

*Environmental Toxicology*EFFECTS OF SUBCHRONIC EXPOSURE OF EARLY LIFE STAGES OF WHITE STURGEON
(*ACIPENSER TRANSMONTANUS*) TO COPPER, CADMIUM, AND ZINC

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Abstract—Populations of sturgeon (Acipenseridae) are declining in many places in the world because of several potential factors, including overharvesting, habitat alteration, and pollution. In North America, populations of the white sturgeon (*Acipenser transmontanus*) have been experiencing poor annual recruitment in major river systems for more than three decades. Metal pollution has been hypothesized as a potential contributing factor to the poor recruitment in some of the water bodies. In general, little is known about the toxicity of metals such as Cu, Cd, and Zn to white sturgeon and their potential influence on survival of embryos and juveniles. The present study was conducted to establish baseline toxicity data for the subchronic exposure of early life stages of white sturgeon to Cu, Cd, and Zn that can be used in metal-related risk assessments. Embryos, larvae, and fry were exposed to increasing concentrations of dissolved Cu, Cd, or Zn for 66 d using laboratory-based flow-through exposure systems. Hatching success was greater than 79% for all controls, and no significant differences were observed among treatment groups or between treatments and controls. Chronic lethal concentrations at which 20% mortality occurred (LC20s) for Cd (1.5 µg/L), Cu (5.5 µg/L), and Zn (112 µg/L) obtained for white sturgeon in the present study were comparable to those of sensitive salmonid species. Based on LC20 values for 19 or 58 d posthatch white sturgeon, the United States national ambient water quality criteria and the Canadian water quality guidelines for the protection of aquatic life that have been established for Cd, Cu, and Zn protect white sturgeon early life stages. Environ. Toxicol. Chem. 2011;30:2497–2505. © 2011 SETAC

Keywords—Sturgeon Early life stages Metal toxicity Water quality criteria

INTRODUCTION

Sturgeon (Acipenseridae) populations are threatened throughout the world and have been decreasing over the past century in Northern Europe, Asia, and North America [1–3]. Factors that are hypothesized to contribute to this decline are overharvesting, habitat alteration, and contamination [1–7]. In North America, populations of white sturgeon (*Acipenser transmontanus*) have been reported to be declining in the northwestern United States and British Columbia, Canada. Populations of white sturgeon have been listed as endangered in parts of Canada (Committee on the Status of Endangered Wildlife in Canada; http://www.cosewic.gc.ca/eng/sct1/searchdetail_e.cfm) and the United States (<http://ecos.fws.gov/speciesProfile/profile/speciesProfile.action?spcode=E087>). Decreases in sturgeon populations in the Columbia (British Columbia, Canada/northwest USA), Fraser (British Columbia, Canada), and Sacramento-San Joaquin Rivers (California, USA) and their tributaries have been attributed primarily to poor annual recruitment [2,6–8]. Some simulation models have predicted that without implementing successful remedial efforts, the white sturgeon will become virtually extinct in these rivers within 50 years [4,6,8,9].

Several hypotheses for recruitment failures of white sturgeon have been suggested. These include habitat alteration, varying flow regimen, decreased water quality (temperature, turbidity, total dissolved gases), poor nutrition, genetic bottlenecks or inbreeding depression, predation by introduced species such as walleye (*Sander vitreus*), interspecific competition, pathogens, and pollution [2,8,10,11]. A limited number of studies have examined the effects of pollutants on white sturgeon, and a general consensus exists that further investigations are needed to assess the relative sensitivity of this species to environmental contaminants [8,10–13]. Most studies involving sturgeon have been conducted with acute exposures, and no studies of the effects of contaminants have been conducted with early life stages from egg through juvenile. Fish are considered to be most sensitive to exposure to contaminants such as metals during this period of development [14,15], and chronic studies of the effects of contaminants on early life stages are needed to assess sturgeon sensitivity.

