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Effects of Maternally Transferred Egg Selenium on Embryo-Larval Survival, Growth, and Development in Arctic Grayling (*Thymallus arcticus*)

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Abstract: Selenium (Se) toxicity to fish is primarily manifested via maternal transfer to the eggs, which may result in adverse effects on larval survival and development. The present study assessed the effects of egg Se concentrations derived via maternal transfer on early life–stage development, survival, and growth of Arctic grayling (*Thymallus arcticus*), a salmonid species not previously assessed for Se sensitivity. Fish gametes were collected from 4 streams in Alaska known to exhibit a range of egg Se concentrations. Eggs were fertilized and reared in the laboratory from hatch through post-swim-up. Larvae were assessed for survival, length, and weight, as well as deformities (skeletal, craniofacial, fin-fold) and edema based on a graduated severity index. Eggs from a total of 47 females were collected, with egg Se concentrations ranging from 3.3 to 33.9 mg kg^{-1} dry weight. No relationships were observed between larval endpoints evaluated and parent females' egg, muscle, or whole-body Se concentrations. Therefore, Se 10% effective concentrations (EC10s) were defined as the maximum measured Se concentrations: >33.9, >17.6, and >19.7 mg kg^{-1} dry weight for eggs, muscle, and whole-body tissue, respectively. Collectively, these data indicate that Arctic grayling are relatively insensitive to maternally transferred Se compared to other fish species. *Environ Toxicol Chem* 2021;40:380–389. © 2020 SETAC

Keywords: Tissue thresholds; Fish; Reproduction; Developmental abnormalities

INTRODUCTION

Selenium (Se) is an essential element in trace amounts but is also known to be a developmental toxicant to vertebrates at elevated concentrations (Janz et al. 2010). In aquatic systems, Se is readily accumulated from water into primary producers, with bioconcentration factors heavily dependent on the particular aqueous species of Se present (Adams et al. 1998; Presser and Luoma 2010; DeForest et al. 2017). Once in primary producers, Se is efficiently transferred through the diet to primary consumers and higher trophic levels, including fish (DeForest et al. 2016).

The liver is the primary organ involved in Se detoxification and storage in vertebrates. In oviparous vertebrates, Se can be maternally transferred from the liver to eggs during vitellogenesis, leading to elevated egg Se concentrations (Janz et al. 2010). There are 2 current hypotheses regarding the mechanism of Se toxicity, neither of which has been robustly tested. The first hypothesis is based on research showing that Se readily substitutes for sulfur in several amino acids (selenomethionine, selenocysteine) and potentially leads to protein misfolding and/or differential interaction with other proteins (Yuan et al. 1998). Selenium-induced protein dysfunction in embryos could lead to a range of developmental abnormalities which, depending on severity, could reduce embryo viability during hatching and early life–stage development. Alternatively, or in addition, the second hypothesis is that Se may exert toxicity via oxidative stress (Spallholz and Hoffman 2002; Palace et al. 2004). The exact mechanisms by which Se-induced oxidative stress exerts toxicity are unknown,

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but several different pathways have been proposed (Palace et al. 2004; Arnold et al. 2016; Kupsco and Schlenk 2016).

Selenium-induced developmental effects were first documented in fish from Belews Lake, North Carolina, USA, where populations of several fish species collapsed in response to Se contamination of the lake by a coal-fired power plant (Cumbie and Van Horn 1978). Since then, multiple studies have been conducted to characterize the Se sensitivity of a range of fish species. A sufficient diversity of species has now been tested that the US Environmental Protection Agency (USEPA) recently developed water quality criteria (WQC) based on egg Se concentrations in fish (US Environmental Protection Agency 2016). Available species mean egg Se 10% effective concentrations (EC10s) range from 15.6 to 56.2 mg kg^{-1} dry weight. Salmonids are the most heavily tested taxonomic group in the data set, with *Salmo* and *Oncorhynchus* being the third and fourth most sensitive genera tested to date, whereas another salmonid, Dolly Varden (*Salvelinus malma*), is the least sensitive species tested to date (US Environmental Protection Agency 2016).

Recently developed egg-based WQC for Se and the high variability in salmonid sensitivity provide context for evaluating Se downstream of a mining operation in northwest Alaska. The Teck Alaska Incorporated Red Dog Mine is located north of Kotzebue, Alaska, USA. The Red Dog Mine is a zinc mine with on-site milling operations that generate tailings which are deposited in an impoundment. All mine drainage water and mining-impacted waters are collected in the tailings impoundment, from which water is treated prior to being discharged to Red Dog Creek.

Selenium is a naturally occurring element in the Red Dog Mine ore body and surrounding environment (Brienne et al. 2000). Processing of the ore body may contribute to Se concentrations in the Red Dog Mine effluent that are above background, although instream Se concentrations in Mainstem Red Dog Creek are indistinguishable from background (Ott and Morris 2015).

The revised USEPA final ambient WQC for Se consists of both fish tissue and water column elements, with the former having precedence over the latter. Selenium concentrations in Arctic grayling (*Thymallus arcticus*) and *Salvelinus malma* ovaries collected downstream of Red Dog Mine intermittently exceed the fish egg/ovary Se criterion of 15.1 mg kg^{-1} dry weight based on individual fish and, in a few cases, based on annual mean concentrations from multiple fish at a given site (Ott and Morris 2015). Consequently, if the Alaska Department of Environmental Conservation adopts the USEPA 2016 WQC for Se as a statewide standard, the Red Dog Mine will potentially be intermittently out of compliance with this standard.

