# UPPER COLUMBIA RIVER

# Semi-Chronic Water Exposures of Cadmium, Copper, and Zinc to Early Life-Stages of White Sturgeon (*Acipenser transmontanus*)

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# 1 PURPOSE AND OBJECTIVES

The purpose of this study was to evaluate the sensitivity of early life-stages (ELS) of white sturgeon (*Acipenser transmontanus*) to water-borne cadmium, copper, and zinc. Specifically, this study was designed to provide data needed to answer the following questions:

1. What concentration of cadmium, copper and zinc are chronically toxic to ELS of white sturgeon?
2. Are ambient water quality criteria (AWQC) for cadmium, copper, and zinc protective of ELS of white sturgeon?

# 2 METHODS

## 2.1 TEST SPECIES AND SOURCE

Fertilized white sturgeon eggs were obtained from the Kootenay Trout Hatchery (KTH) located in Fort Steele, British Columbia on July 15, 2008. Hatchery staff collected eggs from four breeding pairs of adult white sturgeon captured in the Columbia River (CR) near Waneta, Canada (Ron Ek 2008, pers. comm.). Fertilization of the eggs was harmonized in the hatchery by injecting the sturgeon with a gonadotropin analog on two subsequent days. Embryos as collected and provided by the KTH were transported in oxygenated bags and received at the laboratory within 6 hours of fertilization. Embryos were acclimated to test waters for 1 hour, before being incubated in McDonald-type hatching jars (Aquatic Ecosystems, Apopka, FL). Hatching began on July 22, 2008 and ended on July 25, 2008; approximately 7 to 10 days post-fertilization.

## 2.2 TEST MATERIALS - STOCK SOLUTIONS

Stock solutions were prepared using copper (II) sulfate pentahydrate (Chemical Abstracts Service [CAS] number 7758-99-8; purity 99.995 percent), cadmium chloride hemi-pentahydrate (CAS number 7790-78-5; purity 99.999 percent), and zinc chloride (CAS number 7646-85-7; purity 98 percent). Chemicals were obtained from Sigma-Aldrich (Oakville, Ontario, Canada). All chemicals were dissolved in laboratory reverse osmosis (RO) water and allowed to equilibrate for approximately 72 hours prior to testing. Nominal exposure concentrations evaluated were as follows:

Cadmium = (0, 0.02, 0.16, 1.3, 10, and 82) μg/L (micrograms per liter)

Copper = (0, 0.1, 0.6, 3.6, 22, and 130) μg/L

Zinc = (0, 1.0, 6.0, 36, 216, and 1,296) μg/L

## 2.3 EXPOSURE METHODS

Chronic toxicity tests were conducted at the Aquatic Toxicology Research Facility, University of Saskatchewan (U of S), Saskatoon, Saskatchewan. The exposure design consisted of a continuous flow-through system, constructed to provide five serial dilutions for each test metal plus two laboratory controls. All exposures were tested under an illumination cycle of 16 light to 8 dark hours; and at a target temperature of 15 ± 1 degrees Celsius (°C).

Test solutions were adjusted to a water hardness of approximately of 65 to 70 milligrams per liter as calcium carbonate (mg/L as $CaCO_3$) by mixing the RO water with laboratory (i.e., dechlorinated, City of Saskatoon water) in a 1:1 ratio. Nominal concentrations were prepared in dedicated 1,000 L high-density polyethylene (HDPE) holding tanks which were then metered into two 80 L HDPE reservoirs. Each 80 L reservoir was furthered metered into two replicate 40 L HDPE exposure chambers at a rate of approximately 165 mL/min (milliliters per minute); resulting in a total of four replicate exposures per metal concentration. A summary of treatments, associated replicates and study parameters is presented in Tables 1 and 2.

At day 0 of the study (July 15, 2008), approximately 800 embryos were placed within dedicated McDonald-type hatching jars per replicate system until hatch. At the onset of hatch (July 22, 2008), embryos/larvae were placed into respective exposure chambers, 10 individuals at a time, to provide a random distribution. A summary of seeding densities (after correction for culling) per exposure chamber is presented within Table 3. Total test duration extended for 59 days post hatch (dph) and concluded on September 19, 2008.

Table 1. Experimental Design for Chronic Water Toxicity Studies with White Sturgeon Early Life-stages.

| Treatment Group | Water Source | Recirculating System | | Test Chambers | |
|---|---|---|---|---|---|
| | | No. | Volume (L) | No. | Volume (L) |
| Y1 & 2 | Filtered Saskatoon city water mixed 1:1 with RO water | 4 | 200 | 3 | 40 |
| B1 - 5 | Filtered Saskatoon city water mixed 1:1 with RO water spiked with appropriate Zn concentration | 2 | 160 | 2 | 40 |
| O1 - 5 | Filtered Saskatoon city water mixed 1:1 with RO water spiked with appropriate Cu concentration | 2 | 160 | 2 | 40 |
| P1 - 5 | Filtered Saskatoon city water mixed 1:1 with RO water spiked with appropriate Cd concentration | 2 | 160 | 2 | 40 |

**Notes:**
Cd – cadmium
Cu – copper
RO – reverse osmosis
Zn – zinc

Table 2. Study Parameters for Chronic Water Toxicity Studies with White Sturgeon Early Life-stages.

| Parameter | Units |
|---|---|
| Time of exposure initiation | ≤ 12 hours post hatch |
| Exposure duration | 59 days (> 40-day post swim up) |
| Loading density/rate | ≤ 0.1 g/L/24 hours |
| Number of true replicates per treatment/dose | 2 (laboratory studies) |
| Number of fish per treatment at end of study | ≥ 200 |
| Observations | ≥ 2 times per day |
| Feeding | ≥ 3 times per day |

Table 3. Number of Eggs and Seeding Densities (After Correction for Culling) in All Test Chambers.

| Chamber ID | # Eggs | # Fry | Chamber ID | # Eggs | # Fry | Chamber ID | # Eggs | # Fry |
|---|---|---|---|---|---|---|---|---|
| Y1A1 | 1200 | 239 | B4A1 | 800 | 258 | O5A1 | 800 | 260 |
| Y1A2 | | 217 | B4A2 | | 252 | O5A2 | | 281 |
| Y1A3 | | 225 | B4B1 | 800 | 164 | O5B1 | 800 | 239 |
| Y1B1 | 1200 | 232 | B4B2 | | 441 | O5B2 | | 262 |
| Y1B2 | | 238 | B5A1 | 800 | 287 | P1A1 | 800 | 296 |
| Y1B3 | | 235 | B5A2 | | 356 | P1A2 | | 293 |
| Y2A1 | 1200 | 217 | B5B1 | 800 | 309 | P1B1 | 800 | 272 |
| Y2A2 | | 233 | B5B2 | | 335 | P1B2 | | 280 |
| Y2A3 | | 215 | O1A1 | 800 | 256 | P2A1 | 800 | 331 |
| Y2B1 | 1200 | 238 | O1A2 | | 267 | P2A2 | | 224 |
| Y2B2 | | 246 | O1B1 | 800 | 296 | P2B1 | 800 | 265 |
| Y2B3 | | 227 | O1B2 | | 245 | P2B2 | | 290 |
| B1A1 | 800 | 267 | O2A1 | 800 | 266 | P3A1 | 800 | 271 |
| B1A2 | | 297 | O2A2 | | 259 | P3A2 | | 250 |
| B1B1 | 800 | 313 | O2B1 | 800 | 281 | P3B1 | 800 | 289 |
| B1B2 | | 289 | O2B2 | | 280 | P3B2 | | 273 |
| B2A1 | 800 | 280 | O3A1 | 800 | 258 | P4A1 | 800 | 286 |
| B2A2 | | 283 | O3A2 | | 291 | P4A2 | | 312 |
| B2B1 | 800 | 274 | O3B1 | 800 | 258 | P4B1 | 800 | 340 |
| B2B2 | | 284 | O3B2 | | 249 | P4B2 | | 346 |
| B3A1 | 800 | 276 | O4A1 | 800 | 318 | P5A1 | 800 | 381 |
| B3A2 | | 281 | O4A2 | | 333 | P5A2 | | 247 |
| B3B1 | 800 | 251 | O4B1 | 800 | 286 | P5B1 | 800 | 338 |
| B3B2 | | 262 | O4B2 | | 306 | P5B2 | | 294 |

At 7 dph (July 28, 2008), food (i.e., live brine shrimp [*Artemia salina)*] and frozen bloodworms), was introduced into test chambers 4-8 times throughout the day and into the evening; rates were increased when larvae were transitioning to feeding. Fish were allowed to feed *ad libitum.* At 38 dph, to ensure that fish density within test chambers were below 0.5 g/L as defined by the American Society for Testing and Materials (ASTM) guidelines (ASTM 2009), fish were randomly culled to a density of approximately 115 per exposure chamber. Culled fish were euthanized using tricaine methanesulfonate (MS-222), assigned a unique identifier, weighed, and measured.