The potential for exposure to metals and other contaminants in the Columbia River is of concern because of past and present activities of mines, metallurgical facilities, pulp and paper mills, as well as other industrial and municipal sources (Upper Columbia River Work Plan for the Remedial Investigation and Feasibility Study. U.S. Environmental Protection Agency [U.S. EPA]; <http://yosemite.epa.gov/R10/CLEANUP.NSF/sites/Upperc>). Aquatic ecological risk assessment of metals is affected by the fact that toxicity largely depends on each organism's ability to regulate or store the metal, as well as the toxicokinetics and toxicodynamics of specific metals in

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particular environments [16,17]. In general, little is known about the potential toxicity of metals such as Cu, Cd, and Zn to white sturgeon or the tolerance of white sturgeon in comparison with other fish species. Previous studies have shown that some standard test species, such as rainbow trout (*Oncorhynchus mykiss*), are relatively sensitive to certain metals, whereas others, such as fathead minnows (*Pimephales promelas*), are more tolerant [18,19]. Species-specific chronic concentration–response relationships are needed to establish metal toxicity threshold values for white sturgeon that can be used in environmental risk assessments. Therefore, the objective of the present study was to determine the chronic toxicity of Cu, Cd, and Zn to white sturgeon under laboratory conditions. Embryos and early life stages of white sturgeon were exposed for 66 d to a range of concentrations of dissolved Cu, Cd, and Zn that bracketed respective concentrations observed in the Columbia River.

METHODS

Experimental fish

Fertilized white sturgeon eggs were obtained from the Kootenay Trout Hatchery (Fort Steele, BC, Canada). Eggs were collected from four breeding pairs of adult white sturgeon caught in the Columbia River near Waneta, BC, Canada. Fertilization of the eggs was harmonized in the hatchery by injecting adult sturgeon with a gonadotropin analog (luteinizing hormone releasing hormone analog, Syndel International) on two subsequent days. Embryos were transported in oxygenated bags and received at the exposure facility within 6 h of fertilization. Embryos were gradually acclimated to their solution waters for 1 h before being randomly assigned to McDonald-type hatching jars (Aquatic Ecosystems). Embryos were incubated under a low flow velocity (~3 L/min) for 72 h until neurulation occurred. After 72 h, the flow velocity was increased to gently agitate the embryos and prevent the development of fungus. Hatching began approximately 5 to 7 d postfertilization. Toward the end of the hatching period, all remaining eggs and yolk sac larvae from each replicate were enumerated and separated into two experimental chambers.

Test chemicals

Copper (II) sulfate pentahydrate (Chemical Abstracts Service [CAS] 7758-99-8; purity 99.995%), cadmium chloride hemi-pentahydrate (CAS 7790-78-5; purity 99.999%), and zinc chloride (CAS 7646-85-7; purity 98%) were obtained from Sigma-Aldrich. All chemicals were dissolved in laboratory reverse osmosis water.

Exposure design

Toxicity testing was conducted at the Aquatic Toxicology Research Facility, University of Saskatchewan (Saskatoon, SK, Canada). Laboratory water was adjusted to approximate the natural conditions of river waters inhabited by white sturgeon. The target hardness and dissolved organic carbon concentrations of 65 to 70 mg/L CaCO₃ and 2 to 3 mg/L, respectively, were achieved by mixing laboratory water with deionized, reverse osmosis water in a 1:1 ratio. The target temperature of 15°C ± 1°C was achieved by immersing the exposure chambers in chilled water baths. All fish were tested under a 16:8 h light:dark illumination cycle.

The exposure design consisted of a continuous flow-through system constructed to provide 17 treatments: five concentrations each of Cu, Cd, and Zn, plus two references containing only laboratory water and reverse osmosis water (1:1 ratio). Nominal exposure concentrations (Table 1) were prepared separately in individual 1,000-L holding tanks and in turn were metered into corresponding 100-L reservoirs. Each treatment was split into two true replicates (two reservoirs), which in turn were fed into two replicate exposure chambers, for a total of four replicate exposure chambers per metal concentration. Exposure chambers contained 40 L exposure water with 10 L/h exposure water replacement, providing four volume replacements per day.

At day 0 of the study, each true replicate system for every metal concentration contained an egg hatching jar with 800 embryos, loaded within 8 h of fertilization to maximize exposure during embryo development. Posthatch, larvae were divided into the corresponding two replicate chambers for each treatment, 10 individuals at a time, to provide a random distribution. The exposure period consisted of 66 d, and at 38 d posthatch (dph), the fish were culled to 115 individuals per exposure chamber to ensure that the loading density was less than 0.5 g fish/L per American Society for Testing and Materials (ASTM) guidelines [20]. Fish that were removed from chambers were euthanized using tricaine methanesulfonate (MS-222, Sigma-Aldrich), measured, weighed, and then fixed in 10% buffered formalin (Sigma-Aldrich). At 7 dph, food was introduced to the chambers to familiarize the larvae with a food scent. Fish were fed a combination of live brine shrimp (*Artemia salina*) and frozen bloodworms (Hagen, San Francisco Bay Brand), ad libitum four to eight times throughout the day and into the evening. Feeding rates were increased when larvae were transitioning to feeding, because this has been shown to be a critical period for survival [14]. Exposure tanks were cleaned twice per day. Dead fish, if any, were removed, measured, weighed, and fixed in 10% buffered formalin. Measurement endpoints included percent hatched, percent survival, body condition (mass/length), and morphology of

