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Maternal Transfer and Effects of Selenium on Early Life Stage Development of Redside Shiner (*Richardsonius balteatus*)

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Abstract: Maternal transfer of selenium (Se) to developing fish eggs during vitellogenesis can cause larval deformity and mortality. Previous studies have shown wide variation among fish species in both the magnitude of maternal transfer (exposure) and the egg Se concentration causing effects (sensitivity). We studied maternal transfer and effects of Se on early life stage development, survival, and growth of redside shiner (*Richardsonius balteatus*), a small-bodied cyprinid that has been reported to have relatively high ovary:muscle Se concentration ratios. Gametes were collected from lentic areas in southeast British Columbia (Canada) with a range of dietary Se concentrations related to weathering of waste rock from coal mining. Eggs were fertilized and reared in the laboratory from hatch to the onset of exogenous feeding. Larvae were assessed for survival, length, weight, Se-characteristic deformities, and edema. Eggs from a total of 56 females were collected, with egg Se concentrations from 0.7 to 28 mg/kg dry weight. Maternal transfer varied among sites, with egg:muscle Se concentration ratios ranging from <1 to >4. We also found that sampling residual ovaries can overestimate Se concentrations in ripe eggs by up to a factor of 5.7. A correlation between larval weight and egg Se concentration was identified, although the relationship was weak ($r^2 < 0.1$) and appeared to be a site effect. No other relationships were observed between larval endpoints and egg Se concentrations up to the highest concentration tested, indicating that the effects threshold for this species may be >28 mg/kg dry weight in eggs. These data indicate that redside shiner is less sensitive to maternally transferred Se than most other tested fish species. *Environ Toxicol Chem* 2023;42:2350–2357. © 2023 SETAC

Keywords: Selenium; Toxic effects; Developmental toxicity

INTRODUCTION

Selenium (Se) is well known both as an essential trace element required by all animals (Watanabe et al., 1997) and, at higher concentrations, as a teratogen for oviparous vertebrates (Janz et al., 2010). Selenium is mobilized into aquatic environments by weathering of mineral phases, which can be greatly accelerated by activities such as mining and agriculture (Janz et al., 2010; Vriens et al., 2019). Selenium enters water primarily as the oxyanions selenate and selenite (Vriens et al., 2019), where it is actively bioaccumulated by microbes, algae, and other primary producers (DeForest et al., 2016; Riedel et al., 1991), biotransformed into organoselenium species such as the essential amino acid selenomethionine (Bowie et al., 1996; Fan et al., 2002), and transferred via the diet to higher trophic levels (Orr et al., 2012; Presser & Luoma, 2010).

Chronic toxicity in fish occurs when dietary Se concentrations exceed a threshold for direct toxicity (Knight et al., 2016) or when maternal transfer of dietary Se during vitellogenesis results in egg Se concentrations exceeding a threshold for embryotoxicity (DeForest et al., 2012; Janz et al., 2010), with the latter endpoint reported to be more sensitive. Selenium embryotoxicity has been characterized in relatively few fish species, with effects thresholds expressed as egg or ovary Se concentration ranging from 15 to 56 mg/kg dry weight (Brix et al., 2021; DeForest et al., 2012; US Environmental Protection Agency [USEPA], 2016). Vitellogenic provisioning of

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Se into eggs is also variable, with concentration ratios usually >1 and some cases 10 or higher (USEPA, 2021) between egg (or ovary, which tends to be similar to egg in many species; USEPA, 2021) and muscle (or diet, which tends to be similar to muscle in many species; Presser & Luoma, 2010; USEPA, 2021). Because reported variability in egg to diet ratios among fish species (~8-fold) is greater than reported variability in effects thresholds (~4-fold), efficiency of maternal transfer may be as important as sensitivity in determining relative Se risk.

A consequence of the small number of fish species evaluated for Se sensitivity is uncertainty in application of appropriate egg Se thresholds when assessing Se risk for untested species, which was the impetus for our study. Metallurgical coal mining operations in the Elk River valley of British Columbia, Canada have resulted in mobilization and release of Se, primarily as selenate, into the Elk River drainage and subsequently into the Koocanusa Reservoir. Ovary Se concentrations exceeding regulatory guidelines in both Canada (11 mg/kg dry wt) and the United States (15 mg/kg dry wt) have been reported for redside shiner (*Richardsonius balteatus*), prompting concern about potential effects (Teck, 2021). Of particular interest was that Se concentrations in muscle of redside shiners collected from the Koocanusa Reservoir have generally met regulatory guidelines, whereas concentrations in ovaries have not. The risk of effects to this species is uncertain because the sensitivity of redside shiner to Se-induced embryotoxicity is unknown. To address this uncertainty, the present study evaluated the toxicity of maternally transferred Se to early life stages of redside shiners. Because redside shiners have not previously been studied in this way, we also undertook method development to obtain ripe gametes from field-collected adults, fertilize and transport embryos to the laboratory, and rear them through hatch until yolk absorption was complete at the onset of feeding.