However, several important issues regarding the applicability of the USEPA WQC for Se to the site-specific conditions of Red Dog Creek require consideration. Most importantly, Red Dog Creek naturally supports a limited fish community consisting of Arctic grayling and Dolly Varden. Slimy sculpin (*Cottus cognatus*) have also been collected intermittently in Red Dog Creek at very low numbers and were uncommon in the Red Dog Creek drainage during pre-mine conditions (Ott and Morris 2015). Consequently, the species sensitivity distribution on which the USEPA WQC is based has limited applicability to Red Dog Creek. In fact, whereas the sensitivity of Arctic grayling to Se is unknown, Dolly Varden, as noted, is the least sensitive species for which an egg/ovary tissue threshold is available (56.2 mg kg^{-1} dry wt; McDonald et al. 2010; US Environmental Protection Agency 2016), and Se concentrations in Dolly Varden eggs collected downstream of the Red Dog Mine have never exceeded one-half this threshold (Ott and Morris 2015).

Given the above, the primary uncertainty in applying the USEPA Se WQC to the Red Dog Mine site is the sensitivity of Arctic grayling to Se and whether the USEPA's fish egg–based Se criterion is appropriate for this species. To address this uncertainty, we conducted an early life–stage survival, growth, and development study with Arctic grayling eggs originating from adults at sites with differing levels of Se exposure.

METHODS

Experimental design overview

The general experimental design for the present study was similar to previous studies used to derive egg Se concentration–response data for fish (Rudolph et al. 2008; McDonald et al. 2010). The overall objective was to collect eggs from fish with a range of egg Se concentrations, fertilize them, and then monitor embryo hatching success as well as early larval development, survival, and growth as a function of measured egg Se concentrations. Endpoints were also evaluated as a function of muscle and whole-body Se concentrations in parent females. To accomplish this, Arctic grayling were collected during peak spawning from several locations within the Wulik River drainage near Red Dog Mine. However, because of the mineralized nature of the region, it was unclear if we could obtain low egg Se concentrations from local reference streams. To address this, we also collected Arctic grayling eggs from the Chena River drainage in central Alaska, from a population of Arctic grayling previously demonstrated to exhibit low egg Se concentrations (Ott et al. 2016).

Adult fish and gamete collection

Fish sampling was conducted in cooperation with the Alaska Department of Fish and Game. Fish sampling was conducted in Fish Creek, a tributary to the Chena River, near Fairbanks, Alaska, USA, and at 3 locations in the Wulik River drainage near the Red Dog Mine (Figure 1). Arctic grayling spawning typically occurs when the water temperature reaches 3 to 4 °C. The spawning window for Arctic grayling is typically only 3 to 4 d. Each location had a different spawning window, depending on water temperature, with the Chena River location sampled the first week in May, whereas the sites in the Wulik River drainage were sampled over a 2-wk period at the end of May and beginning of June.

Primary sampling areas targeted in the Red Dog Mine area were the confluence of the Mainstem Red Dog Creek and

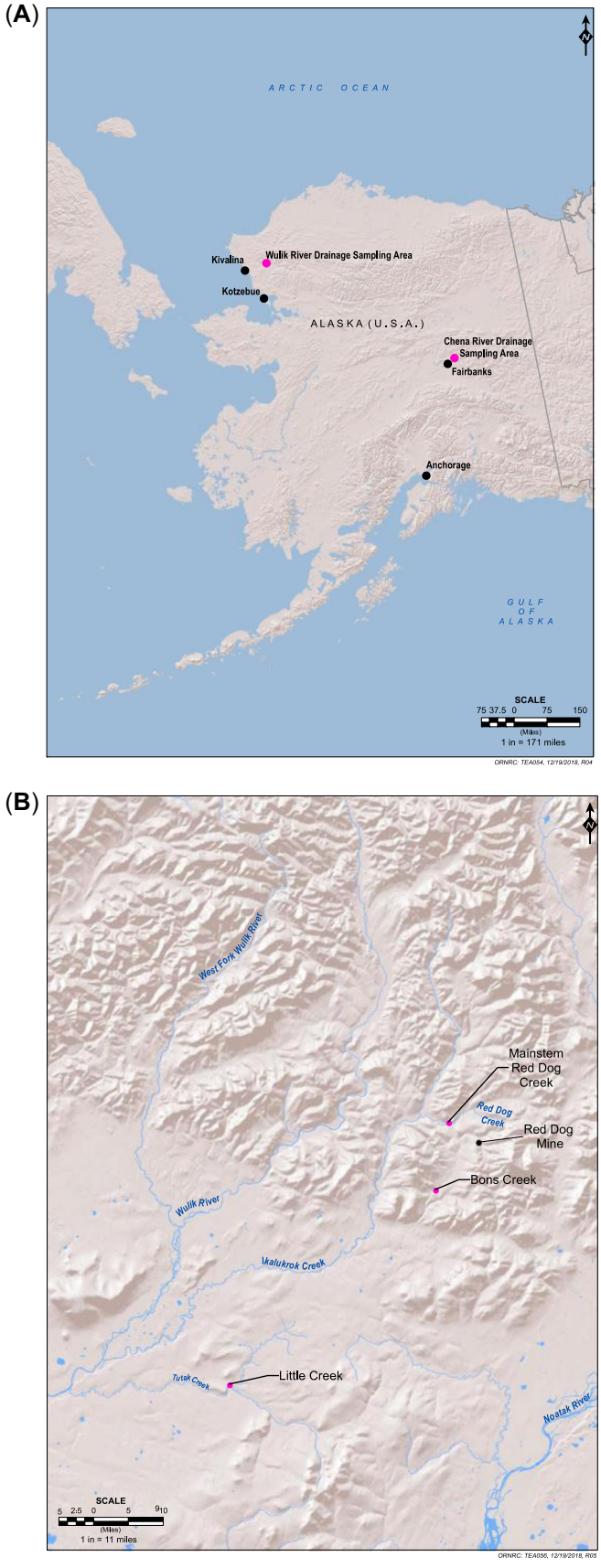

FIGURE 1: (**A**) Locations of the Wulik and Chena River drainage areas in Alaska, USA. (**B**) Locations of Mainstem Red Dog Creek, Bons Creek, and Little Creek sampling locations within the Wulik River drainage.