Table 1. Nominal, mean ± standard deviation (SD; numbers in brackets) measured exposure concentrations (concn.) and gram-atomic weight concentrations (GAW) for Cu, Cd, and Zn during the 66 d flow-through exposure experiments with white sturgeon (*Acipenser transmontanus*)

Treatment	Cu concn.			Cd concn.			Zn concn.		
	Nominal (µg/L)	Measured (µg/L)	GAW (mol/L)	Nominal (µg/L)	Measured (µg/L)	GAW (mol/L)	Nominal (µg/L)	Measured (µg/L)	GAW (mol/L)
Reference	0	0.6 (± 0.1)	9.4E-9	0	0.05 (± 0.02)	4.4E-10	0	7.2 (± 1.8)	1.1E-7
[1]	0.1	0.7 (± 0.1)	1.1E-8	0.02	0.06 (± 0.01)	5.3E-10	1	5.8 (± 0.6)	8.9E-8
[2]	0.6	1.8 (± 0.4)	2.8E-8	0.16	0.17 (± 0.02)	1.5E-9	6	9.3 (± 2.9)	1.4E-7
[3]	3.6	5.9 (± 0.1)	9.3E-8	1.28	1.1 (± 0.02)	9.8E-9	36	34 (± 3.0)	5.2E-7
[4]	21.6	36 (± 1.4)	5.7E-7	10.24	8.3 (± 0.11)	7.4E-8	216	198 (± 5.3)	3.0E-6
[5]	129.6	217 (± 42.0)	3.4E-6	81.92	69 (± 2.02)	6.1E-7	1,296	1,214 (± 39.0)	1.9E-5

larvae and juveniles. Behavior (mobility, swimming performance) was also observed and noted during times of cleaning and feeding.

Water chemistry analyses

Basic water quality parameters (temperature, pH, dissolved oxygen, and conductivity) were recorded daily with symphony electrodes (VWR). Hardness, alkalinity, ammonia, nitrate, nitrite, chlorine, sulfate, sulfide, and phosphate were recorded weekly using LaMotte colorimetric and titrator test kits; except during the first two weeks posthatch, when measurements were performed twice a week while the fish were transitioning from yolk sac to free feeding. Water samples were collected weekly from each chamber using acid-cleaned polyethylene bottles, filtered through 0.45- μm polycarbonate filters (Whatman) in Nalgene filter holders and receivers, and then acidified with ultrapure nitric acid to $\text{pH} < 2$. Quantification of metals was performed weekly by inductively coupled plasma mass spectrometry, following U.S. EPA method ILM05.2D. Cation and anion analyses were performed weekly based on U.S. EPA method 300.1 by Dionex ICS-3000 dual-ion chromatography system equipped with dual pump, eluent generation, dual ion exchange column, dual conductivity detector, dual cation/anion self-regenerating suppressor, and X-Y autosampler. The software Chromeleon 6.80 (Dionex) was used to simultaneously quantify cations and anions. Dissolved organic carbon analysis was performed weekly using a total organic carbon analyzer (TOC-5050A, Shimadzu, Mandel Scientific).

Data analyses

Mortality data. Mortality in the present study was dependent on initial seeding density. This was determined based on results from a concurrent field experiment conducted in 2008 (A. Tompsett et al., University of Saskatchewan, Saskatoon, SK, Canada, unpublished results) and reference data from the present laboratory experiment. A least squares linear regression was performed with initial seeding density as the independent variable and total number of fish that died during the experiment as the dependent variable. The relationship between these two parameters was statistically significant ($R^2 = 0.985$; $p < 0.001$). This function (Equation 1) was then applied to all treatments at 58 dph in the present experiment to account for seeding density mortality.

$$Y = 0.9138x - 68.97 \quad (1)$$

Before calculating lethal concentrations (LCs) from bioassays, one must correct for control, or natural mortality to minimize biased estimations [21]. Accordingly, all data were normalized for reference mortality using Abbott's formula (Equation 2) [22].

