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Relationship Between Embryo Selenium Concentration and Early Life Stage Development in White Sucker (*Catostomus commersoni*) from a Northern Canadian Lake

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The Cluff Lake uranium mine in northern Saskatchewan has been in operation since 1980. Over time, some contamination of downstream waterbodies has occurred (e.g., Island Lake). One contaminant of concern is selenium (Se). Selenium is known to cause teratogenic effects in fish at very low concentrations and dietary uptake is believed to be the most important exposure pathway (Lemly 1997, 2002; Hamilton 2002, 2003). It has also been proposed that selenium concentrations in fish eggs/embryos can be used to predict teratogenicity, since toxicity to early life stages seems to occur when alevins undergo yolk absorption (Lemly 1997). Lemly (1997), using established terata-mortality relationships for two fish families, Centrarchidae and Cyprinidae, developed a teratogenic deformity index and proposed that once selenium concentrations in fish eggs exceeded 10 μg/g dry weight the frequency of teratogenic deformities should increase rapidly. This approach has been evaluated by other researchers, with both supporting (Holm et al. 2003) and contradicting (Kennedy et al. 2000) evidence, and often with varied viewpoints (Chapman 1999; DeForest et al. 1999; Lemly 1999; Sappington 2002) being presented. Furthermore, the US EPA (2004) recently proposed a chronic water quality criterion for selenium of 7.91 μg/g dry weight based on whole-body tissue concentration. A whole-body concentration was chosen largely for practicality, although it was recognized that ovaries may be the best tissue to link selenium to reproductive effects.

Selenium concentrations in Island Lake, a 181-ha shallow lake (≤2.2 m) directly downstream of the Cluff Lake uranium mine, have been in the range of 1-11 μg/L for many years (unpublished company monitoring data), with more recent (1994 - 2000) levels reported in the 4-5 μg/L range (COGEMA Resources Inc. 2000). It was therefore decided that an assessment of possible selenium-induced teratogenic (malformations of the embryo) and developmental (abnormalities in the alevin and fry) effects in the early life stages of fish native to Island Lake would be conducted. Ideally, fish would also have been obtained from an uncontaminated reference lake (e.g. Cluff Lake) for comparison, but this could unfortunately not be accomplished. Only white sucker, *Catostomus commersoni*, a species common in the region, was captured in adequate numbers of both sexes to accomplish the assessment. Whether contaminants, including selenium, have reduced the abundance of other fish species in Island Lake is not known.

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The objective of the research described here was to evaluate the early life stage development of white sucker collected from Island Lake near the Cluff Lake uranium mine, a lake with both historical and present contamination from uranium mining activities, including contamination with selenium. Emphasis was placed on the assessment of teratogenic/developmental abnormalities in embryos reared in uncontaminated water, especially those abnormalities that could be associated with chronic parental exposure to selenium.

MATERIALS AND METHODS

A 50-m gill net (8.9 cm mesh size) was set multiple times in ~2 m deep water in the southern section of Island Lake, SK, Canada, on June 2, 2002. Sexually mature male and female white sucker (*C. commersoni*) ranging in size from 35 to 40 cm were caught in the net. A dry fertilization method (Environment Canada 1998), conducted on the shore of Island Lake, was used to maximize the fertilization rate. Eggs were stripped individually from four females, hereby considered replicate fish, by applying hand pressure to the abdomen and gently squeezing towards the vent (Rottmann et al. 1991). Eggs were deposited in an egg fertilization pan and the milt from two males was expressed onto the eggs. Milt and eggs were mixed for 2-3 min using a natural fibers brush. Water (~200-500 mL) from Cluff Lake was added to the eggs and milt, followed by constant stirring for 2-3 min. Water was then decanted, fresh water added, and eggs and milt stirred once again for 2-3 min. This procedure was repeated, if necessary, to remove residual fecal material or blood in the eggs/milt mixture and "water harden" the eggs.

After fertilization, eggs were gently poured onto a Nitex® screen, rinsed with a bentonite clay solution twice, and then rinsed with fresh Cluff Lake water twice. The bentonite solution was added to prevent egg clumping (Rottman et al. 1991) and was prepared by adding bentonite clay to water until a stable suspension formed. Eggs from the four females were kept separate and poured into separate 1-L flexible plastic containers and gently shaken every 5-10 min to minimize egg clumping. The containers with the eggs were transported to the environmental laboratory at the Cluff Lake Mine, placed on ice, and gently aerated. Containers were air transported on ice to the Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada, on June 3, 2002, approximately 12 h after fertilization was completed. The aerator lines were removed and containers sealed 2 h prior to transport; there was no head space during transport. Eggs remained unaerated for ~5 h.