North Fork Red Dog Creek, Bons Creek, and Little Creek (Figure 1). Fish from Red Dog Creek and Little Creek spend their lives solely in the lotic habitats within the Wulik River drainage. In contrast, Bons Creek fish spend the majority of their lives in the lentic habitat of Bons Reservoir, an impoundment built in 1988 to provide potable water for the mine, with lotic habitat within Bons Creek primarily used during spawning. Arctic grayling were introduced to the reservoir in 1994 and 1995 by the Alaska Department of Natural Resources (Ott and Townsend 2003). The population is closed because upstream access from the Wulik River drainage to the reservoir is restricted by a 10-m waterfall. However, tagging studies have demonstrated that some fish are able to migrate out of the pond over the waterfall, and depending on the year, up to 15% of fish collected at Mainstem Red Dog Creek/North Fork Red Dog Creek may be from Bons Reservoir (Ott and Morris 2015). Little Creek, which is intended to provide a local reference condition, is located in the Tutak Creek drainage.

All fish sampling was conducted using fyke nets that were checked once or twice every 24 h over a 3- to 4-d period. All fish captured were removed from the nets and placed in tubs filled with fresh water from the stream. Captured fish were identified by species and measured to the nearest millimeter in fork length, and all species other than Arctic grayling were immediately released at the site of capture. Captured adult Arctic grayling were assessed for spawning condition and segregated for gamete collection. Gametes from individual males and females were collected separately into Ziploc® bags and placed in coolers for transport back to the Red Dog temporary laboratory or Owl Ridge Natural Resource Consultants facilities (Fairbanks sampling).

Eggs harvested from females were further subsampled for fertilization and for determination of Se concentration for individual fish. In addition to collecting gametes from female Arctic grayling, a subsample of females was euthanized at the site of capture and retained for determination of muscle and whole-body Se concentrations. These samples, along with the egg subsamples, were frozen at −20 °C and archived for future analysis.

Egg fertilization and initial holding

For each sampling location, pooled milt obtained from up to 4 males was used for fertilization. Once in the laboratory, the mass of eggs collected from each female was measured and milt quality evaluated by placing a subsample on a microscope slide, adding a drop of water, and observing motility (Environment Canada 1998). Only milt with highly active sperm was used in testing.

Fertilization was conducted under low-level light provided with an LED lantern-style flashlight. Approximately 400 eggs from each female fish were transferred using a clean plastic spoon to plastic weigh boats. Remaining eggs were weighed, transferred to Whirl-Pak® bags and frozen for Se analysis. Two or 3 drops of pooled milt were delivered to the eggs in each weigh boat and gently mixed by manually stirring the eggs with

TABLE 1: Water quality measured during exposure

Water quality parameter	Range
Temperature (°C)	6.0–7.5
pH	7.1–7.9
Conductivity (μS cm^{-1})	218–262
Dissolved oxygen (mg L^{-1})	10.9–12.5
Total ammonia (average, mg L^{-1}), new solutions	0.03
Total ammonia (average, mg L^{-1}), old solutions	0.04

an inflated clean laboratory glove. The eggs were left for 20 min to allow for fertilization to occur, following which weigh boats were filled with culture water. The eggs were allowed 20 min to begin water hardening, rinsed twice with test water to remove milt, and then transferred to 4-L food-grade plastic containers.

Rearing containers were filled with an artificial water that approximates the ionic composition and pH of North Fork Red Dog Creek in the May/June timeframe (Tables 1 and 2). The eggs were left to complete water hardening, following which any damaged or opaque eggs were recorded and removed, and the total number of eggs was reduced to 300 per container. Rearing containers with fertilized embryos were placed in refrigerators able to maintain a temperature of 6.0 ± 3.0 °C at both the Owl Ridge and Red Dog Mine field laboratories. Rearing containers were provided with continuous aeration to maintain dissolved oxygen concentrations close to saturation and gentle mixing of water without disturbing the eggs.

The day following fertilization, embryos were inspected for mortalities, and the water quality (temperature, dissolved oxygen, pH, and conductivity) of solutions was recorded. Developing embryos were then transferred into a small fish net and placed in an iodophor disinfectant (Ovadine™) solution (1000 mg L^{-1} prepared in culture water) for 10 min. The eggs were rinsed with test water and returned to the rearing containers refilled with fresh test water.

After fertilization, water quality was measured, and water changes (~80% renewal) were conducted daily. Eggs from Fish Creek were maintained at the Owl Ridge field laboratory for less than 24 h before transport, and eggs from the Red Dog Mine locations were kept at the Red Dog Mine field laboratory for 24 to 96 h before transport to Nautilus Environmental.

Each batch of eggs was transported in a 4-L Cubitainer® filled with test water with no headspace. Cubitainers were transported in coolers containing ice packs, bubble wrap, and/or foam padding to protect from physical movement causing stress to the embryos. The coolers were transported overnight by plane and then by vehicle to the laboratory.

A permit was obtained from Fisheries and Oceans Canada to transport Arctic grayling embryos from the site to the Nautilus Environmental facility. The Introductions and Transfers License (no. 14022) permitted the transfer of live eggs from up to 80 Arctic grayling from the British Columbia border crossing to the Nautilus Environmental laboratory.

TABLE 2: Mean water chemistry in test water during exposure[a]

	(mg L^{-1})
Major anions	
Alkalinity, total (as $CaCO_3$)	46.8 ± 2.8
Chloride	14.9 ± 2.7
Sulfate (SO_4)	32.1 ± 5.1
Major cations	
Calcium	20.3 ± 2.0
Magnesium	2.4 ± 0.4
Potassium	1.5 ± 0.6
Sodium	18.7 ± 1.4

[a] $n = 4$; mean ± standard deviation.