Adjusted % test response

$$= \frac{\% \text{ test response} - \% \text{ control response}}{100 - \% \text{ control response}} \times 100 \quad (2)$$

Effective concentrations at which 50% and 20% mortality occurred (LC50 and LC20) at critical time periods (19 and 58 dph) were compared to assess potential confounding of metal-induced mortalities by seeding densities. In addition, multivariate analysis was performed using the statistical program R (Comprehensive R Archive Network, Vienna University of Technology, Vienna, Austria) to investigate the interactions between chemical concentration and seeding

density on fish mortality, in relation to the critical time periods during white sturgeon early life stage development.

General data analysis. All data were expressed as the mean ± 1 standard deviation (SD). Mortality was analyzed by comparing the proportion of fish dead in each of the four exposure chambers of a given metal concentration with that of the reference. Mortality was adjusted for fish that were removed during culling. Hatchability was analyzed by comparing percent of hatched eggs per egg-hatching jar. Data were tested for normality by use of the Shapiro-Wilks test. When data were normally distributed or approximated normal distribution, analysis of variance and a Dunnett's post hoc test were used to detect significant differences between treatment and reference groups. In cases in which data were not normally distributed, statistical analyses were conducted using the Kruskal-Wallis test followed by the Mann-Whitney U test. Systat statistical software (Systat Software) was used for these analyses. The LC50 and LC20s were estimated using TOXSTAT software [23]. A body condition index was calculated for each surviving fish at the termination of the experiment (Equation 3). Differences in body conditions between treatment groups and references were analyzed using the same statistical methods as those for mortalities.

$$\text{Condition factor} = \frac{\text{body weight (g)}}{\text{length (mm)}^3} \times 100,000 \quad (3)$$

Statistical significance was accepted when $p < 0.05$.

RESULTS

Exposure verification

Measured concentrations of all target metals were comparable to nominal concentrations (Table 1). However, measured concentrations of Cu, Cd, and Zn in the references were greater than zero, and the actual concentrations measured in the test vessels were slightly greater than the target nominal concentrations in the two lowest doses of each metal (Table 1). As a consequence, the control is considered to be and referred to as the reference exposure for each metal. Concentrations within the exposure chambers remained constant throughout the exposure period. All calculations and reported values pertaining to concentrations of metals are based on measured concentrations. In addition, gram-atomic weight concentrations are presented to compare relative potencies of Cu, Cd, and Zn (Table 1).

Water quality

The average water temperature over the 66-d exposure period for all treatment groups was 15°C (± 0.5). The average dissolved oxygen concentration, pH, and conductivity for all treatment groups were 8.9 mg/L (± 0.9), 7.9 (± 0.2), and 220 $\mu\text{S}/\text{cm}$ (± 40), respectively. The average hardness was 70 mg/L CaCO_3 (± 9.8), and the average total concentration of ammonia nitrogen for all treatment groups was 0.04 mg/L (± 0.03). The mean dissolved organic carbon concentration for all treatment groups was 2.5 mg/L (± 0.5).

Hatchability

No significant differences ($p > 0.05$) in hatching success were observed among treatment groups or between any of the treatments and the reference exposure ($> 79\%$ hatchability). All exposure chambers, including the reference group, contained a few deformed fish (between 1 and 3% of the original seeding density per exposure chamber) that exhibited kyphosis; an axial

defect characterized by ventral spinal curvature around the yolk sac. These deformities were distributed randomly and could be attributed, in part, to transporting the embryos from the hatchery to the laboratory by air. All deformed fish were identified, removed, and euthanized within the first week posthatch.