METHODS

Experimental design

Our general experimental design was similar to that of previous studies that have evaluated the toxicity of maternally transferred Se to fish early life stages (see Brix et al., 2021; McDonald et al., 2010; Rudolph et al., 2008). To develop egg Se concentration–response relationships, adult redside shiner were collected and spawned from multiple locations in the Elk River drainage with differing dietary Se concentrations. Fertilized embryos were then brought into the laboratory and monitored through several early life stages.

Fish collection and spawning

Redside shiner is a small-bodied western cyprinid found throughout British Columbia and in the northwestern United States west of the Rocky Mountains. They occur in schools of thousands in large lakes, ponds, and moderately fast-moving streams, tolerating a wide variety of temperatures and trophic conditions. Other than during spawning migration, the species exhibits little directed movement aside from on and off shoals in lacustrine environments related to changes in water temperature (Scott & Crossman, 1998). Redside shiner usually reach sexual maturity by their third year and spawn in the spring in streams and lakes when water temperatures reach approximately 10 °C (Scott & Crossman, 1998). Eggs hatch 3 to 15 days post spawning, depending on water temperature. Fry feed mainly on plankton and demersal crustaceans, whereas larger fish are mainly insectivorous but also feed opportunistically on fish eggs (Scott & Crossman, 1998).

Study sites were selected to provide a range of dietary Se exposures for invertivorous fish. Composite benthic invertebrate samples were collected between May and August 2018 from 44 lentic areas in and near the Elk Valley (see *Tissue sampling and analysis* below). Field monitoring of potential redside shiner spawning areas began in early May 2019. Redside shiner collection occurred between May 6 and June 24, 2019. Mature males and females were collected from a reference lake (Loon Lake), two locations in Koocanusa Reservoir, and three off-channel lentic areas in the Elk Valley close to mining operations (Table 1 and Supporting Information, Figure S1). Fishing was also conducted at a second reference lake (Grave Lake) and two additional lentic areas close to mining (Elk River oxbow and Goddard Marsh), but no mature females were found. A total of 56 mature females were caught across all sampled areas. All fish were collected under British Columbia Fish Collection Permits CB19-474596 and CB19-471215.

Fish were captured using short-set gill nets, minnow traps, mini hoop nets, angling, and seine nets. Gill nets (15–23 m length, 0.5–1″ mesh) were deployed extending from the shoreline outward and set for a maximum of 15 min. Minnow traps were baited with cat food in a screened (<1-mm mesh)

TABLE 1: Redside shiner sampling areas

Area description	UTM[a] easting	UTM northing	Total catch	Mature females	Invertebrate (Se; mg/kg dry wt)[b]
Loon Lake	638 220	5 441 850	3508	10	3.7[c]
Koocanusa at Englishman Creek	627 997	5 447 625	1548	3	7.7
Koocanusa at Gold Creek	630 804	5 436 413	4185	11	3.0
Elk River impoundment in Fernie	640 447	5 486 898	3513	13	2.7–3.7
Elk River wetland south of Fernie	639 138	5 484 622	66	3	3.2–4.8
Stanford Pond	639 864	5 483 139	730	16	14–35

[a]Universal Transverse Mercator Zone 11, NAD83.
[b]Ranges are shown for sites with additional sampling events in 2019.
[c]Invertebrate samples were not available from Loon Lake; value shown is for the Grave Lake reference area.

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container, set on the substrate in shallow areas near the shore, and checked after approximately 24 h. Mini hoop nets (24 × 24″ frame, 0.5″ mesh) were deployed for 24 to 48 h. Angling was conducted near schools of congregating redside shiner using a size 18 hook baited with an artificial maggot. Hooked fish were pulled out of the water, dehooked, and allowed to recover in a cooler with clean, aerated water from the collection site. Seining (15 × 0.9 m, 0.3-cm mesh) was conducted by wading to corral fish into the net. Nontarget species and immature fish were released at the point of capture.