Laboratory rearing

Laboratory rearing of Arctic grayling was adapted from the methodology described by Environment Canada (1998) for conducting early life–stage tests with salmonids, with modifications appropriate for Arctic grayling as further described. On receipt at the laboratory, eggs from individual females were randomly distributed to 4 replicates of 60 eggs; any mortality associated with egg transport was noted. Eggs were held in food-grade plastic high-density polyethylene containers and randomly distributed in an environmental chamber maintained at 7 ± 1 °C.

Prior to hatching, the chamber was kept dark, and sufficient low-level lighting was provided during water renewals using a single incandescent bulb. Low-level light was introduced on an 18:6-h light:dark photoperiod as the eggs approached swim-up. Test chambers were provided with continuous aeration sufficient to maintain dissolved oxygen concentrations close to saturation (>80%).

Rearing was conducted under static-renewal conditions, with daily 80% water renewal using dechlorinated Metro Vancouver drinking water amended with reagent-grade salts to achieve the ionic composition and pH of North Fork Red Dog Creek, as described. Chlorine was measured weekly, and total metals and metalloids were measured monthly throughout the rearing period to ensure that the water supply was of a suitable quality. In addition, temperature, dissolved oxygen, conductivity, and pH were measured daily in a subset of test chambers. Fish were inspected daily, mortalities were removed and recorded, and the number of hatched fish was recorded.

Deformity assessments

The study design involved assessing larval deformities once fish reached the swim-up stage, which coincides with the onset of feeding. Arctic grayling reach swim-up approximately 5 d following hatch (Baker et al. 2015). Consequently, for fish from Fish Creek, which were tested first, hatched fish were removed from each replicate every 2 to 3 d once swim-up began and assessed for deformities. However, it was noted during the deformity assessment of these fish that some larvae had not completely absorbed their yolk sac. To address this, a subset of larvae from each female (range = 19–60, mean = 47) from the Red Dog Mine sites were allowed to develop for 5 to 7 d posthatch to ensure that the assessment included fish that had

completely absorbed their yolk sac. These fish are identified as post-swim-up.

Once larvae reached the swim-up or post-swim-up stage, they were removed from each test replicate, terminated using clove oil, and evaluated for length, weight, and deformities. Total length to the nearest 0.5 mm was determined for each fish and dry weight was determined following 24 h in a drying oven at 60 °C.

All deformity assessments were provided blind and conducted by a single observer, with 8% of samples scored by a second observer for quality assurance. Deformity assessments were conducted on fresh, unpreserved fish to avoid possible morphological artifacts associated with preservation. Deformities were assessed using a graduated severity index (GSI), as described (Rudolph et al. 2008). This approach involved assigning a score of 0, 1, 2, or 3 based on the severity of edema and 3 types of deformities: skeletal, craniofacial, and fin-fold (Supplemental Data, Table S1). A GSI score was then assigned to each fry and summed across these 4 categories. Thus, GSI scores ranged from 0 for no edema or deformities to 12 for fry with gross edema and deformities in all categories. Establishing a threshold to determine that a level of edema or deformity falls into a specific category of 0, 1, 2, or 3 required some degree of best professional judgment. Briefly, scores were assigned as follows: 0 = fish with no signs of edema or deformity; 1 = any minor deviation from natural phenotypic variability (e.g., minor deformity or edema that is detectable but not expected to adversely affect mobility, sight, or feeding); 2 = a significant deviation that is expected to result in ecologically significant impairment, such as reduced feeding or swimming efficiency (e.g., malformed fins that may inhibit mobility and ability to feed); and 3 = a gross deviation likely to result in mortality due to an inability to swim, feed, or evade predators (e.g., severe skeletal deformities, missing eyes or jaws).

Skeletal deformities include lordosis (i.e., concave curvature in the lumbar region of the spine), scoliosis (i.e., lateral curvature of the spine), and kyphosis (i.e., convex curvature of the thoracic region of the spine). Craniofacial deformities include missing or deformed eyes, shortened or otherwise deformed jaw or operculum, and/or abnormalities in the shape of the head. Fin-fold deformities include missing or deformed fins or abnormalities in the articulation of pectoral fins.

Analytical chemistry

All tissues were frozen and shipped on ice-packs to Brooks Applied Labs, where Se was measured using modified USEPA Method 3050B and modified Method 6020A. The analyses were performed on a triple quadrupole inductively coupled plasma-mass spectrometer. Method detection limits for Se across all tissue samples ranged between 0.017 and 0.030 mg kg^{-1} dry weight. A certified standard reference material (DORM-4 fish protein certified reference material for trace metals) was measured concurrently with each batch of samples for total Se ($n = 3$, recovery = 102 ± 0%). Matrix spikes (recovery = 97–101%, $n = 3$), duplicate analyses (relative percentage of difference = 0.7–7%, $n = 5$), and method blanks (0.009–0.012 mg kg^{-1}, $n = 4$) were also performed assess accuracy and precision. Percent moisture was determined for each batch of eggs and used to convert the wet weight Se measurement to a dry weight concentration.

Whole-body Se concentrations were determined based on measured Se concentrations in muscle, remainder tissue, and eggs and their corresponding masses. Because dry weight Se concentrations were of interest, tissue masses, which were measured on a wet weight basis, had to be converted to dry weight masses based on measurement of percentage of solids.

