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Original Article

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Effects of maternally transferred egg selenium on embryo-larval survival, growth, and development in mountain whitefish (*Prosopium williamsoni*)

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Abstract

This study assessed the effects of egg selenium (Se) concentrations derived via maternal transfer on early life stage survival, growth, and development of mountain whitefish (*Prosopium williamsoni*), a species not previously assessed for Se sensitivity. Over four reproductive seasons, fish gametes were collected from multiple locations in the Elk Valley watershed in British Columbia, Canada which has elevated Se concentrations associated with mining activities. Eggs were collected from 65 females with egg Se concentrations ranging from 15.5–54.0 mg kg^{-1} (dry wt). Fertilized eggs were reared in the laboratory from hatch through post-swim-up. Larvae were assessed for survival, length, weight, developmental deformities, and edema. Integrating data from all 4 years, a 10% effect concentration (EC10) of > 54 mg kg^{-1} (dry wt) egg Se was determined for the embryo-larval deformity endpoint, which is characteristic of maternally transferred Se effects. However, a statistically significant relationship between larval growth (length and weight) and egg Se was observed, with a shallow linear response relationship over the entire egg Se concentration range evaluated. An EC10 of 34 mg kg^{-1} (dry wt) was estimated but is uncertain because the lower limit of the apparent effect is undefined (nonconservative error), there is evidence a size-dependent maternal provisioning effect could be contributing or driving the relationship (conservative error), and this endpoint has not been diagnostic of Se-induced effects in other fish species tested. Overall, the weight of evidence provides more support for size-dependent effects on maternal provisioning of eggs as the cause of these observations, but an effect of Se cannot be ruled out. Regardless of which endpoint is used, this study demonstrates that mountain whitefish are less sensitive than generic Se guidelines.

Keywords: tissue thresholds, fish, reproduction, developmental abnormalities

Introduction

Selenium (Se) is an essential element in trace amounts but, at elevated concentrations, can cause developmental effects on the embryo-larval life stages of fish (Janz et al., 2010). Selenium is accumulated from water into primary producers and consumers at the base of the food chain with accumulation varying as a function of the aqueous Se species present (Adams et al., 1998; de Bruyn et al., 2025; DeForest et al., 2017; Presser & Luoma, 2010). Once it has entered the base of the food chain, Se is transferred through the diet to primary consumers and higher trophic levels, including fish (DeForest et al., 2016).

In fish, Se is preferentially accumulated in the liver, where it can be incorporated into vitellogenin, the primary precursor to egg yolk (Kroll & Doroshov, 1991). Under the current paradigm, Se is then transferred to developing oocytes during vitellogenesis, although recent analyses suggest the details of this pathway are more complex (Brix et al., 2025). The mechanism(s) by which Se causes effects on embryo-larval fish development is not well studied. One potential mechanism involves Se causing oxidative stress (Palace et al., 2004; Spallholz & Hoffman, 2002), whereas another involves Se substituting for sulfur in amino acids (e.g., selenomethionine, selenocysteine), leading to protein misfolding and/or differential interaction with other proteins (Yuan et al., 1998).

Regardless of the mechanism, Se-induced developmental effects are well documented in multiple watersheds in North America (Janz et al., 2010) and multiple studies have been conducted to characterize the sensitivity of different fish species to maternally transferred Se using egg Se concentrations as a measure of exposure. A sufficient diversity of species has now been tested that it is possible to develop water quality guidelines (we use the term "guidelines" herein to include criteria and standards) based on egg Se concentrations (British Columbia Ministry of Environment and Climate Change Strategy [BCMOE], 2014; Environment and Climate Change Canada [ECCC], 2022; U. S. Environmental Protection Agency [USEPA], 2016). Available species mean egg Se 10% effect concentrations (EC10s) range from

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15.6 mg kg^{-1} (dry wt) for white sturgeon (*Acipenser transmontanus*) to 56.2 mg kg^{-1} (dry wt) for Dolly Varden (*Salvelinus malma*). 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, *Thymallus* being of intermediate sensitivity, and *Salvelinus* being the least sensitive genus tested to date (Brix et al., 2021; USEPA, 2016).

The high variability in salmonid sensitivity to maternally transferred Se provides context for evaluating Se concentrations in ovaries from another salmonid, the mountain whitefish (*Prosopium williamsoni*; MWF) in the Elk River watershed of southeastern British Columbia, Canada. Mining of metallurgical-grade coal in Elk Valley has accelerated the release of selenium into this watershed, leading to elevated waterborne Se concentrations (primarily selenate; de Bruyn et al., 2025). Selenium concentrations in MWF ovaries have been monitored since 2006 and in some locations proximate to mining activities, individual and in some cases mean ovary Se concentrations exceed provincial (11 mg kg^{-1} dry wt) and federal (14.7 mg kg^{-1} dry wt) egg Se guidelines.

Given the range in sensitivity to Se of other salmonid taxa, we hypothesized that MWF are less sensitive to maternally transferred Se than the provincial and federal guidelines. To test this hypothesis, we undertook a study to determine the effects of maternally transferred Se on embryo-larval survival, development, and growth as a function of egg Se concentrations.

Methods

Experimental design overview

The general experimental design for this study was similar to previous studies used to derive egg Se concentration-response data for fish (Brix et al., 2021; de Bruyn et al., 2023; McDonald et al., 2010; Rudolph et al., 2008). 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 Se concentrations in parent females.

