EFFECT OF SELENIUM ON EARLY LIFE-STAGE DEVELOPMENT OF WESTSLOPE CUTTHROAT TROUT

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ABSTRACT

As part of on-going efforts by the Elk Valley Selenium Task Force to characterize benchmarks for selenium that are protective of environmental quality, the effect of egg selenium burden on early life-stage development of westslope cutthroat trout was investigated for fish from the Elk Valley, BC. The investigation was designed to provide data to derive a Tissue Residue Guideline for selenium for this species and region, and to resolve apparent differences in results from prior studies. Eggs collected from adult fish from lentic and lotic habitats were reared to 28-days beyond swim-up to establish whether habitat type resulted in differences in expression of selenium-related effects, including larval survival, rates of deformities and growth. Speciation of selenium in eggs from lentic and lotic environments was measured, and effects on study results assessed. The study demonstrated that larval mortality was the primary adverse effect of elevated selenium, with deformities generally limited to surviving fry from egg batches that also elicited significant mortalities. A sharp dose-response curve was obtained, with no evidence of differing sensitivities for eggs obtained from fish from lentic and lotic habitats. The EC10, EC20 and EC50 values for larval survival were 19.0, 22.8 and 29.9 µg/g Se (egg, dry weight). Speciation of selenium was shown to be similar in eggs from lentic and lotic sites, although analytical complications prevented an assessment of the relative concentrations of individual seleno-amino acids present.

Key Words: Selenium, cutthroat trout, tissue residue guideline, deformities

INTRODUCTION

Selenium toxicology has been an issue of considerable scientific discussion as a result of the complexity of the substance with respect to environmental speciation, as well as its unusual toxic mode of action. In the case of fish, toxicity of greatest interest is typically observed in the progeny of exposed organisms, rather than in exposed adults. Consequently, the risk of adverse effects is generally associated with a specific body burden in the adult female, resulting in accumulation of selenium in eggs and deformities and/or mortality in the developing offspring, due to mobilization of selenium during development. As a

result of this mechanism of effect, efforts to establish regulatory guidelines have typically focused on estimating a threshold concentration for selenium in the tissues of exposed fish.

Selenium can be taken up by organisms in both inorganic and organic forms; however, it is generally recognized that organo-selenium compounds, particularly seleno-amino acids, are the most toxic and readily accumulated. Selenium forms seleno-analogues of sulfur-containing amino acids, such as methionine and cysteine, by replacing sulfur. These seleno-amino acids are found predominantly in organs whose tissues are associated with protein synthesis and storage, such as muscle, liver and eggs.

Selenium is present in the Elk Valley in naturally-occurring seleniferous rock. Open pit coal mining has increased mobilization of selenium into the receiving environment. Consequently, there is considerable interest in the potential for adverse effects associated with selenium exposure to fish populations in the Elk Valley.

Two previous investigations have been conducted on westslope cutthroat trout collected from the Elk Valley river system in an attempt to identify threshold effect concentrations of selenium in the tissues of this species. One of these investigations was conducted using fish from lotic (i.e., flowing water) environments (Kennedy et al., 2000), and resulted in no relationship between selenium tissue concentrations in fish from the Fording River and deformities or survival of offspring. Eggs with selenium concentrations as high as 81.3 µg/g dry weight (dw) Se did not exhibit adverse effects on survival or deformity rate of juvenile fish hatched from those eggs, although mortalities were observed in offspring from a number of fish with intermediate concentrations of selenium. A follow-up study, commissioned by the Elk Valley Selenium Task Force (EVSTF), evaluated development of offspring of fish from Clode Settling Pond, a lentic (i.e., still water) exposure site on Teck Coal's Fording River Operations mine property, in which selenium body burdens were expected to be high (Rudolph et al. 2008). The results from this study appeared to contradict those reported by Kennedy et al. (2000). In particular, eggs with 46.8 µg/g dw Se or higher body burdens exhibited mass mortality of developing embryos. Another interesting result from the Rudolph et al. (2008) study was that eggs from four fish with very high selenium burdens (i.e., >86 µg/g dw Se) were entirely non-viable and died within hours of fertilization.