Throughout the entire course of the study, exposure chambers were cleaned twice daily; any mortalities were removed, measured, weighed, and fixed in formalin. At the conclusion of the study, all fish were euthanized, assigned a unique identifier, weighed, and measured. In addition to the above-mentioned measures, behavior was also observed and recorded during times of cleaning and feeding.

## 2.4 WATER CHEMISTRY AND WATER QUALITY

Routine water quality parameters (i.e., temperature, pH, dissolved oxygen (DO), and conductivity) were recorded daily with symphony electrodes (VWR, Cat # 11388-328). Following the first two weeks of the study where bi-weekly measurements were initially made, weekly measures of hardness, alkalinity, ammonia, nitrate, nitrite, chlorine, sulfate, sulfide, and phosphate were recorded using LaMotte colorimetric and titrator test kits for the duration of the study within each exposure chamber.

In addition to the above-mentioned routine water quality measurements, weekly water samples were collected from each exposure chamber and analyzed for dissolved metals (i.e., Target Analyte List [TAL] metals at the initiation of the study and test analytes [cadmium, copper, and zinc] weekly thereafter) and major cations/anions (calcium, magnesium, potassium, sodium, nitrate, sulfate, chloride, fluoride, bromide). All samples were collected using acid-cleaned polyethylene bottles, filtered through a 0.45 μm polycarbonate filter with Nalgene® filter holders and receivers as required (e.g., dissolved metals); acidified with ultrapure nitric acid to a pH <2 standard units (s.u.), and maintained at approximately 4°C for shipment to the analytical laboratory (Liber Laboratory, Toxicology Centre, U of S).

Dissolved TAL metal analyses were performed using inductively coupled plasma mass spectrometry (ICP-MS) following U.S. Environmental Protection Agency (EPA) Method ILM05.2D (Creed et al. 1994); while major cations and anions were analyzed using EPA Method 300.1 by Dionex ICS-3000 Dual Ion Chromatography (Dionex, Sunnyvale, California, USA). Dissolved organic carbon (DOC) analysis was performed weekly using a total organic carbon (TOC) analyzer (TOC-5050A, Shimadzu, Mandel Scientific, Guelph, Ontario). A summary of the samples collected, blanks, analytical methods and associated method detection limits is presented within Tables 4 through 6.

Table 4. Summary of Analytical Methods and Associated Method Detection Limits for Water Quality Parameters

| Parameter | Dates | Method | Unit | LOD |
|---|---|---|---|---|
| Temperature | 6/18/2008 through 9/19/2008 | VWR Symphony 14002-860 | ˚C | 0 |
| pH | 6/18/2008 through 9/19/2008 | VWR Symphony 14002-860 | s.u. | 0 |
| DO | 6/18/2008 through 9/19/2008 | VWR Symphony 11388-374 | mg/L | 2 |
| Conductivity | 6/18/2008 through 9/19/2008 | VWR Symphony 11388-372 | µS/cm | 1 |
| Ammonia-Nitrogen | 6/18/2008 through 9/19/2008 | LaMotte Kit 3304 | mg/L | 0.02 |
| Nitrate | 6/18/2008 through 9/19/2008 | LaMotte Kit 3319 | mg/L | 0.25 |
| Nitrite | 6/18/2008 through 9/19/2008 | LaMotte Kit 7674 | mg/L | 0.02 |
| Hardness | 6/18/2008 through 9/19/2008 | LaMotte Kit 4824-DR-LT | mg/L | 20 |
| Alkalinity | 6/18/2008 through 9/19/2008 | LaMotte Kit 4419-DR | mg/L | 20 |
| Sulfate | 6/18/2008 through 9/19/2008 | LaMotte Kit 7778 | mg/L | 20 |
| Phosphate | 6/18/2008 through 9/19/2008 | LaMotte Kit 7416-01 | mg/L | 0.05 |
| Total Chlorine | 6/18/2008 through 9/19/2008 | LaMotte Kit 6905 | mg/L | 0.2 |
| DOC | 7/24/2008 through 9/16/2008[a] | EPA 415.4 | mg/L | 0.01 |
| TOC | 7/24/2008 through 9/16/2008[a] | EPA 415.3 | mg/L | 0.01 |
| DOC | 8/4/2008 | EPA 415.4 | mg/L | 0.7 |
| TOC | 8/4/2008 | EPA 415.3 | mg/L | 0.7 |

**Notes:**
Measurements conducted in one randomly selected test chamber within each treatment group
DO – dissolved oxygen
DOC – dissolved organic carbon
LOD - limit of detection
TOC – total organic carbon
[a] Except 08/04/08

Table 5. Summary of Limits of Detection (LOD) and Method Blank (MB) Concentrations (µg/L) for Target Analyte List and Test Analyte Metals (Cu, Zn and Cd)

| Dissolved Metal | Date Analyzed | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 6/30/2008 | | 7/15/2008 | | 7/22/2008 | | 9/3/2008 | | 10/9/2008 | | 10/21/2008 | | 11/5/2008 | | 11/7/2008 | |
| | LOD | MB | LOD | MB | LOD | MB | LOD | MB | LOD | MB | LOD | MB | LOD | MB | LOD | MB |
| Be | 1.5388 | <LOD | | | | | | | | | | | | | | |
| B | 0.000 | 0.150 | | | | | | | | | | | | | | |
| Al | 0.5961 | <LOD | | | | | | | | | | | | | | |
| Ti | 0.000 | <LOD | | | | | | | | | | | | | | |
| V | 0.0994 | <LOD | | | | | | | | | | | | | | |
| Cr | 0.0154 | <LOD | | | | | | | | | | | | | | |
| Mn | 0.0169 | <LOD | | | | | | | | | | | | | | |
| Fe | 0.189 | <LOD | | | | | | | | | | | | | | |
| Co | 0.0054 | <LOD | | | | | | | | | | | | | | |
| Ni | 0.0481 | <LOD | | | | | | | | | | | | | | |
| Cu | 0.0533 | <LOD | 0.0397 | 0.084 | 0.0217 | <LOD | 0.0682 | <LOD | 0.1222 | <LOD | 0.0737 | <LOD | 0.0239 | <LOD | 0.0193 | <LOD |
| Zn | 0.2756 | <LOD | 0.15 | <LOD | 0.1501 | 0.136 | 0.1592 | 0.743 | 0.0924 | 0.142 | 0.2583 | <LOD | 0.0699 | <LOD | 0.0419 | 0.199 |
| As | 0.1237 | <LOD | | | | | | | | | | | | | | |
| Se | 6.577 | <LOD | | | | | | | | | | | | | | |
| Sr | 0.0118 | 0.852 | | | | | | | | | | | | | | |
| Mo | 0.0105 | <LOD | | | | | | | | | | | | | | |
| Ag | 0.0254 | <LOD | | | | | | | | | | | | | | |
| Cd | 0.0092 | 0.072 | 0.0055 | <LOD | 0.0034 | <LOD | 0.0025 | 0.005 | 0.0034 | 0.094 | 0.0026 | <LOD | 0.0016 | 0.008 | 0.0009 | 0.005 |
| Sn | 0.1401 | <LOD | | | | | | | | | | | | | | |
| Sb | 0.0056 | 0.016 | | | | | | | | | | | | | | |
| Ba | 0.0301 | <LOD | | | | | | | | | | | | | | |
| Hg | 0.0259 | <LOD | | | | | | | | | | | | | | |
| Tl | 0.0026 | 0.005 | | | | | | | | | | | | | | |
| Pb | 0.0034 | 0.007 | | | | | | | | | | | | | | |
| U | 0.000 | 0.007 | | | | | | | | | | | | | | |

**Note:**
Method = EPA ILM05.2D

Table 6. Summary of Dissolved Organic Carbon (DOC) and Total Organic Carbon (TOC) Blanks and Spiked Samples.