To accomplish this, MWF were collected during peak spawning from several locations within the Kootenay region of southeastern British Columbia (Figure 1). The first round of collection and testing occurred in 2010 ($n = 6$) with subsequent rounds in 2011 ($n = 9$) and 2013 ($n = 27$) to increase sample size. A final round of sampling and testing was conducted in 2020 ($n = 22$) with the objective of expanding the range of egg Se concentrations in the data set.

Adult fish and gamete collection

Mountain whitefish are distributed throughout the Pacific drainages from the Peace River in northern British Columbia to the Lahontan Basin in Nevada (Scott & Crossman, 1973). The species occurs in both lentic and lotic environments and is adapted to bottom feeding, typically on larval stages of insects, although opportunistic feeding on fish eggs, small fish, and midwater plankton has also been documented (DosSantos, 1985; Pontius & Parker, 1973; Stalnaker & Gresswell, 1974). Spawning occurs in the fall, typically in small cobble or gravel habitat, with populations in southern British Columbia spawning when water temperatures drop to < 10 °C, with peak spawning occurring at approximately 6 °C (Boyer, 2016; Irvine et al., 2017). In the Kootenay region, this typically occurs in late October through mid-November.

Starting in late September or early October each year, site reconnaissance was conducted to monitor field conditions (i.e., temperature, spawning condition) and identify target sampling locations. The reconnaissance strategy included water temperature monitoring and periodic snorkel surveys to detect congregations of spawning-sized MWF. On identification of potential MWF spawning locations and preferred spawning conditions, additional field crews were mobilized to begin fish sampling.

Angling and fyke nets were used to capture adult males and females using methods consistent with fish collection permits. Angling was the preferred method, as it is considered minimally invasive to fish and fish health. However, when water levels and flow conditions were low, fyke nets were utilized to aid in capture. Fyke nets fitted with wings were installed parallel to the shoreline with the capture chamber extending upstream, and the arms extending to the banks to guide fish into the fyke net opening. Fyke nets were used during overnight sets (< 24 hr) and checked regularly throughout the day and first thing each morning.

Mountain whitefish were inspected to determine sex and ripeness, and target individuals were held in live-wells with flow-through systems (i.e., placed within the watercourse) or in holding tanks equipped with battery-powered bubblers and lids until processing. Fork and total lengths were measured to the nearest millimeter using a standard measuring board. Overall body weights were measured using appropriately sized spring scales (e.g., 500 g, 1,000 g, or 5,000 g).

Gamete collection and fertilization

Fish were anaesthetized using dilute clove oil, after which eggs or milt were expressed from each captured fish by application of gentle pressure to the abdomen. Care was taken not to overexpress fish to avoid collecting unripe eggs. The total mass of eggs expressed was measured to support calculation of gonadosomatic index and a subsample (target mass > 100 mg wet wt) of eggs from each female was collected for tissue analysis and placed in separately labelled polyethylene Whirl-Pak bags.

Once gametes were expressed, male fish were placed in a separate live-well where the fish could acclimate with river conditions before release. Mature female MWF retained for tissue sampling were euthanized using a lethal dose of clove oil. Meristic data (total and fork length, weight) were recorded for each fish. Muscle samples were not collected in 2010 and only from a subset of fish in 2011. In 2013, muscle plugs were collected but there was insufficient sample mass to determine moisture content. Muscle fillets were collected in 2020, allowing for determination of both Se and moisture content. Egg and muscle samples were stored frozen (–20 °C) pending shipment for chemical analysis.

Milt and eggs from each fish were transported in a cooler with ice packs to the Nautilus Environmental laboratory in Burnaby, BC. Receipt of gametes and fertilization of eggs were completed on the same day as collection except in 2010, when a flight delay resulted in fertilization being conducted the following day. Sample names for each batch of eggs were assigned sequentially to ensure laboratory staff were blind to sample location. On arrival, each batch of eggs was inspected and observations relating to gamete quality were made prior to fertilization. Observations of blood in the ovarian fluid, unusually large or limited amounts of ovarian fluid, and the presence of water-hardened or broken eggs were noted. A subset of approximately 10 eggs was inspected under a dissecting microscope and the appearance of oil droplets on the periphery of the eggs was recorded, because