Mortality and body condition

Reference exposure and seeding density mortality. Relatively great overall mortality occurred within the reference group during the transition to feeding stage. This mortality was largely dependent on seeding density (A. Tompsett et al., University of Saskatchewan, Saskatoon, SK, Canada). Between 21 and 34 dph, when the fish were transitioning from yolk sac to exogenous feed, 43% mortality occurred (of the total number of fish seeded in the reference group; Fig. 1). When mortalities in the reference and metal treatment groups were stratified into four life stages (yolk sac, swim-up, transition to feed, and juvenile), mortalities in the transition to exogenous feeding stage were near equivalent among treatments (Fig. 2). The greatest remaining concentration of Cu (5.9 $\mu\text{g/L}$), however, did not result in greater mortality during the transition life stage, but this can be attributed to the fact that a proportion of fry from this treatment group ($28\% \pm 3.2$) died during the previous swim-up life stage, leaving a relatively small number of fish in this group. In addition, the greatest remaining concentration of Cd appeared to have had slightly delayed mortality, with the greatest percentage of deaths during the subsequent juvenile life stage. Furthermore, a significant positive relationship between seeding density and mortality was observed for all groups regardless of treatment during the transition of larvae to exogenous feeding (Fig. 3). When compared with other life stages, no such significant relationships existed in the yolk sac and swim-up stages. A significant positive relationship between seeding density and mortality was observed during the juvenile life stage, but this was driven by Cd 8.3 $\mu\text{g/L}$ treatment that exhibited delayed mortality effects, as previously mentioned.

The overall mean mortality of the reference group was 63%, and when seeding density mortality was accounted for, the overall mean mortality was adjusted to 4% (Table 2). No noticeable changes in physical appearance or behavior were observed among the reference group fish during the exposure period.

Multivariate analysis. Concentrations of metals and seeding density influenced fish mortality to varying degrees during the different early life stages of white sturgeon development. For each metal, significant mortalities were observed early in the exposure at the greatest concentration, whereas significant

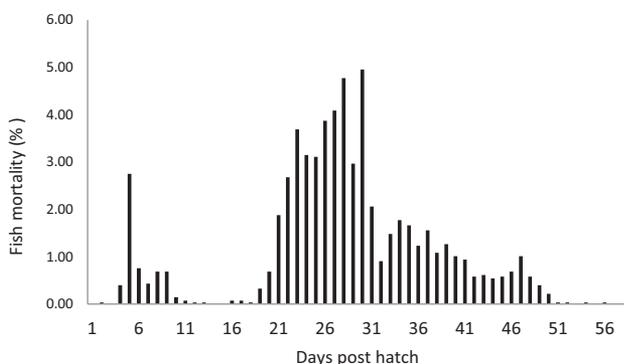


Fig. 1. Mean reference group mortality throughout the exposure period. Expressed as average % mortality of fish seeded in reference group per day.