| Sample Type | Parameter | Date Analyzed | Method | Value (mg/L) |
|---|---|---|---|---|
| RO Water | DOC | 7/30/2008 | EPA 415.3 | 0.22 |
| RO Water | TOC | 7/30/2008 | EPA 415.3 | 0.22 |
| Tap Water | DOC | 7/30/2008 | EPA 415.3 | 2.98 |
| Tap Water | TOC | 7/30/2008 | EPA 415.3 | 3.04 |
| Spiked 2 | DOC/TOC | 8/4/2008 | EPA 415.3 | 1.99 |
| Spiked 4 | DOC/TOC | 9/9/2008; 9/16/2008 | EPA 415.4 | 4.46-4.69 |
| Spiked 6 | DOC/TOC | 8/17/2008-9/9/2008 | EPA 415.5 | 5.57-6.77 |
| Spiked 7 | DOC/TOC | 8/17/2008 | EPA 415.6 | 6.72 |

**Notes:**
RO – reverse osmosis
Tap Water - UofS laboratory water
Spiked 2 - blank spiked with 2 mg /L organic carbon
Spiked 4 - blank spiked with 4 mg /L organic carbon
Spiked 6 - blank spiked with 6 mg /L organic carbon
Spiked 7 - blank spiked with 7 mg /L organic carbon

## 2.5 DATA ANALYSIS AND STATISTICS

Differences in percent mortality among treatment groups was analyzed by comparing the proportion of dead fish in each of the four exposure chambers of a given metal concentration to that of the controls at four different life-stages; yolk-sac larvae, swim-up, transition to feeding and juvenile. Percent mortality was adjusted for fish that were removed during culling. All data were tested for normality using a Shapiro Wilk's test and were found to be normally distributed or approximated a normal distribution. Therefore, analysis of variance (ANOVA) and a Bonferroni post hoc test were used to detect significant differences between treatment and control groups. Statistical significance was accepted when $p < 0.05$.

Concentrations at which 50 percent and 20 percent mortalities occurred (LC50 and LC20, respectively) were calculated either using a PROBIT model or a log-linear regression model, as appropriate. Prior to these calculations, data were further normalized by adjusting for initial seeding density (i.e., number of fish put into the test chambers at the start of the study). Based on work being completed by Tompsett et. al. (in preparation) it appears that seeding density adversely influences fish survival during the critical transition to feeding life stage. The linear model shown in Figure 1 was used to adjust the overall mortalities to account for mortality that resulted from seeding density. For each exposure chamber the number of individual fish that died due to seeding density was calculated:

$$N_{SD} = 0.9138 \times SD - 68.97 \qquad \text{Eq. (1)}$$

$$P_{SD} = 100 \times \frac{(N_M - N_{SD})}{(SD - N_{SD})} \qquad \text{Eq. (2)}$$

Where:

$N_{SD}$ = Estimated number of mortalities expected based on the original seeding density

SD = Original seeding density

$P_{SD}$ = Percent mortality adjusted for seeding density

$N_M$ = Observed number of dead fish

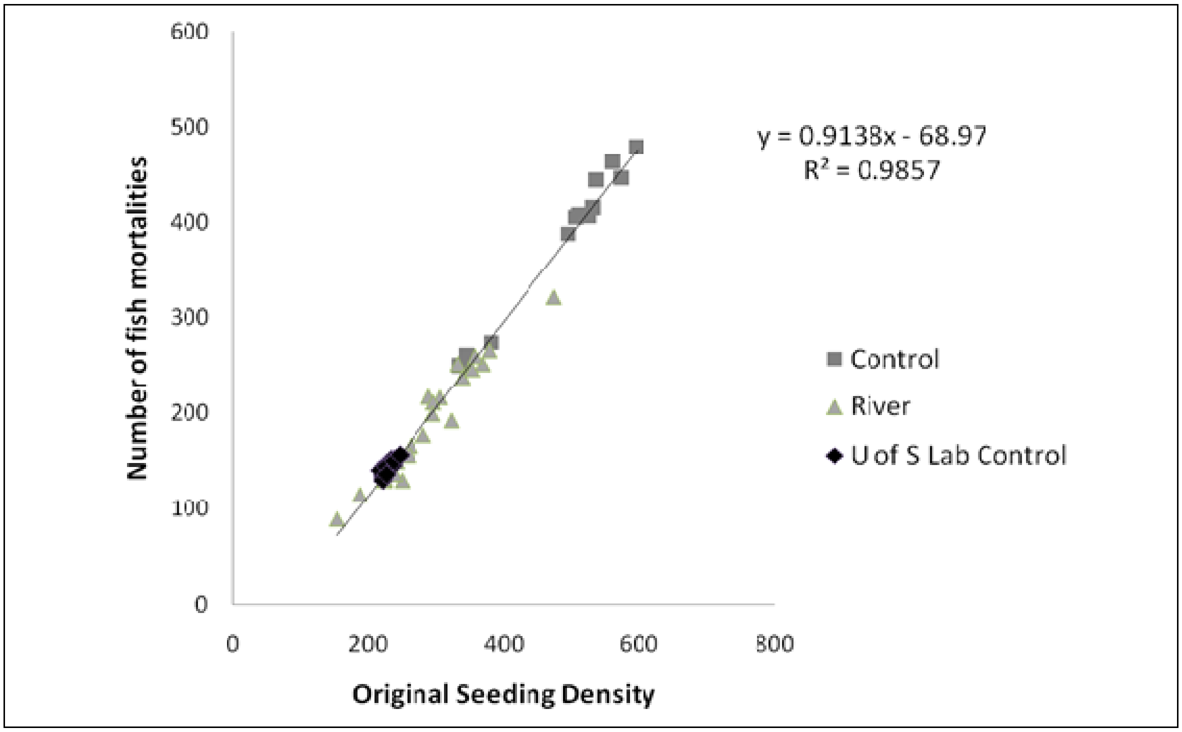


Figure 1. Linear Regression Model as Developed by Tompsett et. al. (in preparation) Applied to Laboratory Controls for Present Study.

The adjustment as described in equations 1 and 2 accounts for a significant portion of the observed mortality, as can be seen in the dose-response relationships with and without adjustment (Figure 2). After considering mortality associated with seeding density, the adjusted dose-response relationships for all three metals have control mortality below 10 percent. Additional adjustment to normalize for control mortality using Abbott's formula was also examined (Figure 2). It was apparent that this additional adjustment is small relative to the seeding density adjustment and has little or no affect on the estimated LC20 and LC50 values. Therefore, normalization to control mortality using Abbott's formula was not used in the data analysis.