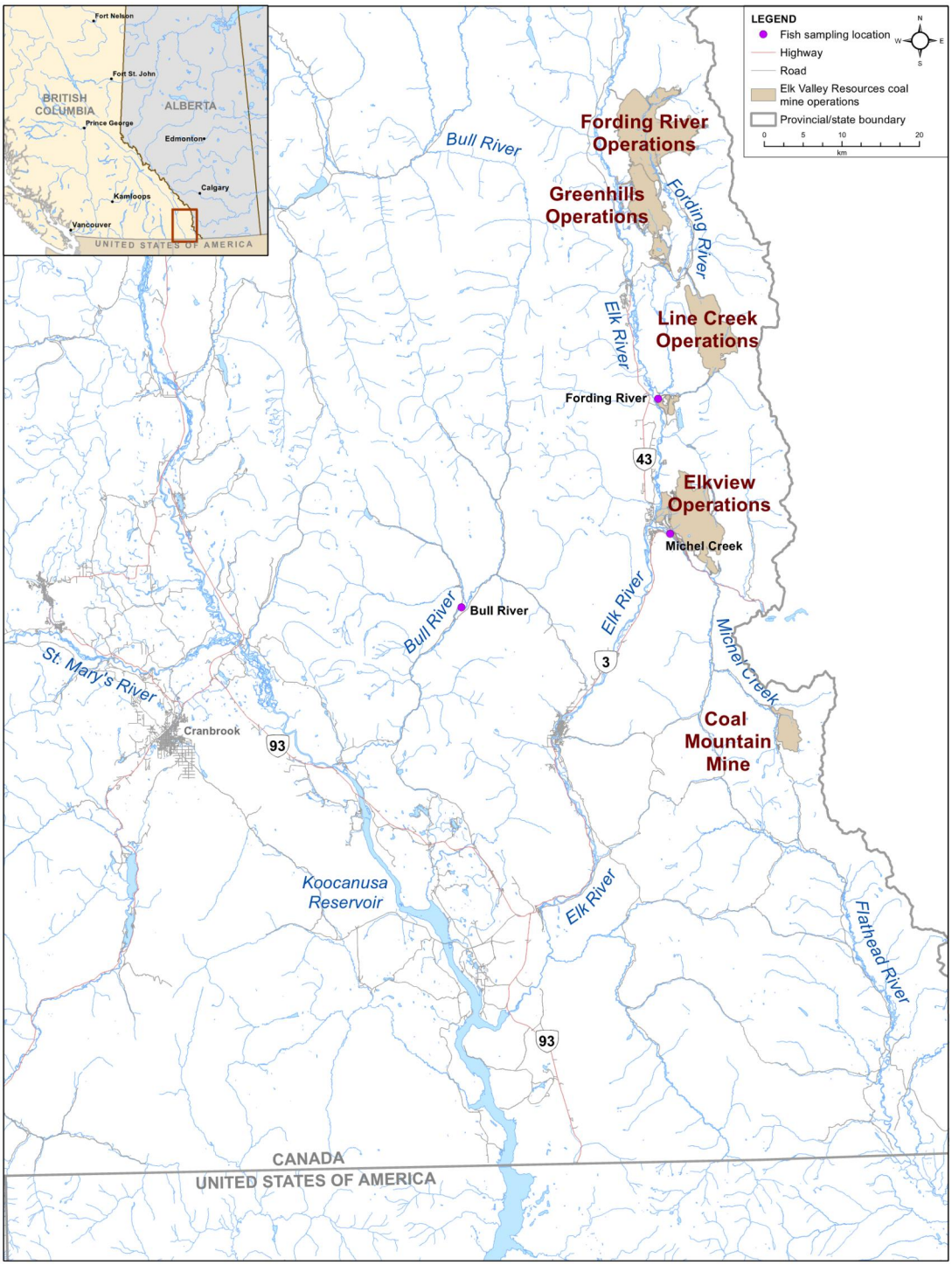

Figure 1. Sampling locations in the Elk River drainage of British Columbia.

the distribution of oil droplets is indicative of egg development (Mansour et al., 2008; Mansour et al., 2007).

Milt from each male was inspected under a microscope to determine sperm viability. Milt was selected from males that produced sufficient volume of sperm with good motility and where blood or fecal material was not present (Environment Canada, 1998). Milt from three or four males was pooled for fertilization. Eggs from each female were fertilized with one or two drops of milt delivered using a glass Pasteur pipette. Eggs and milt were gently mixed by manual stirring, after which they were covered and allowed 20–25 min for fertilization.

Containers were then filled with metropolitan Vancouver dechlorinated tap water (see online supplementary material Table S1 for ionic composition) and the eggs allowed to water harden. Finally, 60 eggs were transferred into each of four replicates, for a total of 240 eggs per female, although in a few cases there were insufficient eggs and the number of replicates was adjusted.

Laboratory rearing

Embryos were reared in 4-L food-grade plastic tubs held in a walk-in chamber with air conditioning and a digital thermostat

able to maintain room temperature at 7 ± 1 °C. The chamber was initially maintained in continuous dark, except for low-level lighting during water renewals. Following hatching, a 16:8-hr light:dark low intensity photoperiod was used consistent with Environment Canada (1998) procedures for early life stages of salmonids. Test chambers were provided with continuous aeration just below the surface at approximately 100 bubbles min^{-1}, sufficient to maintain dissolved oxygen levels close to saturation (11.8 to 12.4 mg L^{-1} for 7 ± 1 °C) and provide gentle mixing of the water in the test containers.

Dechlorinated Vancouver municipal tap water in the test containers was renewed three times per week prior to hatch by gently pouring or siphoning out approximately half of the water from the containers and replacing with fresh water. Water replacement was increased to a daily replacement of 80% following hatch, consistent with requirements for rearing juvenile rainbow trout fry (Environment Canada, 2007). Fungus was observed on some eggs in 2010 and a prophylactic treatment regime using an iodophore (1% Ovadyne) was performed twice per week starting on Day 27 until hatch to prevent fungal growth. In subsequent testing years, iodophore treatments were initiated at the beginning of the experiment.

The fish were observed prior to water replacements and mortalities removed and recorded. Once hatching began, hatched fish were removed from the test containers on a daily basis and pooled (per female) into a new container. Hatched fish were pooled into new containers daily for a 1-week period, after which new containers were used for fish that hatched in the following week. This process was necessary, as embryo hatching occurs over a relatively long time period (~4 weeks) and MWF fry are ready to actively feed shortly after hatch.