Whole-body Se concentrations, on a dry weight basis, were then calculated per Equation 1:

$$C_{WB} = \frac{C_{muscle} \times Mass_{muscle} + C_{egg} \times Mass_{egg} + C_{remainder} \times Mass_{remainder}}{Mass_{muscle} + Mass_{egg} + Mass_{remainder}} \quad (1)$$

Data analysis

Toxicity data for hatching success, growth, survival, and deformities were analyzed by nonlinear regression to evaluate whether EC10s could be calculated for each endpoint based on egg, muscle, and calculated whole–body Se concentrations. The USEPA's Toxicity Relationship Analysis Program (Ver 1.30) was used to analyze toxicity data. Linear regression analysis of relationships between Se concentrations in different tissues (e.g., eggs and muscle) using both raw and ln-transformed data were conducted in R (R Development Core Team 2017). Differences between sites in Se tissue concentrations were determined by a Brown-Forsythe analysis of variance (ANOVA) to account for unequal variance between sites, followed by Dunnett's T3 multiple comparisons test. The ANOVA was conducted in GraphPad Prism (Ver 8.3).

RESULTS

Water quality

Water quality at both the field laboratories and the Nautilus Environmental laboratory are summarized in Table 1. Concentrations of major ions in test water were analyzed 4 times throughout the testing period and were relatively stable (Table 2).

Se concentrations in eggs and other tissues

A total of 47 female fish were successfully spawned and evaluated in the present study: Bons Creek ($n = 20$), Mainstem Red Dog Creek ($n = 5$), Little Creek ($n = 10$), and Fish Creek ($n = 10$). No fish were collected from North Fork Red Dog Creek because of substantial aufeis in the creek through the entire spawning window for Arctic grayling. Although sample sizes were uneven across sites, egg Se concentrations across all locations ranged from 3.3 to 33.9 mg kg^{-1} dry weight, achieving the objective of collecting fish with a range of egg Se concentrations (Figure 2). Muscle and whole-body Se concentrations were measured in 34 females (13 females were released following egg collection). Muscle Se concentrations ranged from

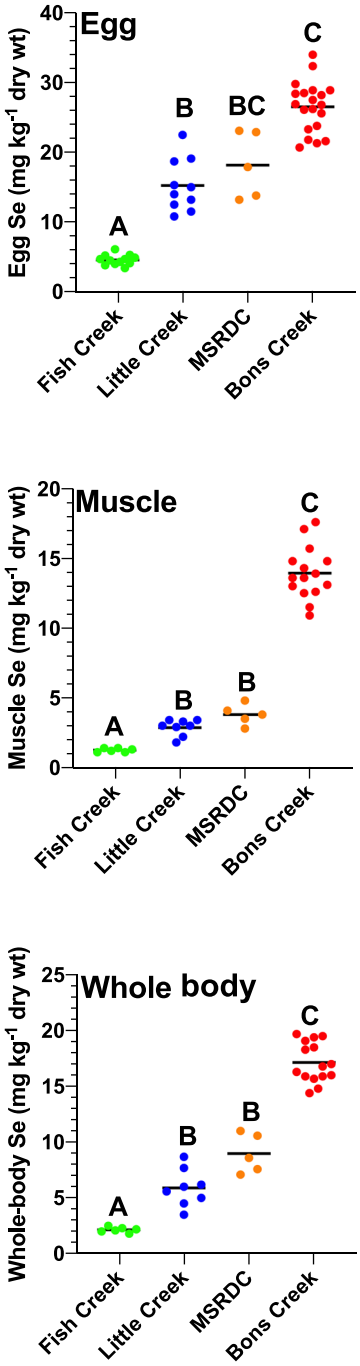

FIGURE 2: Measured Se concentrations in female Arctic grayling by site eggs ($n = 47$), muscle ($n = 34$), and whole body ($n = 34$). Different letters indicate statistically significant differences ($p < 0.05$). MSRDC = Mainstem Red Dog Creek.

1.07 to 17.6 mg kg^{-1} dry weight, and whole-body Se concentrations ranged from 1.78 to 19.7 mg kg^{-1} dry weight. All tissue Se data are provided in Supplemental Data, Table S2.

For the 13 females that were released after egg collection, muscle and whole-body Se concentrations were estimated from egg Se concentrations based on relationships developed from the other 34 females. Selenium concentrations in both muscle and egg were significantly higher (except compared to egg Se in Mainstem Red Dog Creek) in Bons Creek compared with other creeks (Figure 2). These higher concentrations stand apart from the relationship between muscle and egg Se concentrations in fish collected from Fish Creek, Little Creek, and Mainstem Red Dog Creek (Figure 3A). There was no significant relationship between muscle and egg Se concentrations for Bons Creek fish using either raw or ln-transformed data ($p = 0.49$ and 0.41, respectively). In contrast, a statistically significant relationship was observed across the other sites ($p < 0.0001$), with ln-transformed data providing the best linear model ($R^2 = 0.84$; Figure 3A). Thus, the model based on ln-transformed concentrations was selected to estimate muscle Se concentrations for fish collected from Fish Creek, Little Creek, and Mainstem Red Dog Creek (see Equation 2):

$$\ln(\text{Muscle Se}[\text{mg kg}^{-1}\text{ dry wt}]) = 0.687 \times \ln(\text{Egg Se}[\text{mg kg}^{-1}\text{ dry wt}]) - 0.781 \quad (2)$$

Because the regression was not significant for Bons Creek fish, the mean (±standard deviation) muscle-to-egg Se ratio of 0.54 (±0.10) was used to estimate muscle Se concentrations from egg Se concentrations for Bons Creek fish (Equation 3):

$$\text{Muscle Se}(\text{mg kg}^{-1}\text{dry wt}) = 0.54 \times \text{Egg Se}(\text{mg kg}^{-1}\text{ dry wt}) \quad (3)$$

The best-fitting linear regression of whole-body versus egg Se concentrations was significant for pooled Fish Creek, Little Creek, and Mainstem Red Dog Creek fish and, separately, for