These two studies produced conflicting results and major questions remained regarding the potential toxicity of selenium to westslope cutthroat trout in the Elk Valley, including: (1) were differences in habitat (i.e., lentic versus lotic) in which the adult fish were residing responsible for the different results observed in the two studies?; and (2) what is the tissue concentration effects threshold for selenium for westslope cutthroat trout in the Elk Valley? The latter question remained unanswered by these two investigations, in part because of their differing results, but also because of the small sample size employed in both studies and data gaps in critical areas of the dose-response relationship.

The primary objective of the current study was to obtain empirical data to be used as a basis for establishing a site-specific effects threshold for westslope cutthroat trout on the basis of tissue selenium concentrations. The study was also designed to resolve whether differences in the results observed by Kennedy et al. (2000) and Rudolph et al. (2008) might relate to differential use of fish originating from

lotic and lentic habitats, respectively. It is not unreasonable to anticipate that conditions might differ in lentic and lotic environments as a result of differing biogeochemical cycles (Simmons and Wallschläger, 2005; Orr et al., 2006). Approaches used to assess the potential effects of habitat types included: rearing fry produced from non-overlapping populations of lentic and lotic adults that represented a range of selenium concentrations; and distinction and measurement of selenium speciation in the eggs to determine whether the forms/species of selenium accumulated in the eggs differed between habitat types.

METHODS

Fish from lentic environments were captured in East Clode Pond and as they moved from East Clode Pond up into the spawning area in Clode Creek (between East and Primary Clode Ponds); these sites are referred to collectively throughout this document as Clode Pond. The Clode Ponds were constructed as a treatment facility for water that was impacted by mining of Clode Pit, which commenced in 1971. A viable population of westslope cutthroat trout has utilized the ponds since installation. Fish were able to move freely between the Fording River and Clode Pond system until 2004, at which time gates were installed in the culverts draining the ponds. A significant relocation effort was undertaken at that time, involving capture and movement of approximately 6000 fish from the ponds into the Fording River.

Fish captured from lotic environments were caught predominantly in the Fording River (at the Multiplate Culvert) and two tributaries (Clode Settling Pond South Exfiltration [referred to here as Clode Pond Exfiltration] and Clode Settling Pond Discharge [referred to here as Clode Pond Outlet]), all of which are proximate to Fording River Operations. Fish from these locations do not have access to Clode Pond as a result of gates that prevent movement of fish into, or out of, Clode Pond. All fish collected from habitat that was defined as lotic had free access to the Fording River, and the vast majority of these fish were collected either in the Fording River, or in traps at the confluence of tributaries to the Fording River, as the fish moved up from the river into these tributaries to spawn.

Fish were primarily captured by angling and trapping, but dip netting and electro-shocking were opportunistically applied in a small number of cases. Fyke traps were utilized in areas where fish would be expected to be cruising along a shoreline and V-weir traps were established in tributaries, just upstream of their confluence with the Fording River, and in areas where the entire width of a stream could be barricaded so that fish moving upstream would have to enter the trap. Traps were inspected every one to three days and fish were then transported to the holding area, as described below.

Fish were tagged with individually numbered Floy tags, injected into the fish below the dorsal fin, and released into one of two holding areas in Clode Creek (one for fish from lentic and the other for fish from lotic habitats). The holding areas were each approximately 30m in length and provided natural habitat, including spawning gravels. Fences at the upstream and downstream ends prevented escape of the fish and camouflage netting provided overhead cover.

