as sodium selenate (ACS Reagent grade, 95%), CAS number 13410-01-0 (Sigma, St. Louis, MO). Upon receipt in the laboratory, the test substance was assigned a unique identification number (C04-025). This material was used to prepare a primary stock (volumetrically) with Milli-Q® grade water (2,262 mg Se/L). The primary stock was used to prepare a secondary stock (2,262 µg Se/L, prepared from Milli-Q® grade water), which was used to add selenium into the dilutor (see below).

2.1.2 Dietary Study

The selenium source for the dietary portion of the study was a selenium-yeast product, Seleno*Source*™ *AF* 600 (Diamond V Mills, Inc., Cedar Rapids, IA). The product is used as a supplement to livestock feed. The product was purchased from Ranch-Way Feeds (Fort Collins, CO), and was designated FCETL lot #F08-008. The major component of the Seleno*Source*™ *AF* 600 is selenomethionine, an organic form of selenium. The target concentration of selenium in the Seleno*Source*™ *AF* 600 was 600 mg/kg, while the measured concentration was 580 mg/kg (Appendix A).

2.2 Dilution Water

The dilution/control water used in the study was FCETL process water obtained from Horsetooth Reservoir. The ambient incoming water is coarse-filtered (through a sand filter and polypropylene core filters [10 and 1 micron]) to remove indigenous organisms, particulate matter, and contaminants. Water then passes through an ultraviolet light disinfection system before being stored in large holding tanks. This water is periodically analyzed for contaminants. Horsetooth Reservoir process water is very soft to soft water according to USEPA (2002), with both hardness and alkalinity typically 20 - 30 mg/L as $CaCO_3$. The background sulfate level in unaltered Horsetooth water is ~5.0 mg/L. This water served as the base water for the dilutor panel used in the ELS study to expose trout to aqueous selenium and in the chambers used to culture *Lumbriculus* fed yeast diets with a range of selenium concentrations.

In the ELS study, the ambient (unheated) laboratory Horsetooth reservoir water was passed through a 1 µm filter prior to use in the dilutor. In order to increase the background sulfate concentration, calcium sulfate and magnesium sulfate were added to the filtered Horsetooth water to achieve a target hardness of ~50 mg/L as $CaCO_3$ and sulfate concentration of ~20 mg/L (see Exposure System for more detail).

2.3 Test Organisms

2.3.1 Trout

Test organisms were Yellowstone cutthroat trout (*Oncorhynchus clarki*) eyed-up eggs obtained from Henry's Lake Fish Hatchery, Henry's Lake, ID. The eyed eggs, which were obtained late in the spawning season, were shipped via overnight courier and delivered at ENSR's FCETL on 14 May 2008. Viable eyed eggs were impartially distributed among the 35 egg cups by adding no more than 5 eggs at a time into each egg cup and repeating the process until each cup contained the required number. Egg cups were then placed in their randomly assigned test chambers. Twenty eyed eggs were placed in replicates A through D for each treatment, while 34 eggs were placed in each E replicate to maximize the available organisms for whole body selenium analysis.

Egg mortality was higher than anticipated due to receiving eggs at the end of the spawning season for Henry's Lake and possibly due to shipping and handling or the fact that these were wild fish. Eggs had been treated with formalin while at the hatchery to reduce fungal growth but once received at ENSR were not treated for fungus. Any eggs that were observed dead the first day were replaced with remaining viable eyed eggs.

After all eggs in a cup had hatched, yolk-sac fry were removed from the egg cup to the floor of the test chamber. This occurred within a day of the last egg hatching (or being noted as not viable, day 8). Swim-up of fry was first noted on Day 16 in all treatments while the day of swim-up was Day 22 (swim-up date was defined as the date that >70% of the sac fry had swum-up). The feeding study was delayed from swim-up to Day 38 because younger fish were not eating the *Lumbriculus* diet, presumably due to gape-limited predation (i.e., *Lumbriculus* were too large for swim-up fry to eat). A discussion of the feed schedule during the test is presented in more detail below.

2.3.2 *Lumbriculus*

Oligochaetes (*Lumbriculus variegatus*, commonly known as blackworm or California blackworm) were chosen as the diet for the YTC for a number of reasons. This species is commercially available, inexpensive, and relatively easy to mass-culture in the laboratory (Mount et al. 2006). It has the ability to bioaccumulate metals both through its diet and via waterborne exposure. *L. variegatus* has been used by other researchers as a natural live diet for fish when studying the effects of diet-borne metals on fish (Besser et al. 2006, Hansen et al. 2004, Hockett et al. 2003, Ivey et al. 2007, McIntyre et al. 2007).

L. variegatus were purchased from Bayou Aquatic Foods in Ontario, California, USA. The first batch (~470 g) was received on April 22, 2008 (FCETL lot 08-020). The worms were held in a 20-gallon glass aquarium containing a substrate of shredded brown paper toweling, through which laboratory process water from Horsetooth Reservoir flowed at a rate of approximately 100 ml/min. Target temperature was ~20°C. Worms in the aquarium were fed 6 g of starter trout chow #1 (Zeigler Brothers, Gardners, Pennsylvania) three times per week. The aquarium was aerated to maintain dissolved oxygen above 3.5 mg/L. These organisms were used to confirm that we were able to maintain healthy oligochaetes with a flow-through system, to evaluate substrate, and to serve as a backup source of organisms. Due to the impact of the paper towel substrate on dissolved oxygen, and difficulty in separating the worms from the substrate during harvesting, future culture tanks

utilized clean sand as substrate. On April 30, 2008, 7.7 g (wet weight) of these worms were transferred to a 2.5-gallon glass "control" aquarium which contained ~50 ml of cleaned "play" sand, and which was plumbed flow-through with Horsetooth Reservoir water. *L. variegatus* in this tank were fed 1.25% (dry weight yeast / wet weight of worms) daily of nutritional yeast (FCETL lot# F08-007; aka, brewer's yeast; Whole Foods, Fort Collins, CO). The initial concentration of selenium in the nutritional yeast was 1.9 µg/g dwt (Appendix A).

Additional *L. variegatus* were received from Bayou Aquatic Foods on two other occasions: approximately 2 pounds (~991 g) on May 1, 2008 (FCETL lot 08-022), and approximately 4 pounds (~1,869 g) on May 23, 2008 (FCETL lot 08-026). Organisms from these two lots were primarily used to develop the control and selenium-supplemented diets for the YCT study. Pictures from this portion of the study are included in Appendix B.

2.4 Exposure System

2.4.1 ELS Study

A continuous-flow serial diluter (after Benoit et al. 1982) with a dilution factor of 0.5 (except for the 15 µg Se/L treatment) was used for the ELS portion of the test. The diluter system was constructed out of glass, silicone adhesive, and silicone stoppers. A diagram of the diluter system is provided in Figure 2-1. Test solutions were delivered to the test chambers through (3/8 I.D. x ½ O.D. x 1/16 thickness, inch) polyethylene tubing (Nalgene® 489, Nalgene, Rochester, NY, USA). The dilutor system delivered six test concentrations and a dilution water control to the test chambers (i.e., 0, 2.5, 5.0, 10, 15, 20, and 40 µg Se/L). The polyethylene tubing was routed to test chambers in random positions within the water bath. From the mixing chamber, the solution flowed into one end of the serial diluter box. Additional dilution water flowed into the serial diluter box at various points, providing additional dilution for the lower test concentrations. From the diluter box, each test solution flowed into a flow-splitting cell to promote mixing of the test solution and to equally allocate the test solution between the four replicate test chambers. Flow rate was adjusted to deliver a target rate of 20 ml of test solution per minute to each exposure chamber. Overflow from the flow-splitting cell provided water for the E replicates for each treatment, and was measured weekly along with other test chamber flows.

Sodium selenate was metered from a Marriotte bottle into the mixing chamber and diluter. The Mariotte bottle consisted of a 5-gallon glass bottle; the sodium selenate test stock solution (~2,262 µg Se/L) was delivered into the test system at a target rate of 4.5 ml/min. As stated, the nominal selenium concentrations were: 0, 2.5, 5.0, 10, 15, 20, and 40 µg Se/L (Newfields 2007). The test substance and dilution water delivery rates to the diluter were checked at least once daily; adjustments were made as necessary. In instances when the flow rate was outside the target value, additional analytical samples were collected and measured to document the change in potential exposure concentration.

The test chambers were 4.5-L aquaria constructed of plate glass and silicone adhesive. Each test chamber drain consisted of a piece of 5-mm ID glass tubing inserted through a silicone stopper which was pressed into a small hold drilled in the side of the aquarium. The test solution volume

was maintained at the level of the top of the drain. With the test solution volume of 4 L and a flow rate of 20 ml/min, each test vessel received ~ 7.2 volume additions per day.

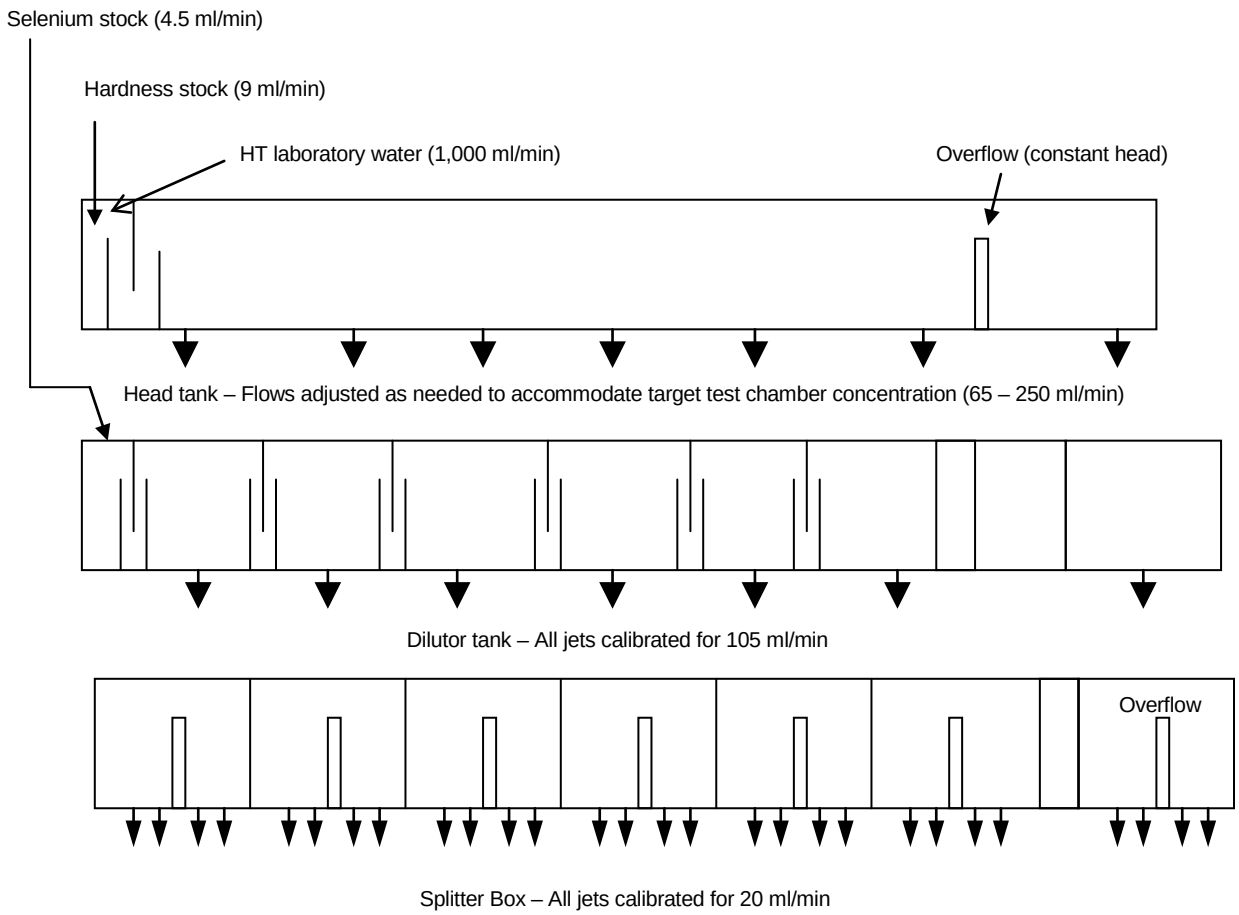

Figure 2-1 Schematic diagram of the diluter system used to provide continuous selenium concentrations to the flow-through early life stage study using Yellowstone cutthroat trout (*Oncorhynchus clarki*)

A super hardness stock was metered from a 5-gallon Marriotte bottle into the head tank (Figure 2-1). Our laboratory Horsetooth water was adjusted to increase the hardness and sulfate so that it would be higher than ambient levels and more similar to the field conditions, similar to previous parental studies with YCT and brown trout collected from the field (Newfields 2007). Given the soft water conditions of the laboratory Horsetooth water and the volume of water used on a daily basis, it was impractical to match the water quality characteristics of the site. Therefore, a hardness of ~50 mg/L as $CaCO_3$ and sulfate concentration of ~20 mg/L were targeted. Calcium sulfate ($CaSO_4.2H_2O$; Ben Franklin® Aquacal™, Plaster City, CA) and magnesium sulfate ($MgSO_4.7H_2O$; The PQ Corporation, Valley Forge, PA) were added at a ratio of 1.82:1 calcium: magnesium (molar basis) to deionized water to prepare the super hardness stock solution of ~2,000 mg/L as $CaSO_4$. This super stock was metered into the head tank (~ 9 ml/min) to achieve the target levels. Water hardness was measured daily during the study, while sulfate was monitored periodically. Batches of the super hard stock solution were prepared as needed (~ every three days), while the Mariotte bottle was filled daily

throughout the study. Flows on the main dilution water (unadjusted Horsetooth water) and the hardness stock solution drip flowing from the head tank (Mariotte bottle fed this tank) were measured at least once daily throughout the study.

2.5 Test Conditions

The exposure chambers were housed in a temperature-controlled water bath with a target of maintaining the temperature in each chamber at 10°C. Embryos and fry were maintained under dim lighting (approximately 3.7 foot-candles) until swim-up occurred, after which they were held in ambient lighting (approximately 52 ft-c) with 16 hours of light per 24-hour period. The flow rate and exposure chamber volume were selected and adjusted based upon the size of the test organisms in order to provide a loading rate of not more than 0.5 g of biomass per liter of water passing through each chamber in a 24-hour period at test end.

2.6 Dietary Study

While the workplan outlined that the *Lumbriculus* would be exposed to selenium via water and diet (i.e., yeast), work by Besser et al. (2006) indicated that target selenium levels could be adequately achieved using dietary exposure alone. For the sake of simplicity, we altered the exposure of selenium to *Lumbriculus* using diet only (i.e., yeast) in order to achieve the target selenium concentrations in *Lumbriculus*. Each group of *Lumbriculus* was fed a nutritional yeast diet supplemented with a commercial selenized yeast product (Section 2.1.2) to target selenium concentrations. Target selenium concentrations in *Lumbriculus* tissue (dry weight basis) were 0 (control), 2.5 µg/g, 5 µg/g, 10 µg/g, 15 µg/g, 20 µg/g, and 40 µg/g. According to the work conducted by Besser et al. (2006) the amount of selenium accumulated in the oligochaetes was expected to reach a plateau (equilibrium) after approximately 28 days. At equilibrium (for non-toxic levels of selenium), the selenium concentration (on a dry weight basis) in the worm tissue would be approximately one half the concentration of selenium in the yeast diet. Therefore, the target selenium concentrations for the yeast diets were: 0 (control), 5 µg/g, 10 µg/g, 20 µg/g, 30 µg/g, 40 µg/g, and 80 µg/g (dry weight basis).

L. variegatus (Lot 08-022) for the first dietary batch was divided among seven, 2.5-gallon aquaria (May 2, 2008) so that each aquarium contained ~140 g (wet weight) of organisms. Each tank contained ~50 ml of clean “play” sand as substrate, and was plumbed for flow-through of Horsetooth Reservoir water. Originally, shredded paper towels were used as the substrate; however, an initial in-house study with an earlier batch of *Lumbriculus* demonstrated that it was difficult to separate the *Lumbriculus* from the paper towel substrate. Therefore, sand was used as the substrate in the feeding studies to simplify isolation of the organisms for the feeding study. Standpipes for drainage were set so that the tanks held ~6 L of water. Tanks were designated (in order of increasing Se concentration) control, A, B, C, D, E, F (Figure 2.2). These were considered the “small” tanks, since larger ones were initiated later. Approximately one week later (May 8-9, 2008), it appeared that there had been substantial mortality in the control and F tanks. Therefore, an appropriate wet weight amount of additional *Lumbriculus* were transferred from the holding tank (Lot 08-020) to the control and F tanks.

To ensure that sufficient amounts of *Lumbriculus* were available to feed to the YCT as they became larger, additional, larger, *Lumbriculus* tanks were initiated. An additional batch (Lot 08-026) of *Lumbriculus* was received on May 23, 2008 and divided among seven, 10-gallon aquaria on the day of receipt so that each aquarium contained ~240 g (wet weight) of organisms. Each tank contained ~100 ml of clean "play" sand as substrate, and was plumbed for flow-through of Horsetooth Reservoir water. Standpipes for drainage were set so that the tanks held ~27 L of water. Tanks were designated (in order of increasing Se concentration) control-2, A-2, B-2, C-2, D-2, E-2, F-2, to distinguish them from the 2.5-gallon tanks control and A-F (Figure 2.2).

Lumbriculus in each tank were fed the yeast diet mixed for that tank (i.e., target Se level) at the 1.25% daily rate (dry wt yeast / wet wt of worms; yeast for each tank was weighed out for each day's feeding). Wet weight of *Lumbriculus* in each tanks was estimated, and yeast amounts adjusted as needed (e.g., food reduced if it appeared that worms had died and as worms were removed to feed to trout). Cultures were continued until July 24, 2008, at which point they were terminated after sub-sampling for selenium and nutritional analyses.

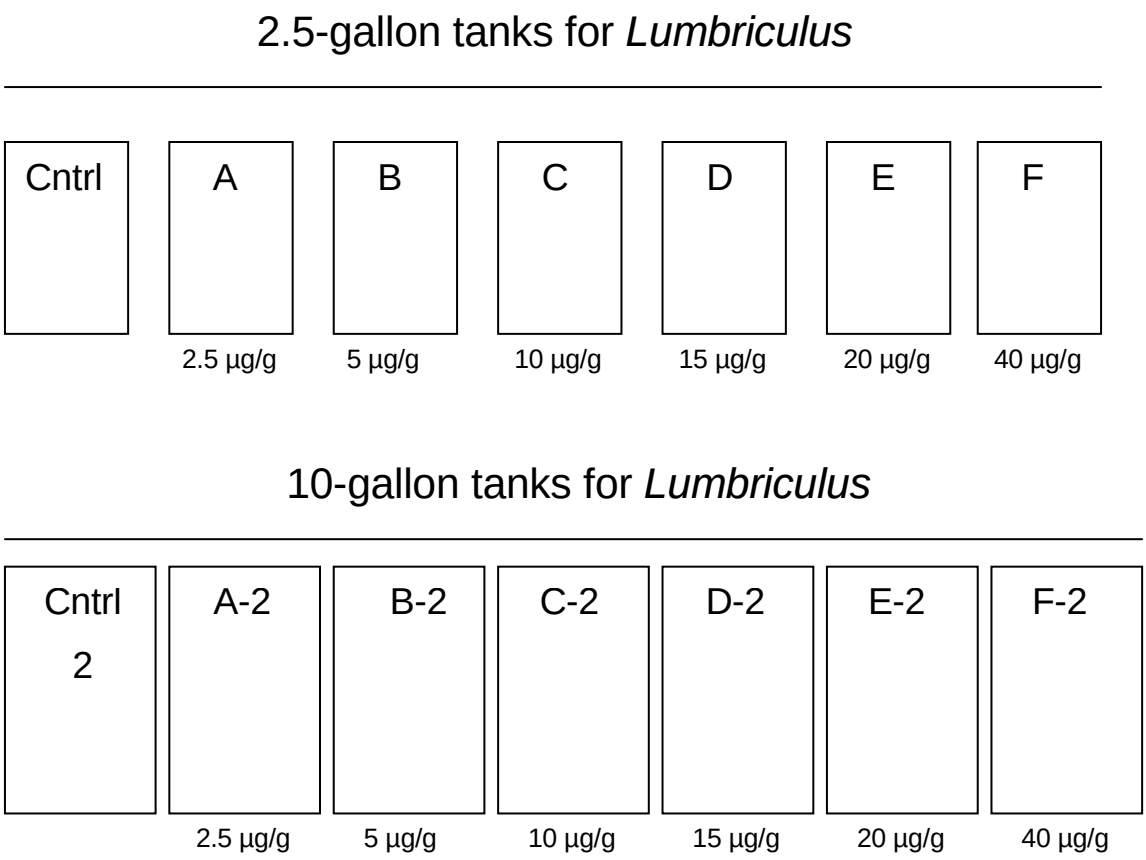

Figure 2-2 Schematic diagram and nomenclature of the test chambers used for the dietary exposure of selenium to *Lumbriculus variegatus*

2.6.1 Feeding ration

The original plan was to start feeding the larval YCT the live dosed *Lumbriculus* diet at swim-up; however, after several feeding attempts it was determined that the fish would not eat the *Lumbriculus* presumably because the worms were too large. After confirming with Newfields, it was decided to delay the start of the dietary study to allow the larval fish to get larger so they could eat the *Lumbriculus*. In the interim (test days 16-17), two different food types (freshly hatched brine shrimp nauplii and salmon starter #1) were offered to the larval fish. The diet of choice was the brine shrimp nauplii because we wanted the fish to get accustomed to eating a live food.

Larval fish were fed a diet of a concentrated slurry of newly hatched brine shrimp 3X per day (i.e., morning, noon, evening). The brine shrimp diet was offered to the swim-up fry once each day starting on Day 18 for two days and then going to three times a day until initiation of the dietary study. The volume typically consisted of either 0.75 ml or 1.0 ml three times per day, which amounted to 55.2 – 73.5 mg per chamber per day. Given the estimated weight of 0.119 g based on a subsample of the test organisms from the E reps in all treatments (measured at thinning on June 10, 2008; Appendix C), this translated to a 3.4 to 4.6% food ration per body (wet) weight of the fish until fish were fed the live *Lumbriculus* diet. The brine shrimp is analyzed for contaminants by lot; the results of the contaminant analysis are on file at ENSR.

The weight of an adult *Lumbriculus* was estimated at 5-12 mg each (ASTM 2006). Given that the plan was to feed fish at a 4% body weight ration, (i.e., 4.8 mg per fish), the initial feeding rate was calculated at 1 worm per every two fish (wet wt of fish = 0.119 mg). By test day 38 trial studies indicated that most fish were large enough to eat the *Lumbriculus* and; therefore, the dietary study was initiated (June 21, 2008). While we were concerned about the trout getting enough food (i.e., how two trout would share one worm), we were primarily concerned with having enough worms for the entire study because the worms had to be maintained for at least one month to reach the targeted Se dietary values (Besser et al., 2006). Changes in the feeding ration to YCT during the study are presented in the following table.

Table 2-1 Timeline of changes to feeding ration during the dietary portion of the Yellowstone Cutthroat trout ELS study

Date[a]	Day of dietary study	Overall test day	Feeding ration	Feeding ration (dwt)
6/21 -	0	38	1 worm / 2 fish	~5 mg / fish
6/24 -	3	41	1 worm / 1.5 fish	~6 mg / fish
6/27 -	6	44	1 worm / 1 fish	~10 mg / fish
6/30 -	9	47	2 worms / 1 fish	~20 mg / fish
7/1 -	10	48	5 worms / 1 fish	~50 mg / fish
7/2[b] -	11	49	2 worms / 1 fish	~20 mg / fish
7/11 -	20	58	2.5 worms / 1 fish	~25 mg / fish
7/14 -	23	61	3 worms / 1 fish	~30 mg / fish
7/17 -	25	64	4 worms / 1 fish	~40 mg / fish
7/22 – 7/24	30 – 32	69 – 72	5 worms / 1 fish	~50 mg / fish

[a]Feeding period started on the initial date and then continued until the subsequent date (i.e., line below).
[b]Observed overeating in fish which lead to ruptured stomachs; so reduced feeding to previous feeding level.

Chambers were siphoned daily once feeding was initiated, and typically all worms were eaten during the study. Any uneaten worms (only observed early on) were left in the test chambers and were subsequently eaten. During the study, we observed that the larger YCT were very territorial and would guard uneaten worms from other fish in the chamber. Because of this behavioral pattern and also because some of the fish were small and appeared to be underfed; we increased the feeding ration from 2 worms per 1 fish (per chamber) to 5 worms per fish (day 10 of dietary exposure). We thought that increasing the amount of available food would help curtail the spotty mortality that we were observing at this point in the study. After increasing the amount of food to this level, we noticed that some of the fish overate resulting in distended stomachs and in several cases ruptured stomachs, resulting in death. In other fish, *Lumbriculus* were observed sticking out of the fish's gill aperture (see adjacent figure). Our best explanation of this phenomenon was that the fish ate too fast or that the *Lumbriculus* were too large (long) for the larval YCT. Whatever the explanation, these fish tended to be found dead the next day, possibly due to gill damage (adjacent figure). Because of the concern for losing more fish due to these unanticipated feeding phenomena, we reduced the feeding rates to the previous level (Table 2-1). However, we made some slight modifications in attempts to ensure that organisms were getting enough food during this period. For smaller organisms that did not look like they were getting enough food, we isolated these organisms with their food to give them a chance to eat. Secondarily, we also supplemented dietary *Lumbriculus* feedings with additional feedings of brine shrimp nauplii. The idea was that while some of the larger fish could guard uneaten worms, adding food that dispersed within the test chamber and isolated feedings would help reduce the concern for malnourishment so that these smaller fish could return to the *Lumbriculus* diet.

Feeding rates were adjusted during the dietary portion of the study to account for the expected increase in fish size. By the end of the test, each fry was fed approximately 50 mg of worms per day (5 worms at 10 mg each), resulting in a feeding ration of ~9.0% (based on wet weight of control fish at test termination of 552.7 mg). As mentioned, feeding rates were based on the number of surviving fish in each chamber. Fish were not fed within 24 hours of test termination. Other pictures taken during the study are included in Appendix D.

2.7 Test Monitoring

After the hatching phase, alevins (recently hatched young with yolk sacs) were monitored daily for mortality which continued throughout the study. Dead organisms were removed and placed in Davidson's solution. As alevins approached swim-up, food was offered to the organisms to determine if they were actively feeding. The swim-up date was set based on absorption of the yolk sac and active feeding by at least 70% of the alevins. After the swim-up stage and before the dietary study had begun, a subset of fish (16) from the E replicates were sacrificed for measurement of whole-body selenium concentrations, dry weight (via freeze drying), and percent solids at Columbia Analytical Laboratories (CAS; Kelso, WA). One additional fish from each replicate was also sacrificed for wet weights to estimate feeding rates.

At the end of the dietary study (test day 71), five randomly selected organisms from each treatment were obtained for whole-body analysis of selenium (analysis was performed on individual fish). Fish were sacrificed using isopropyl alcohol, rinsed with deionized water, blotted dry, and then measured for standard lengths (tip of snout to caudal peduncle) and wet weights. These organisms were then frozen and sent via overnight courier to CAS for measurement of whole body selenium concentration, dry weight, and percent solids.