Upon arrival at the Toxicology Centre, the temperature of the water in the egg containers was 9 °C. The containers were placed in a 10 °C water bath and the air temperature of the environmental chamber set at 12 ± 2 °C for the first 18 h, and then increased to 14 ± 2 °C for the remainder of the study.

On June 4, approximately 36 h after fertilization, eggs were placed in test chambers designed according to an Environment Canada test protocol for early life stage tests

with salmonid fish (Environment Canada 1998). Before transfer, eggs were placed in a betadine solution (Betadine Surgical Scrub, Purdue Pharma, Pickering, ON, Canada; final concentration of povione-iodine was 0.000075%) for 10-20 min to discourage fungal growth on the developing embryos (Alberta Government 2000). Following the iodine treatment, eggs were visually examined and only those that had clumped and adhering fecal/blood contamination were removed (Environment Canada 2000). Since the emphasis was on early life-stage development, not fertilization success, only eggs that appeared to have been fertilized and begun to demonstrate cleavage were used. These eggs appeared to be between the morula and blastula stage of embryo development (Kelso and Rutherford 1996). Two hundred embryos were randomly chosen from each batch collected from each of the four replicate fish, divided into groups of 100, and placed into individual test chambers (8 chambers in total). Test chambers contained ~2.5 L of Cluff Lake water and were aerated through 0.7-mm dia. plastic tubing. A separate subsample of embryos (5.2 ± 0.2 g wet weight; ~500 embryos) was collected from each replicate fish for Se analysis. Selenium analysis of embryos was conducted by Saskatchewan Research Council, Saskatoon, SK, Canada, using inductively coupled plasma-mass spectrometry, following pressurized microwave digestion of eggs in 8N nitric acid. Certified reference material samples (DORM-2, National Research Council, Ottawa, ON, Canada) were extracted and analyzed in duplicate as a quality control check. Results were within specified limits.

To reduce potential confounding effects from aqueous selenium exposure, fish embryos were hatched and reared in water from Cluff Lake, an uncontaminated lake near the Cluff Lake mine. This would ensure that any developmental effects that could be observed would be the result of parental exposure to contaminants, and not exposure during the early life stage development period evaluated in this experiment. Cluff Lake water was transported by air to the Toxicology Centre, University of Saskatchewan, in 20-L plastic containers once per week and stored at 4 °C until used. Prior to use, the water was vacuum filtered through 20-25 µm Whatman® filter papers (Whatman International Ltd., Maidstone, England), placed in the environmental chamber (14 ± 2°C), and aerated for at least 4 h. Water in each test chamber was changed every 3-4 d by siphoning old water out and replacing it with new Cluff Lake water. At the beginning of each water change, pH, dissolved oxygen (DO) concentration, temperature, alkalinity, total hardness, conductivity, and ammonia were measured. The DO, temperature, pH, and ammonia concentrations were also measured in all test chambers at the end of each water change. Table 1 contains a summary of these measurements. Only total ammonia showed a significant change between water renewals, but the mean level recorded at the end of water changes was still below Canadian water quality guidelines for the protection of aquatic life (0.715 mg/L at pH 8.0 and 15 °C; CCME 2000) and should therefore not have influenced embryo viability or development. Water temperature and DO concentrations were measured with an Orion Dissolved Oxygen Meter Model 835 (Orion Research, Beverly, MA, USA) and pH with an Orion PerpHecT LogR Meter Model 370. Water hardness and alkalinity were measured with a Hach Digital Titrator Model 16900

(Hach Company, Loveland, CO, USA). Ammonia was measured with an Orion Aquafast II Ammonia Photometer and conductivity was measured with an Orion Conductivity Meter Model 170 ATI.

Every 24 to 48 h, fish embryos were visually inspected for signs of development and fungal infections. Eggs that did not show signs of development (e.g. opaque), or that appeared to have experienced fungal infection, were removed from the test chambers to minimize any further potential for fungal infection of other embryos (Environment Canada 1998). When approximately 50 % of the embryos had hatched, all remaining embryos and larvae in each chamber were divided approximately in half to reduce loading stress and placed in clean chambers (16 chambers in total). Larval development continued to be monitored every 24-48 h until 33 d post-fertilization at which time yolk absorption appeared to be complete. Observations during this period focused on visual signs of health and deformities, as well as on general swimming and feeding behaviour. Dead larvae were removed as they were observed and, if possible, visually inspected for deformities. Fish embryos that appeared to develop deformities during hatching were identified as having embryological deformities, which were considered different from developmental deformities that only became evident as the alevins absorbed their yolk sacs.

Table 1. Physical and chemical characteristics (mean ± SD) of Cluff Lake water at the beginning and end of water changes over the course of the white sucker early life stage development study.