Post-swim-up test methods varied across study years. In 2010, the experiment was terminated at swim-up, whereas in subsequent years, fish were fed recently hatched *Artemia nauplii* ad libitum. The fry in each container were fed for an additional 7 days following the end of the week, at which time the fish were considered swim-up fry and anaesthetized using clove oil. Thus, depending on whether the fry hatched at the beginning or the end of the week, euthanized swim-up fry were reared and fed for between 7 and 14 days following hatch. Fry were assessed for deformities and individual lengths (to the nearest 0.5 mm) and combined wet weight of the fry from test containers.

Deformity assessments

Deformity assessments were conducted immediately following euthanization to avoid morphological changes that can occur when preservatives are used. Deformities were assessed using a Graduated Severity Index (GSI), as described and used by Holm et al. (2005) and Rudolph et al. (2008). This approach involved assigning a score to deformities of 0, 1, 2, or 3 based on the severity of deformity in each of four categories: skeletal, craniofacial, finfold, and edema. Graduated Severity Index scores were then assigned for each fry by summing scores for each of these four categories. Thus, GSI scores could range from 0 for no deformities to 12 for fry with gross deformities in each category (see online supplementary material Table S2).

Skeletal deformities included lordosis, scoliosis, and kyphosis. Craniofacial deformities included missing or deformed eyes, shortened or otherwise deformed jaw or opercle, and/or abnormalities in the shape of the head. Finfold deformities included missing or deformed fins or abnormalities in the articulation of pectoral fins. Edema is not considered to be a true teratogenic effect but rather is a sign of toxicological stress and has been reported as a common sign of Se exposure (Janz et al., 2010), although it is also commonly associated with exposure to other toxicants. Edema involves accumulation of fluid in the visceral cavity and is recognized as a clear fluid typically anterior or posterior to the yolk sac. Exophthalmus was also scored as edema.

Deformity analyses were conducted on all fish by a single individual who was not aware of the fish identity with respect to capture location or Se concentration. A second observer conducted deformity analyses on a subset (10%) of fish as a component of quality assurance/quality control

Analytical chemistry

Egg and muscle samples were submitted to ALS (Burnaby, BC) or Brooks Applied Laboratories (Bothell, WA) for analysis. Selenium concentrations were measured by either collison/reaction cell-inductively coupled plasma mass spectrometry (CRC-ICP-MS) or dynamic reaction cell (DRC-ICP-MS) consistent with USEPA 200.3/6020B. The percentage of moisture was determined on subsamples for conversion of wet weight Se concentrations to dry weight concentrations or samples were first dried then microwave digested following USEPA 3052 prior to analysis. Detection limits varied as a function of sample mass but were ≤ 0.01 mg kg^{-1} (dry wt) in all cases.

A certified standard reference material (DORM-4 Fish Protein Certified Reference Material for Trace Metals) was measured concurrently with each batch of samples along with matrix spikes, duplicate analyses, and method blanks to assess accuracy and precision.

Data analysis

Toxicity data for survival, growth, and deformities as a function of egg Se concentrations were analyzed by linear and/or nonlinear regression as appropriate for the data distribution to estimate the EC10, the standard statistical endpoint used by regulators in Canada and the United States for Se water quality guidelines/criteria (BCMOE, 2014; ECCC, 2022; USEPA, 2016). All statistical analyses were conducted using GraphPad Prism (Ver. 10.3).

Results

Fish collection and selenium concentrations in eggs and muscle

A total of 64 female fish were spawned and evaluated over the course of the four study years. Egg Se concentrations ranged from 15.5 to 54.0 mg kg^{-1} (dry wt) across all samples (Figure 2A). Muscle Se concentrations in a subset of female fish ($n = 55$) ranged from 3.1–8.4 mg kg^{-1} (dry wt). Corresponding egg-muscle ratios were highly variable (2.1–10.2) with no significant relationship between the two tissues (Figure 2B).

Toxicity studies: general

Temperature, dissolved oxygen, and pH during larval rearing remained within ranges recommended by Environment Canada (1998) throughout exposures in all four study years (see online supplementary material Table S3). In 2010, fungus was observed on some eggs in Week 3 of the exposure leading to mortalities. An iodophore treatment (1% Ovadine) was initiated twice weekly, which effectively controlled fungal growth. This treatment was initiated at the beginning of the study in subsequent years and stopped just prior to hatch.