Remaining fish were sacrificed via immersion in isopropyl alcohol, rinsed with deionized water, blotted dry and measured for standard length. Wet weights were taken on a subset of organisms. Organisms were then preserved in Davidson's solution and saved for deformities assessment by CSU. After deformities assessment was performed on all these organisms, they were returned to the FCETL and then measured for dry weight. For dry weight analysis, each fish was transferred to a tared weight boat and dried at 100 °C for at least 48 hours. After removal from the drying oven, the weigh boats were placed in a dessicator to prevent absorption of moisture from the air, until weighed (dry weight) to the nearest 0.01 mg.

2.8 Analytical Chemistry

Temperature (°C), pH (s.u.), dissolved oxygen (mg/L) concentrations, and conductivity (μS/cm) were measured and recorded in one alternating chamber for each test treatment daily. Hardness (as mg/L $CaCO_3$) was measured from the dilutor panel or from a test chamber daily during the study. Sulfate concentration was measured from water collected from the dilutor panel or from test chambers. Determinations of waterborne sulfate concentrations were made at Paragon Analytics, Inc. (Fort Collins, Colorado, USA) using ion chromatography (EPA Method 300.0).

Water samples for total recoverable and "dissolved" selenium analyses were collected, prepared, and preserved from selected test chambers during the course of the study. Briefly, approximately 50 to 250 ml of test solution was collected for analysis of either dissolved or total selenium analysis. Aqueous analytical samples were analyzed at ACZ Laboratories (Steamboat Springs, CO). Dissolved selenium samples were filtered through 0.45 μm filters (GHP Acrodisc Syringe Filters, Pall Gelman Scientific, Ann Arbor, MI, USA) prior to placing in the polypropylene sample containers and preserved with 1% nitric acid. Samples were analyzed using an ICP-MS (EPA Method 200.8).

Tissue samples (*Lumbriculus* and trout) were sub-sampled and sent to CAS for analysis of total selenium concentration, percent solids, and dry weight.

A sub-sample of *Lumbriculus* was collected at test termination and sent to Warren Analytical Laboratory (Greeley, CO) for analysis of nutritional parameters including: fat, protein, carbohydrates, ash, moisture, and calories.

2.9 Deformities Assessment

All fry at the end of the study (excluding the 5 organisms selected for whole body Se analysis) were examined for deformities. Dead fish and alevins that had been removed during the study were not saved for deformity assessment because many of these organisms were in various states of decay

and did not preserve well. All samples for deformity analysis were sent to Dr. Kevin Bestgen at CSU. Data from these samples were incorporated in the deformity assessment performed.

2.10 Endpoints

Test endpoints that were utilized for this test include: survival (at different points during the study), growth (length and weight [wet and dry wt], condition factor, and whole-body selenium concentrations at two different times during the study. The condition factor was determined by dividing the mean wet weight (mg) per surviving fish by the cubed mean standard length (mm) per surviving fish and multiplying by 100. The first whole body selenium measurement was at thinning to measure pre-dietary influence (i.e., aqueous exposure only) and the second was at test termination to measure both dietary and aqueous selenium exposure. Deformities (craniofacial, skeletal, finfold, and edema) were also assessed in all organisms at test termination, except those sub-sampled for whole-body selenium analysis.

3.0 Results

3.1 Water Quality Analyses

The water quality parameters (pH, temperature, conductivity, and dissolved oxygen [DO]) monitored daily in alternating replicates from each treatment during the study were within acceptable ranges for the survival of Yellowstone cutthroat trout (Table 3-1).

Table 3-1 Water hardness (avg ± SD), dissolved oxygen (low and % saturation), pH (range), temperature (avg ± SD and range), and conductivity (range) measured in each treatment during the Yellowstone cutthroat trout (*Oncorhynchus clarki*) dietary study

Treatment	Water Hardness (mg/L)	Minimum Dissolved Oxygen (mg/L) & % Saturation	pH (s.u.)	Avg ± SD Temp (°C)	Temp. Range (°C)	Conductivity (µS/cm)
Control	46.0 ± 3.4	7.1 / 80	7.3 – 8.0	11.8 ± 1.2	9-14	101-164
2.5 µg/L	--	7.1 / 80	7.2 – 7.9	11.8 ± 1.1	9-14	100-132
5 µg/L	--	7.1 / 80	7.1 – 7.9	11.8 ± 1.2	9-14	100-140
10 µg/L	--	7.2 / 81	7.2 – 7.9	11.7 ± 1.1	9-14	102-132
15 µg/L	--	7.0 / 79	7.2 – 7.9	11.6 ± 1.1	9-13	97-133
20 µg/L	--	6.9 / 78	7.3 – 7.9	11.7 ± 1.1	9-14	99-127
40 µg/L	--	7.0 / 79	7.3 – 7.9	11.8 ± 1.1	9-14	97-125

Water hardness averaged 46.0 mg/L (as $CaCO_3$) in the source water for the study. Alkalinity was measured at least weekly in the laboratory Horsetooth dilution water and it averaged 30.4 ± 6.0 mg/L (as $CaCO_3$) between May 20, 2008 and July 22, 2008. A summary of the water quality data is included in Appendix E.

3.2 Aqueous Test Concentrations

Samples were collected for total and dissolved selenium analyses on Days 1, 8, 15, 23, 29, 36, 43, 50, 57, and 64. Time-weighted average concentrations for total recoverable and dissolved selenium concentrations are presented (Table 3-2). Measured total recoverable selenium concentrations were 101 to 112% of nominal concentrations, while measured dissolved values were 115 to 119% of nominal values (Table 3-2). This indicated that measured values were very similar to target aqueous concentrations. Dissolved values ranged from 107 to 116% of total recoverable selenium values, indicating strong agreement between dissolved and total recoverable values.

Additional samples were collected for specific treatments on days 13, 48, and 61 to capture possible differences in selenium concentration because the drip rate was outside of the target range on these days. Values were taken into account for the TWA concentration for the 20 (one event) and 40 (two events) µg/L nominal treatments. The slight decreases in selenium concentration did not have a

significant affect on the selenium concentration for these treatments. For instance, the calculated TWA values for the 20 and 40 µg/L treatments not including these out-of-range values were 20.4 and 41.4, respectively. Given the low impact on theses concentrations, the adjustments were not made to other concentrations or to dissolved selenium concentrations.

Initial reported values from the analytical laboratory for dissolved selenium concentrations through day 23 were 1.5 – 2.0X higher than total recoverable selenium concentrations. Because we believed these dissolved values to be suspect, we had the lab re-analyze a subset of these samples, which confirmed values closer to nominal and total recoverable values (i.e., that the initial values were too high). Unfortunately, the analytical laboratory disposed of the dissolved selenium samples from days 1 and 8 prior to re-analysis of all these suspect samples. Therefore, in order to use data from this time period, we developed a model to estimate dissolved selenium concentrations in these samples (Appendix F).

Table 3-2 Measured aqueous selenium concentration in the trout dietary study

Nominal Se Treatment (µg/L)	TWA Total Recoverable Se	% of Nominal	TWA Dissolved Se	% of Nominal	Diss./TR (%)
Control	0.1 U	--	0.1 U	--	--
2.5	2.8	112	3.0	118	107
5.0	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

Note: Values in the table were calculated and presented in the spreadsheet (Appendix F)

A summary of all the individual selenium measurements including min-max total recoverable and dissolved selenium values are included in Appendix F.

3.3 Dietary Test Concentrations

Selenium concentrations were measured in yeast treatments and in *L. variegatus* including small and large tanks. The average (±SD) selenium concentration in yeast and *L. variegatus* are presented below (Table 3-3).

Table 3-3 Measured selenium concentration in yeast and *Lumbriculus variegatus* during the trout dietary study

Target Se Conc. (µg/g) Yeast / L.v.	Avg. Se conc. in Yeast (µg/g dwt)	Avg. Se conc. in L.v. (µg/g dwt)
Control	<0.05	1.69 ± 0.39
5.0 / 2.5	1.115 ± 0.39	3.82 ± 0.39
10/ 5.0	4.34 ± 0.20	5.94 ± 0.14
20 / 10	11.53 ± 6.5	10.6 ± 0.81
30/ 15	23.95 ± 7.1	17.4 ± 3.0
40/ 20	37.1 ± 6.8	22.0 ± 0.28
80/ 40	85.15 ± 11.2	45.2 ± 2.7

Note: Target Se concentration in yeast was 2X higher than in *L. variegatus* (L.v.)

Measured selenium concentrations were lower than target values in most of the yeast diets but very similar to target values in *L. variegatus*. A summary of the analytical measurements for these samples are included in Appendix G, including specific concentrations in *Lumbriculus* from small and large tanks.

The nutritional content of the *Lumbriculus* was also measured to evaluate whether the selenium diet could have resulted in any potential differences to the trout due to nutritional differences in the diet. *L. variegatus* were measured for fat, protein, carbohydrates, ash, moisture and calories (Table 3-4).

Table 3-4 Measurement of nutritional parameters in *Lumbriculus variegatus* from different selenium treatments during the trout dietary study

Target treatment (µg/g Se)	Fat (g/100g)	Protein (g/100g)	Carbohydrate (g/100g)	Ash (g/100g)	Moisture (g/100g)	Calories
Control	2.17	8.14	1.27	1.68	86.74	57
2.5	0.85	7.62	7.78	1.90	81.85	69
5.0	< 0.8	8.33	3.76	1.54	86.37	48
10	< 0.8	8.13	5.09	1.70	85.08	53
15	< 0.8	9.10	4.61	1.78	84.51	55
20	0.89	8.72	3.32	1.23	85.84	56
40	0.81	8.71	2.67	1.37	86.44	53

Note: the sum of fat, protein, carbohydrates, ash, and moisture for each sample = 100 g. Therefore fat, protein, carbohydrates, and ash are expressed on a wet weight basis. The detection limit for fat was 0.8% (or g/100g).

Only one subsample of *L. variegatus* was taken for analysis of these parameters so slight differences observed (i.e., higher fat in the control organisms) could not be substantiated. Overall, values were similar among different selenium treatments. Raw data are included in Appendix G.

3.4 Trout Response

3.4.1 Survival

Survival of YCT at different stages of the study are presented below (Table 3-4). Survival at hatch may have been lower than expected because the eyed eggs were received late in the spawning

season. Survival from hatch was fairly consistent through thinning (±5.6%), day 27. At this point, survival averaged 74.7% and ranged from 67.9% to 79.3%.

Table 3-5 Percent survival of Yellowstone cutthroat trout at different stages during the aqueous-dietary selenium ELS study

Nominal Se Treatment (µg/L)	% Survival				
	Hatch (Day 6)	Swim-up (Day 22)	Thinning (Day 27)	Se Diet start (Day 38)	Termination (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
5.0	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

Survival at the start of the initiation of the selenium *L. variegatus* diet (test day 38) averaged 68.5% and ranged from 60.9 to 73.6% but by test termination ranged from 28.5 to 50.6%. Survival in individual replicates at test termination is presented in the table below (Table 3-6).

Table 3-6 Percent survival of Yellowstone cutthroat trout at test termination in individual replicates during the aqueous-dietary selenium ELS study

Nominal Se Treatment (µg/L)	% Survival (test termination)						
	Rep A	Rep B	Rep C	Rep D	Rep E	Overall Mean	Std. Dev.
Control	45	55	45	55	53	50.6	5.2
2.5	55	35	30	70	24	42.7	19.3
5.0	30	55	40	55	47	45.4	10.6
10	35	25	50	35	29	34.9	9.4
15	30	45	45	60	24	40.7	14.3
20	20	15	35	55	18	28.5	16.7
40	25	60	35	35	18	34.5	16.0

Percent survival and the actual number of fish alive at the various stages of the study are included in Appendix H). Control survival from hatch to test termination averaged 74%.

As discussed above, we believe that the lower survival was related to several factors including; obtaining eyed eggs late in the spawning season, using a non-standard test organism, territorial behavior of the trout (e.g., larger fish guarding excess food), and fish death due to overeating or from the *Lumbriculus* coming out the fish's gills. While these issues prevented higher survival rates, we believe that useful information can be gleaned from the organisms that did live in these experiments. This will be discussed in more detail below.

3.4.2 Growth

Dry weights were measured on all fish at termination, either at CAS or ENSR. Dry weight determinations were presented on a per surviving and on a per original weight basis. Dry weights on a per surviving weight basis exclude any potential survival effect, whereas dry weight

measurements on a per original basis take into account any potential survival and / or growth effects.

Table 3-7 Summary of dry weight per surviving number of Yellowstone cutthroat trout during the aqueous-dietary selenium ELS study

Nominal Se 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	79.40	81.17	82.52	4.86
2.5	71.31	71.76	78.64	64.78	77.65	72.83	5.59
5.0	87.94	69.64	76.22	68.42	81.59	76.76	8.20
10	65.73	80.75	67.72	78.25	89.80	76.45	9.89
15	90.30	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	87.40	11.9

Dry weights on a per surviving basis ranged from 72.83 to 87.40 mg per fish, with the highest weights observed in the highest selenium treatment.

Table 3-8 Summary of dry weight per original number of Yellowstone cutthroat trout during the aqueous-dietary selenium ELS study

Nominal Se 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
5.0	26.38	38.30	30.49	37.63	59.34	38.43	12.7
10	23.01	20.19	33.86	27.39	89.80	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	24.00	8.16
40	22.47	44.99	28.49	29.51	53.22	35.74	12.8

Mean dry weights on a per original basis ranged from 24.00 to 45.17 mg per fish, with the highest weights observed in the control treatment. A summary of dry weight measurements on a per surviving and per original basis is included in Appendix I.

3.4.3 Wet Weight, Length, and Condition Factor

Wet weight of fish at termination was measured for a subsample of fish primarily in organisms sent to CAS for whole-body selenium concentrations. Length determinations were made on all surviving fish at test termination. Condition factor could only be calculated on fish for which both wet weight and length were measured.

Table 3-9 Summary of standard lengths per surviving number of Yellowstone cutthroat trout during the aqueous-dietary selenium ELS study

Nominal Se Treatment (µg/L)	Standard Length (mm)						
	Rep A	Rep B	Rep C	Rep D	Rep E	Overall Mean	Std. Dev.
Control	34.0	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
5.0	33.3	31.3	32.5	31.6	34.2	32.6	1.24
10	30.4	33.6	31.2	32.1	34.0	32.3	1.53
15	34.7	31.8	32.2	31.0	33.0	32.5	1.40
20	31.0	34.3	31.6	31.6	34.7	32.6	1.72
40	33.0	33.3	33.6	33.6	35.3	33.8	0.91

Mean standard length ranged from 32.3 to 33.8 mm, with the longest average fish observed in the highest Se treatment.

Table 3-10 Wet weights and condition factor for a subset of Yellowstone cutthroat trout during the aqueous-dietary selenium ELS study

Nominal Se Treatment (µg/L)	Avg Wet wt (g)	Overall Condition Factor	n
Control	0.5527	1.49	16
2.5	0.4810	1.10	5
5.0	0.4329	1.12	6
10	0.5494	1.32	5
15	0.4573	1.28	5
20	0.5867	1.38	5
40	0.5697	1.29	5

Wet weights and condition factors were performed on a limited number of organisms. Therefore, these values are provided for general informational purposes. A summary of these measurements can be found in Appendix I.

3.4.4 Whole Body Trout Selenium Concentration

Whole body selenium concentration in Yellowstone cutthroat trout was measured in a subset of organisms on day 27 (at thinning, prior to introduction of selenium diet) and at test termination. The results of these analyses are presented below (Table 3-11).

Table 3-11 Summary of whole body selenium concentrations measured in Yellowstone cutthroat trout prior to dietary selenium exposure (test day 27) and at test termination (test day 71)

Nominal Se Treatment (µg/L)	Whole Body fish concentration	
	Avg. Se conc. (µg/g dwt) Day 27	Avg. Se conc. (µg/g dwt) Day 71
Control	1.50 ± 0.051	1.44 ± 0.42
2.5	1.47 ± 0.235	2.65 ± 0.62
5.0	1.70 ± 0.020	4.46 ± 0.57
10	1.67 ± 0.071	5.40 ± 1.72
15	1.84 ± 0.208	14.77 ± 6.83
20	1.71 ± 0.029	12.90 ± 1.85
40	1.71 ± 0.145	34.48 ± 7.38

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

Selenium exposure measured at day 27 resulted in a very low and flat response across all aqueous selenium treatments. Whole-body selenium in these treatments ranged from 1.47 to 1.84 µg/g dwt. For fish measured at test termination, whole-body selenium concentrations in the control were similar to measurements at day 27, but increased to an average of 34.48 µg/g dwt in the highest selenium treatment. From these results, it is clear that the body burden of selenium increased in YCT due to dietary selenium exposure from *Lumbriculus*. A summary of the analytical results associated with these data is included in Appendix J.

3.4.5 Deformity Assessment

Results of the deformity assessment are included in Appendix K. This assessment included categorization of individual fish into one or more types of deformities (i.e., cranio-facial, skeletal, fin or finfold, or edema) if present and ranking the severity of deformity. Specific ranking methods are presented together with a summary of the scoring for each fish in each treatment. Finally, a series of summary graphics showing the percentage of fish in each treatment for the deformity type are shown by deformity severity. Generally, very few fish showed any type of deformity, and those that did, were ranked as slightly deformed.

3.5 Lessons Learned

While the overall survival of the fish in this study was less than desirable, there were several factors that, in hindsight, may have contributed to this.

- The test species (YCT) used in this dietary study at hatch were smaller than other species used in dietary studies which used older, larger fish (~0.3 g)
- Trout are territorial fish and this caused feeding competition within chambers (compared to other species)
- Increased number of fish per chamber, although within appropriate loading rates, may have lead to increased competition and negatively impacted survival
- YCT death due to gorging (stomach rupturing through the body wall) was observed when feeding rates were increased

- Prey / food size to alevin size appears to have contributed to death due to (possible) gill damage from *Lumbriculus* coming out the YCT gill opercula after eating
- A recommended feeding rate of 4% was utilized (ASTM 2006), other studies utilized a feeding rates of 4 – 20%

The results of this study provide valuable data. Rationale for this follows.

- Results at test end were based on ~ 40 fish per treatment
- Aqueous selenium exposures were achieved for the test duration and ranged from 3.0 to 47.4 µg/L (as dissolved Se)
- Whole-body selenium concentrations in *Lumbriculus* approached target values, ranging from 3.82 to 45.2 µg/g dwt
- Whole-body selenium concentrations in YCT at day 27 following aqueous only exposure ranged from 1.47 to 1.84 µg/g dwt
- Whole-body selenium concentration in YCT at day 71 following dietary and aqueous exposure ranged from 2.65 to 34.48 µg/g dwt
- Aqueous and dietary exposures in this study are consistent with the literature that demonstrate dietary exposure is the primary pathway of concern for selenium
- The lack of effect from YCT exposure to selenium in the ELS study supports data generated in the parental Se transfer study (although the modes of exposure were different).

4.0 References

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Appendix A

Initial selenium concentration in yeast samples used to prepare diets for *Lumbriculus*

Paragon Analytics

TOTAL METALS CASE NARRATIVE

ENSR Consulting and Engineering

YCT diet -- 12699-002

Order Number - 0804209

1. This report consists of 2 solid samples.
2. The samples were received intact at ambient temperature on 04/22/08.
3. The samples were prepared for analysis based on SW-846, 3rd Edition procedures.

   For analysis by Trace ICP, the samples were digested following method 3050B and PA SOP 806 Rev. 13.
4. The samples were analyzed following SW-846, 3rd Edition procedures.

   Analysis by Trace ICP followed method 6010B and PA SOP 834 Rev. 7.

   The relationship between intensity and concentration for each element is established using at least four standards, one of which is a blank solution.

   During sample analysis concentrations are computed by the software and the results are printed in mg/L. The instrument software does not provide a printout which gives both intensity and concentration. The validity of the calibration equation is tested by analyzing the following solutions: a blank, a low level check solution with concentrations near the reporting limit, an Initial Calibration Verification (ICV) standard from a 2nd source standard solution with concentrations near the middle of the analytical range, a Continuing Calibration Verification (CCV) standard with concentrations at two times those in the ICV, and a readback of the highest calibration standard.

   These solutions provide verification that the calibration equations are functioning properly throughout the analytical range of the instrument. During sample analysis dilutions are made for analytes found at concentrations above the highest calibration standard. No results are taken from extrapolations beyond the highest standard.
5. All standards and solutions are NIST traceable and were used within their recommended shelf life.
6. The samples were prepared and analyzed within the established hold times.

All in house quality control procedures were followed, as described below.

7. General quality control procedures.
   - A preparation (method) blank and laboratory control sample were digested and analyzed with the samples in this digestion batch. There were not more than 20 samples in the digestion batch.
   - The preparation (method) blank associated with this digestion batch was below the practical quantitation limit for the requested analyte.
   - The laboratory control sample associated with this digestion batch was within the acceptance limits. This indicates complete digestion according to the method.
   - All initial and continuing calibration blanks associated with this analytical batch were below the practical quantitation limits for the requested analyte.
   - All initial and continuing calibration verifications associated with this analytical batch were within the acceptance criteria for the requested analyte. This indicates a valid calibration and stable instrument conditions.
   - The interference check samples and high standard readbacks associated with Method 6010B analyses were within acceptance criteria.
8. Matrix specific quality control procedures.

   Per method requirements, matrix QC was performed for this analysis. Since a sample from this order number was not the selected quality control (QC) sample, matrix specific QC results are not included in this report.
9. Sample 0804209-2 required a dilution to bring selenium into the analytical range of the Trace ICP.

The data contained in the following report have been reviewed and approved by the personnel listed below. In addition, Paragon Analytics certifies that the analyses reported herein are true, complete and correct within the limits of the methods employed.

Emily Knodel — 04-23-08
Emily Knodel — Date
Inorganics Primary Data Reviewer

Inorganics Final Data Reviewer — 4/23/08
Date

Inorganic Data Reporting Qualifiers

The following qualifiers are used by the laboratory when reporting results of inorganic analyses.

- Result qualifier -- A “B” is entered if the reported value was obtained from a reading that was less than the Practical Quantitation Limit but greater than or equal to the Method Detection Limit (MDL). If the analyte was analyzed for but not detected a “U” is entered.

- QC qualifier -- Specified entries and their meanings are as follows:

  E - The reported value is estimated because of the presence of interference. An explanatory note may be included in the narrative.

  M - Duplicate injection precision was not met.

  N - Spiked sample recovery not within control limits. A post spike is analyzed for all ICP analyses when the matrix spike and or spike duplicate fail and the native sample concentration is less than four times the spike added concentration.

  Z - Spiked recovery not within control limits. An explanatory note may be included in the narrative.

  * - Duplicate analysis (relative percent difference) not within control limits.

  S - SAR value is estimated as one or more analytes used in the calculation were not detected above the detection limit.

Paragon Analytics

Sample Number(s) Cross-Reference Table

Paragon OrderNum: 0804209
Client Name: ENSR Consulting and Engineering
Client Project Name: YCT diet
Client Project Number: 12699-002
Client PO Number: 2055116

Client Sample Number	Lab Sample Number	COC Number	Matrix	Date Collected	Time Collected
Nutritional yeast	0804209-1		SOLID	22-Apr-08	12:30
Se-yeast	0804209-2		SOLID	22-Apr-08	12:30

0804209

ENSR INTERNATIONAL

CHAIN OF CUSTODY RECORD

Page ____ of ____

Client/Project Name: (JR Simplot)

Project Location: FCETL

Project Number: 12699-002

Field Logbook No.:

Sampler (Print Name)/(Affiliation): BJN/AM

Chain of Custody Tape No.:

Signature:

Send Results/Report to: ~~[illegible]~~ A. Rohner

Analysis Requested: Total Se (as rec'd)

	Field Sample No./ Identification	Date	Time	Grab	Comp	Sample Container (Size/Mat'l)	Sample Type (Liquid, Sludge, Etc.)	Preservative	Field Filtered	Total Se (as rec'd)	Lab I.D.	Remarks
1)	National yeast	4/22/08	1230	X		50 ml	solid	~~ice~~		X	Lot# F08-007	
2)	Se yeast	4/22/08	1230	X		50 ml	↓			X	F08-008	

Relinquished by: (Print Name)/(Affiliation)	Date	Received by: (Print Name)/(Affiliation)	Date
Jennifer Niswender, ENSR Signature: [signature]	4/22/08 Time: 1250	[signature] Signature: Shari Lafferty	4.22.08 Time: 1250
Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:	Received by: (Print Name)/(Affiliation) Signature:	Date: Time:
Relinquished by: (Print Name)/(Affiliation) Signature:	Date: Time:	Received by: (Print Name)/(Affiliation) Signature:	Date: Time:

Analytical Laboratory (Destination):

ENSR Toxicology Lab
4303 W. Laporte Avenue
Fort Collins, CO 80521
(970) 416-0916
(970) 493-8935 (FAX)

Paragon

Serial No. 47038

5

FM01008b_902

CONDITION OF SAMPLE UPON RECEIPT FORM

Paragon Analytics

Client: ENSR Workorder No: 0804209

Project Manager: DF Initials: SL Date: 4-22-08

1. Does this project require any **special handling** in addition to standard Paragon procedures?		YES	(NO)
2. Are custody **seals** on **shipping containers** intact?	(NONE)	YES	NO
3. Are Custody seals on **sample containers** intact?	(NONE)	YES	NO
4. Is there a **COC (Chain-of-Custody) present** or other representative documents?		(YES)	NO
5. Are the **COC and bottle labels complete and legible**?		(YES)	NO
6. Is the **COC in agreement** with samples received? (IDs, dates, times, no. of samples, no. of containers, matrix, requested analyses, etc.)		(YES)	NO
7. Were **airbills / shipping documents** present and/or removable?	(DROP OFF)	YES	NO
8. Are all aqueous **samples requiring preservation preserved correctly?** (excluding volatiles)	(N/A)	YES	NO
9. Are all aqueous **non-preserved samples pH 4-9**?	(N/A)	YES	NO
10. Is there **sufficient sample** for the requested analyses?		(YES)	NO
11. Were all samples placed in the **proper containers** for the requested analyses?		(YES)	NO
12. Are all samples within **holding times** for the requested analyses?		(YES)	NO
13. Were all sample containers received **intact**? (not broken or leaking, etc.)		(YES)	NO
14. Are all samples requiring no headspace (VOC, GRO, Rx CN/S, radon), headspace free? **Size of bubble**: ____ < green pea ____ > green pea	(N/A)	YES	NO
15. Were samples checked for and free from the presence of **residual chlorine?** (Applicable when PM has indicated samples are from a chlorinated water source; note if field preservation with sodium thiosulfate was not observed.)	(N/A)	YES	NO
16. Were the samples **shipped on ice**?		YES	(NO)
17. Were cooler temperatures measured at 0.1-6.0°C?		YES	(NO)

IR gun used*:	#2	#4	RAD ONLY
Cooler #:	1		
Temperature (°C):	Amb		
No. of custody seals on cooler:	Ø		
DOT Survey/ Acceptance Information — External µR/hr reading:	NA		
Background µR/hr reading:	12		

Were external µR/hr readings ≤ two times background and within DOT acceptance criteria? (YES) / NO / NA (If no. see Form 008.)