Redside shiner with spawning coloration and swollen abdomens (females) or visible spermeation (males) were placed in coolers with clean, aerated water from the collection site. Males and females were kept separate. Water temperature and dissolved oxygen were monitored to confirm that fish were not under stress prior to processing. Fish that appeared to be ripe were removed individually, anesthetized using clove oil, measured (total length, fork length, total weight), and subjected to gentle abdominal pressure to express eggs and milt. Initial attempts to obtain eggs from fish from a reference lake (Loon Lake) and one of the exposure locations (Elk River impoundment in Fernie) resulted in expression of under-ripe eggs. Whereas these eggs initially appeared to be successfully fertilized, it became apparent after a few days that they were not developing successfully. Thereafter, multiple fish were gently handled to attempt to express eggs until a fish that was notably fully ripe was obtained; fully ripe fish expressed eggs surrounded by ovarian fluid that flowed freely from the vent, and eggs were more consistent in size than those obtained initially. Females that easily expressed eggs were euthanized for Se analysis and removal of aging structures. All other fish were placed in an aerated recovery bath and released at the point of capture.

Eggs were fertilized on site using a combination of dry and wet fertilization. At each site and on each sampling date, milt from two to six males was pooled in a small plastic vial that was kept cool and dark by placing it under a padded icepack. Milt was collected from inverted male fish using a Pasteur pipette and rubber bulb, taking care to ensure that water and feces did not combine with the milt. Eggs were expressed by gentle pressure on the abdomen of inverted females onto a glass slide that was then placed in a plastic Petri dish. A small drop of pooled milt was placed on each clutch of eggs in the absence of water and gently mixed using the tip of a clean pipette. After a short dry-fertilization period, eggs were covered with a small amount of reconstituted moderately hard water (USEPA, 2002) and allowed to wet-fertilize for 10 to 15 min. Initial attempts at fertilization with Loon Lake and Elk River impoundment in Fernie eggs using a 10- to 15-min dry-fertilization period resulted in variable and often low (40% ± 24%) success. A shorter dry-fertilization period was found to result in a higher success rate, and thereafter a 2-min dry-fertilization period was used, resulting in more consistent and higher (69% ± 19%) fertilization rates. Following the wet-fertilization period, eggs were transferred to a plastic Ziploc transport container with 500 to 1000 ml of reconstituted moderately hard water. Eggs were not adhesive following the wet fertilization period, which allowed them to be transferred freely to containers.

Toxicity testing

Transportation of eggs occurred between 3 h and 2 days post fertilization. Eggs were held prior to transport at 14 ± 3 °C, within the range measured at the collection areas, and then transported by air to the laboratory in Burnaby (BC, Canada). Care was taken to minimize mechanical disturbance from handling and to limit temperature fluctuations. A visual inspection was requested at airport security so that x-ray of the shipment was not needed.

Temperature was measured on arrival at the laboratory, and eggs were inspected to confirm they were undamaged by transport. Eggs were held for at least 12 h to allow equilibration to laboratory conditions, placed in an environmental chamber at 14 ± 2 °C, and reared in reconstituted moderately hard water with a daily water renewal of approximatey 80%. Temperature, dissolved oxygen, conductivity, and pH were measured before and after each water renewal, and eggs were inspected daily for fungal infection. Chlorine was measured weekly and total metals monthly by inductively coupled plasma–mass spectrometry (ICP–MS) scan to characterize the rearing water quality.

Embryonic development was evaluated periodically with a dissecting microscope. When gastrulas were observed, which generally occurred 3 to 4 days following arrival, any eggs that had not developed to gastrula were assumed to be unfertilized. Fertilized eggs were counted and divided into groups of 50 into as many replicate containers as needed. Containers were randomly distributed within the environmental chamber and provided with continuous gentle aeration. Embryos were monitored daily for mortality and hatching, and any mortalities were removed. Hatched larvae were transferred to a 2-L rearing container and reared under the same conditions.

When yolk sac absorption was complete at 20 to 21 days post fertilization, larvae were euthanized using tricaine methylsulfonate (CAS 886-86-2) and assessed the same day without preservation for skeletal, craniofacial, and fin deformities and edema consistent with Se toxicity (Lemly, 1997; Maier & Knight, 1994) using a dissecting microscope. Deformities were scored using a graduated severity index (GSI) according to Holm et al. (2005) and Rudolph et al. (2008). After deformity assessment, 20 fish from each replicate were randomly selected and measured to assess growth. Larvae from each replicate were then pooled, dried at 60 °C for 24 h, and weighed. Deformity scoring was conducted by technicians with training and experience in fish deformity analyses. All test containers were labeled blind. Photographs of normal larvae and a representative selection of each type and severity of deformity were used as a guide for the GSI scoring. A minimum of 10% of larvae were reassessed by an observer not involved in the original scoring.

Statistical analysis

Data were analyzed by ordinary least squares (OLS) regression using Systat™ Ver. 13.1 (Systat Software) and nonlinear regression using the Toxicity Relationship Analysis Program, Ver. 130a (TRAP; USEPA, 2015) to evaluate relationships between

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egg Se concentration and survival, fertilization, growth (length and weight), and deformity. Analyses of deformity prevalence were conducted for a GSI of ≥1 (i.e., any deformity in any category) and a GSI of ≥2 (i.e., significant deformity in one category or a minor or greater deformity score in two or more categories).