Embryo-larval survival

Embryo-larval survival was monitored through swim-up (2010) or for 7–14 days after swim-up (2011, 2013, 2020). As just described, in 2010 embryo survival was affected by a fungal

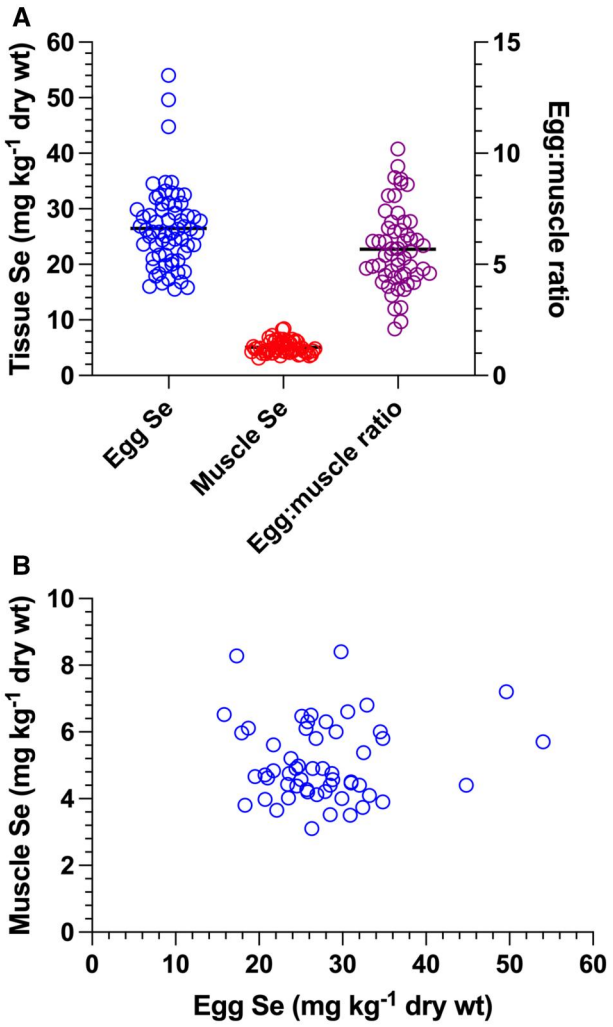

Figure 2. (A) Distribution of egg Se concentrations, muscle Se concentrations, and the egg-muscle ratio in spawning females. (B) Relationship between egg Se and muscle Se concentrations.

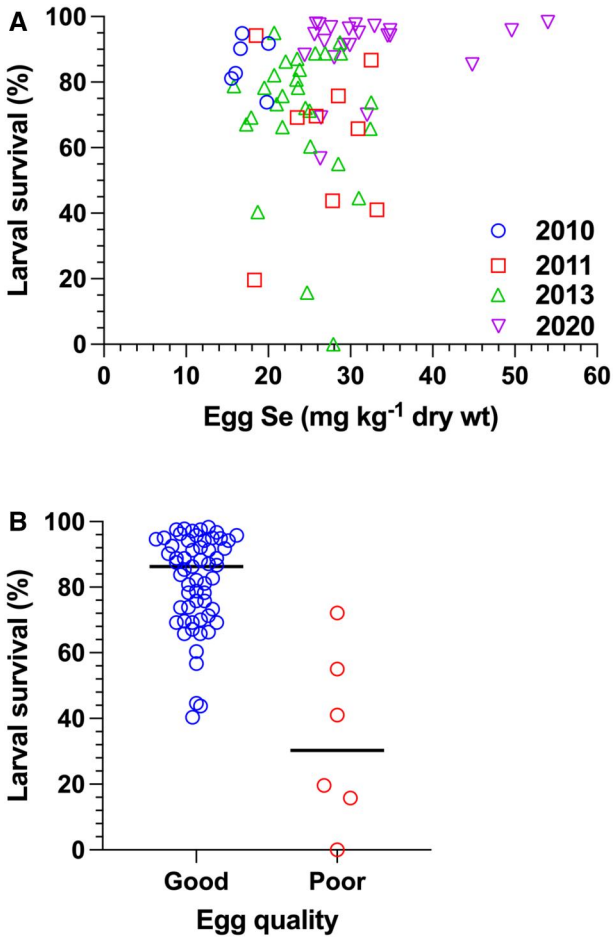

Figure 3. (A) Relationship between egg Se concentrations and larval survival at swim-up. (B) Larval survival as a function of egg quality.

infection and infected embryos were removed from survival calculations. Survival was variable, ranging from 0%–98% across all data (Figure 3A; online supplementary material Table S4). A few egg samples were qualitatively identified as being of low quality due to the presence of blood in the sample or low quantities of ovarian fluid. Consideration of qualitative egg quality prior to spawning explains the lowest (< 40%) survival results (Figure 3B). A significant effect of study year was observed on survival (Kruskal Wallis; $p < 0.0001$), with 2020 having a significantly higher survival than 2011 and 2013 (Kruskal Wallis; $p < 0.001$). No significant relationship between egg Se concentration and embryo-larval survival was observed in any year or for all years combined ($p > 0.17$).

Embryo-larval growth

Embryo-larval growth was evaluated using both total length and wet weight at swim-up (see online supplementary material Table S4). A significant ($p < 0.02$) negative linear relationship between egg Se and larval length was observed in 2013 and 2020 but not the two earlier experimental years (Figure 4A). Comparatively evaluating the 2013 and 2020 data, no significant difference in the y-intercepts or slope were detected (F-test; $p > 0.05$), although visually there is an apparent separation between the data sets. Consistent with this observation, a regression model of the pooled data set did not adequately describe both data sets (F-test; $p = 0.017$) because the model regressed across the two data sets with a resulting slope substantially steeper than models for either separate data set. Consequently, we suggest a weighted mean slope for the two experimental years (m=–0.0353) best represents the relationship.