Fish were successfully spawned on five occasions between June 9 and July 1, 2008. The trout were retrieved from the holding area by seining, and ripe fish were anesthetized using clove oil. Fish were spawned by manual stripping inside a portable ice-fishing hut, which provided cover from weather. Milt

and eggs samples were collected into labeled Whirl-pack bags and hard-plastic Ziploc containers, respectively. Sample containers were filled with oxygen using a compressed oxygen cylinder, re-sealed, and placed into a cooler with ice-packs and bubble-wrap to prevent direct contact with the ice packs.

In addition to collection of fish from the Fording River system, eggs from four female fish were collected from Connor Lake by staff of the Kootenay Trout Hatchery as part of brood-stock collection efforts. Fish were collected using a weir-type trap and were held for one to two days before spawning. Eggs were collected by manual stripping on June 18, 2008 and transported by helicopter to the hatchery, where they were packaged with other samples collected that day from the Elk Valley. Eggs from this site were used as a methodological control, to ensure the procedures used in the laboratory were appropriate for supporting early life stage development of this species.

Coolers were sealed with tape and shipped by Air Canada Cargo or Pacific Coastal Airlines on the next available flight, and arrived at the Nautilus Environmental laboratory within approximately 12 - 16 hours of spawning. Upon arrival at the laboratory in Burnaby, BC, eggs were inspected and observations relating to gamete quality were made. Eggs were also observed under a dissecting microscope and the appearance of oil droplets on the periphery of the eggs recorded. Milt from each male was inspected under a microscope to determine sperm viability; a small amount of milt was mixed with saline solution and observed under 100 X magnification to determine the degree and duration of sperm motility.

Approximately 250 eggs from each female fish were transferred into plastic weighboats where they were fertilized with a drop of milt, and the eggs and milt were gently mixed. The weighboats were covered and left to fertilize for approximately 15 to 20 minutes, at which time water was added and the eggs allowed to water harden, prior to counting and transfer to the replicate test containers. Four replicate containers were used for each adult fish, each containing 60 eggs, unless insufficient eggs were available from a particular adult, in which case only 30 eggs were reared and/or replication was reduced to three containers.

In a few cases, breakage of all or a significant proportion of the eggs occurred immediately upon addition of water, as evidenced by a significant degree of milky and viscous material in the weighboats. In cases where this occurred, counting the eggs became difficult since the broken eggs tended to stick together. Nonetheless, approximately 60 eggs were transferred into a single test container using a plastic spoon and left until the following morning, to confirm that there were, indeed, no surviving eggs.

The fish were reared in the dark in 4-L food-grade plastic tubs in a walk-in cooler at 11 ± 1°C. Dim lighting was provided during water renewals using a single incandescent bulb. Water used for culturing of fish was municipal tap water that had been treated with activated carbon to remove chlorine and other residual contaminants. Approximately half of the water was renewed three times per week and the test chambers were provided with continuous gentle aeration to maintain dissolved oxygen levels close to saturation. The fish were observed daily and mortalities were removed and recorded.

In replicates in which there were at least 40 surviving fish at swim-up, half of the surviving fish were impartially separated and cultured for an additional 28 days, and the remainder were terminated for deformity assessment. All fish in an individual replicate were terminated for deformity assessment in cases where there were fewer than 40 surviving fry at swim-up.

During the extended 28-day rearing period, the fish were exposed to a 16:8 light:dark photoperiod and were fed daily with trout chow that had been ground into fine crumbs. Water was renewed and fecal material was siphoned from the bottom of the containers daily during this period. These fish were terminated following the 28-day period and evaluated for deformities.

Fish were sacrificed using clove oil and total lengths were measured to the nearest 0.5 mm. 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 by Holm et al. (2003) 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. The total GSI score was the sum of scores for each category. Thus, GSI scores could range from zero for a normal fry to twelve for fry with gross deformities in each category. Deformity analyses were conducted on all fish by a single individual who was blind to the identity of the fish with respect to capture location. In addition, a second observer also conducted deformity analyses on a subset (approximately 10%) of fish.