Tissue sampling and analysis

Composite benthic invertebrate samples were collected in September 2018 from all sampling areas except the Loon Lake reference area. Additional sampling was conducted in 2019 at Stanford Pond (May and September), at Elk River wetland south of Fernie (May, June, and September), and at Elk River impoundment in Fernie (June). A net with 400-μm mesh was used to collect three replicate composite-taxa tissue samples by sweeping the shoreline of each lentic area. Replicate samples were spaced at least 10 m apart and distributed throughout the sampling area. The outside of the net was rinsed with site water to concentrate the sample at the cod end of the net, and the contents were placed on a white plastic tray. Clean forceps were used to separate invertebrates from debris and a sample of 1 to 2 g wet mass was transferred to a white enamel tray to be photographed. Samples were transferred to labeled cryovials, placed in a cooler with ice packs until they could be transferred to a freezer at the end of the day, and stored frozen until analysis. Samples were shipped frozen to Saskatchewan Research Council Environmental Analytical Laboratories (SRC; Saskatoon, SK, Canada). Samples were freeze-dried and homogenized and total metal concentrations were measured using high-resolution (HR) ICP–MS.

All reproductive tissue remaining after ripe eggs were expressed (referred to in the present study as residual ovaries) and two skinless dorsal muscle samples were dissected from each euthanized fish and placed in Whirl-Pak bags. An aliquot of unfertilized ripe eggs from each female was placed in a 15-ml plastic vial. Heads were removed and submitted to AAE Tech Services (La Salle, MB, Canada) for aging via otolith sectioning. Samples were immediately frozen and stored at −20 °C.

To maximize the number of eggs that could be incubated, small aliquots of 2.6 to 334 mg wet mass were taken for Se analysis. Some egg sample masses were too small for routine analysis by HR ICP–MS, and therefore were shipped frozen to TrichAnalytics (Saanichton, BC, Canada) where they were dried at 50 to 55 °C, homogenized into a powder, and compressed into pellets. Total Se in eggs was measured by laser ablation (LA) ICP–MS using an NWR-213 laser ablation system (New Wave Research) coupled to an iCAP RQ Series ICP–MS (ThermoFisher Scientific). Residual ovary and muscle samples were split and shipped to TrichAnalytics, where one subsample was prepared and analyzed as just described, and to the SRC, where the other subsample was freeze-dried and total Se measured by HR ICP–MS. Detection limits for HR ICP–MS were 0.02 to 0.5 mg/kg dry weight Se in muscle and residual ovary. Detection limits for LA ICP–MS were 0.165 mg/kg dry weight Se in muscle, 0.32 mg/kg dry weight Se in eggs, and 0.2 to 1.5 mg/kg dry weight Se in residual ovary. Data quality was evaluated by analysis of laboratory duplicates by LA ICP–MS (13% egg samples; 25% ovary samples; 7% muscle samples) and field duplicates by HR ICP–MS (5% egg samples; 5% ovary samples). Both analytical methods used DORM-4 certified reference material.

RESULTS

Mature female redside shiner were 5.6 to 12.1 cm total length and 2.3 to 23 g wet weight (Supporting Information, Table S1). Mature females from the Loon Lake and Koocanusa Reservoir sites tended to be larger (average 10.2–16.6 g) compared with the smaller and higher elevation Elk River sites (average 4.6–5.9 g). Gonadosomatic indices were 5% to 26%, with median values in each area between 14% and 18%, consistent with previous observations of spawning redside shiner in the Columbia River, Washington (USA; ≥8%; Gray & Dauble, 2001). Mature females were 1 to 5 years old, a wider age range than previously reported (2–4 years; Gray & Dauble, 2001; Lindsey & Northcote, 1963). All mature females used in the present study had residual ovaries with underdeveloped eggs, consistent with previous observations that redside shiner is a fractional spawner (Weisel & Newman, 1951). No fish used in our study had visible external anomalies or deformities.

Selenium concentrations in Loon Lake fish ($n = 10$) were 0.71 mg/kg to 1.2 mg/kg dry weight in eggs, 1.5 mg/kg to 2.6 mg/kg dry weight in residual ovary, and 0.60 mg/kg to 0.90 mg/kg dry weight in muscle (Supporting Information, Table S2). Fish from mine-influenced areas had Se concentrations ranging from 2.5 mg/kg to 28 mg/kg dry weight in eggs, 5.0 mg/kg to 47 mg/kg dry weight in residual ovary, and 1.4 mg/kg to 23 mg/kg dry weight in muscle. Maternal transfer varied among areas, with relatively low egg:muscle ratios in Stanford Pond (0.9–1.8), Loon Lake (1.0–2.0), Elk River impoundment in Fernie (1.3–2.9), and Elk River wetland south of Fernie (1.9–2.1) and higher and more variable ratios in Koocanusa Reservoir at Gold Creek (1.9–4.0) and Englishman Creek (2.1–3.7; Figure 1). The mean muscle:diet concentration ratio across sites was 1.0 ± 0.33 (±SD), consistent with the ratio of 1.08 reported in Table 3.11 in USEPA (2021).