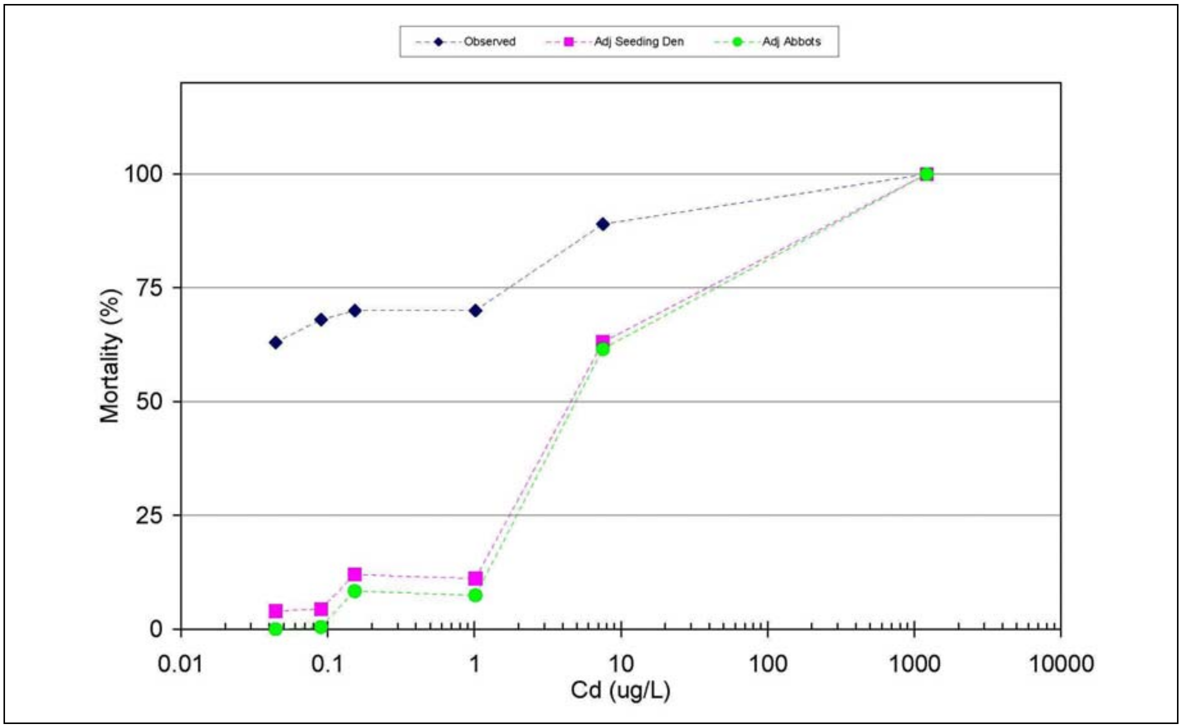


Figure 2, Panel A: Dose Response Relationships for Cadmium to ELS Sturgeon at 60 dph Showing Percent Mortality as Originally Observed in the Chronic Tests (Blue), After Adjustment to Account for Mortality Associated with Seeding Density (Pink), and After Adjustment by Abbott's Formula to Normalize to Control Mortality.

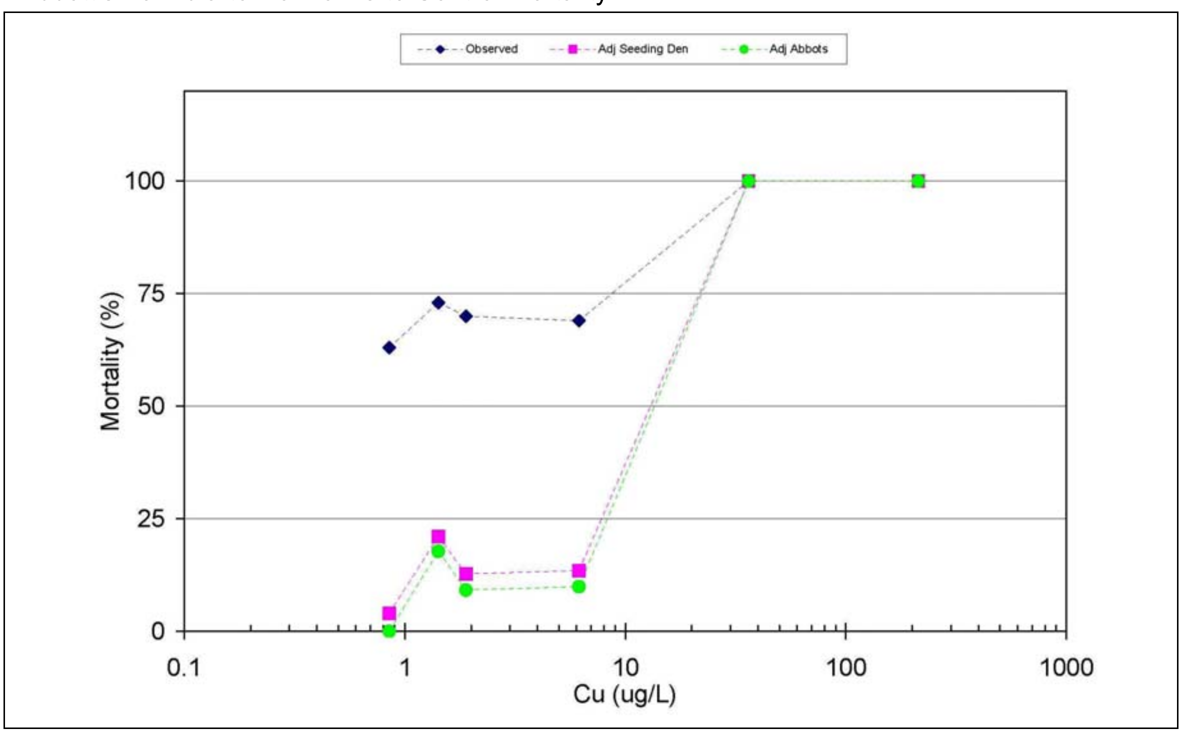


Figure 2, Panel B: Dose Response Relationships for Copper to ELS Sturgeon at 60 dph Showing Percent Mortality as Originally Observed in the Chronic Tests (Blue), After Adjustment to Account for Mortality Associated with Seeding Density (Pink), and After Adjustment by Abbott's Formula to Normalize to Control Mortality.

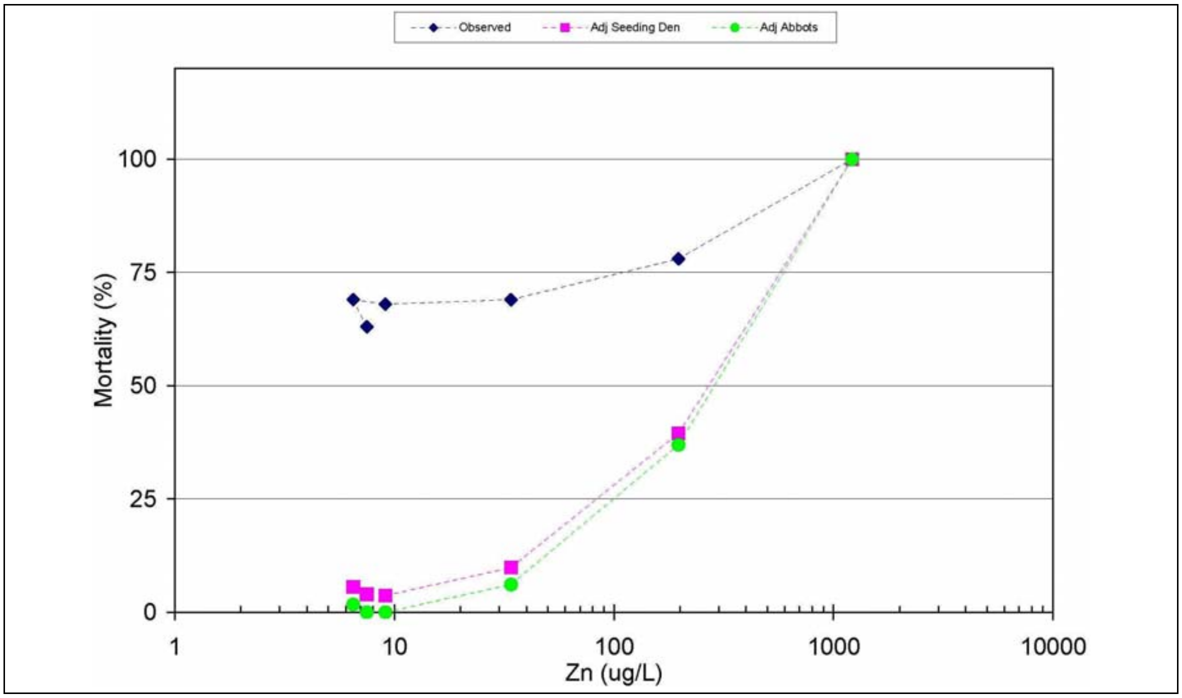


Figure 2, Panel C. Dose Response Relationships for Zinc to ELS Sturgeon at 60 dph Showing Percent Mortality as Originally Observed in the Chronic Tests (Blue), After Adjustment to Account for Mortality Associated with Seeding Density (Pink), and After Adjustment by Abbott's Formula to Normalize to Control Mortality.

In addition to the above-mentioned, fish condition was evaluated using Fulton's Condition Index (K) as follows:

$$K = \frac{W}{L^3} \times SF \qquad \text{Eq. (3)}$$

Where:

W = weight of fish (g)

L = Total length of fish (mm)

SF = Scaling factor @ 100,000

Statistical software packages used for these analyses were Systat (Systat Software Inc., Chicago, Illinois, USA) or SPSS (SPSS Inc., Chicago, Illinois, USA).

# 3 RESULTS AND DISCUSSION

Results for endpoints described above are summarized below.