Characteristic	At beginning of water changes	At end of water changes
Temperature (°C)	15.3 ± 0.4	14.8 ± 0.5
DO (mg/L)	8.57 ± 0.28	8.39 ± 0.37
pH	8.10 ± 0.08	8.13 ± 0.09
Total ammonia (mg/L)	0.02 ± 0.03	0.35 ± 0.25
Hardness (mg/L) [1]	70.9 ± 4.8	Not measured
Alkalinity (mg/L) [1]	68.4 ± 3.0	Not measured
Conductivity (µS/cm)	157.7 ± 5.0	Not measured

[1] Reported in mg/L as $CaCO_3$.

Fish larvae started feeding ~4-5 d after 50 % hatching was observed. Each test chamber received 1 mL of newly hatched (≤36 h old) brine shrimp (*Artemia franciscana*) for the first week, and thereafter 1 mL of brine shrimp that were between 24 and 96 h old (cultured in Cluff Lake water). Fish were fed once daily for the first 4 d and twice daily thereafter.

On day 30 of the study (three days prior to test termination), all fish larvae that exhibited macroscopic deformities (i.e. kyphosis, lordosis, scoliosis and edema visible to the naked eye) were removed from the test chambers, photographed and preserved in Lillie's neutral buffered formalin (Lillie 1965). The study was terminated on day 33, 21 d after 50 % hatching was observed. On the final day of the test, 10 larvae from each test chamber (a total of 40 larvae from each replicate fish) were anaesthetized with MS-222 (tricaine methanesulphonate, Argent Chemical Laboratories, Redmond, WA, USA). The length and weight of each larvae were recorded, and observations for microscopic developmental deformities were conducted under a dissecting microscope at 5-9X magnification. Microscopic developmental deformities were similar in nature to macroscopic developmental deformities, but were not clearly visible to the naked eye.

RESULTS AND DISCUSSION

Embryos began hatching on day 10; by day 12 approximately 61 % of the embryos had hatched. Hatching was complete by day 15. At this time, 5.1 ± 2.5 % of the hatched larvae exhibited embryological deformities. It is not unusual to see up to ~5 % embryological deformities in newly hatched fish larvae unrelated to contaminant exposure (Lemly 1997). Between days 21 and 23 (11-13 d post-hatch), 1-7 larvae per chamber were found dead on the bottom of the chambers (it is thought that this period may have represented the transition between alevin and swim-up larvae). It was evident that some of these larvae were deformed prior to death, while others appeared to be curled up and twisted. It was not possible to discern if all deaths were related to developmental (post-hatch) deformities. It is possible that some fish may simply have curled up after death (i.e. conventional mortality).

By the end of the study (~21 d post-hatch), the mean number of macroscopic developmental deformities for the four replicates was 7.2 ± 3.3 % (ranging from 3.4 to 11.4 %; Table 2). The mean number of fish with microscopic deformities was estimated at 5.6 ± 2.4 % (9 out of 160 larvae); the majority of these larvae had slight curvatures of the spine (Table 2). This low percentage may not have led to a significant number of additional macroscopic deformities that could have influenced fish survival as the fry matured (Lemly 1997).

The average wet weight selenium concentration in embryos was 1.8 ± 1.3 µg/g and the average dry weight selenium concentration was 25.6 ± 19.9 µg/g (based on embryos from each of the four replicate females; measured moisture content 92.8 ± 0.4 %). These concentrations are not substantially different from those reported by Kennedy et al. (2000) for fertilized eggs from a wild population of Canadian cutthroat trout (*Oncorhynchus clarki lewisi*; 21.0 ± 18.3 µg/g dry weight) collected from a coal mining area. There was no correlation between selenium concentration in white sucker and frequency of developmental deformities, either macroscopic ($Y = 7.135 + 0.003X$; $r^2 < 0.001$; $p = 0.985$), microscopic ($Y = 2.960 + 0.107X$; $r^2 = 0.755$; $p = 0.131$), or combined ($Y = 10.095 + 0.110X$; $r^2 = 0.201$; $p = 0.551$). Using the

relationship between ovary and whole-body selenium concentration provided in US EPA (2004), the draft whole-body criterion concentration of 7.91 µg/g dry weight translates to an ovary concentration of 17.03 µg/g dry weight, a value only slightly exceeded in the white sucker eggs studied here.

Table 2. Summary of macroscopic and microscopic deformities of white sucker (*C. commersoni*) from Island Lake assessed 21 d post-hatch (end of study).