The data were analyzed on the basis of the proportion of fish in each replicate that exhibited deformities (i.e., fish that were scored a 1 or higher for any of the four categories, resulting in a GSI score of 1 or more). The data were also analyzed on the basis of the proportion of fish having gross or multiple deformities (resulting in an overall GSI score of 2 or more); deformities were considered to be "gross" deformities if they were scored a 2 or a 3. This latter analysis was conducted because the most difficult determination in conducting deformity assessment involves distinguishing minor deformities from phenotypic variation. In comparison, fry that were scored a 2 or a 3 had clearly defined deformities, likely to impinge on the survival or fitness of the fry. Multiple deformities were included in this category since it would be unlikely for an individual fry to be incorrectly scored with two minor deformities.

Total metals and metalloids were measured by Maxxam Analytics (Burnaby, BC) on samples of unfertilized eggs from all fish using Collision Reaction Cell Inductively Coupled Plasma Mass Spectrometry. In addition, speciation of selenium was measured in eggs from ten lentic and ten lotic adults using Ion Chromatography ICP-DRC-MS and Size Exclusion Chromatography ICP-DRC-MS; these analyses were conducted by Applied Speciation (Tukwila, WA).

The fish and associated offspring were identified throughout the study using the Floy tag number assigned to the adult fish following collection in order to ensure that analysts were blind to the collection locations of the fish until the testing was complete.

RESULTS AND DISCUSSION

Fish were caught before the peak of freshet (between May 12 and 22), and after the peak of freshet (between June 3 and July 1, 2008). Of the female fish used in the study, nine were captured pre-freshet (all from Clode Pond), and the remainder were captured after the peak of freshet. Of these fish, 37 were caught in fish weirs, 10 by angling, 6 in fyke traps, 2 by dip net, 3 by electrofishing, and 8 were "volunteers" that moved into the Clode Creek holding area from Clode Pond prior to, or during, construction of the holding pen.

Based on behavioural observations during holding, the fish appeared to be comfortable in the fish holding areas. Some spawning activity was noted during holding, including pairing, exploratory digging and apparent spawning. Thus, the fish appear to have been provided appropriate cues and environmental conditions to facilitate ripening of the gonads to spawning condition, with no overt indication of stress.

The adults were spawned on five occasions (i.e., June 9, 13, 18, 24 and July 1, 2008). The holding period for the fish between capture and spawning ranged from 0 to 47 days, and averaged 8.1 (± 12.3) days; 42 of 66 fish were successfully spawned within one week of capture. Characteristics of the female fish used in this study are summarized in Table 1.

Table 1. Selenium concentrations (µg/g dw) in westslope cutthroat trout eggs.

	Lentic		Lotic		
	Connor Lake	Clode Pond	Clode Pond Outlet	Clode Pond Exfiltration	Fording River (Multiplate)
Sample size	4	33	14	6	6
Mean Se	4.5	72.0	39.0	45.9	20.3
Standard deviation Se	1.0	22.5	29.3	45.6	23.1
Maximum Se	5.4	122.3	91.7	128.3	67.4
Minimum Se	3.2	3.3	5.3	9.5	9.3