Additional Information: PROVIDE DETAILS BELOW FOR A NO RESPONSE TO ANY QUESTION ABOVE. EXCEPT #1 AND #16

Samples received ambient

If applicable, was the client contacted? YES / NO / (NA) Contact: _____ Date/Time: _____

Project Manager Signature / Date: Debbie Fogio 4/22/08

*IR Gun #2: Oakton, SN 29922500201-0066 *IR Gun #4: Oakton, SN 2372220101-0002

Form 201r20.xls (12/27/07)

Total SELENIUM

Method SW6010

Sample Results

Lab Name: Paragon Analytics
Client Name: ENSR Consulting and Engineering
Client Project ID: YCT diet 12699-002
Work Order Number: 0804209
Reporting Basis: As Received
Final Volume: 100 ml
Matrix: SOLID
Result Units: mg/kg

Client Sample ID	Lab ID	Date Collected	Date Prepared	Date Analyzed	Percent Moisture	Dilution Factor	Result	Reporting Limit	MDL	Flag	Sample Aliquot
Nutritional yeast	0804209-1	4/22/2008	4/22/2008	04/23/2008	N/A	1	1.9	0.5	0.26		1.01 g
Se-yeast	0804209-2	4/22/2008	4/22/2008	04/23/2008	N/A	5	580	2.5	1.3		1.01 g

Comments:

1. ND or U = Not Detected at or above the client requested detection limit.

Data Package ID: *IT0804209-1*

ICP Metals

Method SW6010B
Method Blank

Lab Name: **Paragon Analytics**
Work Order Number: 0804209
Client Name: ENSR Consulting and Engineering
ClientProject ID: YCT diet 12699-002

Lab ID: IP080422-4MB

Sample Matrix: SOIL
% Moisture: N/A
Date Collected: N/A
Date Extracted: 22-Apr-08
Date Analyzed: 23-Apr-08

Prep Batch: IP080422-4
QCBatchID: IP080422-4-1
Run ID: IT080423-2A4
Cleanup: NONE
Basis: N/A
File Name: 080423A.

Sample Aliquot: 1 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1

CASNO	Target Analyte	DF	Result	Reporting Limit	MDL	Result Qualifier	EPA Qualifier
7782-49-2	SELENIUM	1	0.5	0.5	0.27	U	

Data Package ID: *IT0804209-1*

ICP Metals

Method SW6010B

Laboratory Control Sample

Lab Name: **Paragon Analytics**
Work Order Number: 0804209
Client Name: ENSR Consulting and Engineering
ClientProject ID: YCT diet 12699-002

Lab ID: IP080422-4LCS

Sample Matrix: SOIL
% Moisture: N/A
Date Collected: N/A
Date Extracted: 04/22/2008
Date Analyzed: 04/23/2008
Prep Method: SW3050B

Prep Batch: IP080422-4
QCBatchID: IP080422-4-1
Run ID: IT080423-2A4
Cleanup: NONE
Basis: N/A
File Name: 080423A.

Sample Aliquot: 1 g
Final Volume: 100 ml
Result Units: mg/kg
Clean DF: 1

CASNO	Target Analyte	Spike Added	LCS Result	Reporting Limit	Result Qualifier	LCS % Rec.	Control Limits
7782-49-2	SELENIUM	200	180	0.5		90	80 - 120%

Data Package ID: *IT0804209-1*

Appendix B

Select Photographs of Different Phases of the *Lumbriculus* Exposure System

Photo 1: Obtaining organisms from culture tanks of *Lumbriculus variegatus* (blackworm) to feed to Yellowstone cutthroat trout in the early life stage (ELS) study.

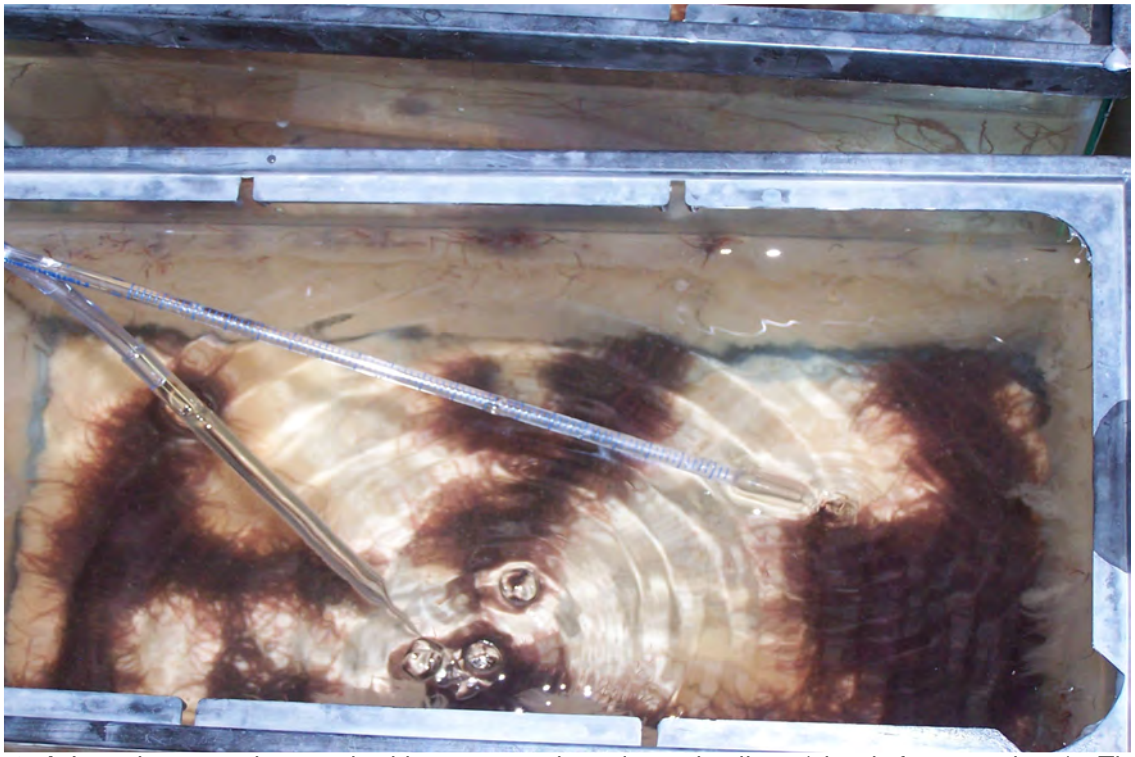


Photo 2: A *L. variegatus* culture tank with water supply and aeration lines (view is from top down). The dark masses are clumps of *L. variegatus*.

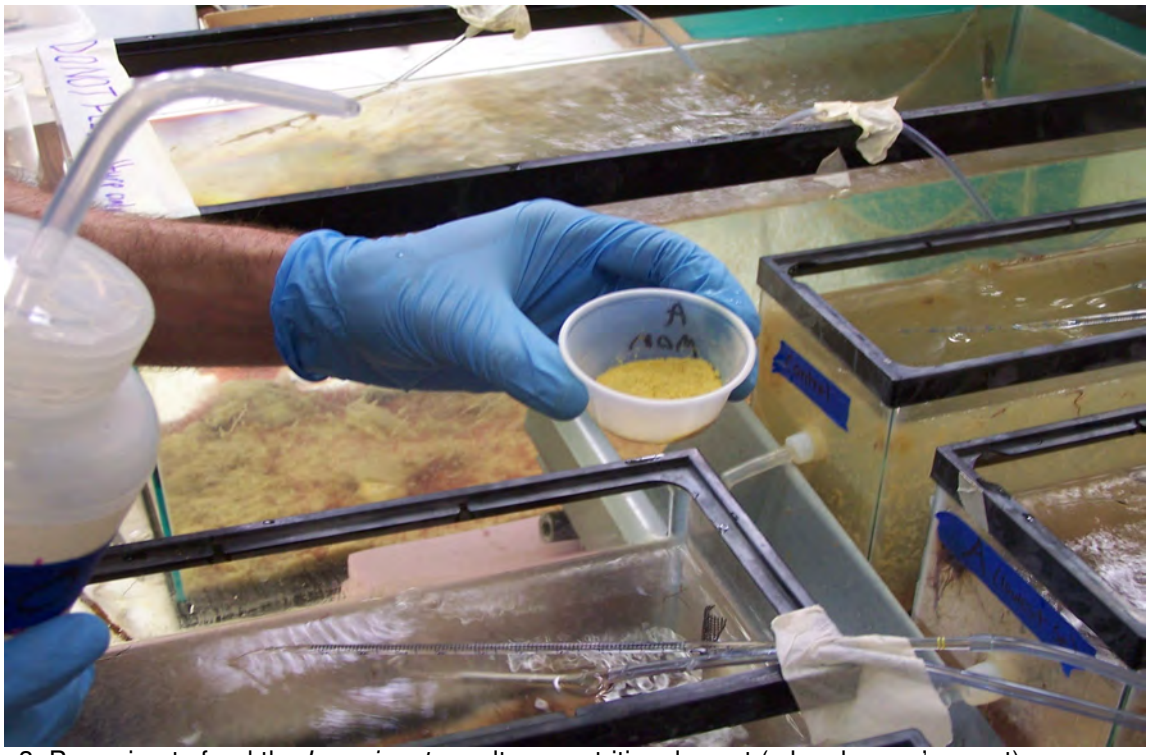


Photo 3: Preparing to feed the *L. variegatus* cultures nutritional yeast (a.k.a. brewer's yeast).

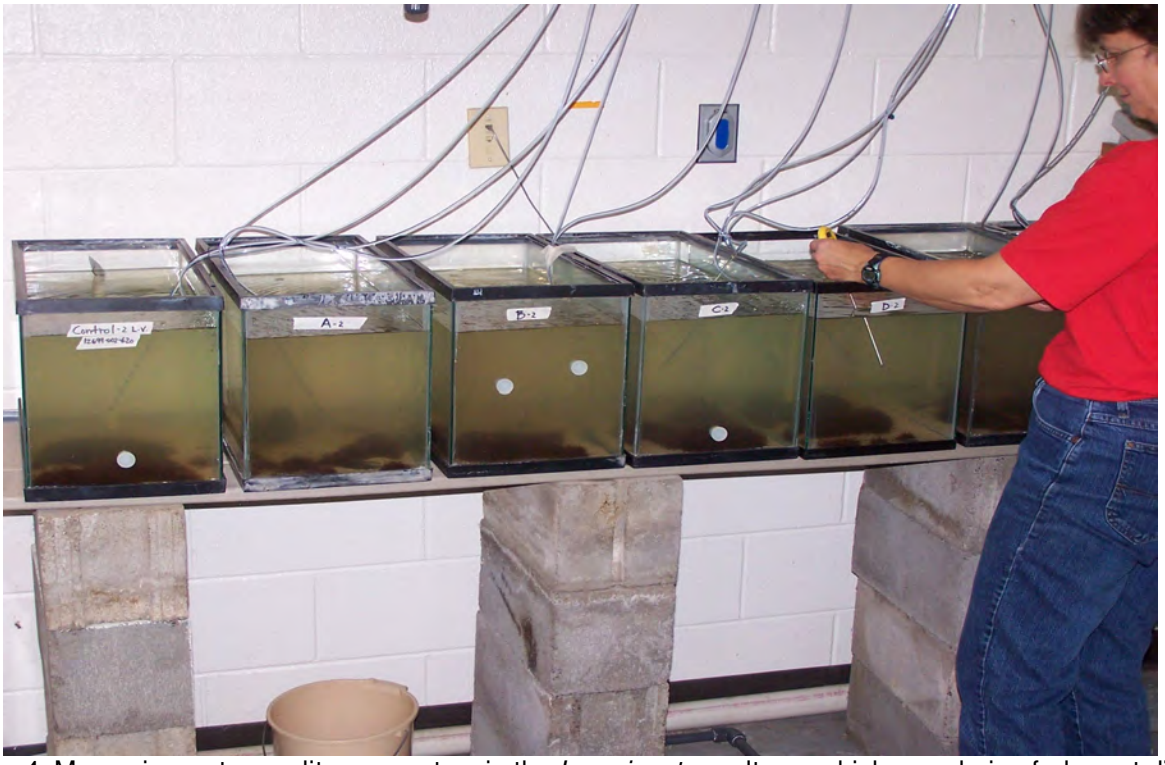


Photo 4: Measuring water quality parameters in the *L. variegatus* cultures which were being fed yeast diets with a range of selenium concentrations.

Appendix C

Wet Weight of Juvenile Yellowstone Cutthroat Trout at Thinning (Test Day 27) and Wet Weight Determination of Brine Shrimp

TEST ORGANISM LENGTHS, WEIGHTS, AND LOADING

Day 27

Project Number: 12699-002-610
Test Substance: Se
Species: ~~S. trutta~~ O. clarki
Analyst Tare: AMC Analyst Gross: AMC
Date/Time of Tare Wt.: 6/10/08 @ 1440
Date/Time of Gross Wt.: 6/10/08 1510
Comments:
Analytical Balance ID: Sart #1
Dried in Oven # ___ from Date: _____ Time: _____
to Date: _____ Time: _____

Weight Type (Circle): Wet Blot Dry Dry (>100°C) AFDW (>500°C)
Lot or Batch Number:

Boat No.	Treatment	Rep.	Length Units:	Tare Weight (g)	Gross Weight (g)	Net Weight (g)	Adjusted Net Weight (g)[1]	No. of Orig. Organisms	Mean Wt. per Original Organism (mg)	Mean Wt. per Treatment (mg) (Original)	No. of Surv. Organisms	Mean Wt. per Surviving Organism (mg)	Mean Wt. per Treatment (mg) (Surviving)
	Cont.	E		2.67412	2.79631	0.12219		1					
	2.5	E		2.63877	2.75671	0.11794		1					
	5	E		2.56195	2.67180	0.10985		1					
	10	E		2.58046	2.70503	0.12407		1					
	15	E		2.65175	2.77122	0.11947		1					
	20	E		2.61817	2.73758	0.11941		1					
	40	E		2.67862	2.79900	0.12038		1					
					K=	0.11919			119.19				
Blank													
Range													
Mean													

Test Solution Volume:
Loading Rate:

[1] Add in weight loss of blank boat, if appropriate.

AMC 6/10/08 @

FCETL QA Form No. 094
Revision 0
Effective 06/95

0.75 ml of BS/H2O on each filter
air dried overnight
AR 10/16/09

RAINBOW TROUT LENGTHS, WEIGHTS, AND LOADING OF CONTROL ORGANISMS

Project Number: 12699-002	Test Substance: BS.	Comments:
Species: Brine shrimp nauplii	Analyst:	
Date/Time of Measurements: air dried on filter paper for 5 min on		10/15/09 to 9am on 10/16/09
Weight Type (Circle): Wet Blot Dry ~~Dry (>100°C)~~ AFDW (>500°C) Other:		Lot Number:
Measurement Type (Circle): Pre-weight Post-weight		Organism age: < 24 h

No.	Treatment/ Replicate	Length (mm)	Net Weight (g) (w/o)	No.	Treatment/ Replicate	Length (mm)	Net Weight (g) (w/ BS)	No.	Treatment/ Replicate	Length (mm)	Net Weight (g)
1	Control A		~~0.0~~	1	Control B			1	Control C		
2			0.7070	2			0.7397	2			
3	mod hard only		0.7184	3	Brine shrimp nauplii only		0.7171	3			
4			0.7150	4			0.7388	4			
5				5				5			
6			$\bar{X}$= 0.7135	6			$\bar{X}$= 0.7319	6			
7				7				7			
8				8	diff =	0.0184 g		8			
9				9				9			
10				10				10			

Length Calculations	
Max:	Min:
Mean:	
Ratio (long/short):	

Weight Calculations	
Max:	Min:
Mean:	
Test Solution Volume:	Loading Rate (g/L):

RJN 10/16/09
AR 10/16/09

Determination of Brine shrimp nauplii wet weights.

squirted 0.75 ml (3 x 0.25 ml) of MH (blk) or brine shrimp slurry onto filter paper
let water evaporate overnight (5 pm on 10/15 to 9 am on 10/16), then weighed filter paper

water blanks	(g)	brine shrimp	(g)
1	0.7070		0.7397
2	0.7184		0.7171
3	0.7150		0.7388
avg	0.7135		0.7319

Difference 0.0184 g
18.4 mg per 0.75 ml

DZN 10/16/09
AR 10/16/09

Determination of wet wt feeding rates for YCT fed brine shrimp nauplii

wet wt of brine shrimp ~~(per 0.75 ml)~~ ①: 18.4 mg / 0.75 ml 24.5 mg / 1 ml of slurry

wet wt of BS per feeding rate 3X daily 55.2 mg / 0.75 ml 73.5 mg / 1 ml of slurry

avg wet wt (per fish) of YCT at thinning 119.19 mg

474 fish alive on D 28 (6/11/08), day after thinning, 35 tanks
13.5 avg # fish / chamber

1614.2 biomass (mg) of fish / chamber

3.42% food ration (wet wt BS /wet wt fish) when feeding 0.75 ml BS / chamber 3X daily

4.55% food ration (wet wt BS /wet wt fish) when feeding 1.0 ml BS / chamber 3X daily

① DZN 10/16/09 E

Appendix D

Select Photographs of Different Phases of the Yellowstone Cutthroat Trout Dietary Study

Photo 1: Carefully unwrapping, upon arrival, the eyed-up Yellowstone cutthroat eggs received from Henry's Lake Fish Hatchery.

Photo 2: Impartially distributing the eyed-up eggs into the egg cups using a bulb and fire-polished pipette. Eggs were added five at a time per cup until the required number was achieved.

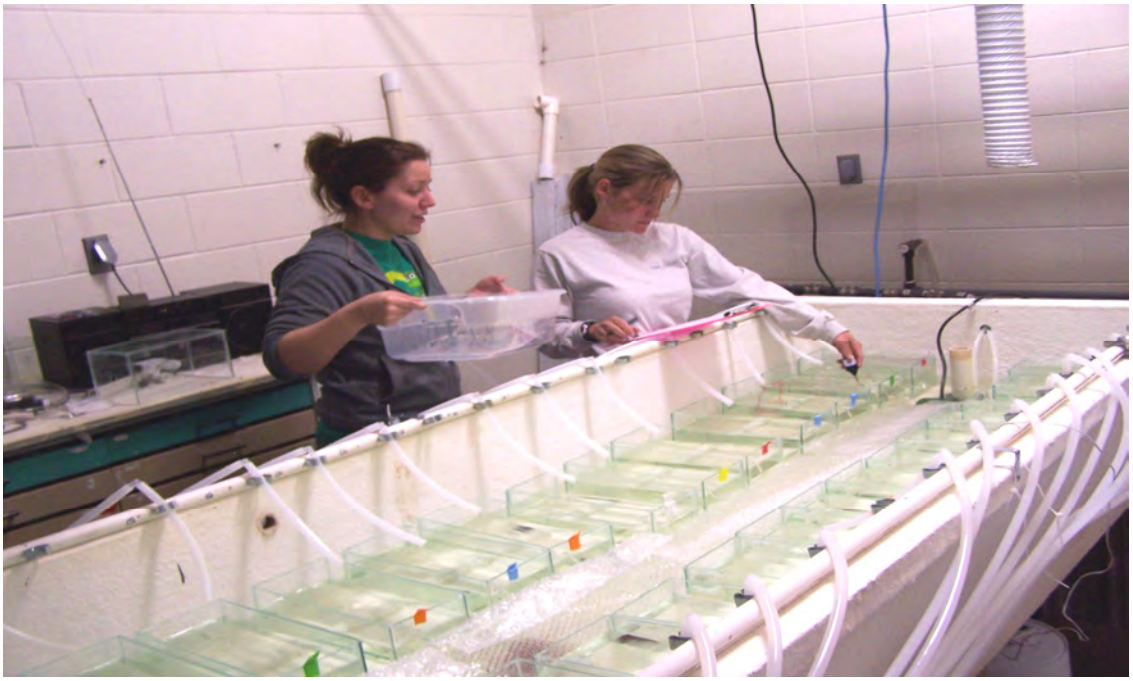


Photo 3: Measuring water quality parameters in test chambers while placing egg cups (with eggs) into the randomly assigned test chambers.

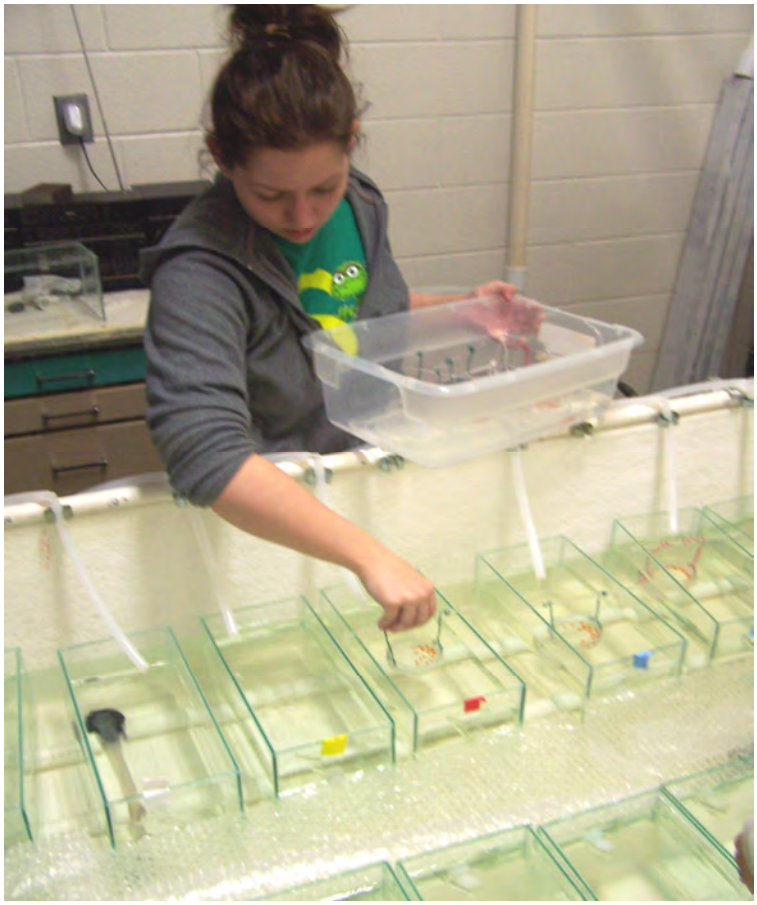


Photo 4: Distributing the egg cups into the test chambers at test initiation.

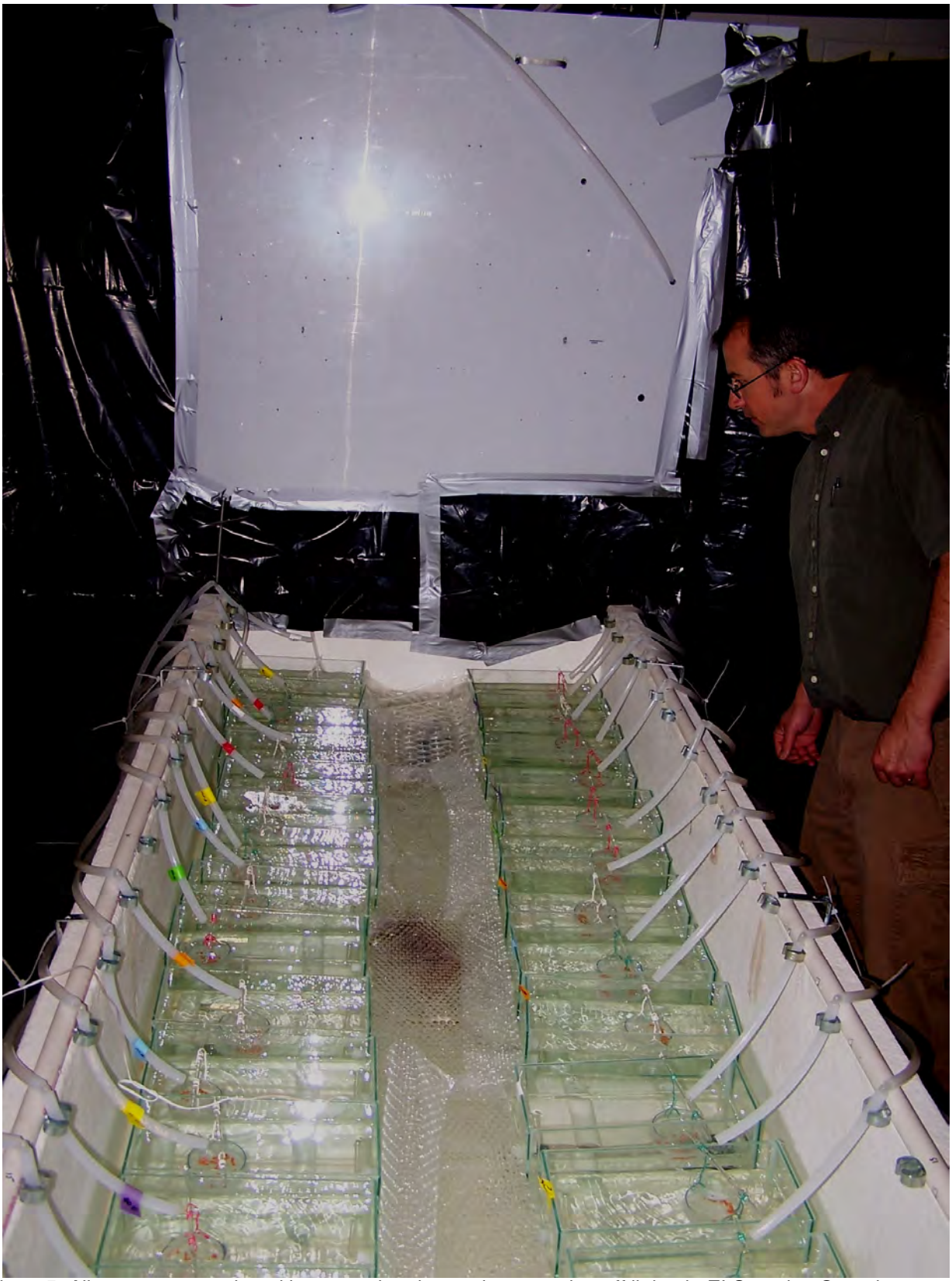


Photo 5: All egg cups are placed into test chambers prior to turning off lights in ELS study. Organisms remained in the dark from test initiation until swim-up stage.

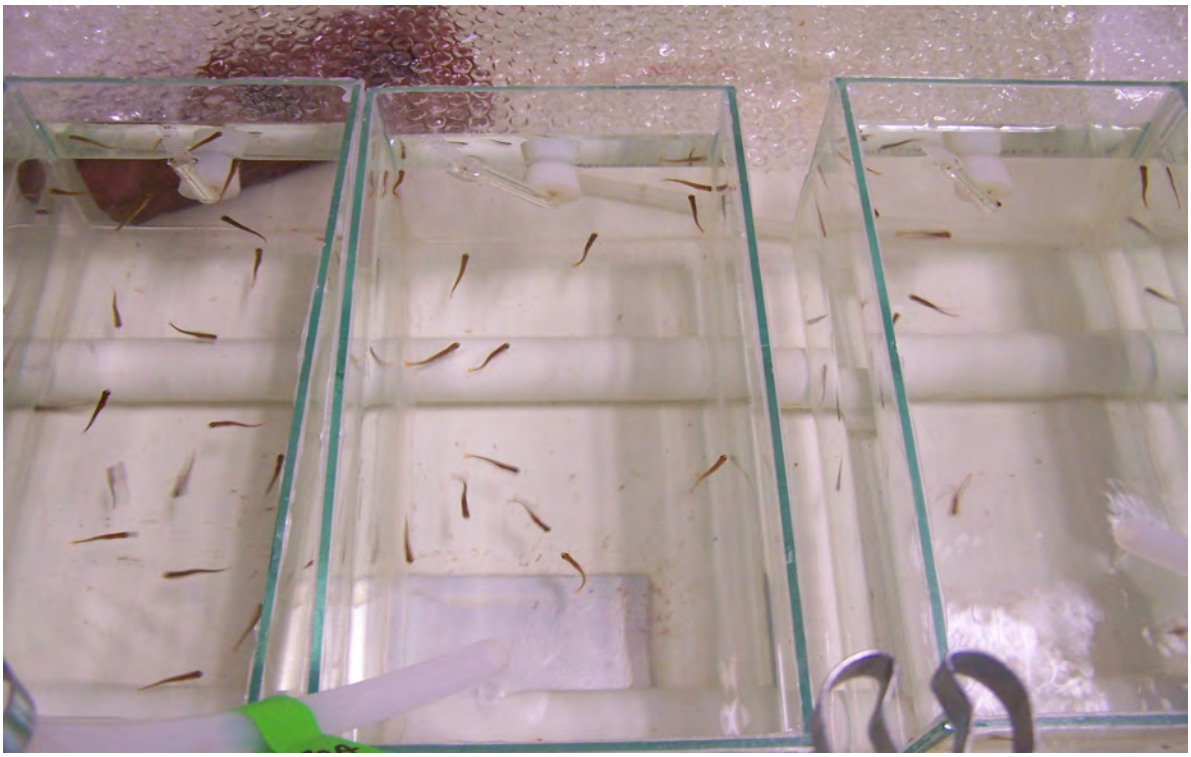


Photo 6: Test chambers in the ELS study with Yellowstone cutthroat trout.