The two analytical methods showed good agreement of Se concentrations for split samples of dorsal muscle (OLS regression: $r^2 = 0.98$, slope = 1.05, $n = 56$; Supporting Information, Figure S1) and residual ovary (OLS regression: $r^2 = 0.98$, slope=1.05, $n = 56$; Supporting Information, Figure S2). For reported concentrations more than 10 times the method detection limit, relative percent difference between methods was on average 14% for muscle and 17% for ovary.

To the best of our knowledge, manual expression and fertilization of redside shiner gametes had not been conducted prior to our study. Therefore some method development between fertilization events was necessary. The initial four rounds of fertilization used a 10-min to 15-min dry-fertilization period and 10-min to 15-min wet-fertilization period, which resulted in a 40% mean fertilization rate. It was hypothesized that the small size of the eggs made them susceptible to drying, and that

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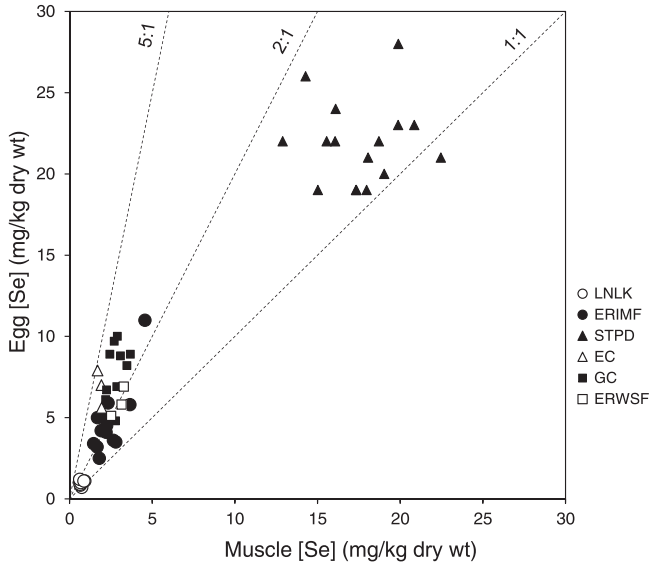

FIGURE 1: Selenium concentration ratios (values shown on dashed lines) between egg and muscle of redside shiner collected from Loon Lake (LNLK; ○), Koocanusa Reservoir at Englishman Creek (EC; △), Koocanusa Reservoir at Gold Creek (GC; ■), Elk River impoundment at Fernie (ERIMF; ●), Elk River wetland south of Fernie (ERWSF; □), and Stanford Pond (STPD; ▲).

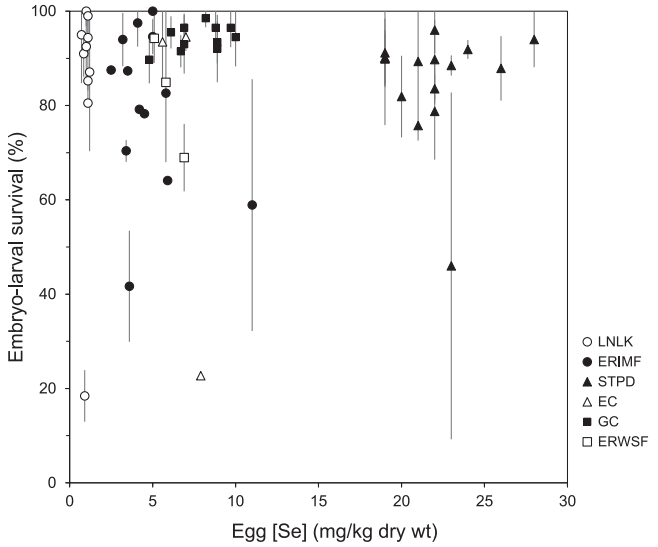

FIGURE 2: Cumulative survival (mean ± SD) from fertilized egg to swim-up larvae relative to egg selenium concentration in redside shiner collected from Loon Lake (LNLK; ○), Koocanusa Reservoir at Englishman Creek (EC; △), Koocanusa Reservoir at Gold Creek (GC; ■), Elk River impoundment at Fernie (ERIMF; ●), Elk River wetland south of Fernie (ERWSF; □), and Stanford Pond (STPD; ▲).

fertilization might be improved by a shorter dry-fertilization period. To test this hypothesis, eggs from one newly expressed female were separated into two groups: one group was dry-fertilized for approximately 2 min and the second group for approximately 15 min. The shorter dry-fertilization period resulted in 68% fertilization, whereas the longer period resulted in 25% fertilization. Based on these results, the dry fertilization period was reduced to 2 min, and the mean percentage of fertilization thereafter was 69 ± 19%.