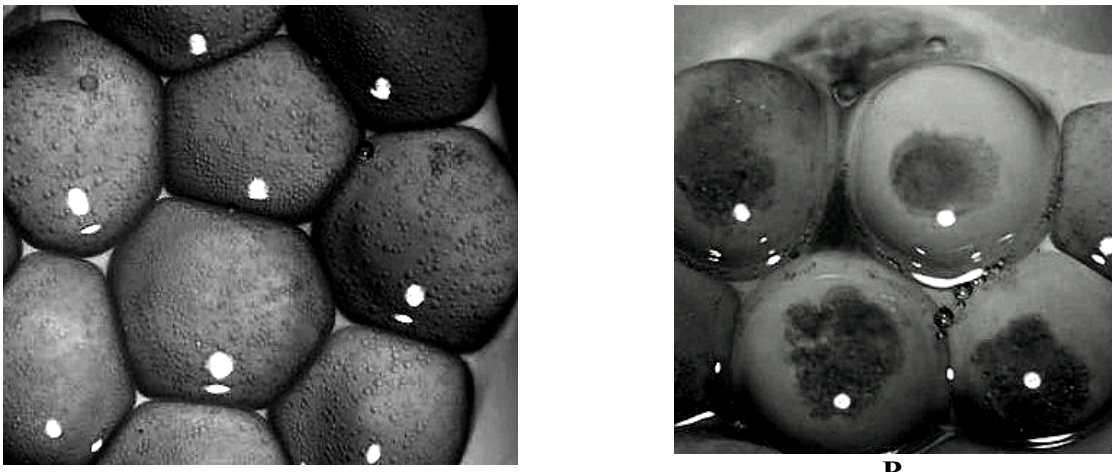
Collectively, egg selenium concentrations encompassed the range of interest. However, with the exception of one fish with surprisingly low selenium (3.3 µg/g dw), eggs from all Clode Pond fish all contained 39.2 µg/g dw Se, or higher. There were no data gaps in the overall dataset that exceeded 50% between adjacent concentrations, and only three data gaps of between 25 and 50% (between 5.7 and 9.3 µg/g dw Se; between 14.7 and 19.7 µg/g dw Se; and between 22.1 and 31.5 µg/g dw Se). This reflects a good distribution of concentrations in comparison to a typical laboratory toxicity test, which commonly use a 50% dose series to establish point estimates (i.e., where each concentration is a factor of two apart).

In general, the quality of gametes appeared to be good, with the exception of partial freezing of eggs from seven fish during shipping; data for these eggs were excluded from analysis. Milt from between two and five male fish was pooled and used for fertilization of the eggs. Milt that was used generally had excellent motility when observed under the microscope. Good fertilization success (>90%) was achieved

on all five test initiation dates in fish with low selenium concentrations, indicating that the collection, transportation and fertilization steps had been conducted appropriately on all occasions.

The appearance of unfertilized eggs varied considerably when observed through a dissecting microscope. In particular, the distribution of lipid vesicles in the eggs ranged from small and evenly distributed to completely coalesced. In a few cases, the amalgamated lipid droplet appeared to have broken into the cytoplasm of the egg. These differences are shown in the photographs provided in Figure 1, which shows examples of eggs in which the oil droplets were evenly distributed (scored 0%) and fully coalesced (scored 100%). In general, eggs from a single female appeared to be similar, and generally scored across no more than a 20 percentage-point range (e.g., 40 to 60% aggregated). However, in one case, eggs from a single female included eggs in which the lipid vesicles were evenly distributed, and others that were 100% aggregated. These eggs produced an unusual result with respect to proportion of surviving offspring, as described below.

Figure 1. Unfertilized eggs scored as (A) 0%, and (B) 100% aggregated lipid vesicles.