## 3.1 EXPOSURE VERIFICATION

Measured concentrations of all target metals were comparable to nominal concentrations. As indicated by the data (Table 7), trace levels of copper, cadmium, and zinc (bracketing the lowest dose of each treatment) were recorded in the controls. As indicated within Table 7, actual exposure concentrations were typically similar to nominal concentrations. Concentrations within exposure chambers remained consistent throughout the exposure period. Multi-element analyses for non-target metals were within acceptable ranges. All calculations and reported values pertaining to metal concentrations are based on measured concentrations, unless otherwise stated.

Table 7. Nominal and Measured (Mean ± SD) Exposure Concentrations of Test Analyte Metals

| Treatment | Copper (µg/L) | | Cadmium (µg/L) | | Zinc (µg/L) | |
|---|---|---|---|---|---|---|
| | Nominal | Measured | Nominal | Measured | Nominal | Measured |
| Control (0) | 0 | 0.9 (± 1.1) | 0 | 0.04 (± 0.05) | 0 | 7.5 (± 2.9) |
| 1 | 0.1 | 1.4 (± 2.2) | 0.02 | 0.09 (± 0.13) | 1 | 6.5 (± 1.4) |
| 2 | 0.6 | 1.9 (± 1.1) | 0.16 | 0.15 (± 0.06) | 6 | 9.1 (± 3.2) |
| 3 | 3.6 | 6.2 (± 0.7) | 1.3 | 1.0 (± 0.11) | 36 | 34 (± 4.0) |
| 4 | 22 | 36 (± 2.4) | 10 | 7.5 (± 2.4) | 216 | 197 (± 7.4) |
| 5 | 130 | 213 (± 38) | 82 | 68 (± 4.9) | 1296 | 1216 (± 28) |

## 3.2 WATER QUALITY

Average water temperature over the 66-day exposure period for all treatment groups was 15.4 °C (± 0.53). Average dissolved oxygen concentration, pH, and conductivity for all treatment groups were 8.90 milligrams per liter (mg/L) (± 0.88), 7.91 s.u. (± 0.24) and 222 microSiemens per centimeter (µS/cm) (± 40.3), respectively. Average hardness was 70 mg/L as calcium carbonate (± 9.75), and the average total ammonia nitrogen concentration for all treatment groups was 0.04 mg as nitrogen per liter (± 0.03). Mean DOC was 3.0 mg/L (± 0.5). Nitrate and nitrite concentrations averaged 1.8 (± 0.85) and 0.04 (± 0.03) mg/L, respectively, across the entire study period.

## 3.3 HATCHABILITY

Eggs hatched 7 to 10 days after transfer to hatching jars and the start of exposure. Hatching success was greater than 70 percent for all treatments. No significant differences in hatching success were seen among treatment groups or between treatments and controls. All exposure chambers, including controls, were observed to contain fish (i.e., 5 to 10 individuals) with curved spines; an axial defect characterized by a downward curve around the yolk sac. This deformity was randomly distributed and could be due to transportation from the hatchery to the laboratory. Any and all such 'deformed' fish were identified, removed, and euthanized.

## 3.4 BEHAVIOR

There were no noticeable changes in behavior among the control group, copper group, or zinc group fish during the exposure period. Most fish exposed to 8.3 μg Cd/L, the greatest cadmium exposure with surviving fish at termination, appeared lethargic and to eat less throughout the exposure.

## 3.5 MORTALITY AND DOSE RESPONSE RELATIONSHIPS

For all metals, there were concentration-dependent and statistically significant increases in mortalities at the highest two or three concentrations tested. One hundred percent mortality occurred in the highest treatment groups of cadmium (68 μg/L) and zinc (1216 μg/L), and in the two highest treatment groups of copper (36 and 213 μg/L). Exposure to 7.5 μg/L cadmium and 197 μg/L zinc caused 91 percent and 78 percent mortality, respectively. Results from one of the exposure chambers in the 7.5 μg/L cadmium treatment were excluded due to loss of fish through equipment failure. Exclusion of this replicate chamber changed the mortalities observed in this treatment group from 91 to 89 percent. Significant ($p < 0.001$) mortalities relative to the controls were seen in the two highest exposure groups for copper (36 and 213 μg/L) and cadmium (7.5 and 68 μg/L). All zinc exposure groups produced statistically significant increases in mortality, although the overall difference among the three lowest concentrations and the controls did not exceed 7 percent. A summary of the overall percent mortalities observed during the test are presented in Table 8.

As indicated within Table 8, controls experienced a relatively high mortality with an average of 63 percent. Consistent with work by others (Bennett and Farrell 1998; Mohler et al. 2000; Tompsett et. al. in preparation), it would appear that relatively high mortality is not uncommon in sturgeon culturing, in large part due to a sensitive period when the fish are transitioning from yolk sac to exogenous feeding. An evaluation of mortality rate in the control group over the course of the experiment identifies the greatest number of fish dying between 21 and 34 dph (Figure 3). This phase coincided with the transition of sturgeon to exogenous food. Similar "die offs" have been reported by other groups that routinely spawn and breed sturgeon such as the Kootenay Trout Hatchery, Canada, the Columbia Basin Hatchery, USA, and the University of California, USA (Ek 2008, pers. comm.; Lyon 2008, pers. comm.; Van Eenennaam 2008, pers. comm.). Based on observations made by the majority of these institutions, it appears that the transition to exogenous feeding represents a sensitive period during the early development of white sturgeon, characterized by natural high mortality rates.

Table 8. Mean (± Standard Deviation) Percent Mortality of Early Life-stages (ELS) of White Sturgeon Exposed to Copper, Cadmium, and Zinc Treatment Concentrations

| Treatment[a] | Copper | Cadmium | Zinc | Control |
|---|---|---|---|---|
| 0 | | | | 63 (± 2.3) |
| 1 | 73 (± 8.8) | 68 (± 3.6) | 70 (± 2.3)*** | |
| 2 | 70 (± 1.7) | 70 (± 6.5) | 68 (± 1.3)** | |
| 3 | 70 (± 0.6) | 69 (± 3.8) | 69 (± 3.2)*** | |
| 4 | 100 *** | 89 (± 1.9) *** | 78 (± 6.8)*** | |
| 5 | 100 *** | 100 *** | 100 *** | |

**Notes:**
[a] Refer to Table 7 for an explanation of exposure concentrations associated with these treatments for each of the metals.
Asterisks indicates significant mortality relative to controls (Bonferroni test):
*=p < 0.05; **=p < 0.01; ***=p < 0.001 ***.
Based on 12 and 4 replicate chambers per treatment for the control and metal exposure groups, respectively.