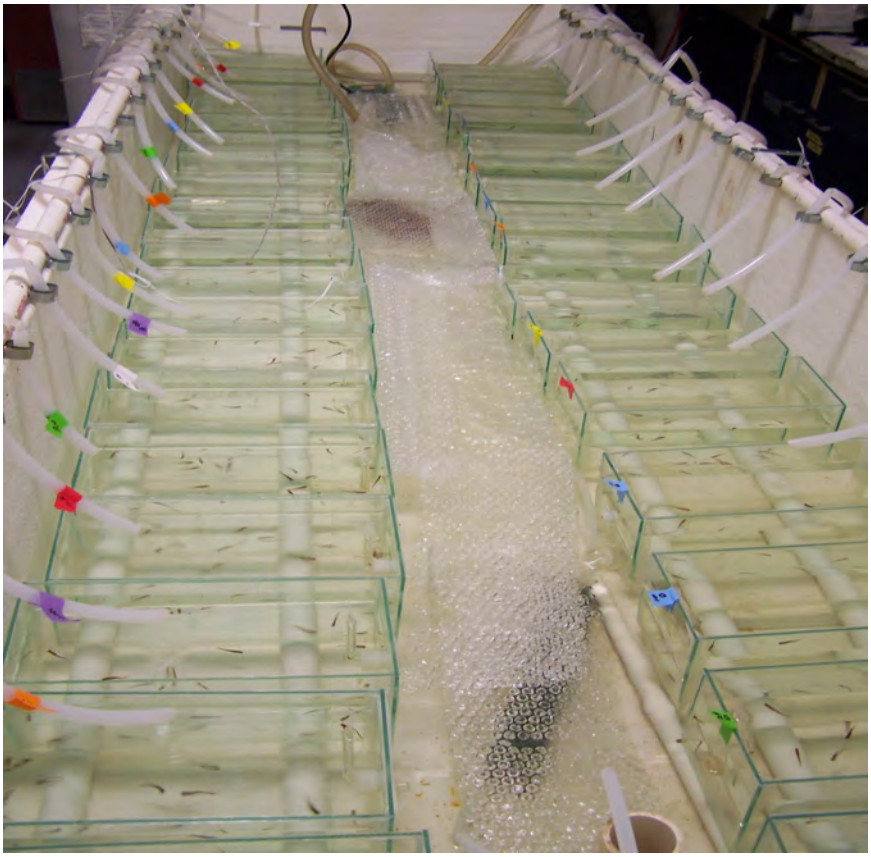


Photo 7: All test chambers.

Photo 8: A close-up of two Yellowstone cutthroat trout in their test chamber.

Photo 9: An example of some of the observed size differences among fish in the same chamber.

Photo 10: Yellowstone cutthroat trout feeding on *Lumbriculus variegatus*.

Photo 11: Another photo of the fish feeding on blackworms (*L. variegatus*).

Photo 12: The fish in the center had a blackworm coming out of its gill.

Photo 13: A test chamber with fish after feeding on *L. variegatus*.

Photo 14: Removing fish from their test chamber at termination. Organisms were either sacrificed for whole-body selenium analysis or measured for standard length and wet weight and then frozen for further analyses.

Photo 15: Euthanized Yellowstone cutthroat trout at termination of the early life stage study.

Appendix E

Summary of Water Quality Data Measured During the Yellowstone Cutthroat Trout Dietary Study

pH & D.O. measurements in Yellowstone Cutthroat trout study (12699-002) Controls

filename:pH-DO.xls

Date	Test Day	Water pH		D.O.		Conductivity	
14-May	0	NM		NM		NM	
15-May	1	7.8		7.8		114	
16-May	2	7.8		7.6		116	
17-May	3	7.8		7.4		120	
18-May	4	7.8		7.2		110	
19-May	5	7.8		7.3		101	
20-May	6	7.7		7.4		129	
21-May	7	7.7		7.4		112	
22-May	8	7.5		7.4		119	
23-May	9	7.6		7.5		118	
24-May	10	8.0		7.4		121	
25-May	11	7.8		7.6		107	
26-May	12	7.8		7.8		109	
27-May	13	7.8		7.8		118	
28-May	14	7.6		7.6		134	
29-May	15	7.6		7.7		120	
30-May	16	7.7		7.7		116	
31-May	17	7.8		7.8		107	
1-Jun	18	7.8		7.7		115	
2-Jun	19	7.6		7.6		117	
3-Jun	20	7.6		7.5		129	
4-Jun	21	7.4		7.5		122	
5-Jun	22	7.7		7.6		133	
6-Jun	23	7.8		7.9		134	
7-Jun	24	7.9		7.8		117	
8-Jun	25	7.4		7.7		128	
9-Jun	26	7.4		7.8		112	
10-Jun	27	7.3		7.6		108	
11-Jun	28	7.4		7.9		119	
12-Jun	29	7.3		8.0		102	
13-Jun	30	7.3		7.6		118	
14-Jun	31	7.7		7.7		120	
15-Jun	32	7.9		7.4		123	
16-Jun	33	7.6		7.6		124	
17-Jun	34	7.5		7.6		126	
18-Jun	35	7.5		7.6		121	
19-Jun	36	7.5		7.6		148	
20-Jun	37	7.6		7.5		113	
21-Jun	38	7.8		7.6		109	
22-Jun	39	7.9		7.6		112	
23-Jun	40	7.7		7.4		120	
24-Jun	41	7.6		7.6		122	
25-Jun	42	7.6		7.6		119	
26-Jun	43	7.6		7.7		120	
27-Jun	44	7.8		7.5		125	
28-Jun	45	7.8		7.1		125	
29-Jun	46	7.9		7.5		115	
30-Jun	47	7.6		7.5		129	
1-Jul	48	7.6		7.2		134	
2-Jul	49	7.7		7.2		123	
3-Jul	50	7.5		7.3		146	
4-Jul	51	7.6		7.5		146	
5-Jul	52	7.5		7.3		134	
6-Jul	53	7.5		7.3		133	
7-Jul	54	7.8		7.5		130	
8-Jul	55	7.7		7.3		164	
9-Jul	56	7.6		7.6		119	
10-Jul	57	7.6		7.5		126	
11-Jul	58	7.4		7.5		123	
12-Jul	59	7.5		7.4		110	
13-Jul	60	7.4		7.1		119	
14-Jul	61	7.4		7.5		121	
15-Jul	62	7.6		7.7		112	
16-Jul	63	7.4		7.2		122	
17-Jul	64	7.5		7.7		126	
18-Jul	65	7.5		7.4		122	
19-Jul	66	7.5		7.6		126	
20-Jul	67	7.6		7.3		136	
21-Jul	68	7.4		7.4		127	
22-Jul	69	7.5		7.3		157	
23-Jul	70	7.5		7.4		123	
24-Jul	71	7.6		7.7		124	
	Average	**7.62**	Average	**7.53**	Average	**122.52**	
	StDev	**0.166**	StDev	**0.194**	StDev	**11.447**	
	Min	**7.3**	Min	**7.1**	Min	**101**	
	Max	**8**	Max	**8**	Max	**164**	

pH & D.O. measurements in Yellowstone Cutthroat trout study (12699-002) Concentration 2.5

filename:pH-DO.xls

Date	Test Day	Water pH		D.O.		Conductivity
14-May	0	NM		NM		NM
15-May	1	7.8		7.8		124
16-May	2	7.8		7.6		118
17-May	3	7.8		7.4		117
18-May	4	7.8		7.3		110
19-May	5	7.8		7.2		103
20-May	6	7.7		7.3		115
21-May	7	7.7		7.5		103
22-May	8	7.6		7.5		112
23-May	9	7.6		7.6		118
24-May	10	7.9		7.4		112
25-May	11	7.8		7.6		100
26-May	12	7.8		7.8		113
27-May	13	7.8		7.9		117
28-May	14	7.7		7.7		125
29-May	15	7.7		7.6		120
30-May	16	7.8		7.7		115
31-May	17	7.8		7.9		109
1-Jun	18	7.8		7.9		110
2-Jun	19	7.6		7.7		122
3-Jun	20	7.7		7.6		124
4-Jun	21	7.5		7.4		128
5-Jun	22	7.7		7.8		122
6-Jun	23	7.8		7.9		121
7-Jun	24	7.8		7.9		122
8-Jun	25	7.5		7.8		118
9-Jun	26	7.4		7.9		115
10-Jun	27	7.4		7.7		110
11-Jun	28	7.5		8.0		112
12-Jun	29	7.3		8.1		103
13-Jun	30	7.2		7.8		111
14-Jun	31	7.6		7.4		110
15-Jun	32	7.8		7.3		120
16-Jun	33	7.6		7.6		117
17-Jun	34	7.6		7.7		127
18-Jun	35	7.6		7.6		126
19-Jun	36	7.6		7.6		122
20-Jun	37	7.6		7.4		115
21-Jun	38	7.8		7.7		119
22-Jun	39	7.9		7.3		115
23-Jun	40	7.7		7.4		120
24-Jun	41	7.7		7.5		117
25-Jun	42	7.6		7.6		120
26-Jun	43	7.7		7.6		119
27-Jun	44	7.8		7.6		128
28-Jun	45	7.8		7.2		127
29-Jun	46	7.9		7.5		108
30-Jun	47	7.6		7.3		119
1-Jul	48	7.7		7.3		115
2-Jul	49	7.7		7.3		121
3-Jul	50	7.6		7.4		127
4-Jul	51	7.7		7.3		120
5-Jul	52	7.5		7.1		120
6-Jul	53	7.5		7.5		120
7-Jul	54	7.8		7.5		125
8-Jul	55	7.8		7.4		125
9-Jul	56	7.6		7.7		123
10-Jul	57	7.5		7.6		129
11-Jul	58	7.5		7.6		126
12-Jul	59	7.5		7.5		107
13-Jul	60	7.3		7.2		113
14-Jul	61	7.5		7.5		115
15-Jul	62	7.6		7.4		108
16-Jul	63	7.5		7.4		112
17-Jul	64	7.5		7.7		114
18-Jul	65	7.5		7.4		115
19-Jul	66	7.6		7.7		114
20-Jul	67	7.6		7.2		118
21-Jul	68	7.4		7.4		119
22-Jul	69	7.6		7.4		132
23-Jul	70	7.6		7.4		120
24-Jul	71	7.6		7.5		122
	Average	**7.64**	Average	**7.55**	Average	**117.44**
	StDev	**0.151**	StDev	**0.218**	StDev	**6.815**
	Min	**7.2**	Min	**7.1**	Min	**100**
	Max	**7.9**	Max	**8.1**	Max	**132**

pH & D.O. measurements in Yellowstone Cutthroat trout study (12699-002) Concentration 5

filename:pH-DO.xls

Date	Test Day	Water pH		D.O.		Conductivity
14-May	0	NM		NM		NM
15-May	1	7.8		7.8		115
16-May	2	7.8		7.7		126
17-May	3	7.8		7.5		118
18-May	4	7.8		7.4		120
19-May	5	7.8		7.2		102
20-May	6	7.7		7.2		114
21-May	7	7.8		7.5		102
22-May	8	7.7		7.5		106
23-May	9	7.7		7.7		112
24-May	10	7.9		7.4		102
25-May	11	7.8		7.7		100
26-May	12	7.8		7.9		127
27-May	13	7.8		7.9		116
28-May	14	7.8		7.8		126
29-May	15	7.7		7.6		122
30-May	16	7.8		7.8		115
31-May	17	7.8		7.9		107
1-Jun	18	7.8		7.9		102
2-Jun	19	7.7		7.6		120
3-Jun	20	7.7		7.6		122
4-Jun	21	7.6		7.6		124
5-Jun	22	7.7		7.8		124
6-Jun	23	7.8		8.0		120
7-Jun	24	7.8		7.9		124
8-Jun	25	7.5		7.9		117
9-Jun	26	7.4		7.9		112
10-Jun	27	7.1		7.6		112
11-Jun	28	7.5		8.1		118
12-Jun	29	7.4		8.2		101
13-Jun	30	7.2		7.8		104
14-Jun	31	7.6		7.8		106
15-Jun	32	7.7		7.4		120
16-Jun	33	7.5		7.6		111
17-Jun	34	7.6		7.7		127
18-Jun	35	7.6		7.7		117
19-Jun	36	7.6		7.7		121
20-Jun	37	7.6		7.4		114
21-Jun	38	7.7		7.7		118
22-Jun	39	7.8		7.6		119
23-Jun	40	7.7		7.4		120
24-Jun	41	7.8		7.6		117
25-Jun	42	7.6		7.5		127
26-Jun	43	7.7		7.6		120
27-Jun	44	7.8		7.6		129
28-Jun	45	7.8		7.2		126
29-Jun	46	7.9		7.6		105
30-Jun	47	7.6		7.2		118
1-Jul	48	7.7		7.3		114
2-Jul	49	7.7		7.3		118
3-Jul	50	7.7		7.4		133
4-Jul	51	7.7		7.6		119
5-Jul	52	7.6		7.1		119
6-Jul	53	7.5		7.4		124
7-Jul	54	7.8		7.4		124
8-Jul	55	7.7		7.3		140
9-Jul	56	7.6		7.8		122
10-Jul	57	7.6		7.5		124
11-Jul	58	7.5		7.7		126
12-Jul	59	7.7		7.5		107
13-Jul	60	7.3		7.3		114
14-Jul	61	7.6		7.5		117
15-Jul	62	7.6		7.4		110
16-Jul	63	7.5		7.3		114
17-Jul	64	7.4		7.5		119
18-Jul	65	7.5		7.3		115
19-Jul	66	7.6		7.7		115
20-Jul	67	7.6		7.2		115
21-Jul	68	7.5		7.5		113
22-Jul	69	7.7		7.3		126
23-Jul	70	7.6		7.3		121
24-Jul	71	7.7		7.5		120
	Average	**7.65**	Average	**7.57**	Average	**117.10**
	StDev	**0.154**	StDev	**0.238**	StDev	**8.071**
	Min	**7.1**	Min	**7.1**	Min	**100**
	Max	**7.9**	Max	**8.2**	Max	**140**

pH & D.O. measurements in Yellowstone Cutthroat trout study (12699-002) Concentration 10

filename:pH-DO.xls

Date	Test Day	Water pH		D.O.		Conductivity
14-May	0	NM		NM		NM
15-May	1	7.8		7.7		115
16-May	2	7.8		7.7		126
17-May	3	7.8		7.4		109
18-May	4	7.8		7.4		119
19-May	5	7.8		7.2		102
20-May	6	7.7		7.2		114
21-May	7	7.7		7.5		103
22-May	8	7.7		7.4		108
23-May	9	7.7		7.7		116
24-May	10	7.9		7.5		104
25-May	11	7.8		7.7		107
26-May	12	7.8		7.8		120
27-May	13	7.8		7.9		114
28-May	14	7.8		7.8		125
29-May	15	7.8		7.5		122
30-May	16	7.8		7.8		116
31-May	17	7.8		7.8		105
1-Jun	18	7.8		7.8		107
2-Jun	19	7.6		7.5		112
3-Jun	20	7.7		7.6		122
4-Jun	21	7.6		7.7		122
5-Jun	22	7.6		7.6		116
6-Jun	23	7.8		7.8		119
7-Jun	24	7.8		7.8		122
8-Jun	25	7.5		7.9		109
9-Jun	26	7.6		8.0		111
10-Jun	27	7.2		7.5		120
11-Jun	28	7.5		8.1		107
12-Jun	29	7.4		8.2		106
13-Jun	30	7.2		7.8		104
14-Jun	31	7.7		7.8		114
15-Jun	32	7.7		7.4		120
16-Jun	33	7.5		7.4		119
17-Jun	34	7.6		7.5		127
18-Jun	35	7.6		7.7		120
19-Jun	36	7.6		7.7		119
20-Jun	37	7.6		7.4		114
21-Jun	38	7.7		7.6		114
22-Jun	39	7.8		7.6		112
23-Jun	40	7.7		7.4		120
24-Jun	41	7.8		7.6		117
25-Jun	42	7.6		7.4		128
26-Jun	43	7.7		7.4		121
27-Jun	44	7.8		7.5		126
28-Jun	45	7.8		7.2		126
29-Jun	46	7.9		7.6		111
30-Jun	47	7.6		7.3		118
1-Jul	48	7.7		7.3		114
2-Jul	49	7.7		7.2		118
3-Jul	50	7.6		7.4		132
4-Jul	51	7.7		7.6		117
5-Jul	52	7.6		7.3		117
6-Jul	53	7.5		7.3		121
7-Jul	54	7.7		7.3		125
8-Jul	55	7.8		7.3		121
9-Jul	56	7.6		7.8		106
10-Jul	57	7.6		7.5		123
11-Jul	58	7.5		7.5		122
12-Jul	59	7.6		7.4		107
13-Jul	60	7.3		7.3		116
14-Jul	61	7.5		7.4		118
15-Jul	62	7.7		7.5		109
16-Jul	63	7.5		7.2		113
17-Jul	64	7.4		7.5		114
18-Jul	65	7.5		7.2		114
19-Jul	66	7.6		7.7		114
20-Jul	67	7.6		7.2		116
21-Jul	68	7.5		7.4		118
22-Jul	69	7.7		7.2		124
23-Jul	70	7.6		7.2		118
24-Jul	71	7.6		7.6		131
	Average	**7.65**	Average	**7.54**	Average	**116.28**
	StDev	**0.147**	StDev	**0.234**	StDev	**6.914**
	Min	**7.2**	Min	**7.2**	Min	**102**
	Max	**7.9**	Max	**8.2**	Max	**132**

pH & D.O. measurements in Yellowstone Cutthroat trout study (12699-002) Concentration 15

filename:pH-DO.xls

Date	Test Day	Water pH		D.O.		Conductivity	
14-May	0	NM		NM		NM	
15-May	1	7.9		7.7		122	
16-May	2	7.8		7.7		124	
17-May	3	7.8		7.4		108	
18-May	4	7.8		7.3		120	
19-May	5	7.8		7.1		102	
20-May	6	7.7		7.2		114	
21-May	7	7.7		7.4		102	
22-May	8	7.8		7.4		110	
23-May	9	7.8		7.7		110	
24-May	10	7.9		7.5		118	
25-May	11	7.8		7.7		100	
26-May	12	7.8		7.7		117	
27-May	13	7.8		7.9		114	
28-May	14	7.8		7.7		117	
29-May	15	7.7		7.5		113	
30-May	16	7.8		7.7		116	
31-May	17	7.8		7.9		97	
1-Jun	18	7.8		7.7		113	
2-Jun	19	7.6		7.5		116	
3-Jun	20	7.8		7.6		124	
4-Jun	21	7.6		7.6		121	
5-Jun	22	7.6		7.8		113	
6-Jun	23	7.8		7.8		122	
7-Jun	24	7.8		7.8		113	
8-Jun	25	7.5		7.9		113	
9-Jun	26	7.5		7.9		111	
10-Jun	27	7.2		7.5		108	
11-Jun	28	7.5		8.0		111	
12-Jun	29	7.4		8.2		106	
13-Jun	30	7.3		7.7		110	
14-Jun	31	7.7		7.7		116	
15-Jun	32	7.7		7.5		120	
16-Jun	33	7.5		7.4		118	
17-Jun	34	7.6		7.5		119	
18-Jun	35	7.6		7.6		120	
19-Jun	36	7.6		7.7		120	
20-Jun	37	7.6		7.5		124	
21-Jun	38	7.7		7.5		119	
22-Jun	39	7.8		7.5		120	
23-Jun	40	7.6		7.4		129	
24-Jun	41	7.8		7.4		116	
25-Jun	42	7.6		7.4		122	
26-Jun	43	7.7		7.3		120	
27-Jun	44	7.8		7.4		126	
28-Jun	45	7.7		7.0		126	
29-Jun	46	7.9		7.7		104	
30-Jun	47	7.6		7.0		117	
1-Jul	48	7.7		7.3		114	
2-Jul	49	7.6		7.2		121	
3-Jul	50	7.5		7.3		133	
4-Jul	51	7.7		7.5		116	
5-Jul	52	7.6		7.0		118	
6-Jul	53	7.5		7.0		120	
7-Jul	54	7.7		7.3		125	
8-Jul	55	7.7		7.3		122	
9-Jul	56	7.6		7.7		120	
10-Jul	57	7.7		7.6		121	
11-Jul	58	7.5		7.4		122	
12-Jul	59	7.6		7.3		106	
13-Jul	60	7.3		7.2		110	
14-Jul	61	7.6		7.6		119	
15-Jul	62	7.7		7.6		108	
16-Jul	63	7.5		7.1		112	
17-Jul	64	7.5		7.6		112	
18-Jul	65	7.5		7.2		114	
19-Jul	66	7.6		7.8		114	
20-Jul	67	7.6		7.4		117	
21-Jul	68	7.4		7.2		118	
22-Jul	69	7.6		7.3		128	
23-Jul	70	7.6		7.2		118	
24-Jul	71	7.8		7.5		123	
	Average	**7.65**	Average	**7.50**	Average	**116.23**	
	StDev	**0.147**	StDev	**0.257**	StDev	**6.959**	
	Min	**7.2**	Min	**7**	Min	**97**	
	Max	**7.9**	Max	**8.2**	Max	**133**	

pH & D.O. measurements in Yellowstone Cutthroat trout study (12699-002) Concentration 20

filename:pH-DO.xls

Date	Test Day	Water pH		D.O.		Conductivity
14-May	0	NM		NM		NM
15-May	1	7.8		7.8		117
16-May	2	7.8		7.6		123
17-May	3	7.8		7.5		101
18-May	4	7.8		7.4		112
19-May	5	7.7		7.2		101
20-May	6	7.7		7.2		113
21-May	7	7.7		7.5		102
22-May	8	7.8		7.4		105
23-May	9	7.8		7.8		108
24-May	10	7.9		7.5		99
25-May	11	7.8		7.9		105
26-May	12	7.8		7.8		112
27-May	13	7.8		7.9		107
28-May	14	7.8		7.7		109
29-May	15	7.8		7.6		114
30-May	16	7.8		7.6		116
31-May	17	7.8		7.9		101
1-Jun	18	7.8		7.8		101
2-Jun	19	7.7		7.6		114
3-Jun	20	7.8		7.5		122
4-Jun	21	7.6		7.6		120
5-Jun	22	7.6		7.8		119
6-Jun	23	7.8		7.9		120
7-Jun	24	7.8		7.7		121
8-Jun	25	7.5		7.9		116
9-Jun	26	7.5		7.9		104
10-Jun	27	7.3		7.5		108
11-Jun	28	7.4		7.9		113
12-Jun	29	7.4		8.0		106
13-Jun	30	7.3		7.7		110
14-Jun	31	7.6		7.7		117
15-Jun	32	7.7		7.6		120
16-Jun	33	7.5		7.5		118
17-Jun	34	7.6		7.4		119
18-Jun	35	7.6		7.4		121
19-Jun	36	7.6		7.6		122
20-Jun	37	7.6		7.6		115
21-Jun	38	7.7		7.6		114
22-Jun	39	7.7		7.5		115
23-Jun	40	7.7		7.4		119
24-Jun	41	7.8		7.4		116
25-Jun	42	7.7		7.4		122
26-Jun	43	7.7		7.4		118
27-Jun	44	7.8		7.4		127
28-Jun	45	7.7		6.9		126
29-Jun	46	7.9		7.6		105
30-Jun	47	7.6		7.3		116
1-Jul	48	7.6		7.2		117
2-Jul	49	7.7		7.2		120
3-Jul	50	7.5		7.3		125
4-Jul	51	7.7		7.4		118
5-Jul	52	7.6		7.2		119
6-Jul	53	7.6		7.4		120
7-Jul	54	7.7		7.4		124
8-Jul	55	7.8		7.3		122
9-Jul	56	7.6		7.7		117
10-Jul	57	7.7		7.5		122
11-Jul	58	7.5		7.4		123
12-Jul	59	7.6		7.3		108
13-Jul	60	7.3		7.3		108
14-Jul	61	7.6		7.4		111
15-Jul	62	7.7		7.6		105
16-Jul	63	7.6		7.3		112
17-Jul	64	7.5		7.5		111
18-Jul	65	7.5		7.3		114
19-Jul	66	7.6		7.6		114
20-Jul	67	7.7		7.3		115
21-Jul	68	7.5		7.4		117
22-Jul	69	7.7		7.3		123
23-Jul	70	7.5		7.3		119
24-Jul	71	7.8		7.5		119
	Average	**7.66**	Average	**7.52**	Average	**114.54**
	StDev	**0.140**	StDev	**0.223**	StDev	**6.985**
	Min	**7.3**	Min	**6.9**	Min	**99**
	Max	**7.9**	Max	**8**	Max	**127**

pH & D.O. measurements in Yellowstone Cutthroat trout study (12699-002) Concentration 40

filename:pH-DO.xls

Date	Test Day	Water pH		D.O.		Conductivity
14-May	0	NM		NM		NM
15-May	1	7.8		7.7		109
16-May	2	7.8		7.6		124
17-May	3	7.8		7.4		108
18-May	4	7.8		7.4		118
19-May	5	7.7		7.2		100
20-May	6	7.7		7.2		113
21-May	7	7.7		7.5		100
22-May	8	7.8		7.5		105
23-May	9	7.8		7.7		105
24-May	10	7.9		7.5		111
25-May	11	7.9		7.9		101
26-May	12	7.8		7.8		111
27-May	13	7.8		7.8		114
28-May	14	7.8		7.7		112
29-May	15	7.7		7.4		122
30-May	16	7.8		7.7		114
31-May	17	7.8		8.0		102
1-Jun	18	7.7		7.7		103
2-Jun	19	7.8		7.7		110
3-Jun	20	7.8		7.5		121
4-Jun	21	7.6		7.6		118
5-Jun	22	7.6		7.8		117
6-Jun	23	7.8		7.8		119
7-Jun	24	7.7		7.9		121
8-Jun	25	7.5		7.9		115
9-Jun	26	7.6		8.0		108
10-Jun	27	7.3		7.6		108
11-Jun	28	7.4		7.7		104
12-Jun	29	7.4		8.0		98
13-Jun	30	7.3		7.8		103
14-Jun	31	7.6		7.8		114
15-Jun	32	7.7		7.6		120
16-Jun	33	7.5		7.4		117
17-Jun	34	7.6		7.6		117
18-Jun	35	7.6		7.6		123
19-Jun	36	7.6		7.6		119
20-Jun	37	7.6		7.6		115
21-Jun	38	7.7		7.7		108
22-Jun	39	7.8		7.4		108
23-Jun	40	7.7		7.6		121
24-Jun	41	7.8		7.4		114
25-Jun	42	7.7		7.5		121
26-Jun	43	7.7		7.5		118
27-Jun	44	7.8		7.4		123
28-Jun	45	7.8		7.1		125
29-Jun	46	7.9		7.6		97
30-Jun	47	7.7		7.4		114
1-Jul	48	7.7		7.2		114
2-Jul	49	7.6		7.0		118
3-Jul	50	7.5		7.3		125
4-Jul	51	7.7		7.4		115
5-Jul	52	7.7		7.2		116
6-Jul	53	7.6		7.2		120
7-Jul	54	7.8		7.5		121
8-Jul	55	7.8		7.4		121
9-Jul	56	7.6		7.7		112
10-Jul	57	7.7		7.6		119
11-Jul	58	7.4		7.5		117
12-Jul	59	7.6		7.4		107
13-Jul	60	7.3		7.2		120
14-Jul	61	7.6		7.3		109
15-Jul	62	7.7		7.6		106
16-Jul	63	7.6		7.3		110
17-Jul	64	7.5		7.5		116
18-Jul	65	7.6		7.3		114
19-Jul	66	7.6		7.5		112
20-Jul	67	7.7		7.3		113
21-Jul	68	7.5		7.3		110
22-Jul	69	7.7		7.3		124
23-Jul	70	7.6		7.3		117
24-Jul	71	7.8		7.6		117
	Average	**7.67**	verag	**7.53**	verag	**113.54**
	StDev	**0.141**	StDev	**0.226**	StDev	**7.006**
	Min	**7.3**	Min	**7**	Min	**97**
	Max	**7.9**	Max	**8**	Max	**125**