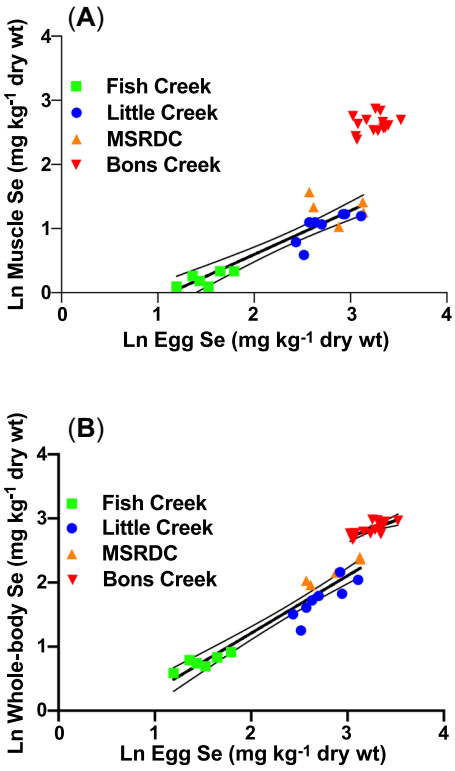

FIGURE 3: Relationships between (**A**) egg and muscle Se ($n = 34$) and (**B**) egg and whole-body Se ($n = 34$) for Arctic grayling. MSRDC = Mainstem Red Dog Creek.

Bons Creek fish (Figure 3B). Models based on ln-transformed data explained a higher percentage of the variance than models based on the raw data ($R^2 = 0.92$ and 0.56, respectively). As for muscle Se concentrations, models based on the ln-transformed data were selected to estimate whole-body Se concentrations for Fish Creek, Little Creek, and Mainstem Red Dog Creek fish per Equation 4:

$$\ln(\text{Whole-Body Se}[\text{mg kg}^{-1}\text{ dry wt}]) = 0.897 \times \ln(\text{Egg Se}[\text{mg kg}^{-1}\text{ dry wt}]) - 0.585 \quad (4)$$

For Bons Creek fish, Equation 5 was used to estimate whole-body Se concentrations from egg Se concentrations:

$$\ln(\text{Whole-Body Se}[\text{mg kg}^{-1}\text{ dry wt}]) = 0.538 \times \ln(\text{Egg Se}[\text{mg kg}^{-1}\text{ dry wt}]) + 1.09 \quad (5)$$

Se toxicity thresholds

The present study generated data for both swim-up and post-swim-up (except Fish Creek) larvae. Of the 1653 larvae from across all sites that were assessed for survival post-swim-up, only one mortality occurred. Consequently, the swim-up and post-swim-up survival data were pooled for analysis. This was obviously not possible for the larval length and weight endpoints because post-swim-up larvae were significantly larger than swim-up larvae, which were assessed separately. For larval length and weight, the analysis focused on swim-up larvae because this allowed inclusion of data from Fish Creek. Except where noted, the same trends and conclusions can be made for both time points for length and weight endpoints (Supplemental Data, Table S2).

In total, 9671 Arctic grayling larvae were assessed for deformities. Assessments were conducted on 157 to 240 larvae from each batch of Arctic grayling, resulting in 950 to 3995 fry assessments per collection site. Photographic examples of craniofacial, skeletal, and edematous deformities as well as fry with no evidence of deformities are provided in Supplemental Data, Figures S1 to S5. Across sampling sites, deformities at swim-up were dominated by skeletal and edematous deformities (approximately 1% incidence each), followed by craniofacial deformities (0.7%), whereas fin-fold deformities were largely absent (<0.2%). In post-swim-up larvae, the incidence of skeletal and craniofacial deformities increased (3.8 and 1.7%, respectively), whereas fin-fold and edematous rates were similar to those at swim-up. Mean moderate/severe deformities were slightly higher for post-swim-up larvae (5.4%) compared to swim-up larvae (2.5%). However, there was no significant relationship between egg Se concentrations and differences in moderate/severe deformities between swim-up and post-swim-up larvae (Supplemental Data, Figure S6). Consequently, for GSI, the analysis focused on swim-up larvae because this allowed inclusion of data from Fish Creek. Except where noted, the same trends and conclusions can be made for both time points for GSI endpoints (Supplemental Data, Table S2).

Egg, muscle, and whole-body Se concentrations were evaluated relative to several different toxicity endpoints: percentage of survival, percentage of normal larvae based on GSI = 0 or ≤1, percentage of larval survival plus percentage of normal larvae based on GSI = 0 or ≤1, larval length and weight at swim-up, and length and weight at post-swim-up (Supplemental Data, Table S2). Effects for the toxicity endpoints across the range of observed Se tissue concentrations were generally <10% relative to responses observed in fish collected from Fish Creek, which had the lowest Se concentrations. This prevented development of concentration–response relationships and EC10 estimates. Similarly, concentration–response relationships could not be developed using data for the post-swim-up larvae either.

No significant relationships between egg Se concentrations and any of the toxicity endpoints evaluated were observed (Figure 4). The largest effect observed was 55.1% embryo-larval survival for eggs collected from a Bons Creek female with an egg Se concentration of 29.7 μg g^{-1} dry weight (Figure 4A). However, 2 females collected from Bons Creek with higher egg Se concentrations (32.3 and 33.9 μg g^{-1} dry wt) had 84 and 95% embryo-larval survival, respectively (Figure 4A). For comparison, mean (±standard error of the mean [SEM]) embryo-larval survival for the Fish Creek and Little Creek reference locations was 90 ± 1 and 95 ± 1%, respectively.

Compared to survival, variability was lower in the percentages of normal larvae within and between sampling sites, when "normal" was defined as those with a GSI of 0 or ≤1 (Figure 4B and C). When percentage of embryo-larval survival and percentage of normal larvae (GSI = 0) were combined into an integrated endpoint, the magnitude and variability of response was largely driven by the percentage of larval survival component of the integrated endpoint (Figure 4D).