Mean cumulative survival from fertilized egg to termination following yolk sac absorption was 81% to 100% for embryos from 9 of 10 females from the Loon Lake reference area and 18% for the remaining female (overall 84% ± 24%). The batch of eggs with low survival had 12% fertilization and yielded only 38 viable eggs, indicating that the eggs in that batch may have been of low quality or may have been damaged. The only other batch of eggs with low survival was from Koocanusa Reservoir, from a female that had been held overnight in a minnow trap. Eggs from that female were sticky and clumped and had a 5.7% fertilization rate. None of the other females were held overnight prior to gamete collection.

Cumulative survival was high across all mine-influenced areas (85% ± 16%). There was no concentration-dependent effect of egg Se concentration on embryo–larval survival across the entire dataset or when egg batches were evaluated separately before and after the refinement of fertilization methods (Figure 2). Neither OLS regression nor nonlinear regression was able to describe a concentration–response relationship between egg Se concentration and larval survival, growth, or the deformity endpoints discussed in the following paragraphs.

Mean larval length was 7.2 mm ± 0.4 mm (6.5 mm–7.9 mm) and was unrelated to egg Se concentration (Figure 3A). Mean larval dry weight was 0.35 mg ± 0.05 mg (0.22 mg–0.46 mg)

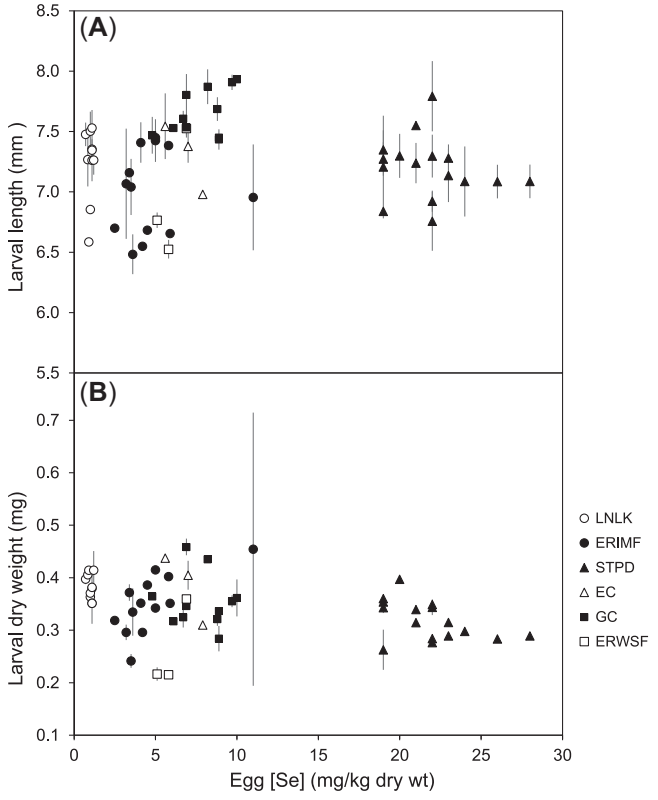

FIGURE 3: Larval length (**A**) and dry weight (**B**; mean ± SD) relative to egg selenium concentration in redside shiner collected from Loon Lake (LNLK; ○), Koocanusa Reservoir at Englishman Creek (EC; △), Koocanusa Reservoir at Gold Creek (GC; ■), Elk River impoundment at Fernie (ERIMF; ●), Elk River wetland south of Fernie (ERWSF; □), and Stanford Pond (STPD; ▲).

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and exhibited a weak but significant negative relationship with egg Se concentration (Figure 3B; OLS regression: $r^2 = 0.098$, $p = 0.011$). No nonlinear relationship could be fit by TRAP. The OLS regression was strongly influenced by the cluster of relatively large larvae from the reference lake (Loon Lake) compared with more variable weights at the other sampling areas, including those with only slightly higher Se exposures (Figure 3B; $p = 0.14$ with Loon Lake excluded). Loon Lake also had relatively large mature females, suggesting that the patterns of larval size might reflect a site effect (e.g., favorable growth conditions in the only sampled lake habitat) rather than Se toxicity.