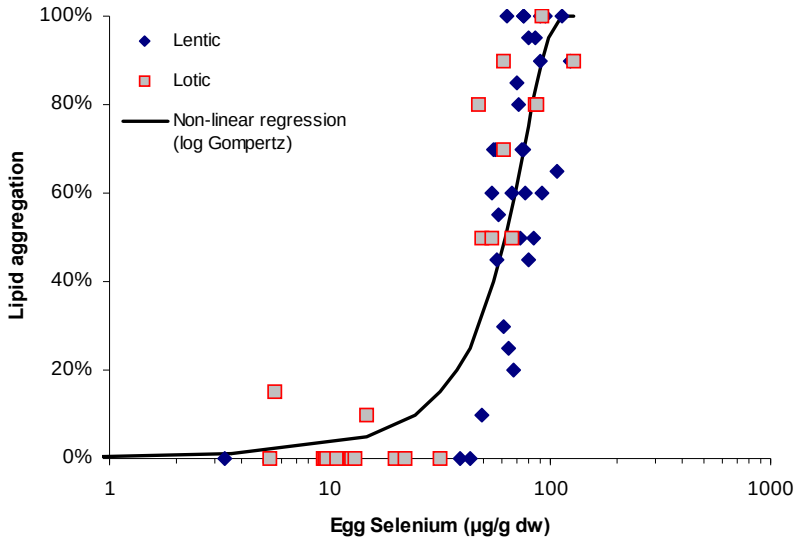
A **B**

The relationship between the distribution of the lipid vesicles in the eggs and measured concentration of selenium is illustrated in Figure 7; the regression line shown in the figure is a non-linear regression for lentic and lotic data combined, using a log-Gompertz model. In general, eggs containing less than 50 µg/g dw Se exhibited less than 25% aggregation of the lipid vesicles, whereas fish with higher selenium concentrations exhibited up to 100% aggregation. On the basis of a non-linear regression of the data, 20 and 50% aggregation of lipid was associated with 37.8 and 62.6 µg/g dw Se, respectively. Thus, it appears that selenium is either responsible for, or correlated with some factor that resulted in aggregation of the lipid vesicles in the eggs. This phenomenon has not been reported in the literature before.

Fifteen of the 21 samples that exceeded 75 µg/g dw Se displayed breakage of a large proportion of eggs immediately upon water-hardening, indicating that the integrity of the chorion was seriously

compromised. Complete mortality was confirmed in these eggs 24 hours after fertilization. These results are consistent with the findings of Rudolph et al. (2008), who observed that eggs from four fish from Clode Pond that exceeded 86.3 µg/g dw Se were non-viable, and died within 24 hours of fertilization.

Figure 2. **Relationship between egg selenium concentration and degree of aggregation of lipid vesicles in eggs prior to fertilization and water-hardening**.



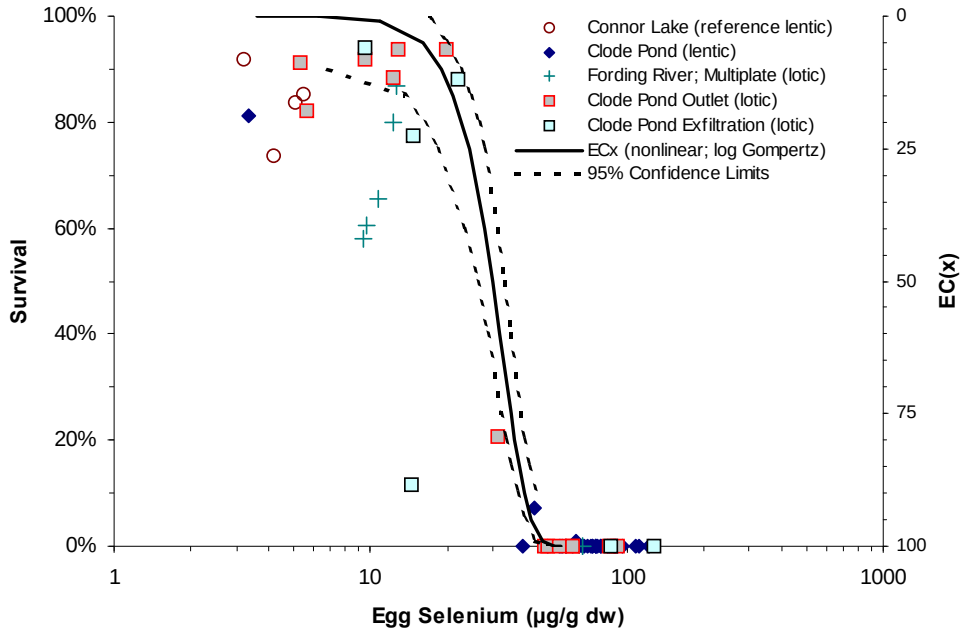
Interestingly, the lipid aggregation score (mean ± SD) of the samples that exceeded 75 µg/g dw Se and exhibited immediate mortality was 90 ± 13%, whereas the aggregation of lipids in the six samples that exceeded 75 µg/g dw Se, but did not show immediate breakage, was 63 ± 15%. Thus, it appears that chorion integrity is associated with the same factors that result in lipid aggregation in the eggs.