Measurement	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Mean ± SD
Successfully hatched larvae [1]	161	140	176	141	154.5 ± 17.3
Deformed larvae [2]	21	25	16	13	18.8 ± 5.3
Dead larvae [3]	6	14	6	4	7.5 ± 4.4
Macroscopic deformities (%):					
- Embryological [4]	6.8	6.4	5.7	1.4	5.1 ± 2.5
- Developmental [5]	6.2	11.4	3.4	7.8	7.2 ± 3.3
Microscopic deformities (%):					
- Developmental [6]	7.5	5.0	2.5	7.5	5.6 ± 2.4
Total developmental deformities (%) [7]	13.7	16.4	5.9	15.3	12.8 ± 4.7
Selenium concentration: [8]					
- Wet weight (µg/g)	2.7	0.7	0.6	3.2	1.8 ± 1.3
- Dry weight (µg/g)	33.6	9.4	8.4	48.3	25.6 ± 19.9

[1] The number of embryos that successfully hatched to larvae by June 15. This total was used to calculated percent deformities. The initial number of embryos per replicate (*n*) was 200.

[2] The total number of deformed larvae removed throughout the study; includes embryological and developmental macroscopic deformities.

[3] The total number of larvae that died throughout the study from unknown causes; larvae were usually found curled or twisted at the bottom of the container.

[4] The percent of curled deformities that appeared to be of a nature that is normal in embryonic fish; deformities were evident immediately after embryos hatched.

[5] The percent of deformities that were designated developmental in nature; these deformities became evident as the larvae grew and absorbed their yolk sac (after experimental day 15).

[6] The percent of microscopic developmental deformities that were evident in the 40 larvae examined per replicate.

[7] The estimated percentage of fish that had developmental deformities; macroscopic and microscopic combined.

[8] Selenium concentration measured in a subsample of embryos collected on day 0.

Since only eggs that appeared to have been successfully fertilized were used in this study (as recommended in Environment Canada 1998), it is not possible to determine if parental selenium exposure influenced fertilization success. However, according to Lemly (1997), elevated selenium concentrations in fish eggs will not affect larval hatching success, or the percent deformity at hatching (embryological deformities). Teratogenic effects are generally observed when larval fish begin to absorb their yolk sac and associated selenium, resulting in disruption of biochemical functions leading

to deformity (Lemly 2002). Once the yolk sac is absorbed and external feeding begins, the potential for tetratogenic effects decline and is soon lost (Lemly 1997). Selenium-induced teratogenic effects should therefore be most evident during the alevin stage. It is acknowledged that the lack of controls (i.e. fish embryos from an adjacent, uncontaminated lake) negates the interpretation of endpoints such as time to hatch and confounds a definitive determination of whether the observed embryological deaths and developmental deformities can be considered normal for this population of white sucker, and unrelated to contaminant exposure.

According to Lemly (1997), less than 5 % population mortality as a result of tetratogenesis indicates a negligible impact due to selenium exposure, whereas 5 to 20 % population mortality indicates that exposure to selenium had a slight to moderate impact. If the worst-case assumption is made that all larvae that exhibited developmental deformities eventually died and that those deaths were due to selenium, then 12.8 % observed deformities would translate to selenium having a slight to moderate effect on the white sucker population in Island Lake.

Lemly (1997) reported that the prevalence of tetratogenic deformities in fish increased rapidly once selenium concentrations in fish eggs exceeded 10 µg/g dry weight. Based on our measured selenium concentrations in embryos and Lemly's index, one would have anticipated a higher frequency of developmental deformities in white sucker from Island Lake. Of course, it is important to recognize the high degree of variability associated with the mean measured selenium concentration in this study, possibly a reflection of the small number of samples analyzed and minor differences in diet of the adult fish collected. Interestingly, Kennedy et al. (2000) found comparable selenium concentrations in the eggs of cutthroat trout (21.0 ± 18.3 µg/g d.w.) and also observed a lack of significant developmental effects, thus supporting these observations for white sucker. Hamilton et al. (2002) reported that razorback sucker (*Xyrauchen texanus*) eggs from two locations near Grand Junction, CO, USA, contained mean selenium concentrations of 40.1 and 54.7 µg/g dry weight, levels that based on Lemly's index should have resulted in much greater levels of deformity than observed. Unfortunately, other selenium data for white sucker do not exist for direct comparison with our results (Lemly's index was based on data for Centrachidae and Cyprinidae), and it is known that there are species-specific differences in selenium-teratogenesis relationships (Lemly 1985, 1997) which could account for at least part of the discrepancy with Lemly's index. Furthermore, it is possible that white sucker in Island Lake have developed some level of tolerance to selenium since this population has been exposed to selenium since the early 1980s. Although some alevin/larvae in this study exhibited developmental deformities with the absorption of their yolk sacs, the low percentage of deformities observed and the lack of correlation with embryo selenium concentration, indicate that selenium in Island Lake may be having at most a slight effect on the resident white sucker population.

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