Water hardness (mg/L) measurements in Yellowstone Cutthroat trout study (12699-002)

filename: TempsELS.xls

Date	Test Day	Water hardness (mg/L)
14-May	0	NM
15-May	1	48
16-May	2	48
17-May	3	32
18-May	4	44
19-May	5	42
20-May	6	46
21-May	7	40
22-May	8	42
23-May	9	44
24-May	10	42
25-May	11	44
26-May	12	48
27-May	13	44
28-May	14	48
29-May	15	46
30-May	16	44
31-May	17	42
1-Jun	18	46
2-Jun	19	46
3-Jun	20	44
4-Jun	21	50
5-Jun	22	48
6-Jun	23	44
7-Jun	24	48
8-Jun	25	48
9-Jun	26	50
10-Jun	27	48
11-Jun	28	42
12-Jun	29	44
13-Jun	30	44
14-Jun	31	44
15-Jun	32	48
16-Jun	33	50
17-Jun	34	50
18-Jun	35	48
19-Jun	36	50
20-Jun	37	44
21-Jun	38	44
22-Jun	39	42
23-Jun	40	48
24-Jun	41	46
25-Jun	42	48
26-Jun	43	50
27-Jun	44	48
28-Jun	45	46
29-Jun	46	48
30-Jun	47	48
1-Jul	48	44
2-Jul	49	46
3-Jul	50	42
4-Jul	51	46
5-Jul	52	46
6-Jul	53	46
7-Jul	54	46
8-Jul	55	48
9-Jul	56	44
10-Jul	57	48
11-Jul	58	48
12-Jul	59	40
13-Jul	60	52
14-Jul	61	40
15-Jul	62	46
16-Jul	63	50
17-Jul	64	42
18-Jul	65	50
19-Jul	66	48
20-Jul	67	54
21-Jul	68	52
22-Jul	69	46
23-Jul	70	46
24-Jul	71	46
Average (overall)		**45.97**
	StDev	**3.439**
	Min	**32**
	Max	**54**

Alkalinity (mg/L) measurements in HT water for Yellowstone Cutthroat trout study (12699-002)

filename: TempsELS.xls

Date	Test Day	Alkalinity (mg/L)
20-May-08	D6	27
27-May-08	D13	27
3-Jun-08	D20	28
10-Jun-08	D27	29
17-Jun-08	D34	28
24-Jun-08	D41	47
4-Jul-08	D51	29
8-Jul-08	D55	31
15-Jul-08	D62	31
22-Jul-08	D69	27
Average (overall)		**30.40**
	StDev	**6.022**
	Min	**27**
	Max	**47**

Temperature measurement for ELS study (Controls)				
12699-002-610				
filename: TempsELS.xls				
Date	Test Day	Temp		
14-May	0	11.0		
15-May	1	10		
16-May	2	10.4		
17-May	3	11		
18-May	4	12.6		
19-May	5	13		
20-May	6	14		
21-May	7	12		
22-May	8	12		
23-May	9	11		
24-May	10	11		
25-May	11	11		
26-May	12	11		
27-May	13	11		
28-May	14	11		
29-May	15	12		
30-May	16	10		
31-May	17	10		
1-Jun	18	11		
2-Jun	19	11		
3-Jun	20	11		
4-Jun	21	11		
5-Jun	22	10		
6-Jun	23	10		
7-Jun	24	11		
8-Jun	25	10		
9-Jun	26	10		
10-Jun	27	11		
11-Jun	28	10		
12-Jun	29	9		
13-Jun	30	10		
14-Jun	31	11		
15-Jun	32	12.0		
16-Jun	33	12.0		
17-Jun	34	11		
18-Jun	35	12		
19-Jun	36	13		
20-Jun	37	13		
21-Jun	38	11		
22-Jun	39	12		
23-Jun	40	13		
24-Jun	41	12		
25-Jun	42	13		
26-Jun	43	13		
27-Jun	44	13		
28-Jun	45	13		
29-Jun	46	12		
30-Jun	47	14		
1-Jul	48	14		
2-Jul	49	14		
3-Jul	50	14		
4-Jul	51	12		
5-Jul	52	13		
6-Jul	53	13		
7-Jul	54	13		
8-Jul	55	13		
9-Jul	56	11		
10-Jul	57	12		
11-Jul	58	12		
12-Jul	59	11		
13-Jul	60	12		
14-Jul	61	13		
15-Jul	62	13		
16-Jul	63	12		
17-Jul	64	13		
18-Jul	65	12		
19-Jul	66	12		
20-Jul	67	12		
21-Jul	68	13		
22-Jul	69	12		
23-Jul	70	13		
24-Jul	71	12		
	Avg	**11.81**		
	std	**1.19**		
	Min	**9**		
	Max	**14**		

Temperature measurement for ELS study (2.5)				
12699-002-610				
filename: TempsELS.xls				
Date	Test Day	Temp		
14-May	0	11.1		
15-May	1	10		
16-May	2	11.0		
17-May	3	12		
18-May	4	12.8		
19-May	5	12		
20-May	6	13		
21-May	7	12		
22-May	8	13		
23-May	9	11		
24-May	10	11		
25-May	11	11		
26-May	12	11		
27-May	13	11		
28-May	14	11		
29-May	15	12		
30-May	16	10		
31-May	17	11		
1-Jun	18	10		
2-Jun	19	11		
3-Jun	20	11		
4-Jun	21	11		
5-Jun	22	10		
6-Jun	23	10		
7-Jun	24	11		
8-Jun	25	10		
9-Jun	26	10		
10-Jun	27	11		
11-Jun	28	10		
12-Jun	29	9		
13-Jun	30	9		
14-Jun	31	11		
15-Jun	32	13		
16-Jun	33	12.0		
17-Jun	34	11		
18-Jun	35	12		
19-Jun	36	12		
20-Jun	37	13		
21-Jun	38	11		
22-Jun	39	12		
23-Jun	40	14		
24-Jun	41	12		
25-Jun	42	14		
26-Jun	43	12		
27-Jun	44	12		
28-Jun	45	13		
29-Jun	46	12		
30-Jun	47	13		
1-Jul	48	13		
2-Jul	49	13		
3-Jul	50	13		
4-Jul	51	11		
5-Jul	52	13		
6-Jul	53	13		
7-Jul	54	13		
8-Jul	55	13		
9-Jul	56	11		
10-Jul	57	13		
11-Jul	58	12		
12-Jul	59	11		
13-Jul	60	13		
14-Jul	61	13		
15-Jul	62	13		
16-Jul	63	12		
17-Jul	64	13		
18-Jul	65	12		
19-Jul	66	12		
20-Jul	67	12		
21-Jul	68	12		
22-Jul	69	12		
23-Jul	70	13		
24-Jul	71	12		
	Avg	**11.75**		
	std	**1.14**		
	Min	**9**		
	Max	**14**		

Temperature measurement for ELS study (5)

12699-002-610

filename: TempsELS.xls

Date	Test Day	Temp
14-May	0	10.94
15-May	1	10
16-May	2	11
17-May	3	12
18-May	4	12.7
19-May	5	14
20-May	6	13
21-May	7	12
22-May	8	13
23-May	9	11
24-May	10	11
25-May	11	10
26-May	12	11
27-May	13	11
28-May	14	11
29-May	15	12
30-May	16	10
31-May	17	10
1-Jun	18	10
2-Jun	19	12
3-Jun	20	11
4-Jun	21	11
5-Jun	22	10
6-Jun	23	10
7-Jun	24	11
8-Jun	25	10
9-Jun	26	10
10-Jun	27	11
11-Jun	28	10
12-Jun	29	9
13-Jun	30	10
14-Jun	31	12
15-Jun	32	12.0
16-Jun	33	12.0
17-Jun	34	12
18-Jun	35	12
19-Jun	36	13
20-Jun	37	12
21-Jun	38	11
22-Jun	39	13
23-Jun	40	13
24-Jun	41	13
25-Jun	42	12
26-Jun	43	13
27-Jun	44	13
28-Jun	45	12
29-Jun	46	12
30-Jun	47	13
1-Jul	48	13
2-Jul	49	13
3-Jul	50	13
4-Jul	51	12
5-Jul	52	12
6-Jul	53	13
7-Jul	54	14
8-Jul	55	12
9-Jul	56	11
10-Jul	57	12
11-Jul	58	13
12-Jul	59	13
13-Jul	60	11
14-Jul	61	13
15-Jul	62	12
16-Jul	63	13
17-Jul	64	14
18-Jul	65	11
19-Jul	66	12
20-Jul	67	11
21-Jul	68	13
22-Jul	69	13
23-Jul	70	13
24-Jul	71	13
	Avg	**11.81**
	std	**1.19**
	Min	**9**
	Max	**14**

Temperature measurement for ELS study (10)				
12699-002-610				
filename: TempsELS.xls				
Date	Test Day	Temp		
14-May	0	10.7		
15-May	1	10		
16-May	2	10		
17-May	3	12		
18-May	4	12.6		
19-May	5	13		
20-May	6	14		
21-May	7	12		
22-May	8	12		
23-May	9	11		
24-May	10	11		
25-May	11	11		
26-May	12	11		
27-May	13	11		
28-May	14	11		
29-May	15	12		
30-May	16	10		
31-May	17	11		
1-Jun	18	10		
2-Jun	19	11		
3-Jun	20	11		
4-Jun	21	11		
5-Jun	22	10		
6-Jun	23	10		
7-Jun	24	11		
8-Jun	25	10		
9-Jun	26	10		
10-Jun	27	11		
11-Jun	28	10		
12-Jun	29	9		
13-Jun	30	11		
14-Jun	31	11		
15-Jun	32	13		
16-Jun	33	11		
17-Jun	34	11		
18-Jun	35	12		
19-Jun	36	13		
20-Jun	37	13		
21-Jun	38	13		
22-Jun	39	12		
23-Jun	40	13		
24-Jun	41	13		
25-Jun	42	13		
26-Jun	43	12		
27-Jun	44	13		
28-Jun	45	12		
29-Jun	46	12		
30-Jun	47	14		
1-Jul	48	13		
2-Jul	49	13		
3-Jul	50	13		
4-Jul	51	12		
5-Jul	52	12		
6-Jul	53	12		
7-Jul	54	13		
8-Jul	55	12		
9-Jul	56	11		
10-Jul	57	11		
11-Jul	58	12		
12-Jul	59	12		
13-Jul	60	11		
14-Jul	61	13		
15-Jul	62	12		
16-Jul	63	12		
17-Jul	64	13		
18-Jul	65	11		
19-Jul	66	12		
20-Jul	67	11		
21-Jul	68	12		
22-Jul	69	13		
23-Jul	70	12		
24-Jul	71	12		
	Avg	**11.68**		
	std	**1.09**		
	Min	**9**		
	Max	**14**		

Temperature measurement for ELS study (15)
12699-002-610
filename: TempsELS.xls

Date	Test Day	Temp
14-May	0	10.8
15-May	1	10
16-May	2	10
17-May	3	12
18-May	4	12.6
19-May	5	13
20-May	6	13
21-May	7	12
22-May	8	12
23-May	9	11
24-May	10	11
25-May	11	11
26-May	12	11
27-May	13	11
28-May	14	11
29-May	15	12
30-May	16	10
31-May	17	10
1-Jun	18	10
2-Jun	19	11
3-Jun	20	11
4-Jun	21	11
5-Jun	22	10
6-Jun	23	10
7-Jun	24	11
8-Jun	25	10
9-Jun	26	10
10-Jun	27	11
11-Jun	28	10
12-Jun	29	9
13-Jun	30	9
14-Jun	31	12
15-Jun	32	12
16-Jun	33	11.0
17-Jun	34	12
18-Jun	35	12
19-Jun	36	13
20-Jun	37	12
21-Jun	38	11
22-Jun	39	12
23-Jun	40	13
24-Jun	41	13
25-Jun	42	13
26-Jun	43	12
27-Jun	44	13
28-Jun	45	12
29-Jun	46	12
30-Jun	47	13
1-Jul	48	13
2-Jul	49	13
3-Jul	50	13
4-Jul	51	11
5-Jul	52	12
6-Jul	53	12
7-Jul	54	13
8-Jul	55	12
9-Jul	56	11
10-Jul	57	12
11-Jul	58	12
12-Jul	59	12
13-Jul	60	11
14-Jul	61	13
15-Jul	62	13
16-Jul	63	13
17-Jul	64	13
18-Jul	65	11
19-Jul	66	12
20-Jul	67	12
21-Jul	68	12
22-Jul	69	12
23-Jul	70	13
24-Jul	71	13
	Avg	**11.64**
	std	**1.10**
	Min	**9**
	Max	**13**

Temperature measurement for ELS study (20)
12699-002-610
filename: TempsELS.xls

Date	Test Day	Temp
14-May	0	10.8
15-May	1	10
16-May	2	11
17-May	3	12
18-May	4	12.5
19-May	5	13
20-May	6	13
21-May	7	12
22-May	8	12
23-May	9	11
24-May	10	11
25-May	11	11
26-May	12	11
27-May	13	11
28-May	14	11
29-May	15	12
30-May	16	10
31-May	17	10
1-Jun	18	10
2-Jun	19	11
3-Jun	20	11
4-Jun	21	11
5-Jun	22	10
6-Jun	23	10
7-Jun	24	11
8-Jun	25	10
9-Jun	26	10
10-Jun	27	10
11-Jun	28	10
12-Jun	29	9
13-Jun	30	11
14-Jun	31	12
15-Jun	32	13
16-Jun	33	12
17-Jun	34	11
18-Jun	35	12
19-Jun	36	13
20-Jun	37	12
21-Jun	38	11
22-Jun	39	12
23-Jun	40	13
24-Jun	41	13
25-Jun	42	12
26-Jun	43	12
27-Jun	44	13
28-Jun	45	12
29-Jun	46	12
30-Jun	47	13
1-Jul	48	13
2-Jul	49	13
3-Jul	50	13
4-Jul	51	12
5-Jul	52	12
6-Jul	53	12
7-Jul	54	14
8-Jul	55	12
9-Jul	56	11
10-Jul	57	11
11-Jul	58	12
12-Jul	59	11
13-Jul	60	11
14-Jul	61	14
15-Jul	62	13
16-Jul	63	12
17-Jul	64	13
18-Jul	65	11
19-Jul	66	12
20-Jul	67	11
21-Jul	68	13
22-Jul	69	13
23-Jul	70	12
24-Jul	71	13
	Avg	**11.67**
	std	**1.11**
	Min	**9**
	Max	**14**

Temperature measurement for ELS study (40)
12699-002-610
filename: TempsELS.xls

Date	Test Day	Temp
14-May	0	11.0
15-May	1	10
16-May	2	11
17-May	3	12
18-May	4	12.6
19-May	5	13
20-May	6	12
21-May	7	12
22-May	8	12
23-May	9	11
24-May	10	11
25-May	11	11
26-May	12	11
27-May	13	11
28-May	14	11
29-May	15	12
30-May	16	10
31-May	17	10
1-Jun	18	11
2-Jun	19	11
3-Jun	20	11
4-Jun	21	11
5-Jun	22	10
6-Jun	23	10
7-Jun	24	11
8-Jun	25	10
9-Jun	26	10
10-Jun	27	11
11-Jun	28	11
12-Jun	29	9
13-Jun	30	10
14-Jun	31	12
15-Jun	32	13
16-Jun	33	13
17-Jun	34	11
18-Jun	35	12
19-Jun	36	13
20-Jun	37	12
21-Jun	38	12
22-Jun	39	11
23-Jun	40	13
24-Jun	41	13
25-Jun	42	12
26-Jun	43	13
27-Jun	44	13
28-Jun	45	12
29-Jun	46	12
30-Jun	47	13
1-Jul	48	14
2-Jul	49	14
3-Jul	50	13
4-Jul	51	12
5-Jul	52	12
6-Jul	53	13
7-Jul	54	14
8-Jul	55	13
9-Jul	56	11
10-Jul	57	12
11-Jul	58	13
12-Jul	59	11
13-Jul	60	12
14-Jul	61	13
15-Jul	62	12
16-Jul	63	13
17-Jul	64	13
18-Jul	65	12
19-Jul	66	12
20-Jul	67	11
21-Jul	68	13
22-Jul	69	13
23-Jul	70	13
24-Jul	71	13
	Avg	**11.83**
	std	**1.13**
	Min	**9**
	Max	**14**

Appendix F

Summary of Aqueous Selenium Concentrations Measured During the Yellowstone Cutthroat Trout Dietary Study

YTC ELS 12699-002-610

Measured Total Recoverable Selenium Concentrations

Date	15-May	22-May	@ 1135 27-May	@1745 27-May	29-May	6-Jun	12-Jun	19-Jun	26-Jun	@0905 1-Jul	@1515 1-Jul	3-Jul	10-Jul	@1010 14-Jul	@1420 14-Jul	17-Jul					
	Day 1	Day 8	Day 13	Day 13	Day 15	Day 23	Day 29	Day 36	Day 43	Day 48	Day 48	Day 50	Day 57	Day 61	Day 61	Day 64	TWA	% Nom.	Min	Max	Max/Min
Control A		0.1 U							0.1 U												
Control B					0.1 B			0.1 U								0.1 U					
Control C						0.1 B							0.1 B								
Control D							0.1 U					0.1 U									
Control E																					
Control comp	0.1 U																				
																	0.1 U	---	0.1 U	0.1 B	
2.5 A		2.5							2.7												
2.5 B					2.9			3.1								2.4					
2.5 C						3.0							2.8								
2.5 D							3.1					2.8									
2.5 E																					
2.5 comp	2.9																				
	2.9	2.5			2.9	3.0	3.1	3.1	2.7			2.8	2.8			2.4	2.80	111.8	2.4	3.1	1.29
5.0 A		4.4							5.2												
5.0 B					5.8			5.8								4.7					
5.0 C						5.6							5.6								
5.0 D							5.7					5.5									
5.0 E																					
5.0 comp	5.5																				
	5.5	4.4			5.8	5.6	5.7	5.8	5.2			5.5	5.6			4.7	5.34	106.9	4.4	5.8	1.32
10 A		8.8							10.2												
10 B					11.2			11.6								10.2					
10 C						10.9							10.1								
10 D							10.6					10.2									
10 E																					
10 comp	9.0																				
	9.0	8.8			11.2	10.9	10.6	11.6	10.2			10.2	10.1			10.2	10.325	103.3	8.8	11.6	1.32
15 A		13.9							15.7												
15 B					16.1			17.8								15.6					
15 C					16.5	16.3							15.3								
15 D							17.0					15.5									
15 E																					
15 comp	13.8																				
	13.8	13.9			16.3	16.3	17.0	17.8	15.7			15.5	15.3			15.6	15.777	105.2	13.8	17.8	1.29
20 A		18.3							19.9					15.4							
20 B					21.3			22.4							17.0	17.2					
20 C						22.5							21.1								
20 D							23.2					21.0									
20 E																					
20 comp	18.5																				
	18.5	18.3			21.3	22.5	23.2	22.4	19.9			21.0	21.1		16.2	17.2	20.302	101.5	15.4	23.2	1.51
																			exclude	15.4	1.365
40 A		35.2							40.1	36.1											
40 B					43.8			44.0			41.9					40.7					
40 C						45.1							40.8								
40 D							44.4					41.1									
40 E																					
40 comp	37.6		15.4	36.5																	
	37.6	35.2	15.4	36.5	43.8	45.1	44.4	44.0	40.1		39.0	41.1	40.8			40.7	40.693	101.7	15.4	45.1	1.281
																			35.2		
			avg	26.0																	

YTC ELS 12699-002-610
Measured Dissolved Selenium Concentrations

Date	2-May Day -12	13 15-May Day 1	22-May Day 8	@ 1135 27-May Day 13	@1745 27-May Day 13	29-May Day 15	6-Jun Day 23	12-Jun Day 29	19-Jun Day 36	26-Jun Day 43	@0905 1-Jul Day 48	@1515 1-Jul Day 48	3-Jul Day 50	10-Jul Day 57	@1010 14-Jul Day 61	@1420 14-Jul Day 61	17-Jul Day 64	TWA	% Nom.	TWA Trmt	% Nom
Control A			0.1 U							0.1 U								0.1 U		0.1 U	
Control B						0.1 U			0.1 U								0.1 U	0.1 U			
Control C							0.1 U							0.1 U				0.1 U			
Control D								0.1 U					0.1 U					0.1 U			
Control E																		0.1 U			
Control comp		0.1 U																0.1 U			
2.5 A			3.6							3.2								3.34	133.7	3.17	126.7
2.5 B						2.9			3.1								2.9	2.97	118.8		
2.5 B						4.9															
2.5 C							3.1							3.3				3.19	127.5		
2.5 C							4.4														
2.5 D								2.7					3.1					2.88	115.1		
2.5 E																					
2.5 comp		4.3																			
		4.3	3.6			2.9	3.1	2.7	3.1	3.2			3.1	3.3			2.9	3.17	126.7		
5.0 A			6.8							6.0								6.29	125.7	6.32	126.5
5.0 B						5.8			5.9								5.9	5.86	117.3		
5.0 B						7.7															
5.0 C							6.1							6.3				6.19	123.7		
5.0 C							8.7														
5.0 D								6.4					6.3					6.36	127.1		
5.0 E																					
5.0 comp		8.9																			
		8.9	6.8			5.8	6.1	6.4	5.9	6.0			6.3	6.3			5.9	6.32	126.5		
10 A			15.0							11.5								12.76	127.6	12.29	122.9
10 B						11.5			11.9								11.3	11.58	115.8		
10 B						17.1															
10 C							11.6							11.5				11.56	115.6		
10 C							16.4														
10 D								12.7					11.2					12.03	120.3		
10 E																					
10 comp		16.9																			
		16.9	15.0			11.5	11.6	12.7	11.9	11.5			11.2	11.5			11.3	12.29	122.9		
15 A			25.1							17.1								19.97	133.2	18.98	126.5
15 B						16.7			18.5								18.2	17.76	118.4		
15 B						24.3															
15 C						16.7	17.7							17.3				17.26	115.1		
15 C						23.7	25.7														
15 D								19.3					17.0					18.28	121.9		
15 E																					
15 comp		25.9																			
		25.9	25.1			16.7	17.7	19.3	18.5	17.1			17.0	17.3			18.2	18.98	126.5		
20 A			39.2							24.2								29.59	147.9	25.34	126.7
20 B						22.0			24.2								20.0	22.17	110.8		
20 B						30.4															
20 C							23.9							23.3				23.64	118.2		
20 C							34.1														
20 D								26.5					23.1					24.99	125.0		
20 E																					
20 comp		32.6																			
		32.6	39.2			22.0	23.9	26.5	24.2	24.2			23.1	23.3			20.0	25.34	126.7		
40 A			76.0							44.9								56.07	140.2	49.59	124.0
40 B						46.1			47.2								46.4	46.57	116.4		
40 B						68.6															
40 C							48.3							44.4				46.60	116.5		
40 C							70.7														
40 D								49.8					42.2					46.43	116.1		
40 D													43.7								
40 E																					
40 comp		66.6																			
40 comp	33.0	51.7																			
	33.0	51.7	76.0			46.1	48.3	49.8	47.2	44.9			43.7	44.4			46.4	49.59	124.0		

yellow shaded & underlined values are original values reported from ACZ
unshaded values are results of re-analysis of sample

YTC ELS 12699-002-610
Measured Dissolved Selenium Concentrations

Date	2-May Day -12	13 15-May Day 1	22-May Day 8	@ 1135 27-May Day 13	@1745 27-May Day 13	29-May Day 15	6-Jun Day 23	12-Jun Day 29	19-Jun Day 36	26-Jun Day 43	@0905 1-Jul Day 48	@1515 1-Jul Day 48	3-Jul Day 50	10-Jul Day 57	@1010 14-Jul Day 61	@1420 14-Jul Day 61	17-Jul Day 64	TWA	% Nom.	Min	Max	Max/Min
Control A			0.1 U							0.1 U												
Control B						0.1 U			0.1 U								0.1 U					
Control C							0.1 U							0.1 U								
Control D								0.1 U					0.1 U									
Control E																						
Control comp		0.1 U																				
		0.1 U	0.1 U			0.1 U	0.1 U	0.1 U	0.1 U	0.1 U			0.1 U	0.1 U			0.1 U	0.1 U	---	0.1 U	0.1 U	---
2.5 A			2.4							3.2												
2.5 B						2.9			3.1								2.9					
2.5 C							3.1							3.3								
2.5 D								2.7					3.1									
2.5 E																						
2.5 comp		2.9																				
		2.9	2.4			2.9	3.1	2.7	3.1	3.2			3.1	3.3			2.9	2.96	118.4	2.4	3.3	1.375
5.0 A			4.7							6.0												
5.0 B						5.8			5.9								5.9					
5.0 C							6.1							6.3								
5.0 D								6.4					6.3									
5.0 E																						
5.0 comp		6.1																				
		6.1	4.7			5.8	6.1	6.4	5.9	6.0			6.3	6.3			5.9	5.94	118.8	4.7	6.4	1.362
10 A			10.5							11.5												
10 B						11.5			11.9								11.3					
10 C							11.6							11.5								
10 D								12.7					11.2									
10 E																						
10 comp		11.8																				
		11.8	10.5			11.5	11.6	12.7	11.9	11.5			11.2	11.5			11.3	11.52	115.2	10.5	12.7	1.210
15 A			17.7							17.1												
15 B						16.7			18.5								18.2					
15 C						16.7	17.7							17.3								
15 D								19.3					17.0									
15 E																						
15 comp		18.2																				
		18.2	17.7			16.7	17.7	19.3	18.5	17.1			17.0	17.3			18.2	17.76	118.4	16.7	19.3	1.156
20 A			27.7							24.2												
20 B						22.0			24.2								20.0					
20 C							23.9							23.3								
20 D								26.5					23.1									
20 E																						
20 comp		23.0																				
		23.0	27.7			22.0	23.9	26.5	24.2	24.2			23.1	23.3			20.0	23.60	118.0	20.0	27.7	1.385
40 A			53.8							44.9												
40 B						46.1			47.2								46.4					
40 C							48.3							44.4								
40 D								49.8					43.7									
40 E																						
40 comp	33.0	51.7																				
	33.0	51.7	53.8			46.1	48.3	49.8	47.2	44.9			43.7	44.4			46.4	47.40	118.5	43.7	53.8	1.231

Note: underlined values are estimated values developed using least square regression analysis. Samples were analyzed because original analyses were ~1.5-2.0 X higher than TR and re-analyzed samples.