Mansour et al. (2007, 2008) reported a similar relationship between the pattern of lipid vesicles in eggs and successful development of subsequent life stages in other species of salmonids. In the context of their studies, the results were attributed to over-ripening of the eggs. Over-ripening appears unlikely here, because attempts were made to spawn the fish in the holding areas regularly during the spawning period.

Survival of the developing larvae at the point of swim-up is illustrated in Figure 3 as a function of selenium. Eggs from only five fish from lentic habitat exhibited good larval survival (>60% survival to swim-up); these larvae were from the eggs from four Connor Lake fish, and from one fish from Clode Pond that had an unusually low egg selenium concentration (3.3 µg/g dw). The remaining eggs from fish from lentic habitat (i.e., from Clode Pond) yielded virtually no surviving larvae. Eggs from fourteen fish from lotic sampling locations showed good larval survival, while those from the remaining twelve fish exhibited poor survival. A strong relationship between survival and egg selenium concentration was apparent, with a sharp dose-response separating concentrations associated with no adverse effects (up to 22.1 µg/g dw Se) from those resulting in significant adverse effects (> 31.5 µg/g dw Se).

There were no significant differences between the survival of offspring from lentic and lotic fish. This can be seen by inspection of Figure 3, in which there is little evidence of departure from the non-linear regression line for fish from lentic or lotic sites. Consequently, it is appropriate to combine the data into a single dataset; the combined data result in EC10, EC20 and EC50 estimates (with 95% confidence intervals) of 19.0 (6.8 – 22.7), 22.8 (16.3 – 26.6) and 29.9 (26.1 – 33.6) µg/g dw Se, respectively. The distribution of point estimates and corresponding 95% confidence intervals are illustrated in Figure 3.

Figure 3. **Survival of westslope cutthroat trout at swim-up stage as a function of egg selenium concentration. The distribution of point estimates (ECx values) with 95% confidence bands for all fish combined is also shown .**



Eggs from three fish with relatively low selenium concentrations (9.3 to 10.6 µg/g dw egg Se) yielded survival of between 58 and 66% at the point of swim-up, compared to 74 to 95% survival for the remainder the eggs with low selenium (i.e., <22 µg/g dw egg Se). These rates of survival are not unusual for an embryo-alevin test encompassing an exposure period of more than six weeks in the laboratory and likely reflects normal variability; in fact, acceptable control performance for a salmonid test encompassing this exposure period is 60% (Environment Canada, 1998).

Eggs containing up to 22 µg/g dw Se generally exhibited >60% survival to swim-up. However, one set of eggs diverged from this relationship with only 11.7% survival to swim-up in eggs containing 14.3 µg/g dw Se. Interestingly, this was the same batch of eggs that displayed an unusual distribution of lipid vesicles, with some eggs showing 100% aggregation and others being completely dissociated. A significant number of these eggs broke during water-hardening, resulting in more than 50% mortality in the first 24 hours of exposure.

A total of 2,100 swim-up fry were assessed for deformities: 537 fry from 7 adults collected from lentic environments (including four from Connor Lake); and 1563 fry from 19 adults from lotic environments. Eggs containing between 3.3 and 22.1 µg/g dw Se exhibited a low incidence of deformities. These fish averaged 6.5 ± 4.0% (range of 2.1 to 15.9%) of fry with a GSI score of 1 or more (i.e., having any deformity), and 3.4 ± 2.5% of fry (range of 0 – 9.6%) with a GSI score of 2 or more (i.e., having gross or multiple deformities). These results reflect a low overall rate of deformity and are similar to the results from the four fish originating from Connor Lake, which averaged 11.3 ± 4.8% and 2.7 ± 0.8% of fry with GSI scores of 1 or more and 2 or more, respectively. There was no relationship between selenium and rates of deformity or edema across the range of 3.3 to 22.1 µg/g dw Se (evaluated using linear regression).