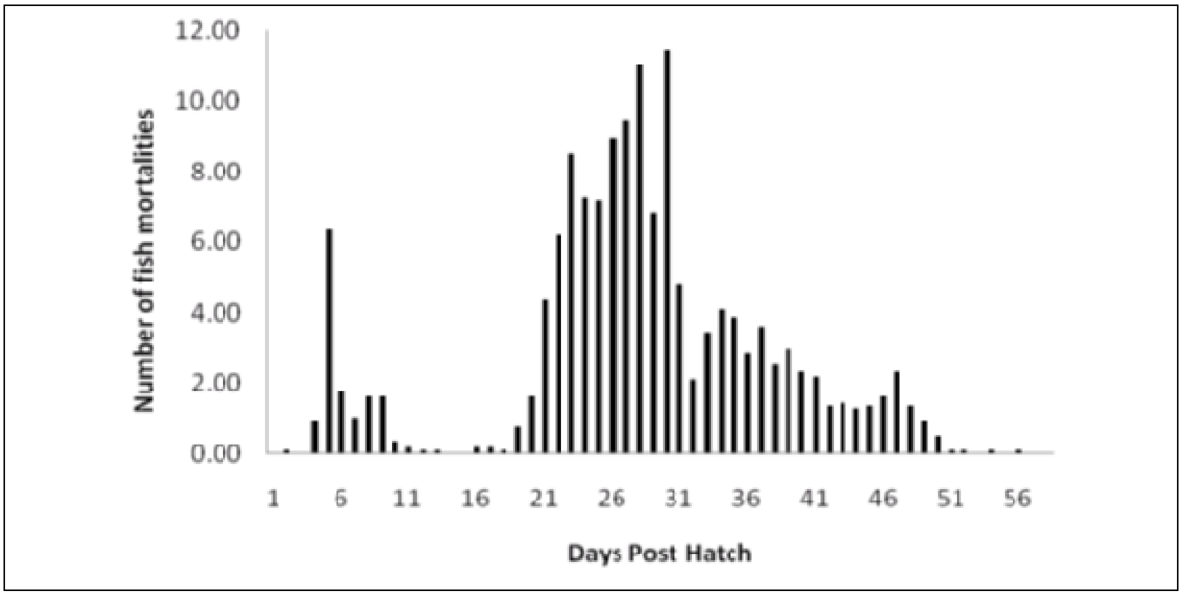


Figure 3. Average Daily Control Mortality Throughout the Exposure Period

When overall mortality for each metal is stratified into four lifestages (yolksac, swim up, transition to feed and juvenile), the sensitive transition phase reveals near equivalent mortality rates for all treatments compared to the control (Figure 4). These calculations are based on an average of 232 (±32) fish per treatment chamber, and 12 and 4 replicate treatment chambers for the controls and metal exposures, respectively. These numbers reflect counts at the initiation of the transition period to feeding, and exclude treatment groups 4 and 5 for copper, 5 for cadmium and 5 for zinc, in which 100 percent mortality occurred prior to this time point. The highest remaining concentration of copper (6.2 µg/L), however, did not result in greater mortality during the transition life-stage but this can be attributed to the fact that a large proportion of fry from this treatment group (28 ± 3.2 percent) died during the previous swim-up lifestage, leaving a relatively small number of fish in this group. In addition, the highest remaining concentration of cadmium appears to have slightly delayed mortality, with the greatest percentage of deaths in the following juvenile lifestage, possibly due to delayed hatching. As a result, mortality in the transition-to-feed stage for cadmium treated fish is predominantly driven by natural mortality.

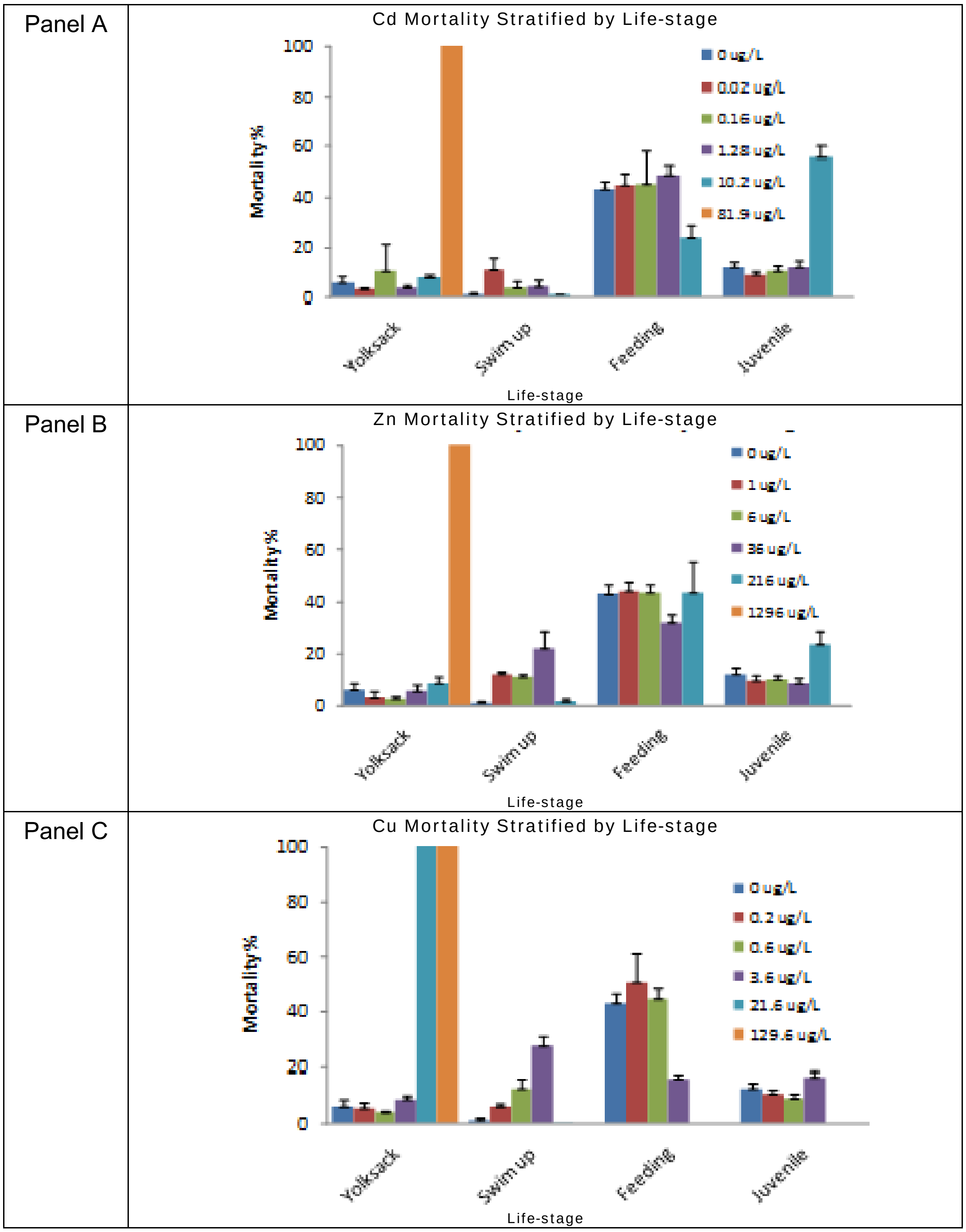


Figure 4. Mortality Stratified into Four Life-stages (Yolksac, Swim Up, Transition to Feed and Juvenile), by Nominal Test Analyte Metal Concentration

Percent mortality adjusted for seeding density is shown in Table 9. The resulting dose-response relationships for cadmium, copper, and zinc are shown in Figure 5, and calculated LC20 and LC50 calculated are in Table 10. The large adjustment required for the seeding density correction does, however, suggest that there may be additional uncertainty from the resulting dose-response relationships and further study with tests designed to avoid the need for seeding density corrections are warranted.

Table 9. Mean Percent Mortality of Early Life-stages of White Sturgeon Exposed to Copper, Cadmium, and Zinc after Adjusting for Mortality Associated with Seeding Density

| Treatment[a] | Copper | Cadmium | Zinc | Control |
|---|---|---|---|---|
| 0 | | | | 4.0 |
| 1 | 22.1 | 4.5 | 5.9 | |
| 2 | 12.9 | 12.0 | 4.2 | |
| 3 | 14.2 | 11.1 | 9.9 | |
| 4 | 100 | 63.1 | 39.5 | |
| 5 | 100 | 100 | 100 | |

**Notes:**
[a] Refer to Table 7 for an explanation of exposure concentrations associated with these treatments for each of the metals
Based on 12 and 4 replicate chambers per treatment for the control and metal exposure groups, respectively

Table 10. Lethal Concentrations (µg/L) for Cadmium, Copper, and Zinc at Which 20% (LC20) and 50% (LC50) of White Sturgeon Fry Died at the End of the Exposure (60 dph), after Adjusting for Seeding Density Effects

| Metal | LC20 | LC50 |
|---|---|---|
| Cadmium | 1.53 | 4.78 |
| Copper | 6.76 | 11.1 |
| Zinc | 111 | 239 |

The Biotic Ligand Model (BLM; Di Toro et al. 2001; Santore et al. 2001, 2002; HydroQual 2007) was calibrated using the metal concentrations (see Table 7) and associated toxicological responses (see Table 8) for cadmium, copper, and zinc effects to white sturgeon. The BLM is a predictive model that can explain how the chemistry of different exposure scenarios can affect metal toxicity. The purpose of this calibration is to develop parameter files that will allow the BLM to predict normalized chronic effect concentrations for white sturgeon to cadmium, copper and zinc, thereby allowing the BLM to translate these effects from laboratory conditions to a wider variety of exposure scenarios where factors such as pH, DOC, hardness, and alkalinity may vary.