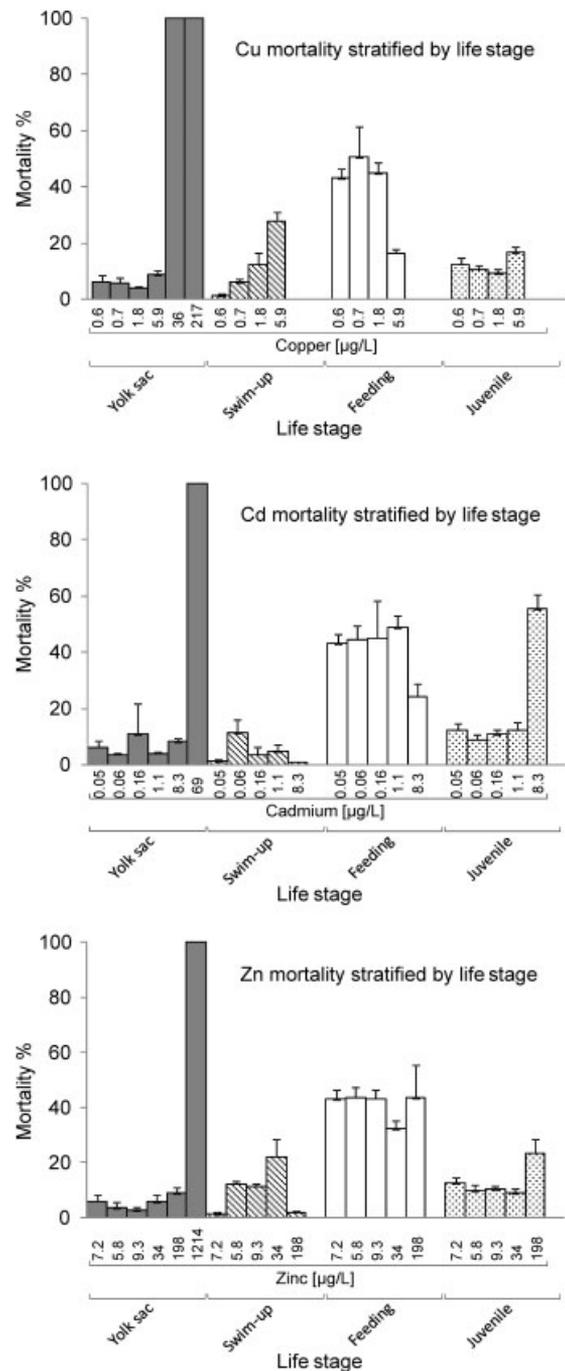


Fig. 2. Mean mortality stratified into four life stages (yolk sac, swim-up, transition to feeding, and juvenile) for Cu, Cd, and Zn. Mortality bars for each life stage represent the different concentrations of the individual metals, with reference group on the left and increasing concentrations to the right. Life stages after yolk sac contain some mortality bars with 0 mortality, because all of the fish in those particular concentrations had already died. Error bars represent standard deviation.

positive relationships between seeding density and mortality were observed during the transition of larvae to exogenous feeding. Results of multivariate analyses indicated significant interactions between concentrations of metals and seeding density on mortality during critical life stages (Table 3). Concentrations of all metals significantly ($p < 0.001$) influenced mortality during the yolk sac life stage; seeding density affected Cu mortality during this life stage ($p < 0.01$), but to a lesser

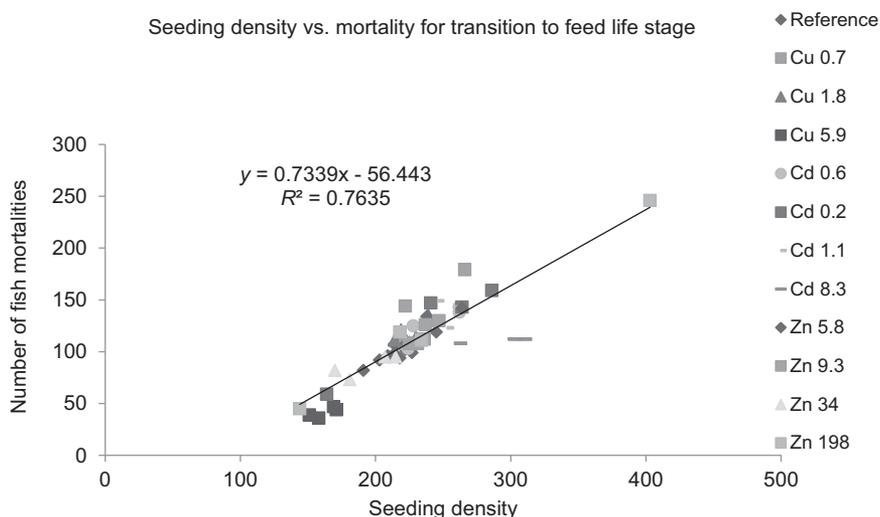


Fig. 3. Seeding density regression for all metal treatments and reference group. Linear regression comparing seeding density at the beginning of the transition to feeding life stage with the number of fish surviving at the end of the life stage. Numbers associated with the individual metals in the legend represent concentrations (µg/L).

degree. In the swim-up life stage, concentration of metal influenced mortality for Cu and Cd ($p < 0.001$, $p < 0.01$, respectively), but not Zn. No interaction was seen between main effects of Zn. Seeding density significantly ($p < 0.001$) influenced mortality during the transition to feeding life stage for Cu and Zn, whereas for Cd a significant interaction ($p < 0.01$) was found between seeding density and chemical concentration that influenced mortality. In the juvenile life stage, results varied from no significant influences for Cu; a slight significant interaction between seeding density and concentration of metal on mortality for Cd ($p < 0.05$); and significant influences of concentration of metal ($p < 0.001$) and seeding density ($p < 0.05$) on mortality caused by Zn.

Copper. Exposure of early life stages of white sturgeon to Cu resulted in concentration-dependent mortalities. Statistically significant greater mortalities relative to the reference group occurred at the two greatest concentrations of Cu (Table 2). Statistically significant increases in mortalities (100% mortality; $p < 0.001$) relative to the reference group occurred within the first 14 dph at the two greatest concentrations of Cu. Surviving juvenile white sturgeon in all of the Cu exposures had no significant differences ($p > 0.05$) in body condition index at termination when compared with the reference group.

No changes in physical appearance or behavior were observed in any of the Cu exposures.