Of 5991 assessed larvae, 296 had a GSI of ≥ 1 for deformity or edema (Supporting Information, Tables S3 and S4). Observed deformities included skeletal curvatures (scoliosis, lordosis, and kyphosis; 222 GSI of ≥ 1, 155 GSI of ≥ 2), fin deformities (56 GSI of ≥ 1, 33 GSI of ≥ 2), and craniofacial deformities (102 GSI of ≥ 1, 70 GSI of ≥ 2). Yolk sac and pericardial edema were also observed (165 GSI of ≥ 1, 107 GSI of ≥ 2). Excluding the batch of eggs from Loon Lake that had low survival and fertilization, from which all seven surviving eggs exhibited deformity, the mean prevalence of any deformity (GSI of ≥ 1) across all areas was 8.8% ± 14%. When only larvae with a moderate to severe deformity or multiple minor deformities were considered (GSI of ≥ 2), the mean prevalence was 6.3% ± 11%. Several batches of eggs from the Elk River impoundment in Fernie with relatively low egg Se concentrations (mean 3.9 mg/kg dry wt) exhibited >10% deformities, whereas the remaining batches from the Elk River impoundment in Fernie with slightly higher egg Se concentrations (mean 6.2 mg/kg dry wt) exhibited <10% deformities, suggesting an influence of some other teratogen or characteristic at this site. No relationship was observed between prevalence of deformity and egg Se concentration for any deformity type or total deformities, for any severity, or for moderate to severe deformities only (Figure 4).

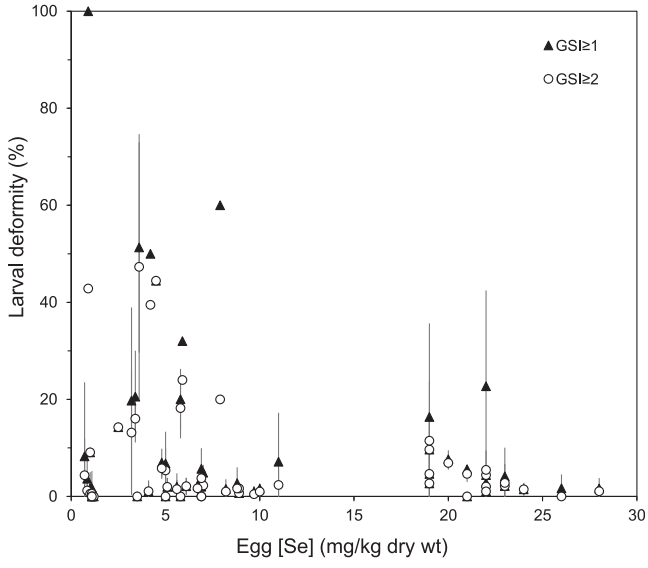

FIGURE 4: Frequency of any deformity (▲) and moderate to severe or multiple mild deformities (○) in redside shiner larvae relative to egg selenium concentration (mean ± SD). Abbreviation: GSI = graduated severity index.

DISCUSSION

We successfully spawned and reared redside shiners, providing the first documented investigation involving spawning and early life stage development with this cyprinid. The methods we developed produced sufficiently high rates of fertilization and survival for study purposes, particularly after we shortened the period of dry fertilization. Handling of adults did not produce excess mortality, and eggs and milt were successfully obtained and fertilized, transported to the laboratory by road and air travel, and reared in a laboratory. The larvae successfully transitioned to feeding on *Artemia* and did not appear to be susceptible to fungal growth.

Redside shiner could be a useful test species in environmental monitoring studies in which existing methods for species such as rainbow trout or fathead minnow do not provide suitable site specificity. For example, fathead minnows do not occur in British Columbia west of the Rocky Mountains, whereas redside shiner could provide a useful alternative that is widely present in coastal streams. Use of redside shiner would be complicated by the need for field collection and availability only during the spawning period. The relatively low frequency of ripe adults obtained in any sampling day also required that a significant number of fish be handled. However, sites like the Loon Lake reference area would be expected to provide access to a significant number of fish that can be readily collected.

Maternally transferred Se has been shown to cause embryotoxicity and larval deformity in fish, often with an effects threshold near 20 mg/kg dry weight in eggs (Deforest et al., 2012; USEPA, 2021). Because many previously studied species have egg:muscle or egg:diet ratios near 1 (Covington et al., 2018; Hamilton et al., 2005a, 2005b; Holm et al., 2005; McDonald et al., 2010) to 2 (Casey & Siwik, 2000; Linville, 2006; Muscatello et al., 2006), these egg-based effects thresholds translate into dietary effects thresholds on the order of 10 to 20 mg/kg dry weight. The present study evaluated the sensitivity to Se of a species with a reportedly higher maternal transfer ratio.