Larvae from the Bons Creek female with a mean egg Se concentration of 29.7 μg g^{-1} dry weight, which had a mean survival of 55%, did not have a corresponding increase in larval deformities; the mean (±SEM) percentage of normal larvae from the survivors was 90 ± 3% based on a GSI score of 0 (Figure 4B). Most of the larval mortalities for the offspring of this fish occurred within the first 7 d (90–96% for the 4 replicates), which was generally not observed for other fish in this experiment. This suggests that the mortalities may have been due to a factor, or set of factors, other than Se toxicity.

There were no negative relationships between egg Se concentrations and larval length or weight. For larvae at swim-up, there were slight, but significant, positive slopes for length ($p < 0.001$) and weight ($p < 0.001$; Figures 4E and F). For post-swim-up larvae (which was not evaluated for Fish Creek fish), a slightly positive slope ($p = 0.01$) was observed between egg Se and weight (data not shown).

As noted, no concentration–response models could be developed from these data, which precluded calculation of EC10s for any of the toxicity endpoints evaluated. Accordingly, the egg Se threshold was defined as >33.9 μg g^{-1} dry weight, the highest egg Se concentration measured.

In general, relationships between muscle Se concentrations and larval endpoints assessed (Supplemental Data, Figure S7) were similar to those observed based on egg Se concentrations

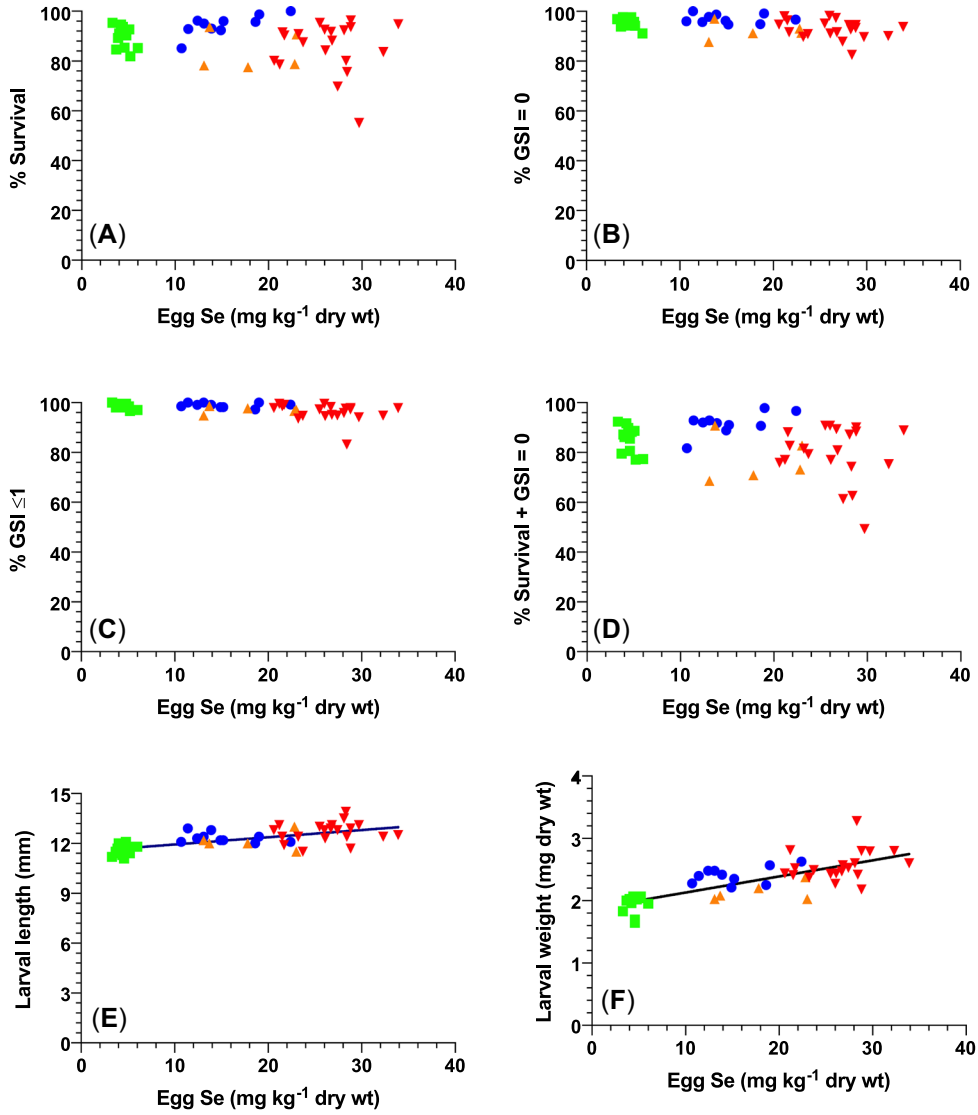

FIGURE 4: Relationship between egg Se concentrations and **(A)** Embryo-larval survival, **(B)** graduated severity index (GSI) = 0, **(C)** GSI ≤1, **(D)** Larval survival + GSI = 0, **(E)** Larval length at swim-up, and **F**. Larval dry weight at swim-up. ■ = Fish Creek, ● = Little Creek, ▲ = Mainstem Red Dog Creek, ▼ = Bons Creek.

(Figure 4). The most conspicuous difference was that muscle Se concentrations for Fish Creek, Little Creek, and Mainstem Red Dog Creek fish cluster separately from muscle Se concentrations for Bons Creek fish. In contrast, egg Se concentrations from the 4 sites represent a more continuous distribution, with the highest Little Creek and Mainstem Red Dog Creek Se concentrations exceeding the lowest Bons Creek egg Se concentrations. This was due to the lower variability in muscle Se concentrations for fish collected from Fish Creek, Little Creek, and Mainstem Red Dog Creek but greater variability in egg Se concentrations for these same fish (i.e., the slope of the relationship between muscle and egg Se concentrations is shallow [Figure 3]).