Cadmium. Exposure of early white sturgeon life stages to Cd resulted in concentration-dependent mortalities. Statistically significant greater mortalities relative to the reference group occurred at the two greatest concentrations of Cd (Table 2). Results from one of the 8.3 µg Cd/L replicates were excluded because of loss of fish (72% of the original number seeded) through equipment failure. Statistically significant increases in mortalities (100% mortality; $p < 0.001$) relative to the reference group occurred within the first 14 dph at the greatest exposure concentration of Cd. Exposure to 8.3 µg Cd/L caused 65% mortality ($p < 0.01$) over the duration of the experiment, with most mortalities observed later (33–43 dph) in the experiment (juvenile stage). Body condition indices of surviving juvenile white sturgeon exposed to 8.3 µg Cd/L, the greatest Cd exposure with surviving fish at termination, were significantly less ($p < 0.001$) at termination than were those in the reference group. Most fish in this treatment lost their pigmentation and became almost translucent by the end of the exposure period. In addition, they were lethargic and appeared to eat less throughout the exposure.

Zinc. Exposure of early white sturgeon life stages to Zn resulted in concentration-dependent mortalities. Statistically

Table 2. Mean ± standard deviation (SD; numbers in brackets) percent mortality of early life stages of white sturgeon (*Acipenser transmontanus*) through 66 d of sub-chronic exposure to Cu, Cd, and Zn^a

Treatment	Cu			Cd			Zn		
	Concn. (µg/L)	Total % dead	Total % dead after seeding density normalization	Concn. (µg/L)	Total % dead	Total % dead after seeding density normalization	Concn. (µg/L)	Total % dead	Total % dead after seeding density normalization
Reference	0.6 (± 0.1)	63 (± 2.0)	4 (± 2.2)	0.05 (± 0.02)	63 (± 2.0)	4 (± 2.2)	7.2 (± 1.8)	63 (± 2.0)	4 (± 2.2)
[1]	0.7 (± 0.1)	73 (± 8.3)	21 (± 27.3)	0.06 (± 0.01)	68 (± 3.2)	2 (± 0.5)	5.8 (± 0.6)	69 (± 1.8)	6 (± 3.3)
[2]	1.8 (± 0.4)	70 (± 1.9)	13 (± 4.2)	0.17 (± 0.02)	70 (± 6.2)	12 (± 9.8)	9.3 (± 2.9)	68 (± 0.7)	4 (± 2.9)
[3]	5.9 (± 0.1)	69 (± 1.1)	14 (± 3.6)	1.1 (± 0.02)	70 (± 3.9)	11 (± 8.1)	34 (± 3.0)	69 (± 3.2)	10 (± 5.5)
[4]	36 (± 1.4)	100 (± 0.0)	100 (± 0.0) ***	8.3 (± 0.11)	89 (± 1.9)	65 (± 3.9) **	198 (± 5.3)	78 (± 6.9)	40 (± 0.0) **
[5]	217 (± 42.0)	100 (± 0.0)	100 (± 0.0) ***	69 (± 2.02)	100 (± 0.0)	100 (± 0.0) ***	1214 (± 39.0)	100 (± 0.0)	100 (± 0.0) ***

^a Asterisks indicate significant mortality relative to reference group (Dunnett's test; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Averages and SDs are based on 12 and four replicate chambers per treatment for the reference and metal exposure groups, respectively. Concn. = metal concentration.

Table 3. Multivariate analyses between seeding density, metal concentration, and mortality during the yolk sac, swim-up, transition to feeding, and juvenile life stages^a

Metal	Life stage											
	Yolk sac			Swim-up			Transition to feeding			Juvenile		
	SD	Concn.	SD × Concn.	SD	Concn.	SD × Concn.	SD	Concn.	SD × Concn.	SD	Concn.	SD × Concn.
Cu	**	***			***		***				#	
Cd		***			**			**	**			*
Zn		***					***			*	***	

^a Asterisks indicate significant mortalities relative to reference group ([#] $p = 0.06$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). SD = seeding density; Concn. = metal concentration; SD × Concn. = combinatory effect.

significant greater mortalities relative to the reference group occurred at the two greatest concentrations of Zn (Table 2). One hundred percent mortality occurred within the first 14 dph at the greatest exposure concentration of Zn. Exposure to 198 $\mu\text{g Zn/L}$ caused 40% mortality ($p < 0.01$). Surviving juvenile white sturgeon exposed to 198 $\mu\text{g Zn/L}$, the greatest Zn exposure with surviving fish at termination, had significantly reduced ($p < 0.001$) body condition indexes at termination when compared with the reference group. Changes in physical appearance and behavior were not noted in any of the Zn exposures.