One notable finding of the present study was that the egg:muscle ratio in redside shiner varied among sites, ranging from <1 in some fish from the Elk River impoundment (in Fernie) to almost 5 in some fish from the Koocanusa Reservoir. Differences among sites in egg:muscle Se ratios (range = 1.3–6.8) were also recently observed in Arctic grayling (*Thymallus arcticus*), with fish collected from lotic environments having a significantly lower ratio than those from lentic environments (Brix et al., 2021). It was hypothesized that differences in habitat (lentic vs. lotic) and corresponding differences in diet and/or Se speciation might be driving differences in Se homeostasis. This seems unlikely for redside shiner (and potentially refutes the hypothesis for Arctic grayling as well), because both the lowest and highest egg:muscle ratios were for fish collected from lentic habitats. Similar to Arctic grayling, this variability also did not appear to be related to concentration (Figure 1).

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An alternative hypothesis for variability in egg:muscle ratios would be a temporal decoupling of Se deposition in muscle versus eggs. Selenium in eggs is the result of vitellogenic transfer from the liver, where vitellogenesis occurs. In synchronous spawners like salmonids, much of the protein utilized for vitellogenesis is thought to be derived from breakdown of muscle (Cleveland & Weber, 2011; Salem et al., 2010). In contrast, for asynchronous spawners that have a more extended, but less intense period of vitellogenesis, direct dietary protein intake plays a greater role. Experiments in fathead minnows (*Pimephales promelas*), an asynchronous spawner, evaluating the biokinetics of Se uptake in different tissues have demonstrated that the liver responds very quickly to dietary Se exposure compared with most other tissues, whereas muscle and adipose tissue are the least responsive (Kleinow & Brooks, 1986). Selenium depuration from liver is biphasic, with approximately 85% of accumulated Se cleared within 6 days followed by a slower depuration phase for remaining Se, whereas Se depuration from muscle is slower, with no rapid depuration phase and a half-life of 42 days (Kleinow & Brooks, 1986).

Consequently, egg Se in asynchronous spawners reflects short-term (days) dietary Se exposure several weeks to perhaps a month earlier depending on the timing of vitellogenesis relative to spawning, whereas muscle Se reflects the average long-term dietary exposure over several to many months prior to fish being sampled. Considering these dynamics, variation in egg:muscle ratios may be the result of variation across sites between the short-term dietary Se exposure immediately prior to vitellogenesis and the long-term average dietary Se exposure prior to fish collection. Potential sources of this variability across sites could include differences in seasonal dynamics of both waterborne Se concentrations and dietary composition relative to the time of vitellogenesis.

A second notable finding was that redside shiner are more tolerant of Se than most previously studied fish species. There was a weak relationship between larval weight and egg Se concentration, although this may reflect a difference in resource provisioning rather than Se toxicity. There was no adverse effect of Se on embryo survival, deformity, or larval length up to the highest tested egg Se concentration, indicating that the reproductive effects threshold for this species may be >28 mg/kg dry weight in eggs. The only species known to be more tolerant are Dolly Varden char (*Salvelinus malma*), for which McDonald et al. (2010) calculated a 10% effective concentration (EC10) of 54 mg/kg dry weight in eggs for larval deformity, brook trout (*Salvelinus fontinalis*), for which Holm et al. (2005) reported no effects on embryo survival or larval deformity up to the maximum tested concentration of approximately 50 mg/kg dry weight in eggs, and Arctic grayling, for which Brix et al. (2021) reported an EC10 of >34 mg/kg dry weight. All other species ($n = 10$) summarized by the USEPA (2021) have EC10 estimates lower than the no-effect concentration observed in the present study.

Our results support growing evidence that some fish species are relatively tolerant of maternally transferred Se, and may suggest a relationship between higher maternal transfer and lower sensitivity to egg Se. Of the species tested to date, the two with the highest egg:muscle ratios (Arctic grayling and redside shiner) are relatively insensitive to maternally transferred Se. Higher egg:muscle ratios were reported in USEPA (2021) for black bullhead (*Ameiurus melas*; median ratio = 6.8), channel catfish (*Ictalurus punctatus*; median ratio = 5.8), and mountain whitefish (*Prosopium williamsoni*; median ratio = 5.8); unfortunately, published Se toxicity data are unavailable for any of these species. Species with lower maternal transfer rates have variable sensitivity to Se: two of the lowest egg:muscle ratios reported in USEPA (2021) were for Dolly Varden char, which is the least sensitive species tested to date, and white sturgeon, which is the most sensitive. Although the dataset is still relatively small, it does suggest that Se risk for species with high egg:muscle ratios in particular should be assessed on a species-specific basis.

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Data Availability Statement—All data used in our study are provided in the Supporting Information.

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