Eggs from only two fish with selenium exceeding 30 µg/g dw produced surviving fry, and both resulted in a high rate of deformities. One of these samples, which contained 31.5 µg/g dw Se in eggs and had 21% survival at swim-up, exhibited a total GSI score of 1 or more in 64.7% of fry and a score of 2 or more in 41.1% of fry. All surviving fry from a sample with 43.5 µg/g dw Se in eggs exhibited gross deformities. There were no eggs evaluated that contained between 22.1 and 31.5 µg/g dw Se.

Sufficient surviving fry were available for rearing for 28 days beyond swim-up from five lentic fish (i.e., four from Connor Lake and one from Clode Pond) and nine lotic fish. Egg selenium concentrations associated with these fish ranged from 3.2 to 19.7 µg/g dw Se. Survival was high in all groups after 28 days; the average survival was 94.6%, and the range for all samples was 87.1 to 99.1% for the 28-day period. Thus, there was no evidence of significant delayed mortalities in the developing fry. The fry increased in weight by approximately two- to three-fold during the 28-day rearing period and there was no relationship between egg selenium concentration and length, weight, or growth rate of the fry. The incidence of deformities of these fry was very low; only 5 out of 369 fry (i.e., 1.4%) from Connor Lake, and 16 out of 866 fry (i.e., 1.8%) from exposure areas had deformities.

Selenium species identified in eggs included: selenite, selenocyanate, methylseleninic acid and selenomethionine. Selenite concentrations comprised an average of 11.7 ± 2.5%, selenocyanate comprised 2.4 ± 1.4%, methylseleninic acid comprised 0.3 ± 0.5%, and "free" selenomethionine comprised 0.8 ± 0.4% of the total selenium reported. In addition to these species, the majority of recovered selenium (58.2 ± 14.9%) was identified as "macromolecular" selenium, and appeared to be bound into proteins. Approximately 25 to 30% of total selenium was unaccounted for on the basis of the sum of measured species, likely as a result of inefficiencies in one of more of the extraction techniques used for measurement of the individual species. The results did not provide any indication that speciation differed in eggs from lentic versus lotic sites, although the analyses were unable to resolve the relative contribution of selenomethionine and other seleno-amino acids to the macromolecular, protein-bound selenium.

Total selenium measurements conducted by the two laboratories used in this study resulted in consistently differing results, with one laboratory (Applied Speciation) reporting an average of 36% more selenium in split samples. Effects endpoints calculated in this study were derived from selenium analyses conducted by Maxxam Analytics (since these results were available for all fish), and would have been higher if data from Applied Speciation had been used.

The results presented in this study provide strong evidence of a relationship between selenium concentration in eggs and adverse effects in developing embryos and larvae, including compromised egg chorion integrity, mortality and deformities. Selenium-related deformities only occurred in batches of larvae in which a significant rate of mortality was also evident, indicating that there is low risk of teratogenic effects at egg selenium concentrations below those associated with mortality in westslope cutthroat trout from the Elk Valley. The data reported here are consistent with those reported by Rudolph et al. (2008), and provides information to better define the shape of the dose-response curve for this species. Conversely, there was no evidence from the current study to support the findings of Kennedy et al. (2000), who reported a lack of effects on development of larvae to swim-up from eggs with approximately 60 and 80 µg/g dw Se collected from adult fish from lotic habitat in the Fording River. It is possible that the fish tested by Kennedy et al. (2000) differed from those in the current study with respect to speciation of selenium in the eggs, or some other factor. However, there was no evidence from the current study that fish collected from lentic and lotic habitats were different in their sensitivity to selenium.

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