Comparison of original and re-analysis of several dissolved Se samples collected from Day 1 - Day 23.

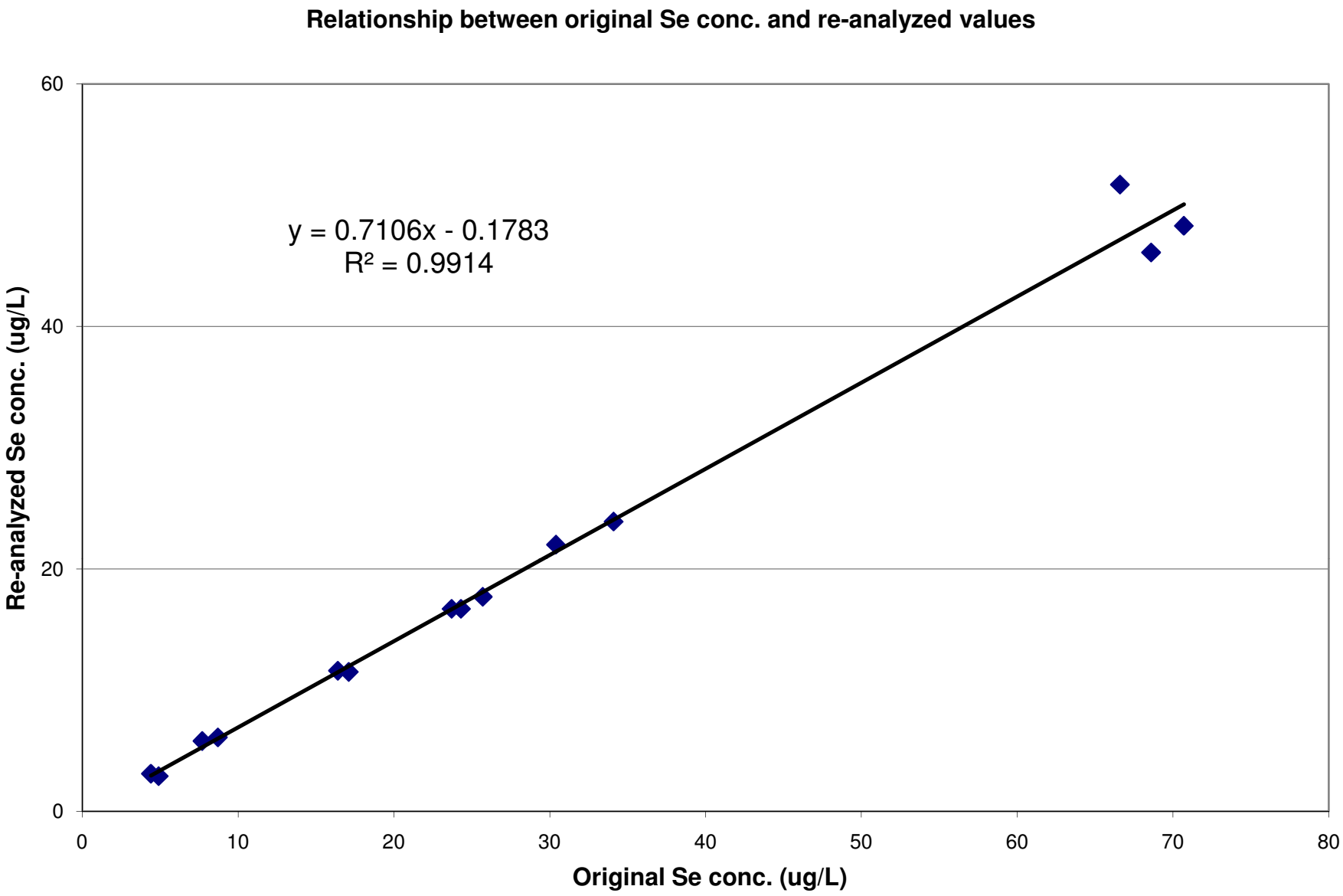
Note: dissolved values were 1.5x - 2.0X higher than co-sampled total recoverable samples.

Not all samples during this time could be re-analyzed because lab terminated these samples prematurely. Therefore, we developed a model to estimate the 'adjusted' dissolved Se concentrations for these samples.

	Day 1, May 15			Day 15, May 29			Day 23, June 6			
	original	re-analyzed		original	re-analyzed		original	re-analyzed		
Nominal Se	ug/l	ug/l	% higher	ug/l	ug/l	% higher	ug/l	ug/l	% higher	% higher for specific treatments
2.5	4.3			4.9	2.9	69.0%	4.4	3.1	41.9%	55.5%
5	8.9			7.7	5.8	32.8%	8.7	6.1	42.6%	37.7%
10	16.9			17.1	11.5	48.7%	16.4	11.6	41.4%	45.0%
15	25.9			24.3	16.7	45.5%	25.7	17.7	45.2%	44.2%
				23.7	16.7	41.9%				
20	32.6			30.4	22.0	38.2%	34.1	23.9	42.7%	40.4%
40	66.6	51.7	28.8%	68.6	46.1	48.8%	70.7	48.3	46.4%	41.3%
						46.4%			43.4%	

	Day 1, May 15		Day 8, May 22	
	original	est. [Se]	original	est. [Se]
Nominal Se	ug/l	ug/l	ug/l	ug/l
2.5	4.3	2.9	3.6	2.4
5	8.9	6.1	6.8	4.7
10	16.9	11.8	15.0	10.5
15	25.9	18.2	25.1	17.7
20	32.6	23.0	39.2	27.7
40	66.6	47.1	76.0	53.8

	original	measured	regression
40 - D1	66.6	51.7	47.15
2.5 - D15	4.9	2.9	3.30
5 - D15	7.7	5.8	5.29
10 - D15	17.1	11.5	11.97
15 - D15	24.3	16.7	17.09
15 - D15 B	23.7	16.7	16.66
20 - D15	30.4	22.0	21.42
40 - D15	68.6	46.1	48.57
2.5 - D23	4.4	3.1	2.95
5 - D23	8.7	6.1	6.00
10 - D23	16.4	11.6	11.48
15 - D23	25.7	17.7	18.08
20 - D23	34.1	23.9	24.05
40 - D23	70.7	48.3	50.06


Relationship between original Se conc. and re-analyzed values
y = 0.7106x - 0.1783
R² = 0.9914
Re-analyzed Se conc. (ug/L)
Original Se conc. (ug/L)
0
20
40
60
0
10
20
30
40
50
60
70
80

Appendix G

Measured Selenium Concentrations in Yeast and *Lumbriculus* Treatments during the Yellowstone Cutthroat Trout Dietary Study

Determination of selenium concentration and total solids (%) in yeast used in dietary YCT study.

12699-002

filename: Se concen in tissue.xls

Nominal Treatment ug Se/g	Se conc. In yeast 6/2/2008 Day 19	7/17/2008 Day 64	Avg	std
0	0.025		0.025	
5	1.39	0.84	1.115	0.389
10	4.48	4.2	4.34	0.198
20	6.96	16.1	11.53	6.463
30	18.9	29.0	23.95	7.142
40	32.3	41.9	37.1	6.788
80	77.2	93.1	85.15	11.243

Nominal Treatment ug Se/g		yeast - total solids 6/2/2008 Day 19	7/17/2008 Day 64	Avg	std
0	Control Yeast	98.9		98.9	
5	A Yeast	98.3	97.8	98.05	0.354
10	B Yeast	98.3	97.7	98	0.424
20	C Yeast	98.4	97.6	98	0.566
30	D Yeast	98.2	97.9	98.05	0.212
40	E Yeast	98.1	97.6	97.85	0.354
80	F Yeast	98.3	96.3	97.3	1.414

Determination of selenium concentration in *Lumbriculus* used in dietary YCT study.

12699-002

filename: Se concen in tissue.xls

Small tank

6/20/2008	7/24/2008 rep A	rep B	rep C	Avg	Avg	stdev
1.82	1.2	0.8	1.0	1.00	1.41	0.580
4.34	3.8	3.9	3.9	3.87	4.10	0.335
5.94	6.3	6.1	6.0	6.13	6.04	0.137
12.3	10.4	9.4	10.2	10.00	11.15	1.626
20.2	18.8	18.4	19.2	18.80	19.50	0.990
22.4	20.4	20.9	22.6	21.30	21.85	0.778
36.6	49.2		50.6	49.90	43.25	9.405
		72.1 (outlier)				

Large tank

Treatment ug nom. Se/L	7/3/2008		7/7/2008	Avg	7/25/2008 rep A	rep B	rep C	Avg	Avg	stdev
0	2.22			2.22	1.7	1.7	1.7	1.70	1.96	0.368
2.5	3.59			3.59	3.4	3.6	3.5	3.50	3.55	0.064
5	6.18		5.92	6.05	5.6	5.5	5.8	5.63	5.84	0.295
10	9.65			9.65	10.2	10.1	10.8	10.37	10.01	0.507
15	14.2	14.8		14.5	16.9	15.7	15.6	16.07	15.28	1.108
20	19.9			19.9	24.7	25.2	23.9	24.60	22.25	3.323
40	48.7			48.7	47.4	44.5	44.7	45.53	47.12	2.239
	(field dup)									

Averge Se concentration between organisms in both tanks.

Treatment ug nom. Se/L	small tank 6/20/2008 worm [Se] ug/g	large tank 7/3-7/2008 worm [Se] ug/g	avg	stdev
0	1.41	1.96	1.69	0.39
2.5	4.10	3.55	3.82	0.39
5	6.04	5.84	5.94	0.14
10	11.15	10.01	10.58	0.81
15	19.50	15.28	17.39	2.98
20	21.85	22.25	22.05	0.28
40	43.25	47.12	45.18	2.73

Determination of total solids in *Lumbriculus* used in dietary YCT study.

12699-002

filename: Se concen in tissue.xls

Small tank Total solids

6/20/2008 solids	7/24/2008 rep A	rep B	rep C	Avg	Avg	stdev
14.8	13.2	12.7	10.8	12.23	13.52	1.815
13.1	12.3	12.4	12.7	12.47	12.78	0.448
9.92	13.0	12.7	13.3	13.00	11.46	2.178
13.1	12.7	12.8	12.7	12.73	12.92	0.259
12.1	14.8	15.3	14.8	14.97	13.53	2.027
12.3	12.3	12.9	13.4	12.87	12.58	0.401
14.4	14.3	15.0	12.1	13.80	14.10	0.424

Averge total solids between organisms in both tanks.

Treatment ug nom. Se/L	small tank 6/20/2008 worm solids ug/g	large tank 7/3-7/2008 ug/g	avg	stdev
0	13.52	14.42	13.97	0.64
2.5	12.78	14.80	13.79	1.43
5	11.46	13.73	12.60	1.61
10	12.92	14.12	13.52	0.85
15	13.53	14.14	13.84	0.43
20	12.58	14.45	13.52	1.32
40	14.10	15.17	14.63	0.75

Large tank

Treatment ug nom. Se/L	7/3/2008 solids	solids	7/7/2008 solids	Avg total solids	7/25/2008 rep A	rep B	rep C	Avg	Avg	stdev
0	14.9			14.9	12.9	14.6	14.3	13.93	14.42	0.684
2.5	14.3			14.3	14.8	15.6	15.5	15.30	14.80	0.707
5	13.3		12.9	13.1	13.9	15.5	13.7	14.37	13.73	0.896
10	11.1			11.1	17.3	16.9	17.2	17.13	14.12	4.266
15	12.9	11.8		12.35	15.7	16.1	16.0	15.93	14.14	2.534
20	13.1			13.1	16.1	15.7	15.6	15.80	14.45	1.909
40	15.1			15.1	15.1	15.8	14.8	15.23	15.17	0.094
		(field dup)								

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Client # OS00530
Fax #: 970-493-8935

Work Order No. : 0810796 ENSR
Receive Date : 08/29/2008 Testing Started : 9/2/2008 Reported : 09/04/2008

SMP. DATE	IDENTIFICATION	TYPE	I.D.
08/28/2008	1 CONTROL LV-2 8/28	Liquid	08CH-014617

Fat - Acid Hydrolysis - AOAC 945.44	2.17	g/100g	Moisture - AOAC 950.46	86.74	g/100g
Protein - AOAC 992.15	8.14	g/100g	Ash - AOAC 920.153	1.68	g/100g
Carbohydrate	1.27	g/100g	Calories	57	

SMP. DATE	IDENTIFICATION	TYPE	I.D.
08/28/2008	2 A LV-2 8/28	Liquid	08CH-014618

Fat - Acid Hydrolysis - AOAC 945.44	0.85	g/100g	Moisture - AOAC 950.46	81.85	g/100g
Protein - AOAC 992.15	7.62	g/100g	Ash - AOAC 920.153	1.90	g/100g
Carbohydrate	7.78	g/100g	Calories	69	

SMP. DATE	IDENTIFICATION	TYPE	I.D.
08/28/2008	3 B LV-2 8/28	Liquid	08CH-014619

Fat - Acid Hydrolysis - AOAC 945.44	0.00	g/100g	Moisture - AOAC 950.46	86.37	g/100g
Protein - AOAC 992.15	8.33	g/100g	Ash - AOAC 920.153	1.54	g/100g
Carbohydrate	3.76	g/100g	Calories	48	

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Client # OS00530
Fax #: 970-493-8935

Work Order No. : 0810796 ENSR
Receive Date : 08/29/2008 Testing Started : 9/2/2008 Reported : 09/04/2008

Testing Methods

Carbohydrate	Calc

Evelyn Silva

Chemistry Supervisor

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Client # OS00530
Fax #: 970-493-8935

Work Order No. : 0811175 ENSR
Receive Date : 08/29/2008 Testing Started : 9/2/2008 Reported : 09/04/2008

SMP. DATE	IDENTIFICATION	TYPE	I.D.
08/28/2008	4 C LV-2 8/28	Liquid	08CH-014620

Fat - Acid Hydrolysis - AOAC 945.44	0.00	g/100g	Moisture - AOAC 950.46	85.08	g/100g
Protein - AOAC 992.15	8.13	g/100g	Ash - AOAC 920.153	1.70	g/100g
Carbohydrate	5.09	g/100g	Calories	53	

SMP. DATE	IDENTIFICATION	TYPE	I.D.
08/28/2008	5 D LV-2 8/28	Liquid	08CH-014621

Fat - Acid Hydrolysis - AOAC 945.44	0.00	g/100g	Moisture - AOAC 950.46	84.51	g/100g
Protein - AOAC 992.15	9.10	g/100g	Ash - AOAC 920.153	1.78	g/100g
Carbohydrate	4.61	g/100g	Calories	55	

SMP. DATE	IDENTIFICATION	TYPE	I.D.
08/28/2008	6 E LV-2 8/28	Liquid	08CH-014622

Fat - Acid Hydrolysis - AOAC 945.44	0.89	g/100g	Moisture - AOAC 950.46	85.84	g/100g
Protein - AOAC 992.15	8.72	g/100g	Ash - AOAC 920.153	1.23	g/100g
Carbohydrate	3.32	g/100g	Calories	56	

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Work Order No. : 0811175 ENSR
Receive Date : 08/29/2008 Testing Started : 9/2/2008 Reported : 09/04/2008

Testing Methods

Carbohydrate	Calc

Evelyn Silva

Chemistry Supervisor

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Client # OS00530
Fax #: 970-493-8935

Work Order No. : 0811176 ENSR
Receive Date : 08/29/2008 Testing Started : 9/2/2008 Reported : 09/04/2008

SMP. DATE	IDENTIFICATION	TYPE	I.D.
08/28/2008	7 F LV-2 8/28	Liquid	08CH-014623

Fat - Acid Hydrolysis - AOAC 945.44	0.81	g/100g	Moisture - AOAC 950.46	86.44	g/100g
Protein - AOAC 992.15	8.71	g/100g	Ash - AOAC 920.153	1.37	g/100g
Carbohydrate	2.67	g/100g	Calories	53	

Testing Methods

Carbohydrate Calc

Notes:

Detection limit for fat = 0.8%. Results lower than that value are reported here as 0.0% for use in calculating Cals/Carbs

(MDC 9/8/08)

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Appendix H

Number of Yellowstone Cutthroat Trout and Survival Rates at Different Stages During the Dietary Study

Survival of YCT in ELS study at different times during the study

12699-002-610

filename: survival_ELS.xls

Treatment	Replicate	# eggs at Test initiation	(5/20/08) (all hatched) Day 6	% survival at hatch		(6/5/08) @ swim-up Day 22	% survival at swim-up		(for analy) (6/10/08) @ thinning Day 27	% survival at thinning		(post-thinning) Revised # of YCT for rep E	6/21/2008 1st d of diet Day 38	% survival 1st d of diet		7/24/2008 test end Day 71	% survival test term.		# dead in dietary test	% died in dietary test	(hatch to term) % survival
	A	20	13	65%		13	65%		13	65%			13	65%		9	45%		4	20%	69%
	B	20	16	80%		16	80%		16	80%			16	80%		11	55%		5	25%	69%
Control	C	20	10	50%		10	50%		10	50%			10	50%		9	45%		1	5%	90%
	D	20	16	80%		14	70%		14	70%			14	70%		11	55%		3	15%	69%
	E	34	29	85%		29	85%		29	85%		17	12	71%		9	53%		3	18%	75%
	Avg & SD			72%	14.5%		70%	13.8%		70%	13.8%			67%	11.0%		51%	5.2%	16	17%	74%
	A	20	17	85%		16	80%		16	80%			15	75%		11	55%		4	20%	65%
	B	20	17	85%		15	75%		15	75%			15	75%		7	35%		8	40%	41%
2.5 ug/L	C	20	18	90%		17	85%		16	80%			16	80%		6	30%		10	50%	33%
	D	20	17	85%		17	85%		17	85%			17	85%		14	70%		3	15%	82%
	E	34	27	79%		26	76%		26	76%		17	9	53%		4	24%		5	29%	40%
	Avg & SD			85%	3.7%		80%	4.7%		79%	3.9%			74%	12.3%		43%	19.3%	30	31%	52%
	A	20	15	75%		15	75%		15	75%			14	70%		6	30%		8	40%	40%
	B	20	17	85%		17	85%		17	85%			17	85%		11	55%		6	30%	65%
5 ug/L	C	20	15	75%		14	70%		13	65%			13	65%		8	40%		5	25%	53%
	D	20	18	90%		18	90%		17	85%			15	75%		11	55%		4	20%	61%
	E	34	30	88%		30	88%		28	82%		17	11	65%		8	47%		3	18%	62%
	Avg & SD			83%	7.2%		82%	8.7%		78%	8.6%			72%	8.4%		45%	10.6%	26	27%	56%
	A	20	14	70%		13	65%		13	65%			13	65%		7	35%		6	30%	50%
	B	20	15	75%		15	75%		15	75%			15	75%		5	25%		10	50%	33%
10 ug/L	C	20	15	75%		15	75%		15	75%			15	75%		10	50%		5	25%	67%
	D	20	16	80%		12	60%		12	60%			12	60%		7	35%		5	25%	44%
	E	34	22	65%		22	65%		22	65%		17	5	29%		5	29%		0	0%	100%
	Avg & SD			73%	5.8%		68%	6.7%		68%	6.7%			61%	18.8%		35%	9.4%	26	26%	59%
	A	20	15	75%		15	75%		15	75%			14	70%		6	30%		8	40%	40%
	B	20	17	85%		16	80%		16	80%			16	80%		9	45%		7	35%	53%
15 ug/L	C	20	14	70%		14	70%		13	65%			13	65%		9	45%		4	20%	64%
	D	20	19	95%		19	95%		19	95%			18	90%		12	60%		6	30%	63%
	E	34	27	79%		24	71%		24	71%		17	7	41%		4	24%		3	18%	40%
	Avg & SD			81%	9.6%		78%	10.3%		77%	11.4%			69%	18.4%		41%	14.3%	28	29%	52%
	A	20	13	65%		13	65%		13	65%			13	65%		4	20%		9	45%	31%
	B	20	16	80%		15	75%		15	75%			15	75%		3	15%		12	60%	19%
20 ug/L	C	20	17	85%		17	85%		17	85%			17	85%		7	35%		10	50%	41%
	D	20	17	85%		16	80%		16	80%			16	80%		11	55%		5	25%	65%
	E	34	28	82%		27	79%		26	76%		17	9	53%		3	18%		6	35%	27%
	Avg & SD			79%	8.4%		77%	7.5%		76%	7.4%			72%	12.8%		29%	16.7%	42	43%	37%
	A	20	12	60%		10	50%		10	50%			10	50%		5	25%		5	25%	42%
	B	20	17	85%		17	85%		17	85%			17	85%		12	60%		5	25%	71%
40 ug/L	C	20	16	80%		16	80%		16	80%			16	80%		7	35%		9	45%	44%
	D	20	17	85%		17	85%		17	85%			15	75%		7	35%		8	40%	41%
	E	34	24	71%		23	68%		23	68%		17	6	35%		3	18%		3	18%	43%
	Avg & SD			76%	10.8%		74%	14.9%		74%	14.9%			65%	21.4%		35%	16.0%	30	31%	48%

Appendix I

Length, Wet and Dry Weight Measurements for Juvenile Yellowstone Cutthroat Trout During the Dietary Study

Length, wet weight, and dry weight data for dietary Yellowstone cutthroat trout for this treatment (2008).
12699-002-610
filename: length & dry wt data_ELS.xls

	C-A std length (mm)	C-A wet wt (g)	C-A dry wt (mg)	C-B std length (mm)	C-B wet wt (g)	C-B wet wt (mg)	C-B dry wt (mg)	C-C std length (mm)	C-C wet wt (g)	C-C dry wt (mg)	C-D std length (mm)	C-D wet wt (g)	C-D dry wt (mg)	C-E std length (mm)	C-E wet wt (g)	C-E wet wt (mg)	C-E dry wt (mg)
A	34		92.21	40	0.7071	707.1	52.36	41		43.780	33		50.280	37			95.81
B	40		84.940	40	0.9535	953.5	140.59	31		50.970	34		100.300	32			60.47
C	29		81.970	36	0.7708	770.8	76.32	33		187.950	27		89.740	33			76.79
D	38		45.350	31	0.5642	564.2	115.23	28		92.580	33		89.490	33	0.4101	410.1	67.00
E	34		132.810	28	0.3590	359.0	93.71	35		109.590	35		72.760	33			56.52
F	35		76.660	32	0.5459	545.9	80.42	27		75.900	32		81.000	34	0.4900	490.0	82.70
G	33		146.430	32	0.6181	618.1	86.84	31		71.270	34		49.610	31	0.3307	330.7	49.50
H	32		62.210	30	0.3898	389.8	52.86	32		72.050	30		70.930	35	0.5606	560.6	96.10
I	31		94.820	32	0.5740	574.0	47.17	28		37.610	32		86.340	40	0.8205	820.5	145.60
J				28	0.3448	344.8	44.49				27		80.780				
K				29	0.4046	404.6	76.93				31		102.170				
L																	
M																	
N																	
O																	
P																	
Q																	
R																	
S																	
T																	
Avg	34.00		90.822	32.55	0.57	566.53	78.8109	31.78		82.41111	31.64		79.40	34.22	0.52	522.38	81.166
Std	3.391		31.767	4.321	0.191	190.805	29.990	4.324		45.747	2.693		17.515	2.774	0.188	187.57	29.261
n	9			11				9			11			9			
			40.87				43.35			37.09			43.67				60.87

NB - underlined values are from CAS (i.e., organisms used for whole-body Se analyses). All other values determined at the FCETL.

	Grand avg	std
length	**32.84**	**1.217**
dry wt (ps)	**82.522**	**4.855**
dry wt (po)	**45.169**	**9.166**

Length, wet weight, and dry weight data for dietary Yellowstone cutthroat trout for this treatment (2008).
12699-002-610
filename: length & dry wt data_ELS.xls

	Se-2.5 A std length (mm)	Se-2.5 A wet wt (g)	Se-2.5 A wet wt (mg)	Se-2.5 A dry wt (mg)	Se-2.5 B std length (mm)	Se-2.5 B wet wt (g)	Se-2.5 B dry wt (mg)	Se-2.5 C std length (mm)	Se-2.5 C wet wt (g)	Se-2.5 C dry wt (mg)	Se-2.5 D std length (mm)	Se-2.5 D wet wt (g)	Se-2.5 D dry wt (mg)	Se-2.5 E std length (mm)	Se-2.5 E wet wt (g)	Se-2.5 E wet wt (mg)	Se-2.5 E dry wt (mg)
A	30			27.17	32		54.28	41		163.45	35		98.54	36	0.5236	523.6	86.80
B	28			97.42	29		43.89	32		81.29	38		44.72	37	0.3475	347.5	48.90
C	34	0.5237	523.7	90.80	32		166.81	28		47.16	37		80.28	30	0.2805	280.5	47.00
D	35			66.82	29		27.57	32		40.48	31		39.69	38	0.7296	729.6	127.90
E	33			50.28	41		72.05	31		71.91	28		36.98				
F	41			40.49	32		64.49	29		67.52	32		77.31				
G	33			52.39	21		73.26				27		62.72				
H	31			61.96							32		59.65				
I	34			75.52							31		23.21				
J	35			147.00							30		124.28				
K	37			74.61							29		56.02				
L											27		115.20				
M											26		56.91				
N											27		31.40				
O																	
P																	
Q																	
R																	
S																	
T																	
Avg	33.73	0.5237	523.7	71.31	30.86		71.76	32.17		78.64	30.71		64.78	35.25	0.4703	470.3	77.65
Std	3.495			32.596	5.928		44.953	4.622		44.308	3.811		30.929	3.594	0.201	201.0	38.187
n	11				7			6			14			4			
				39.22			25.12			23.59			45.35				34.51

NB - underlined values are from CAS (i.e., organisms used for whole-body Se analyses). All other values determined at the FCETL.