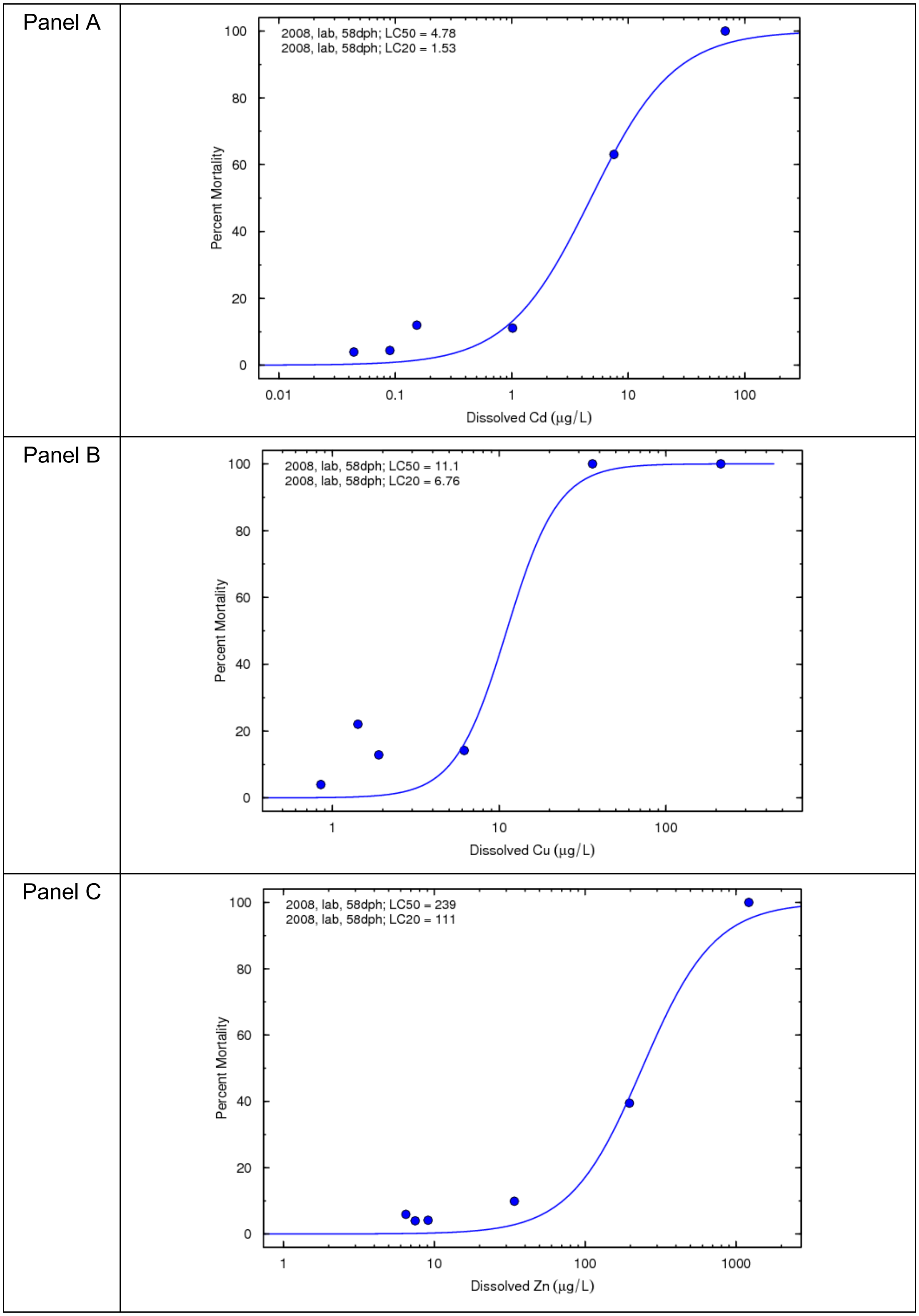


Figure 5. Mortality of White Sturgeon Fry at 60 Days Post Hatch (dph) after Exposure to Cadmium (Panel A), Copper (Panel B), and Zinc (Panel C), Adjusted for Seeding Density

To perform the calibration, the BLM was run with measured water quality parameters required for the model (pH, DOC, temperature, calcium, magnesium, potassium, sodium, chloride, sulfate, and alkalinity). Concentration-response relationships between dissolved metal and observed mortality are shown for cadmium on a dissolved basis (Figure 5, Panel A) or as normalized for bioavailability by considering accumulation on the biotic ligand (Figure 6). Similar figures are shown for dissolved and biotic-ligand bound copper (Figure 5, Panel B and Figure 7) and zinc (Figures 5, Panel C and Figure 8). From the concentration-response on the biotic ligand, the critical accumulation values associated with 20 percent and 50 percent mortality (LA20 and LA50) can be estimated and the value for each metal is shown in the top left corner of Figures 6, 7, and 8.

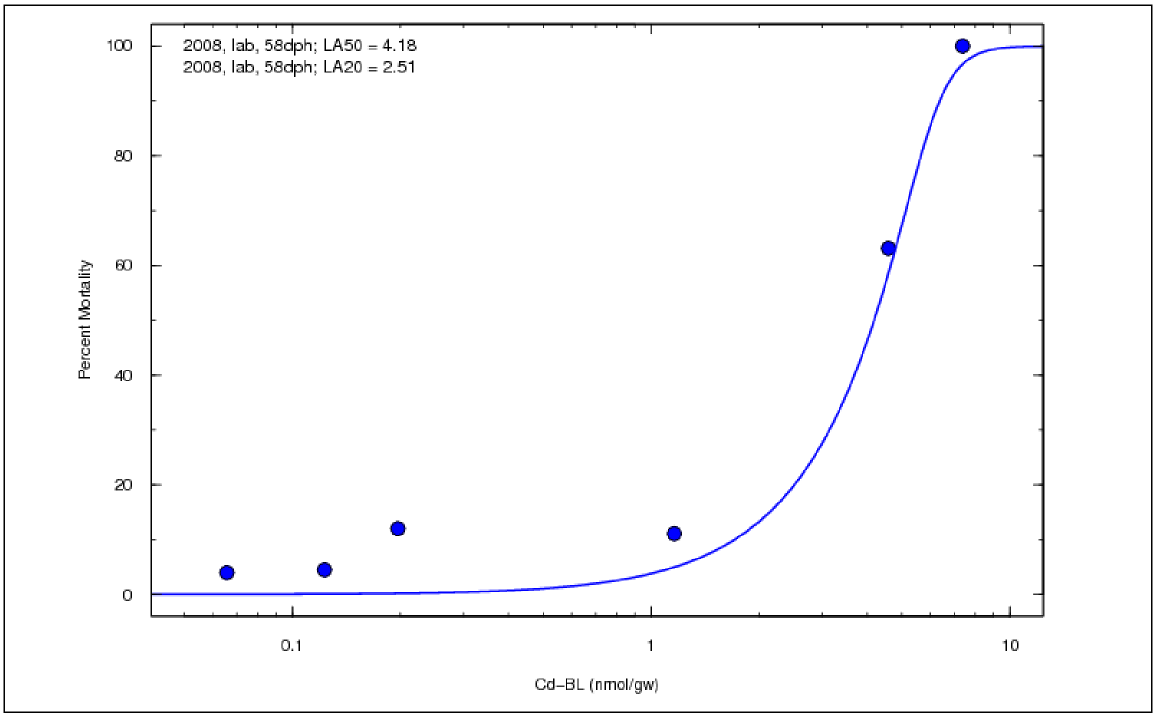


Figure 6. Concentration-response Relationship for Cadmium to White Sturgeon in Chronic Exposures Based on Either BLM Predicted Concentration of Accumulated Cadmium at Biotic Ligand (BL) Sites