To consider this in another way, the mean (±SEM) egg-to-muscle Se ratio is 4.8 ± 0.3 for Fish Creek, Little Creek, and Mainstem Red Dog Creek fish (pooled) and 1.9 ± 0.1 for Bons Creek fish. This suggests that either the egg-to-muscle Se relationship is concentration-dependent or Se is distributed differently between tissues for Bons Creek fish compared with fish from other locations. Regardless of the mechanism, these data highlight differences in between-tissue Se relationships for Arctic grayling and emphasize that direct analysis of egg Se concentrations is the most reliable measure of Se exposure and potential toxicity. As for egg Se, no reliable concentration–response models could be developed from the muscle Se data, and no EC10s could therefore be calculated. Accordingly, the muscle Se toxicity threshold was defined as >17.6 μg g^{-1} dry weight, the highest muscle Se concentration measured.

Similar to egg and muscle Se concentrations, no reliable relationships between whole-body Se concentrations and larval endpoints were identified (Supplemental Data, Figure S8). As observed for muscle Se, a distinct clustering of whole-body Se concentrations for Fish Creek, Little Creek, and Mainstem Red Dog Creek fish was observed compared to Bons Creek fish. Because of the lack of a reliable concentration response for whole-body Se concentrations, a whole-body Se toxicity

threshold was defined as >19.7 μg g^{-1} dry weight, the highest whole-body Se concentration calculated.

DISCUSSION AND CONCLUSION

The objective of the present study was to characterize the sensitivity of Arctic grayling embryos and larvae to maternally transferred Se. Arctic grayling gametes were collected from 2 reference locations (Little Creek and Fish Creek) and 2 locations with Se concentrations elevated above background (Mainstem Red Dog Creek and Bons Creek). Eggs were fertilized and reared in the laboratory through post-swim-up. We observed no statistically significant negative effects related to egg Se concentrations on embryo-larval survival, development, or growth across a wide range of egg Se concentrations. Consequently, Se toxicity thresholds for eggs, muscle, and whole-body tissue were defined based on the maximum concentrations measured: >33.9, >17.6, and >19.7 mg kg^{-1} dry weight, respectively.

A positive relationship between egg Se and larval growth was observed for both weight and length endpoints (Figure 4). It is possible this positive relationship is related to the essentiality of Se, but we think this is unlikely given that Se concentrations are well above essential requirements (Janz et al. 2010). Instead, we noted a relationship between adult female size (fork length) and the size of the corresponding larvae as measured by both weight and length ($p < 0.001$; Supplemental Data, Figure S9). We suggest that the positive relationship between egg Se and larval size is more likely related to maternal size than egg Se.

The egg Se EC10 of >33.9 mg kg^{-1} dry weight indicates that Arctic grayling is at least the second least sensitive fish species tested to date based on species sensitivity distributions for Se (DeForest et al. 2012; US Environmental Protection Agency 2016). Species within Salmonidae represent among the most and least sensitive fish species to Se. *Salmo trutta* is the most sensitive salmonid tested to date, with an EC10 of 21 μg kg^{-1} egg Se, whereas *Salvelinus malma* is the least sensitive species, with an EC10 of 56 mg kg^{-1} egg Se (McDonald et al. 2010; US Environmental Protection Agency 2016). These 2 species are more closely related to each other than to Arctic grayling, which diverged from other salmonids approximately 55 to 75 mya (Crete-Lafreniere et al. 2012; Alexandrou et al. 2013), suggesting that there is no phylogenetic signal associated with Se sensitivity across the Salmonidae. Although the variability in sensitivity within Salmonidae may seem noteworthy, it should be kept in mind that sensitivity varies by only a factor of 2.7, which is not an unusual level of variability in toxicant sensitivity when evaluated at the level of taxonomic family.

Egg Se concentration in fish is generally considered the strongest indicator of potential toxicity because this metric is closely linked to Se exposure for the most sensitive life stage. Maternal muscle or whole-body Se can also be used as indicators of potential toxicity, but these measurements are less reliable because of interspecific variability in how much of a fish's Se body burden is deposited in the eggs. The present study also demonstrates potential intraspecies variability in the relationships between egg Se and muscle or whole-body Se concentrations. To the best of our knowledge, distinct intraspecific differences in concentration ratios between eggs and muscle/whole-body Se have not been previously documented. The US Environmental Protection Agency (2016) conducted a comprehensive review of these ratios for 16 species. In general, it observed relatively consistent ratios for a given species. Although ratios were more variable for some species than others, there is no evidence of a systematic difference in ratios as we observed for fish collected from Bons Pond. However, we note that in most cases the data summarized by the US Environmental Protection Agency (2016) for a given species were all collected from the same or similar habitats.

The mechanism underlying variability in tissue ratios for Arctic grayling is currently unclear. Although grayling from Bons Creek generally had significantly higher tissue Se concentrations, measured egg Se concentrations overlapped with those observed at Mainstem Red Dog Creek and Little Creek (Figure 2). This suggests that the different relationships in Se concentration between tissues are not caused by Se exposure concentration. The other difference between these groups is that Bons Creek fish originate from Bons Reservoir, a closed lentic environment, whereas the other fish originate from lotic environments in the Wulik River drainage. Differences between these 2 habitat types in redox potential and biological productivity are known to strongly influence Se speciation in water and the diet of aquatic organisms (Adams et al. 1998; Brix et al. 2005; Orr et al. 2012; US Environmental Protection Agency 2016). One hypothesis is that these differences may lead to changes in Se homeostasis across tissues for higher–trophic level organisms such as fish. Understanding the mechanisms underlying these differences could provide important insights into differences in Se homeostasis and sensitivity.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at https://doi.org/10.1002/etc.4920.

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Data Availability Statement—Data, associated metadata, and calculation tools are available in the Supplemental Data, and from the corresponding author (kevinbrix@icloud.com).

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