Similar to the length endpoint, significant ($p < 0.05$) relationships between egg Se concentration and larval wet weight were observed in 2013 and 2020 but not earlier experimental years (Figure 4B). Comparatively evaluating the 2013 and 2020 data, a significant difference in model intercepts (F-test; $p = 0.018$) but not slopes (F-test; $p = 0.32$) was detected. As a result, a weighted mean slope for the two experimental years (m=–0.1065) best represents the relationship.

Both endpoints suggest the potential for a Se-dependent effect on larval fish growth, with wet weight being more sensitive as evidenced by the steeper slope. The growth effect does not exhibit a sigmoidal concentration-response relationship but rather a shallow linear relationship with egg Se. Further, because the lower egg Se concentration in the data set is 15.5 mg kg^{-1} (dry wt), it is unknown whether this response extends to lower egg Se concentrations and if it does, at what concentration the effect plateaus. Given this uncertainty, we chose not to extrapolate beyond the available data and used the normalized (slope/intercept) slope for larval weight of –0.0053 to estimate an egg Se EC10 of 34 mg kg^{-1} (dry wt) for larval growth.

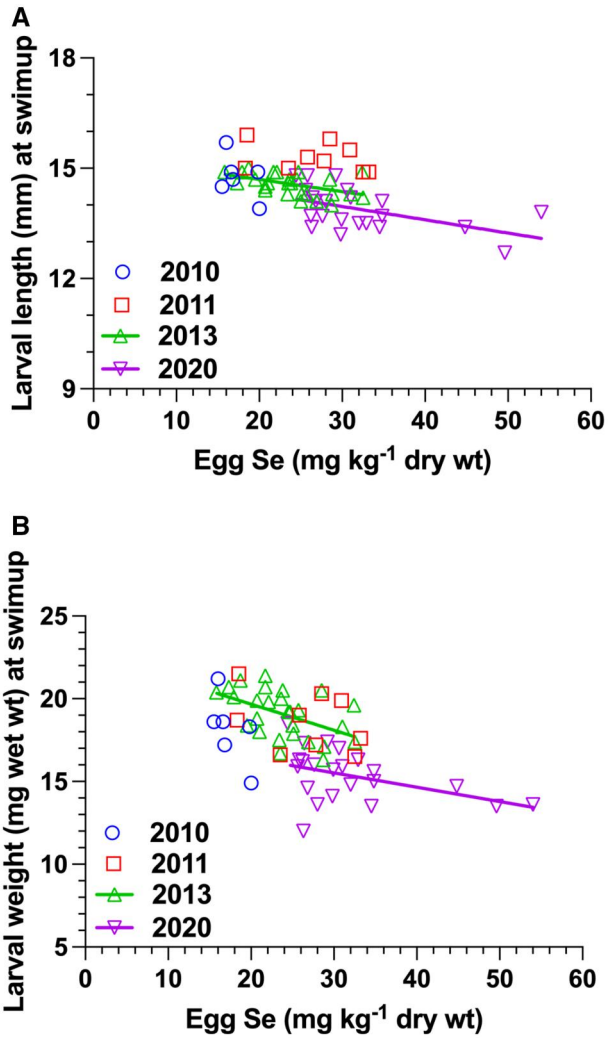

Figure 4. Relationship between egg Se concentrations and (A) Larval length at swim-up. (B) Larval wet weight at swim-up.

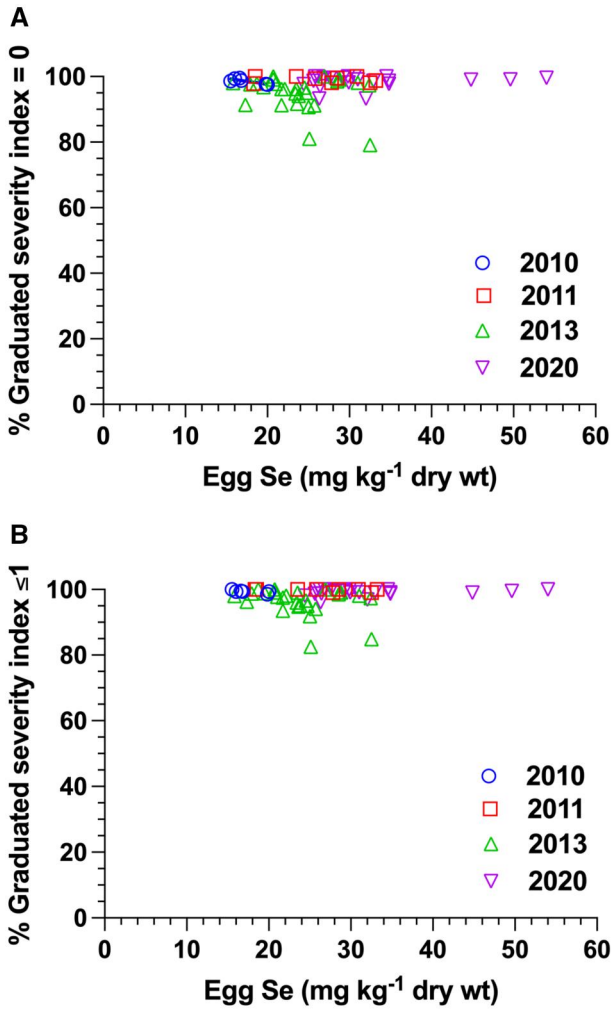

Figure 5. Relationship between egg Se concentrations and (A) Graduated severity index of 0. (B) Graduated severity index ≤ 1.