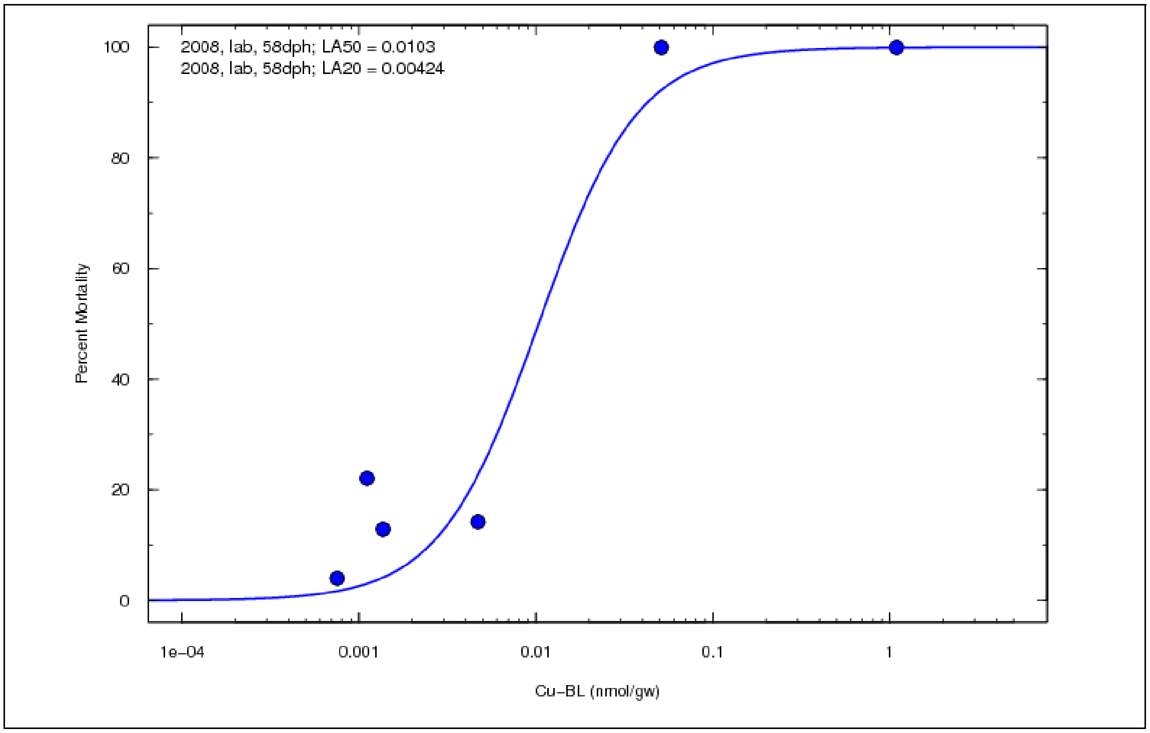


Figure 7. Concentration-response Relationship for Copper to White Sturgeon in Chronic Exposures Based on Either BLM Predicted Concentration of Accumulated Copper at BL Sites

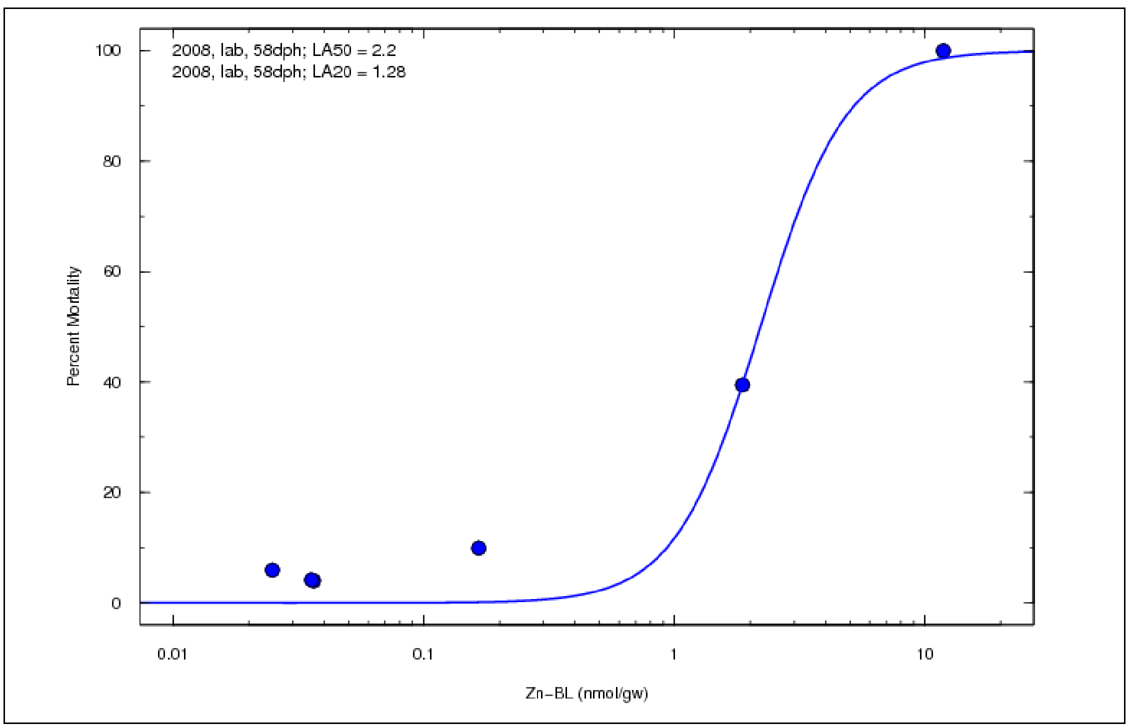


Figure 8. Concentration-response Relationship for Zinc to White Sturgeon in Chronic Exposures Based on Either BLM Predicted Concentration of Accumulated Zinc at BL Sites

Values for chronic water quality criteria for the state of Washington and EPA are shown in Table 11. For cadmium and zinc, the chronic water quality criteria are lower than the chronic LC20 for sturgeon (see Table 10), whereas for copper the chronic criterion is higher than the LC20. While these relative comparisons are instructive, it is also important to note the large adjustments that were required to account for mortality due to seeding density may introduce an additional uncertainty, and that further investigation is warranted to determine the relative protectiveness of the chronic water quality criteria.

Table 11. Chronic Water Quality Criteria for Metals in the State of Washington (WA) (WAC 173-201A-240) and for EPA

| Dissolved Metal | Average Hardness | Chronic Criteria (µg/L) – WA[a] | Chronic Criteria (µg/L) – EPA[b] |
|---|---|---|---|
| Cd | 77 mg/L as $CaCO_3$ | 0.85 | 1.84 |
| Cu | 73 mg/L as $CaCO_3$ | 8.67 | 8.44 |
| Zn | 72 mg/L as $CaCO_3$ | 79.12 | 89.44 |

**Notes:**

Cd – cadmium, Cu – copper, Zn - zinc

[a] Acute criteria for the state of WA are calculated based on an average water hardness using the following equations:
Cd WQC = $0.920(e^{(0.7852[\ln(hardness)]-3.49)})$
Cu WQC = $0.960(e^{(0.8545[\ln(hardness)] - 1.465)})$
Zn WQC = $0.978(e^{(0.8473[\ln(hardness)] + 0.7614)})$

[b] Acute criteria for EPA are calculated based on an average water hardness (Cd, and Zn) using the following equations, or on pH, DOC and major ions using the BLM (Cu):
Cd WQC = $0.920(e^{(0.7852[\ln(hardness)]-2.715)})$
Cu WQC is based on the BLM (USEPA 2007) using average chemistry from exposures near the LC20.
Zn WQC = $0.978(e^{(0.8473[\ln(hardness)] + 0.884)})$

## 3.6 STURGEON SIZE AND BODY CONDITION INDEX

At the termination of the experiment, body condition index for fish in the greatest remaining concentrations of cadmium and zinc (8.28 µg/L and 197.5 µg/L, respectively), were significantly less than controls ($p > 0.001$). No other significant effects were observed.

# 4 CONCLUSIONS

Semi-chronic exposures of cadmium, copper and zinc to ELS of white sturgeon (*A. transmontanus*) resulted in semi-chronic toxicity (LC20s) at concentrations of 1.5, 6.8, and 111 µg/L respectively. For cadmium and zinc these LC20 values are greater than the freshwater chronic water quality criteria for these metals in the state of Washington, which correspond to 0.85 and 79.1 µg/L for cadmium and zinc, respectively. For copper the LC20 of 6.8 µg/L is close to but lower than the hardness equation based freshwater chronic criterion for the state of Washington of 8.7 µg/L. Water quality criteria based on national guidelines from EPA are similar with values of 1.8, 8.4, and 89.4 µg/L for cadmium, copper, and zinc respectively. As noted above, the calculated LC20 values reported in Table 10 relied on large adjustments (Table 9) to correct for mortality due to seeding density. The uncertainty introduced by this adjustment is unknown, and additional information will be needed before definitive comparisons can be made regarding the protectiveness of current water quality criteria.

BLM calibrations were performed for these exposures and concentration-response relationships on the biotic ligand suggest that accumulation on the biotic ligand can be correlated with effects. These concentration-response relationships were used to derive critical accumulation levels (LA50s) that can be used in parameter files that will allow application of the BLM to predict how acute effects for these metals to white sturgeon will change with changing water chemistry.

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