	Grand avg	std
length	**32.54**	**1.940**
dry wt (ps)	**72.829**	**5.594**
dry wt (po)	**33.558**	**9.254**

Length, wet weight, and dry weight data for dietary Yellowstone cutthroat trout for this treatment (2008).
12699-002-610
filename: length & dry wt data_ELS.xls

	Se-5 A std length (mm)	Se-5 A wet wt (g)	Se-5 A dry wt (mg)	Se-5 B std length (mm)	Se-5 B wet wt (g)	Se-5 B dry wt (mg)	Se-5 C std length (mm)	Se-5 C wet wt (g)	Se-5 C dry wt (mg)	Se-5 D std length (mm)	Se-5 D wet wt (g)	Se-5 D dry wt (mg)	Se-5 E std length (mm)	Se-5 E wet wt (g)	Se-5 E wet wt (mg)	Se-5 E dry wt (mg)
A	39		91.52	28		81.03	34		28.43	34		53.18	32	0.348	348.3	56.00
B	33		73.15	32		87.36	37		45.98	31		32.52	26	0.142	142.3	17.86
C	31		57.30	31		81.31	28		72.33	39		92.82	32	0.398	398.0	68.10
D	33		85.23	33		57.10	33		101.28	34		50.77	38	0.709	709.1	121.90
E	30		84.03	33		52.24	27		73.91	31		89.51	37			99.17
F	34		136.38	31		64.05	32		86.25	34		62.07	39			122.49
G				34		84.97	32		113.20	27		92.61	36	0.520	519.5	88.20
H				32		74.12	37		88.36	29		141.17	34	0.480	480.4	79.00
I				30		64.43				30		61.03				
J				30		59.50				31		47.35				
K				30		59.92				27		29.55				
L																
M																
N																
O																
P																
Q																
R																
S																
T																
Avg	33.33		87.935	31.27		69.639	32.50		76.218	31.55		68.416	34.25	0.4329	432.9	81.5900
Std	3.141		26.606	1.737		12.453	3.665		27.934	3.532		32.864	4.234	0.189	189.1	34.960
n	6			11			8			11			8			
			26.38			38.30			30.49			37.63				59.34

NB - underlined values are from CAS (i.e., organisms used for whole-body Se analyses). All other values determined at the FCETL.

	Grand avg	std
length	**32.58**	**1.239**
dry wt (ps)	**76.760**	**8.198**
dry wt (po)	**38.427**	**12.709**

Length, wet weight, and dry weight data for dietary Yellowstone cutthroat trout for this treatment (2008).
12699-002-610
filename: length & dry wt data_ELS.xls

	Se-10 A std length (mm)	Se-10 A wet wt (g)	Se-10 A dry wt (mg)	Se-10 B std length (mm)	Se-10 B wet wt (g)	Se-10 B dry wt (mg)	Se-10 C std length (mm)	Se-10 C wet wt (g)	Se-10 C dry wt (mg)	Se-10 D std length (mm)	Se-10 D wet wt (g)	Se-10 D dry wt (mg)	Se-10 E std length (mm)	Se-10 E wet wt (g)	Se-10 E wet wt (mg)	Se-10 E dry wt (mg)
A	22		122.80	33		62.88	35		57.16	32		53.18	35	0.5257	525.7	86.30
B	26		37.40	29		91.66	37		56.49	35		64.73	34	0.5590	559.0	88.50
C	26		92.22	31		125.43	30		59.45	29		63.03	31	0.3710	371.0	58.20
D	29		35.96	34		42.45	30		50.12	27		31.17	40	0.9755	975.5	169.20
E	35		14.32	41		81.33	29		55.08	30		104.56	30	0.3157	315.7	46.80
F	37		49.67				33		36.42	31		73.03				
G	38		107.75				30		87.26	41		158.04				
H							29		114.89							
I							28		101.09							
J							31		59.27							
K																
L																
M																
N																
O																
P																
Q																
R																
S																
T																
Avg	30.43		65.731	33.60		80.750	31.20		67.723	32.14		78.249	34.00	0.5494	549.38	89.80
Std	6.241		41.461	4.561		31.233	2.898		24.826	4.634		41.524	3.937	0.259	259.161	47.869
n	7			5			10			7			5			
			23.01			20.19			33.86			27.39				89.80

NB - underlined values are from CAS (i.e., organisms used for whole-body Se analyses). All other values determined at the FCETL.

	Grand avg	std
length	**32.27**	**1.526**
dry wt (ps)	**76.451**	**9.887**
dry wt (po)	**38.848**	**28.946**

Length, wet weight, and dry weight data for dietary Yellowstone cutthroat trout for this treatment (2008).
12699-002-610
filename: length & dry wt data_ELS.xls

	Se-15 A std length (mm)	Se-15 A wet wt (g)	Se-15 A wet wt (mg)	Se-15 A dry wt (mg)	Se-15 B std length (mm)	Se-15 B wet wt (g)	Se-15 B dry wt (mg)	Se-15 C std length (mm)	Se-15 C wet wt (g)	Se-15 C dry wt (mg)	Se-15 D std length (mm)	Se-15 D wet wt (g)	Se-15 D dry wt (mg)	Se-15 E std length (mm)	Se-15 E wet wt (g)	Se-15 E wet wt (mg)	Se-15 E dry wt (mg)
A	32			90.27	34		56.42	37		68.92	38		79.82	33	0.4288	428.8	64.40
B	34			85.18	28		129.51	39		54.16	34		106.41	29	0.3934	393.4	60.10
C	37			101.90	28		92.52	30		55.63	26		27.10	38	0.7123	712.3	118.20
D	32	0.4186	418.6	62.40	31		85.25	32		55.53	27		66.57	32	0.3336	333.6	51.10
E	37			99.71	30		49.29	28		36.70	26		82.32				
F	36			102.320	33		88.53	29		112.78	33		87.59				
G					35		55.65	32		87.62	31		127.63				
H					38		48.84	29		34.11	30		29.84				
I					29		61.70	34		130.25	31		35.02				
J											34		58.56				
K											25		69.72				
L											37		39.13				
M																	
N																	
O																	
P																	
Q																	
R																	
S																	
T																	
Avg	34.67	0.4186	418.6	90.297	31.78		74.190	32.22		70.633	31.00		67.476	33.00	0.4670	467.025	73.45
Std	2.338			15.312	3.456		26.931	3.801		33.226	4.369		31.480	3.742	0.168	168.170	30.344
n	6				9			9			12			4			
				27.09			33.39			31.79			40.49				41.97

NB - underlined values are from CAS (i.e., organisms used for whole-body Se analyses). All other values determined at the FCETL.

	Grand avg	std
length	**32.53**	**1.395**
dry wt (ps)	**75.209**	**8.839**
dry wt (po)	**34.943**	**6.209**

Length, wet weight, and dry weight data for dietary Yellowstone cutthroat trout for this treatment (2008).
12699-002-610
filename: length & dry wt data_ELS.xls

	Se-20 A std length (mm)	Se-20 A wet wt (g)	Se-20 A dry wt (mg)	Se-20 B std length (mm)	Se-20 B wet wt (g)	Se-20 B dry wt (mg)	Se-20 C std length (mm)	Se-20 C wet wt (g)	Se-20 C wet wt (mg)	Se-20 C dry wt (mg)	Se-20 D std length (mm)	Se-20 D wet wt (g)	Se-20 D wet wt (mg)	Se-20 D dry wt (mg)	Se-20 E std length (mm)	Se-20 E wet wt (g)	Se-20 E wet wt (mg)	Se-20 E dry wt (mg)
A	28		132.98	33		70.58	38			53.17	29			49.60	32	0.4183	418.3	67.20
B	32		93.66	40		144.14	30	0.4894	489.4	66.80	30			76.13	38	0.7086	708.6	116.70
C	34		50.21	30		68.03	33			58.10	27			39.42	34	0.4820	482.0	81.40
D	30		83.51				31			68.49	32			44.97				
E							30			38.35	33			36.16				
F							27			87.13	40	0.8351	835.1	137.30				
G							32			112.31	28			25.92				
H											35			70.64				
I											30			57.68				
J											34			78.43				
K											30			67.16				
L																		
M																		
N																		
O																		
P																		
Q																		
R																		
S																		
T																		
Avg	31.00		90.09	34.33		94.25	31.57	0.4894	489.40	69.19	31.64	0.8351	835.10	62.13	34.67	0.5363	536.30	88.43
Std	2.582		34.088	5.132		43.225	3.409			24.215	3.722			30.303	3.055	0.153	152.577	25.488
n	4			3			7				11				3			
			18.02			14.14				24.22				34.17				29.48

NB - underlined values are from CAS (i.e., organisms used for whole-body Se analyses). All other values determined at the FCETL.

	Grand avg	std
length	**32.64**	**1.719**
dry wt (ps)	**80.819**	**14.220**
dry wt (po)	**24.004**	**8.163**

Length, wet weight, and dry weight data for dietary Yellowstone cutthroat trout for this treatment (2008).
12699-002-610
filename: length & dry wt data_ELS.xls

	Se-40 A std length (mm)	Se-40 A wet wt (g)	Se-40 A dry wt (mg)	Se-40 B std length (mm)	Se-40 B wet wt (g)	Se-40 B wet wt (mg)	Se-40 B dry wt (mg)	Se-40 C std length (mm)	Se-40 C wet wt (g)	Se-40 C dry wt (mg)	Se-40 D std length (mm)	Se-40 D wet wt (g)	Se-40 D wet wt (mg)	Se-40 D dry wt (mg)	Se-40 E std length (mm)	Se-40 E wet wt (g)	Se-40 D wet wt (mg)	Se-40 E dry wt (mg)
A	32		112.77	29			127.72	35		85.19	36			49.41	36	0.6646	664.6	108.20
B	32		67.17	31			97.05	34		85.46	43			82.29	41	0.9780	978.0	177.00
C	31		111.21	30			170.32	34		69.32	35	0.5965	596.5	103.50	29	0.2463	246.3	34.10
D	36		80.90	35			45.09	32		67.68	29			108.91				
E	34		77.41	38			38.52	32		67.46	35			49.30				
F				40			20.80	37		112.91	27			174.01				
G				33			63.00	31		81.85	30			22.72				
H				43			119.76											
I				30			49.91											
J				31	0.3629	362.9	52.30											
K				36			42.06											
L				24			73.24											
M																		
N																		
O																		
P																		
Q																		
R																		
S																		
T																		
Avg	33.00		89.892	33.33	0.3629	362.90	74.981	33.57		81.410	33.57	0.5965	596.5	84.306	35.33	0.6296	629.63	106.43
Std	2.000		20.801	5.297			44.614	2.070		16.085	5.412			50.433	6.028	0.367	367.10	71.47
n	5			12				7			7				3			
			22.47				44.99			28.49				29.51				53.22

NB - underlined values are from CAS (i.e., organisms used for whole-body Se analyses). All other values determined at the FCETL.

	Grand avg	std
length	**33.76**	**0.909**
dry wt (ps)	**87.404**	**11.918**
dry wt (po)	**35.736**	**12.829**

Condition factor for select organisms (Se analysis) for dietary Yellowstone cutthroat trout for all treatments (2008).
12699-002-610
filename: length & dry wt data_ELS.xls

Fish Id	C-B std length (mm)	C-B wet wt (g)	C-B Condition factor (K)	C-E std length (mm)	C-E wet wt (g)	C-E Condition factor (K)	Se-2.5 A std length (mm)	Se-2.5 A wet wt (g)	Se-2.5 A Condition factor (K)	Se-2.5 E std length (mm)	Se-2.5 E wet wt (g)	Se-2.5 E Condition factor (K)	Se-5 E std length (mm)	Se-5 E wet wt (g)	Se-5 E Condition factor (K)	Se-10 E std length (mm)	Se-10 E wet wt (g)	Se-10 E Condition factor	Se-15 A std length (mm)	Se-15 A wet wt (g)	Se-15 A Condition factor
A	40	0.7071	1.1048	37			30			36	0.5236	1.1223	32	0.348	1.0629	35	0.5257	1.2261	32		
B	40	0.9535	1.4898	32			28			37	0.3475	0.6860	26	0.142	0.8096	34	0.5590	1.4222	34		
C	36	0.7708	1.6521	33			34	0.5237	1.3324	30	0.2805	1.0389	32	0.398	1.2146	31	0.3710	1.2453	37		
D	31	0.5642	1.8939	33	0.4101	1.1412	35			38	0.7296	1.3296	38	0.709	1.2923	40	0.9755	1.5242	32	0.4186	1.2775
E	28	0.3590	1.6354	33			33						37			30	0.3157	1.1693	37		
F	32	0.5459	1.6660	34	0.4900	1.2467	41						39						36		
G	32	0.6181	1.8863	31	0.3307	1.1101	33						36	0.520	1.1135						
H	30	0.3898	1.4437	35	0.5606	1.3075	31						34	0.480	1.2223						
I	32	0.5740	1.7517	40	0.8205	1.2820	34														
J	28	0.3448	1.5707				35														
K	29	0.4046	1.6589				37														
L																					
M																					
N																					
O																					
P																					
Q																					
R																					
S																					
T																					
Avg	32.55	0.57	1.61	34.22	0.52	1.22	33.73	0.5237	1.332	35.25	0.4703	1.044	34.25	0.4329	1.1192	34.00	0.5494	1.317	34.67	0.4186	1.277
Std	4.321	0.191	0.220	2.774	0.188	0.087	3.495			3.594	0.201	0.268	4.234	0.189	0.172	3.937	0.259	0.149	2.338		#DIV/0!
n	11			9			11			4			8			5			6		

NB - underlined values are from CAS (i.e., organisms used for whole-body Se analyses). All other values determined at the FCETL.

	avg	overall X	n	avg wet wt
Control CF (K)	1.416	1.490	16	0.5527
2.5	1.188	1.102	5	0.4810
5	1.119	1.119	6	0.4329
10	1.317	1.317	5	0.5494
15	1.279	1.280	5	0.4573
20	1.461	1.382	5	0.5867
40	1.298	1.293	5	0.5697

Condition factor for select organisms (Se analysis) for dietary Yellowstone cutthroat trout for all treatments (2008).
12699-002-610
filename: length & dry wt data_ELS.xls

Fish Id	Se-15 E std length (mm)	Se-15 E wet wt (g)	Se-15 E Condition factor	Se-20 C std length (mm)	Se-20 C wet wt (g)	Se-20 C Condition factor	Se-20 D std length (mm)	Se-20 D wet wt (g)	Se-20 D Condition factor	Se-20 E std length (mm)	Se-20 E wet wt (g)	Se-20 E Condition factor	Se-40 B std length (mm)	Se-40 B wet wt (g)	Se-40 B Condition factor	Se-40 D std length (mm)	Se-40 D wet wt (g)	Se-40 D Condition factor	Se-40 E std length (mm)	Se-40 E wet wt (g)	Se-40 E Condition factor
A	33	0.4288	1.1932	38			29			32	0.4183	1.2766	29			36			36	0.6646	1.4245
B	29	0.3934	1.6130	30	0.4894	1.8126	30			38	0.7086	1.2914	31			43			41	0.9780	1.4190
C	38	0.7123	1.2981	33			27			34	0.4820	1.2263	30			35	0.5965	1.3913	29	0.2463	1.0099
D	32	0.3336	1.0181	31			32						35			29					
E				30			33						38			35					
F				27			40	0.8351	1.3048				40			27					
G				32			28						33			30					
H							35						43								
I							30						30								
J							34						31	0.3629	1.2182						
K							30						36								
L													24								
M																					
N																					
O																					
P																					
Q																					
R																					
S																					
T																					
Avg	33.00	0.4670	1.2806	31.57	0.4894	1.813	31.64	0.8351	1.305	34.67	0.5363	1.265	33.33	0.3629	1.218	33.57	0.5965	1.391	35.33	0.6296	1.284
Std	3.742	0.168	0.250	3.409		#DIV/0!	3.722		#DIV/0!	3.055	0.153	0.034	5.297		#DIV/0!	5.412		#DIV/0!	6.028	0.367	0.24
n	4			7			11			3			12			7			3		

Appendix J

Summary of Selenium Concentrations Measured in Yellowstone Cutthroat Trout at Thinning and Test Termination

Concentration of Se (mg/kg dry wt) in individual fish (Day 27, prior to feeding) in the ELS study

12699-002-610

filename: ELS fish Se conc.xls

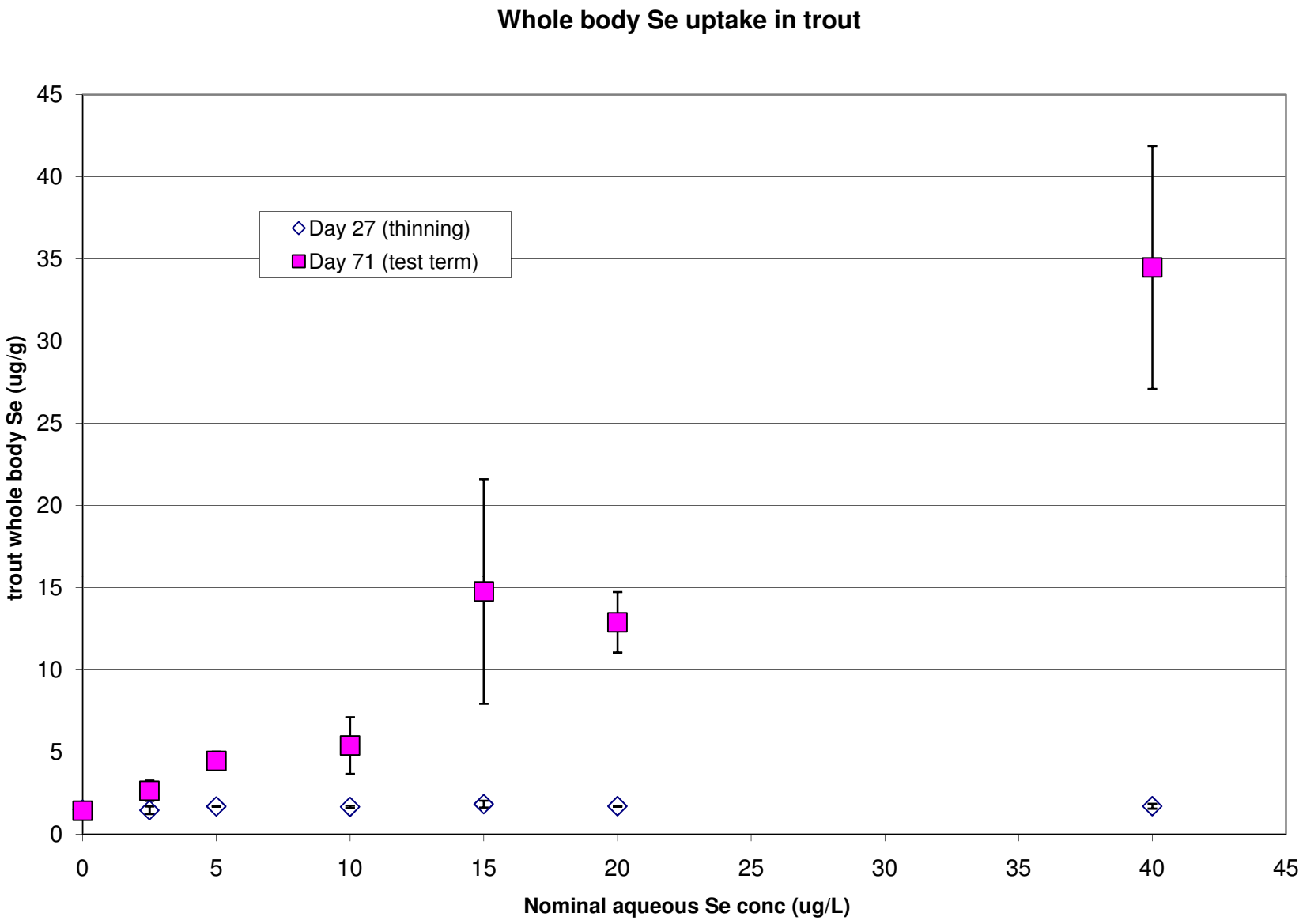
Treatment	Rep	D27 Se mg/kg dwt	Avg	Stdev	CV	Dry mass (g)	Total solids (%)
Control	E.1	1.56				0.0838	24.6
	E.2	1.46				0.0858	21.9
	E.3	1.49	1.503	0.0513	3%	0.0692	23.9
2.5	E.1	1.21				0.0822	23.9
	E.2	1.52				0.0857	24.2
	E.3	1.67	1.467	0.2346	16%	0.0686	26.3
5	E.1	1.72				0.0791	25.8
	E.2	1.68				0.0768	21.8
	E.3	1.70	1.700	0.0200	1%	0.0663	24.2
10	E.1	1.61				0.1001	28.8
	E.2	1.66				0.0932	30.0
	E.3	1.75	1.673	0.0709	4%	0.0750	26.1
15	E.1	1.71				0.0875	25.4
	E.2	1.73				0.0889	24.4
	E.3	2.08	1.840	0.2081	11%	0.0694	24.1
20	E.1	1.73				0.0883	28.2
	E.2	1.68				0.0858	26.8
	E.3	1.73	1.713	0.0289	2%	0.0697	28.3
40	E.1	1.86				0.0804	24.6
	E.2	1.57				0.0850	27.2
	E.3	1.70	1.710	0.1453	8%	0.0760	27.0

Concentration of Se (mg/kg dry wt) in individual fish in the ELS study at test termination

12699-002-610

filename: ELS fish Se conc.xls

Trtmnt	Rep	test term Se mg/kg dwt	whole body Se mg/kg dwt Avg	Stdev	CV	(@ ENSR) Wet wt (g)	(@ CAS) Total solids (%)	Avg	(@ CAS) Dry mass (g)	Avg	dwt (g) Stdev	CV	Dry mass (mg)	Avg	Stdev
Control	E.4	0.98					20.4		0.0670				67.0		
	E.6	1.44					21.5		0.0827				82.7		
	E.7	1.43					19.8		0.0495				49.5		
	E.8	1.23					21.5		0.0961				96.1		
	E.9	2.11	1.438	0.420	29%		21.0	20.840	0.1456	0.0882	0.037	41%	145.6	88.1800	36.519
2.5	E.1	2.47				0.5236	20.9		0.0868				86.8		
	E.2	2.58				0.3475	18.5		0.0489				48.9		
	E.3	2.10				0.2805	22.0		0.0470				47.0		
	E.4	3.71				0.7296	20.2		0.1279				127.9		
	A.3	2.41	2.654	0.617	23%	0.5237	20.7	20.460	0.0908	0.0803	0.034	42%	90.8	80.2800	33.590
5	E.1	3.89					20.6		0.0560				56.0		
	E.3	4.39					23.1		0.0681				68.1		
	E.4	5.41					20.4		0.1219				121.9		
	E.7	4.20					25.3		0.0882				88.2		
	E.8	4.42	4.462	0.570	13%		21.3	22.140	0.0790	0.0826	0.025	30%	79.0	82.6400	25.033
10	E.1	6.19					20.3		0.0863				86.3		
	E.2	3.99					18.4		0.0885				88.5		
	E.3	7.01					18.8		0.0582				58.2		
	E.4	3.16					20.0		0.1692				169.2		
	E.5	6.67	5.404	1.720	32%		18.3	19.160	0.0468	0.0898	0.048	53%	46.8	89.8000	47.869
15	A.4	8.89					20.1		0.0624				62.4		
	E.1	15.90					17.8		0.0644				64.4		
	E.2	9.48					18.2		0.0601				60.1		
	E.3	25.80					19.4		0.1182				118.2		
	E.4	13.80	14.774	6.827	46%		19.9	19.080	0.0511	0.0712	0.027	38%	51.1	71.2400	26.739
20	C.2	12.10					18.6		0.0668				66.8		
	D.6	15.00					19.8		0.1373				137.3		
	E.1	10.50					21.0		0.0672				67.2		
	E.2	12.40					20.2		0.1167				116.7		
	E.3	14.50	12.900	1.845	14%		21.5	20.220	0.0814	0.0939	0.032	34%	81.4	93.8800	31.650
40	B.10	43.60					17.1		0.0523				52.3		
	D.3	37.80					20.3		0.1035				103.5		
	E.1	33.60					18.0		0.1082				108.2		
	E.2	34.00					20.3		0.177				177.0		
	E.3	23.40	34.480	7.382	21%		17.9	18.720	0.0341	0.0950	0.056	59%	34.1	95.0200	55.907


Whole body Se uptake in trout
Day 27 (thinning)
Day 71 (test term)
trout whole body Se (ug/g)
Nominal aqueous Se conc (ug/L)
0
5
10
15
20
25
30
35
40
45

Treatment	Whole body fish Se conc. (mg/kg dwt)			
	Day 27 (prior to Se diet)		Test termination	
ug nom. Se/L	avg.	std	avg.	std
0	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

APPENDIX K

Deformity Assessment ELS

The general scoring 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. The original publication showed pictures of some deformities but others, particularly the intermediate categories were not illustrated or were poorly described. More specific definitions for each of the assessment categories were developed to give better repeatability and consistency across studies, and to aid others in learning the range of deformities possible.

Deformities in each of the categories described above were given a score from 0-3, with 0 being a normal condition and 3 being the most deformed. Some range finding was conducted over the first several samples to find background and severe levels of deformities in each category. Initial samples were rescored as necessary to bring them into compliance with the standards that were used throughout the assessment.

The protocol for assessing damage was to place several fish, head to the left, in a Petri dish and examine them under a dissecting microscope and 10X magnification. The lateral side was examined for spinal deformities (lordosis, kyphosis), appearance of the eye, head and snout shape, edema, and fin deformities. The fish was turned ventrally to look for mouth deformities and further spinal deformities (scoliosis), turned laterally again for the same criteria as the other side, and then dorsally for issues associated with eyes, head size, spinal deformities.

Craniofacial deformities included shortening of the jaw, snout, and missing or poorly developed eye or eyes, and head shape abnormalities. A slightly shortened lower jaw (<= 1 lip width) received a 1, a shortened jaw = 2 lip widths or a slightly shortened and slightly disfigured jaw = 2, and a flat lower jaw or much disfigured (non-functional) jaw = 3. An assessment of fish independent of this study revealed that other Yellowstone cutthroat trout of the same size and developmental state did not have the slight deformity that was assessed as CF =1 for the jaw (J). Thus, the CF = 1 score where the J was concerned were deemed real. A slightly blunted snout (about 50% eye diameter, usually is > than that) = 1, very blunt or flat = 2, deformed or bulbous = 3. Eye deformities were scored as one eye blind or poorly pigmented or poorly developed =1, both poorly developed = 2, both blind = 3. Skulls that were slightly bulbous (1/3 > normal) = 1, moderately bulbous (2/3 > normal) = 2, and bulbous (1x or > than normal) = 3. Usually factors occurred together so a combination of two “1” conditions = 2, three “1” conditions = 3, or a 1 and a 2 = 3, and so on. For example, a deformed jaw and a blind eye = 2, two blind eyes = 2, but a badly deformed jaw (= 2 alone) plus a blind eye (= 1 alone), = 3.

Skeletal deformities included any deformity of the vertebrae or spines. A slight bend of less than 45 degrees (but > than body width off of straight) or a minor body constriction (e.g. a tight rubber band about the body effect) was given a score of 1, 2 slight bends or constrictions anywhere, or bend of > 45-90 degrees was scored a 2, and multi-directional bends > 90 degrees were given a 3. Bends caused by skeletal deformities were usually detectable from normal bending of the body during preservation (these fish were usually well preserved, very straight) by presence of a slight or greater bump below the surface of

the epidermis on the outside of the bend. However, some fish with SD = 1 had just a very slight bend in the range the deformity described but could be due to preservation or the poor condition of the fish. This was sometimes especially true in larger fish, which may be more muscular and undergo stronger contraction during preservation and thus, bend slightly. A score “CF = 1” was a slight deformity, if at all. The scores of SD = 1 involving kyphosis or lordosis were deemed real because that is an unusual preservation deformity. Thin fish difficult to score, and often looked like they were underfed or starving.

Fin deformities included variation in fin or finfold morphology and a slightly smaller or missing fin (in thin fish, the adipose fin was often absent, indicating fat absorption, not uncommon and scored 1) or one with a bend or incomplete ray development (in older fish) was given a 1, 2 fins damaged or malformed = 2, and > 2 fins malformed or if fins were missing (except adipose) was = 3. Often fins were malformed associated with vertebral deformities that did not permit proper development. Folded finfolds as a result of preservation were not counted.