Embryo-larval development

Embryo-larval development, as characterized by the GSI, was assessed in a total of 8,944 individual fry across the four study years. The incidence of deformities was generally low across the full range of egg Se concentrations (Figure 5). Data for GSI was expressed at the percentage of larvae with a GSI = 0 (no deformities) and a GSI ≤1, which are minor deformities unlikely to affect long-term larval survival (see online supplementary material Table S4). A statistically significant relationship was detected between egg Se and GSI = 0 only for the 2010 data ($p = 0.03$), but this was across a narrow range of egg Se concentrations (15.5–20 mg kg^{-1} dry wt; Figure 5A). Relationships were not detected for the other study years or when all of the data were pooled. No significant relationship was detected for any study year or the pooled data set for GSI ≤ 1 ($p > 0.08$; Figure 5B). A total of 124 larvae were identified as having a GSI >1, which equates to 1.4% of all larvae evaluated. Overall, we conclude that egg Se had no effect on embryo-larval development up to the maximum egg Se concentration evaluated resulting in an EC10 > 54 mg kg^{-1} (dry wt).

Discussion and conclusions

The objective of this study was to characterize the sensitivity of larval MWF to maternally transferred Se. Our results indicate there is no significant relationship between egg Se concentrations and either larval survival or embryo-larval development (i.e., incidence of deformities) up to the highest egg Se concentration evaluated (EC10 > 54 mg kg^{-1} dry wt). The latter endpoint has been the most sensitive endpoint for maternally transferred Se in all other species studied to date (Janz et al., 2010). Interestingly, there is some indication that maternally transferred Se may have negatively affected larval growth through swim-up and we explore these data further.

Embryo-larval growth

A concentration-dependent relationship between larval growth (length and wet wt) was observed in the 2013 and 2020 data sets but not in the earlier experimental years (Figure 4). Small differences in larval growth were observed between study years, precluding pooling of the data. This is not surprising, as minor (<1 °C) differences in experimental temperature between years are likely to impart small differences in growth over the approximately 80–100 day study period. The lack of a relationship in the 2010 and 2011 data sets may in part be attributed to small sample sizes.

The apparent growth effect did not exhibit a characteristic sigmoidal concentration-response relationship but rather an unusually shallow linear relationship with egg Se (Figure 4). This is unusual for Se and toxicants in general. For example, a sigmoidal

concentration-response profile has been observed for growth effects on juvenile chinook salmon (*Oncorhynchus tshawytscha*) fed elevated concentrations of dietary Se (Hamilton & Buhl, 1990). Considering this atypical concentration response profile, we considered an alternative hypothesis that the observed pattern is actually the result of a maternal provisioning effect in which larger females better provision oocytes, leading to larger embryos during early development. This effect is well documented in salmonids and other fish species (Green, 2008; Heath et al., 1999; Rollinson & Hutchings, 2010) and typically has a shallow linear response profile consistent with growth effects observed in our study. Plotting larval weight and length as a function of spawning female fork length supports this hypothesis, with significant linear relationships observed for the 2013 and 2020 data (Figures 6A and 6B; $p < 0.003$, $R^2 = 0.38–0.57$).

Although this provides support for the maternal provisioning hypothesis, exploratory data analysis also revealed a shallow linear relationship between spawning female fork length and egg Se concentrations for the 2013 and 2020 data (Figure 6C; $p = 0.03–0.04$; $R^2 = 0.15–0.21$). The relationships are not as strong as observed for the maternal provisioning hypothesis but cannot be ruled out as a potential explanatory variable. It is possible that there is a dietary shift between smaller and larger adult MWF resulting in differential dietary Se exposure. We have previously documented a size effect on ovary Se concentrations in northern pikeminnow (*Ptychocheilus oregonensis*) that corresponds well with a gape limited shift from a primarily insectivorous to piscivorous diet in this species (Brix et al., 2025). We did not observe such a relationship in a similar analysis of MWF ovary Se data, but the MWF data set was not well distributed with respect to female size, which may have influenced the analysis. Studies on age-dependent dietary composition in MWF indicate a shift from a primarily dipteran diet (e.g., chironomids) to a diet approximately evenly distributed between dipterans and Ephemeroptera, Plecoptera, Trichoptera taxa (DosSantos, 1985; Pontius & Parker, 1973; Stalnaker & Gresswell, 1974; Whiteley, 2007). Chironomids can have higher Se concentrations than other taxa at a given location likely due to their preference for bedded sediments with more reducing environments, but this is not always the case and whether this explains observed patterns in MWF is speculative (Adams et al., 1998; de Bruyn et al., 2025).