Edema was assessed because it was evaluated in the previous brown trout and Yellowstone cutthroat trout studies and because it was considered a condition that could affect emergence, mobility, and other factors that may limit survival of fish in the wild. Edema was detected by an obvious swelling and fluid buildup, usually abdominally, and ventrally, which often displaced the gut, and was usually clear fluid that was slightly soft when touched with a blunt probe. The yolk, when present, also created some swelling but was typically yellowish, opaque, and small, and hard to the touch in preservation. Slight edema = 1 was for a fish with up to 1X swelling of the normal body width or depth, up to 2x = 2, and > 2x = 3.

A sample of 50 fish and a sample of 30 fish were scored twice, the same fish for each batch but not necessarily the same order. This sample was characterized by a low incidence of fin deformities (slow development) and a high incidence of jaw deformities. Those cranio-facial traits are difficult to score because they are additive, and subjective as to severity. Thus, the results may be a conservative view of what score replicability should be like for other traits in other samples that are easier to score.

Replicability of frequency of cranio-facial abnormalities was high among assessments. The cumulative sums of the scores were also quite close, but reflecting variability in scoring for all three categories of severity in each sample. Replicability of fin ray development assessments for both frequency and the sum of the scores was similar.

Below we have included tabular and graphical representation of each of the deformities assessed described above, demonstrating scoring values of 0 – 3 for each of the deformities.

Deformity assessment results for Yellowstone cutthroat trout in early life stage study (aqueous and dietary Se exposure). Values represent the number of fish (at swim-up and test termination) in each scoring criterion (i.e., 0-3). See below for a definition of scoring criteria.

filename: deform appendix_ELS.xls

Count of fish #		Craniofacial Deformities (CF)				
Location	Field Sample ID	0	1	2	3	Grand Total
Control	A	9	0	0	0	9
	B	11	0	0	0	11
	C	9	0	0	0	9
	D	10	1	0	0	11
	E	4	0	0	0	4
	Total	43	1	0	0	44
2.5 ug/L	A	9	1	0	0	10
	B	7	0	0	0	7
	C	6	0	0	0	6
	D	11	3	0	0	14
	E					0
	Total	33	4	0	0	37
5 ug/L	A	6	0	0	0	6
	B	11	0	0	0	11
	C	8	0	0	0	8
	D	10	1	0	0	11
	E	3	0	0	0	3
	Total	38	1	0	0	39
10 ug/L	A	5	2	0	0	7
	B	5	0	0	0	5
	C	9	1	0	0	10
	D	7	0	0	0	7
	E					0
	Total	26	3	0	0	29
15 ug/L	A	5	0	0	0	5
	B	9	0	0	0	9
	C	7	1	0	0	8
	D	12	1	0	0	13
	E					0
	Total	33	2	0	0	35
20 ug/L	A	4	0	0	0	4
	B	3	0	0	0	3
	C	6	0	0	0	6
	D	10	0	0	0	10
	E					0
	Total	23	0	0	0	23
40 ug/L	A	5	0	0	0	5
	B	9	2	0	0	11
	C	7	0	0	0	7
	D	6	0	0	0	6
	E					0
	Total	27	2	0	0	29
Grand Total		223	13	0	0	236

Craniofacial deformities included shortening of the jaw, snout, and missing or poorly developed eye or eyes, and head shape abnormailities. A slightly shortened lower jaw (<= 1 lip width) received a 1, a shortened jaw = 2 lip widths or a slightly shortened and slightly disfigured jaw = 2, and a flat lower jaw or much disfigured (non-functional) jaw = 3. An assessment of fish independent of this study revealed that other trout of the same size and developmental state did not have the slight deformity that was assessed as CF =1 for the jaw (J). Thus, the CF = 1 score where the J was concerned were deemed real. A slightly blunted snout (about 50% eye diameter, usually is > than that) = 1, very blunt or flat = 2, deformed or bulbous = 3. Eye deformities were scored as one eye blind or poorly pigmented or poorly developed =1, both poorly developed = 2, both blind = 3. Skulls that were slightly bulbous (1/3 > normal) = 1, moderately bulbous (2/3 > normal) = 2, and bulbous (1x or > than normal) = 3. Usually factors occurred together so a combination of two “1” conditions = 2, three “1” conditions = 3, or a 1 and a 2 = 3, and so on. For example, a deformed jaw and a blind eye = 2, two blind eyes = 2, but a badly deformed jaw (= 2 alone) plus a blind eye (= 1 alone), = 3.

Deformity assessment results for Yellowstone cutthroat trout in early life stage study (aqueous and dietary Se exposure). Values represent the number of fish (at swim-up and test termination) in each scoring criterion (i.e., 0-3). See below for a definition of scoring criteria.

filename: deform appendix_ELS.xls

Count of fish #		Skeletal Deformities (SD)				
Location	Field Sample ID	0	1	2	3	Grand Total
Control	A	9	0	0	0	9
	B	11	0	0	0	11
	C	9	0	0	0	9
	D	11	0	0	0	11
	E	4	0	0	0	4
	Total	44	0	0	0	44
2.5 ug/L	A	10	0	0	0	10
	B	7	0	0	0	7
	C	6	0	0	0	6
	D	14	0	0	0	14
	E					0
	Total	37	0	0	0	37
5 ug/L	A	6	0	0	0	6
	B	11	0	0	0	11
	C	8	0	0	0	8
	D	11	0	0	0	11
	E	3	0	0	0	3
	Total	39	0	0	0	39
10 ug/L	A	6	1	0	0	7
	B	5	0	0	0	5
	C	10	0	0	0	10
	D	7	0	0	0	7
	E					0
	Total	28	1	0	0	29
15 ug/L	A	5	0	0	0	5
	B	9	0	0	0	9
	C	8	0	0	0	8
	D	13	0	0	0	13
	E					0
	Total	35	0	0	0	35
20 ug/L	A	4	0	0	0	4
	B	3	0	0	0	3
	C	6	0	0	0	6
	D	10	0	0	0	10
	E					0
	Total	23	0	0	0	23
40 ug/L	A	5	0	0	0	5
	B	11	0	0	0	11
	C	6	1	0	0	7
	D	6	0	0	0	6
	E					0
	Total	28	1	0	0	29
Grand Total		234	2	0	0	236

Skeletal deformities included any deformity of the vertebrae or spines. A slight bend of less than 45 degrees (but > than body width off of straight) or a minor body constriction (e.g. a tight rubberband about the body effect) was given a score of 1, 2 slight bends or constrictions anywhere, or bend of > 45-90 degrees was scored a 2, and multi-directional bends > 90 degrees were given a 3. Bends caused by skeletal deformities were usually detectable from normal bending of the body during preservation (these fish were usually well preserved, very straight) by presence of a slight or greater bump below the surface of the epidermis on the outside of the bend. However, some fish with SD = 1 had just a very slight bend in the range the deformity described but could be due to preservation or the poor condition of the fish. This was sometimes especially true in larger fish, which may be more muscular and undergo stronger contraction during preservation and thus, bend slightly. A score “CF = 1” was a slight deformity, if at all. The scores of SD = 1 involving kyphosis or lordosis were deemed real because that is an unusual preservation deformity. Some samples were re-examined; most fish were very straight so some samples with higher SD scores (e.g., PSU samples) were determined accurate.

Deformity assessment results for Yellowstone cutthroat trout in early life stage study (aqueous and dietary Se exposure). Values represent the number of fish (at swim-up and test termination) in each scoring criterion (i.e., 0-3). See below for a definition of scoring criteria.

filename: deform appendix_ELS.xls

Count of fish #		Fin Deformities (FD)				
Location	Field Sample ID	0	1	2	3	Grand Total
Control	A	7	2	0	0	9
	B	7	4	0	0	11
	C	8	1	0	0	9
	D	7	4	0	0	11
	E	3	0	1	0	4
	Total	32	11	1	0	44
2.5 ug/L	A	9	1	0	0	10
	B	6	0	1	0	7
	C	4	2	0	0	6
	D	11	3	0	0	14
	E					0
	Total	30	6	1	0	37
5 ug/L	A	4	2	0	0	6
	B	11	0	0	0	11
	C	4	4	0	0	8
	D	7	4	0	0	11
	E	2	1	0	0	3
	Total	28	11	0	0	39
10 ug/L	A	5	1	1	0	7
	B	5	0	0	0	5
	C	10	0	0	0	10
	D	6	1	0	0	7
	E					0
	Total	26	2	1	0	29
15 ug/L	A	5	0	0	0	5
	B	6	3	0	0	9
	C	8	0	0	0	8
	D	12	1	0	0	13
	E					0
	Total	31	4	0	0	35
20 ug/L	A	0	4	0	0	4
	B	2	1	0	0	3
	C	2	4	0	0	6
	D	9	1	0	0	10
	E					0
	Total	13	10	0	0	23
40 ug/L	A	5	0	0	0	5
	B	9	2	0	0	11
	C	6	1	0	0	7
	D	4	2	0	0	6
	E					0
	Total	24	5	0	0	29
Grand Total		184	49	3	0	236

Fin deformities included variation in fin or finfold morphology and a slightly smaller or missing fin (in thin fish, the adipose fin was often absent, indicating fat absorption, not uncommon and scored 1) or one with a bend or incomplete ray development (in older fish) was given a 1, 2 fins damaged or malformed = 2, and > 2 fins malformed or if fins were missing (except adipose) was = 3. Often fins were malformed associated with vertebral deformities that did not permit proper development. Folded finfolds as a result of preservation were not counted.

Deformity assessment results for Yellowstone cutthroat trout in early life stage study (aqueous and dietary Se exposure). Values represent the number of fish (at swim-up and test termination) in each scoring criterion (i.e., 0-3). See below for a definition of scoring criteria.

filename: deform appendix_ELS.xls

Count of fish #		Edema Deformities (ED)				
Location	Field Sample ID	0	1	2	3	Grand Total
Control	A	9	0	0	0	9
	B	11	0	0	0	11
	C	9	0	0	0	9
	D	11	0	0	0	11
	E	4	0	0	0	4
	Total	44	0	0	0	44
2.5 ug/L	A	0	10	0	0	10
	B	7	0	0	0	7
	C	6	0	0	0	6
	D	14	0	0	0	14
	E					0
	Total	27	10	0	0	37
5 ug/L	A	6	0	0	0	6
	B	10	1	0	0	11
	C	8	0	0	0	8
	D	11	0	0	0	11
	E	3	0	0	0	3
	Total	38	1	0	0	39
10 ug/L	A	7	0	0	0	7
	B	5	0	0	0	5
	C	10	0	0	0	10
	D	7	0	0	0	7
	E					0
	Total	29	0	0	0	29
15 ug/L	A	0	5	0	0	5
	B	9	0	0	0	9
	C	8	0	0	0	8
	D	12	1	0	0	13
	E					0
	Total	29	6	0	0	35
20 ug/L	A	3	1	0	0	4
	B	3	0	0	0	3
	C	6	0	0	0	6
	D	0	10	0	0	10
	E					0
	Total	12	11	0	0	23
40 ug/L	A	5	0	0	0	5
	B	7	4	0	0	11
	C	7	0	0	0	7
	D	4	2	0	0	6
	E					0
	Total	23	6	0	0	29
Grand Total		202	34	0	0	236

Edema was assessed because it was common in one early sample and not others, and because it was thought a condition that could affect emergence, mobility, and other factors that may limit survival of fish in the wild. Edema was detected by an obvious swelling and fluid buildup, usually abdominally, and ventrally, which often displaced the gut, and was usually clear fluid that was slightly soft when touched with a blunt probe. The yolk, which was present in some quantity in some study specimens, also created some swelling but was typically yellowish, opaque, and small, and hard to the touch in preservation. Slight edema = 1 was for a fish with up to 1X swelling of the normal body width or depth, up to 2x = 2, and > 2x = 3.

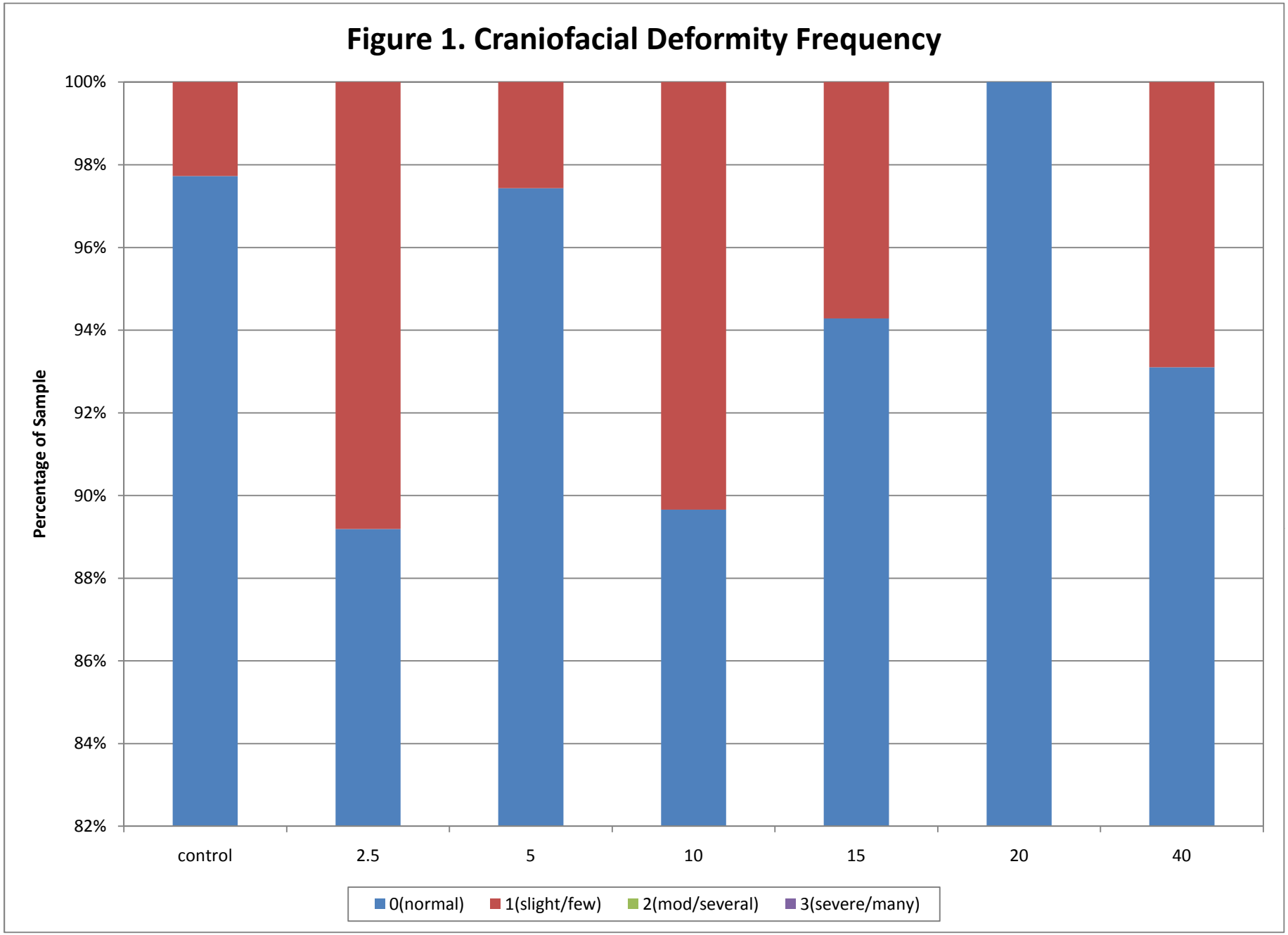


Figure 1. Craniofacial Deformity Frequency
Percentage of Sample
100%
98%
96%
94%
92%
90%
88%
86%
84%
82%
control
2.5
5
10
15
20
40
0(normal)
1(slight/few)
2(mod/several)
3(severe/many)

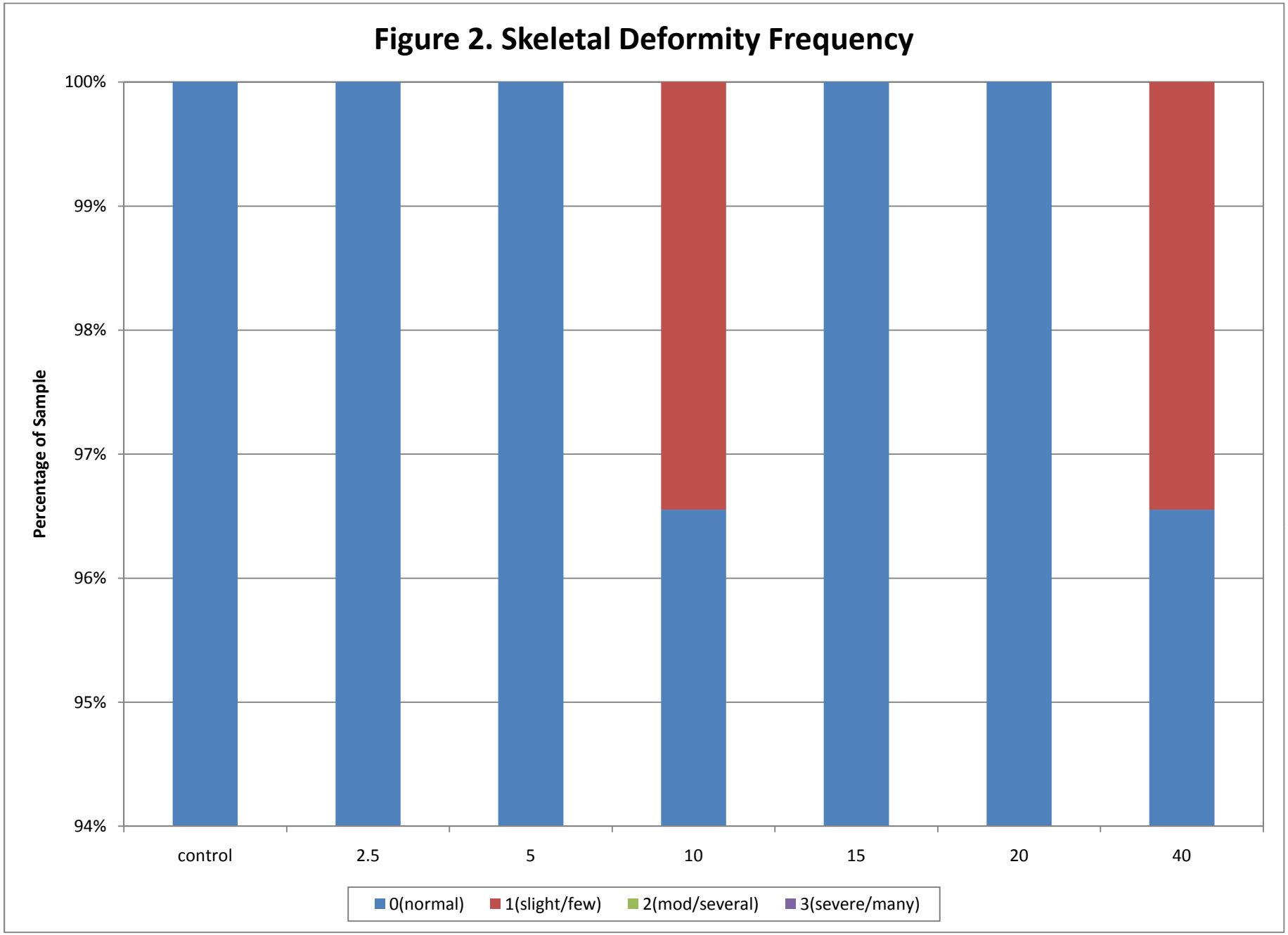


Figure 2. Skeletal Deformity Frequency
100%
99%
98%
97%
96%
95%
94%
Percentage of Sample
control
2.5
5
10
15
20
40
0(normal)
1(slight/few)
2(mod/several)
3(severe/many)

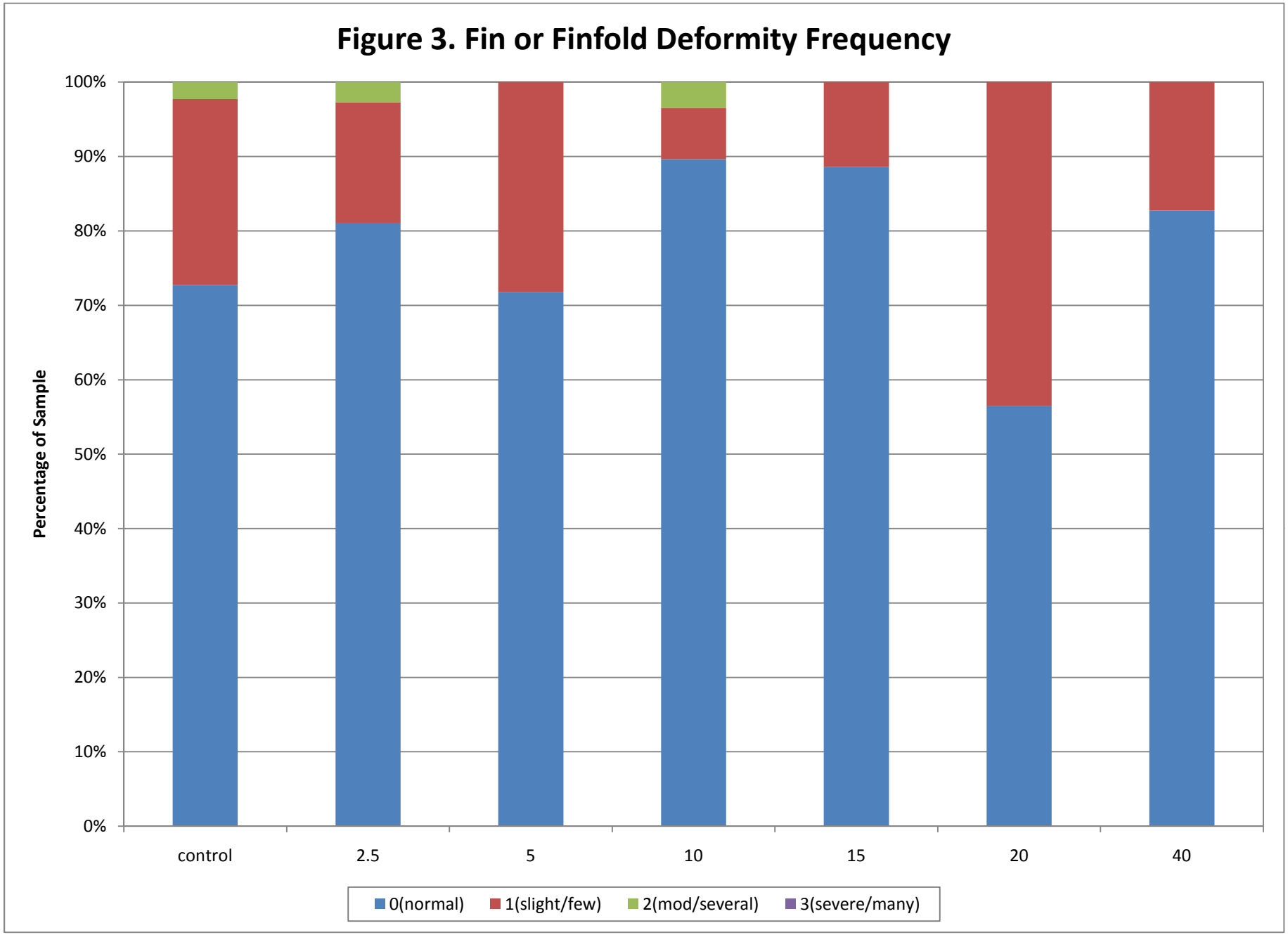


Figure 3. Fin or Finfold Deformity Frequency
Percentage of Sample
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
control
2.5
5
10
15
20
40
0(normal)
1(slight/few)
2(mod/several)
3(severe/many)

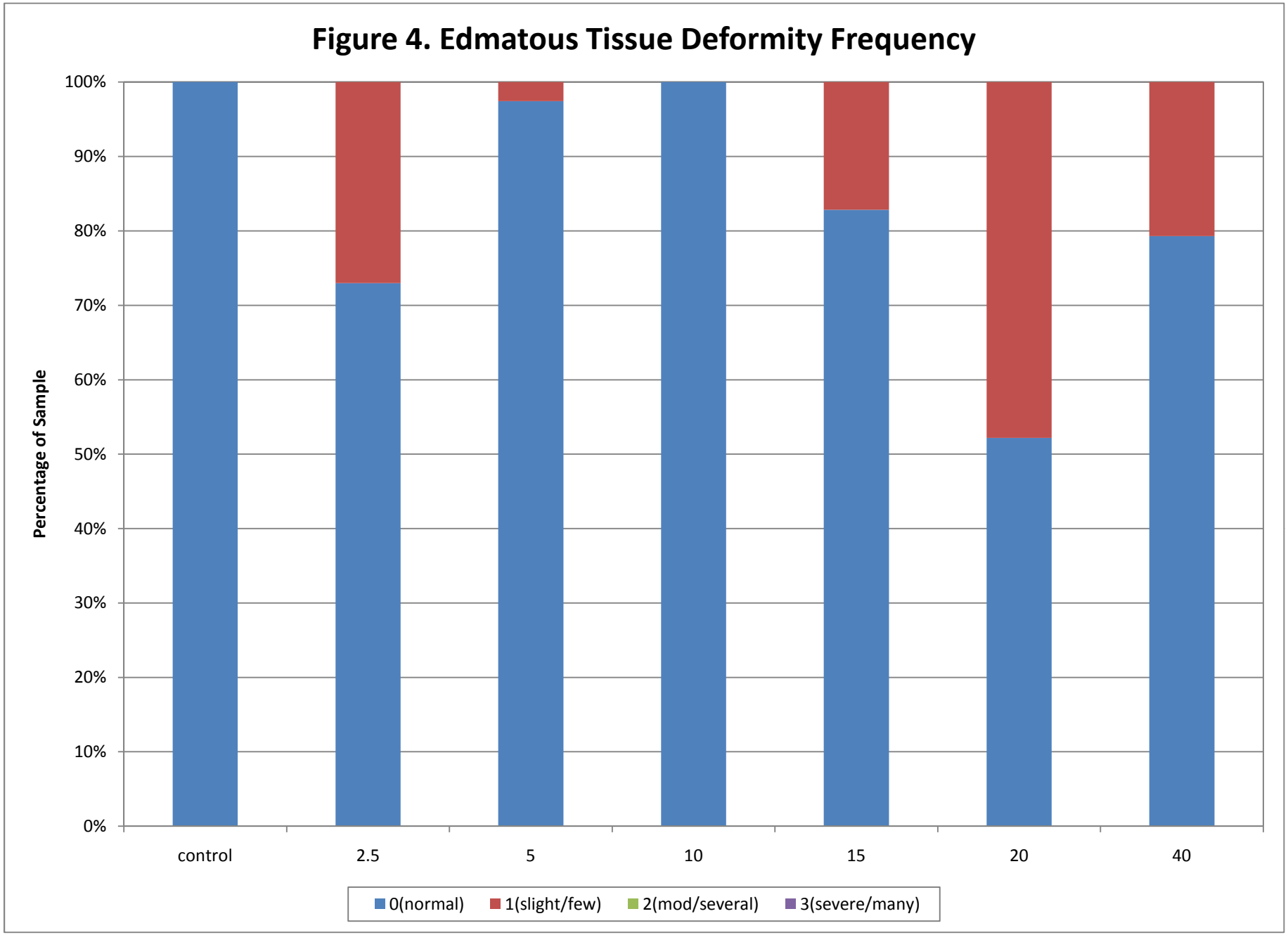


Figure 4. Edmatous Tissue Deformity Frequency
Percentage of Sample
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
control
2.5
5
10
15
20
40
0(normal)
1(slight/few)
2(mod/several)
3(severe/many)