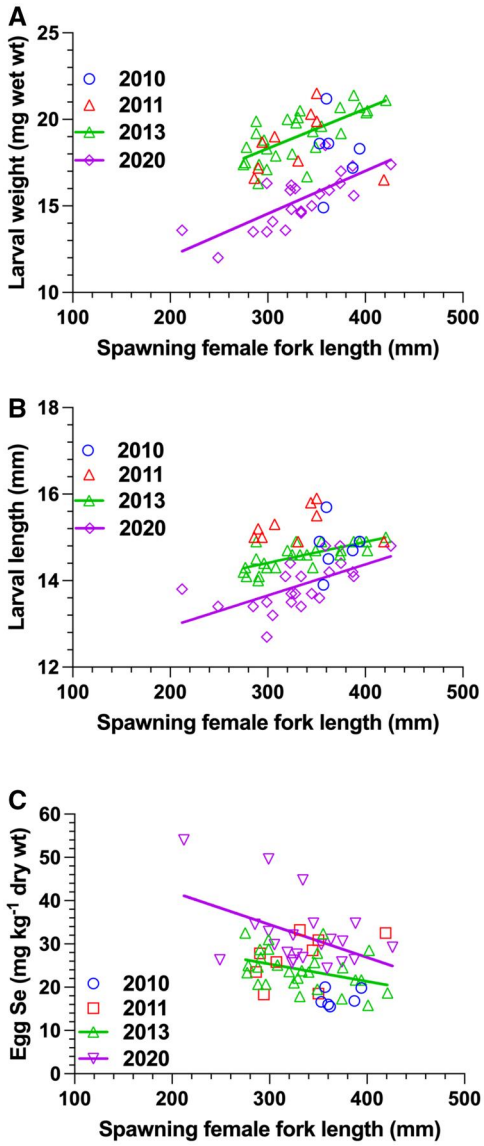

Figure 6. Relationship between spawning female fork length and (A) Larval wet weight. (B) Larval length. (C) Egg Se concentration.

Ultimately, the available data does not allow for a full understanding of the mechanism(s) that might be driving the observed relationship between egg Se and larval growth endpoints. This endpoint is uncertain because the lower limits of the apparent effect are undefined (nonconservative error), there is evidence that a maternal provisioning effect could be contributing to or solely responsible for the observed relationship (conservative error), and because the endpoint has not been shown to be diagnostic of maternally transferred Se effects on other fish species. Overall, the available weight of evidence appears more supportive of a maternal provisioning effect, but a Se effect cannot be conclusively eliminated.

Embryo-larval development

Finally, across all years of study, there were consistently no developmental effects observed on larvae as a function of egg Se concentration resulting in an EC10 of $> 54\,mg\,kg^{-1}$ (dry wt), the highest egg Se concentration tested (Figure 5). This makes MWF, at a minimum, the second least sensitive species tested to date for this endpoint, with only Dolly Varden (*Salvelinus malma*) being potentially less sensitive with an EC10 of $56\,mg\,kg^{-1}$ (dry wt; McDonald et al., 2010).

Egg–muscle tissue ratios

The USEPA (2016) previously reported a significant relationship between MWF muscle and ovary (not egg) Se based on data collected from Elk Valley. However, the relationship slope is extremely steep ($m = 5.8$) compared with most other species (typically slope ~1–2). Data from the current study using egg Se rather than ovary Se suggests the apparent relationship in USEPA (2016) is spurious, as we observed no relationship using a larger data set (Figure 2B). Our observations are also supported by a more recent analysis of fish tissue ratios that evaluated a larger ovary data set and similarly found no relationship with muscle Se concentrations (Detering et al., 2025). Among species that have a sufficiently large data set with ovary/egg Se concentrations spanning at least a factor of 5, MWF appear to be unique in lacking a relationship between these two tissues. The mechanism(s) underlying this fundamental difference in Se homeostasis should be investigated further.

Conclusion

The objective of this study was to determine the sensitivity of embryo-larval MWF to maternally-transferred egg Se, particularly at the higher end of the egg Se distribution observed in the Elk River watershed. Integrating results across study years supports an EC10 of $>54\,mg\,kg^{-1}$ (dry wt) egg Se for the embryo-larval deformity endpoint, which has been the most sensitive endpoint evaluated for all other species tested to date. However, a statistically significant relationship between larval growth (both length and weight) and egg Se was also observed for MWF in half of the experimental years with an atypically shallow linear profile over the entire egg Se concentration range evaluated. An EC10 of $34\,mg\,kg^{-1}$ (dry wt) was estimated for this endpoint, although there are uncertainties given the lack of egg Se data below $15\,mg\,kg^{-1}$ (dry wt). There is also evidence that a size-dependent maternal provisioning effect could be contributing or driving the relationship rather than egg Se concentrations. Regardless of whether the growth endpoint ($34\,mg\,kg^{-1}$ dry wt) or deformity endpoint ($>54\,mg\,kg^{-1}$ dry wt) is used, this study demonstrates that MWF are much less sensitive than the $11\,mg\,kg^{-1}$ (dry wt) egg Se guideline used in British Columbia or the $15.1\,mg\,kg^{-1}$ (dry wt) guideline used on the United States. Any future evaluation of risk to this species should use these species-specific effect concentrations rather than generic guidelines.

Supplementary material

Supplementary material is available online at *Environmental Toxicology and Chemistry*.

Data availability

All data from this study are provided in the online supplementary material.

Author contributions

Kevin V. Brix (Conceptualization, Data curation, Formal analysis, Investigation, Supervision, Visualization, Writing—original draft), Josh Baker (Data curation, Formal analysis, Investigation, Methodology), Adrian M.H. de Bruyn (Conceptualization, Investigation, Supervision), Jennifer Ings (Investigation, Methodology, Supervision), Cait Good (Investigation, Project administration, Supervision), Mariah Arnold (Investigation, Project administration, Supervision), and James R. Elphick (Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision)

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Conflicts of interest

The authors declare they have no conflicts of interest.

Disclaimer

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