Effective concentrations. Significant differences in effective concentrations were observed among development periods (19 and 58 dph) for Cd, but not Cu or Zn exposures (Table 4). The LC50 and LC20 values for Cu exposure at 19 dph (9.9 $\mu\text{g/L}$ and 3.4 $\mu\text{g/L}$, respectively) and 58 dph (12.4 $\mu\text{g/L}$ and 5.5 $\mu\text{g/L}$, respectively) were not significantly different. The LC50 and LC20 values for Cd exposure at 19 dph (21.4 $\mu\text{g/L}$ and 8.7 $\mu\text{g/L}$, respectively) were greater when compared with 58 dph (5.6 $\mu\text{g/L}$ and 1.5 $\mu\text{g/L}$, respectively). The LC50 and LC20 values for Zn exposure at 19 dph (340 $\mu\text{g/L}$ and 102 $\mu\text{g/L}$, respectively) were comparable when compared with 58 dph (250 $\mu\text{g/L}$ and 112 $\mu\text{g/L}$, respectively). In addition, LC50 and LC20 values for Cu, Cd, and Zn were expressed as gram-atomic weight concentrations to compare the relative potencies of each metal with early life stages of white sturgeon (Table 4). At 19 dph, Cu and Cd appeared to be similar in potency, whereas Zn appeared to be 20 to 30 times less potent. At 58 dph, Cd appeared to be approximately five times more potent than Cu and approximately 75 to 130 times more potent than Zn, whereas Cu appeared to be 19 times more potent than Zn.

DISCUSSION

Subchronic laboratory toxicity data were generated to characterize sturgeon sensitivity to selected metals. Toxicity threshold values for Cu, Cd, and Zn were developed for early life stages of white sturgeon. A limited number of studies have worked with early life stages of white sturgeon, and this species was amenable for use in chronic toxicity tests. However, a relatively great percentage of fish died during transition of larvae to exogenous feeding. As a consequence, the reference exposure mortality was greater than would be expected when compared with a toxicity test conducted with a standard test organism [20]. Relatively great larval mortality at transition to exogenous feeding is likely to be an inherent characteristic of white sturgeon biology, resulting in the need to develop independent standard ASTM and U.S. EPA guidelines for white sturgeon toxicity tests.

Mortality observed in the reference group is consistent with the expected mortality of sturgeon during this period when the larvae are transitioning from yolk sac to exogenous feed [14,24,25]. A cumulative assessment revealed mortality rates that were heterogeneously distributed over the course of the experiment, with the greatest number of individuals dying between 21 and 34 dph, coinciding with the transition to feeding on exogenous food. Groups that routinely spawn and breed white sturgeon such as the Kootenay Trout Hatchery, Canada, the Columbia Basin Hatchery, USA, and the University of California, USA, also experience die-offs during this transition phase; sometimes in excess of 70% (R. Ek, Kootenay Trout Hatchery, Fort Steele, BC, Canada, personal communication; B. Lyon, Columbia Basin Hatchery, Moses Lake, WA, USA, personal communication; J. Van Eenennaam, University of California, Davis, CA, USA,

Table 4. Lethal concentrations for Cu, Cd, and Zn at which 20 (LC20) and 50% (LC50) of white sturgeon early life stages died before (19 dph) transition to feeding at the end of the exposure experiment (58 dph)^a

Metal	LC20				LC50				CCC ^b	
	19 dph Concn.		58 dph Concn.		19 dph Concn.		58 dph Concn.		Measured ($\mu\text{g/L}$)	GAW (mol/L)
	Measured ($\mu\text{g/L}$)	GAW (mol/L)								
Cu	3.4 (3.1 – 3.7)	5.4E-8	5.5 (4.9 – 6.3)	8.7E-8	9.9 (9.3 – 10.6)	1.6E-7	12.4 (11.2 – 13.7)	2.0E-7		^c
Cd	8.7 (7.9 – 9.5)	7.7E-8	1.5 (1.2 – 1.8)	1.3E-8	21.4 (19.9 – 23.0)	1.9E-7	5.6 (4.8 – 6.5)	5.0E-8	0.19	1.7E-9
Zn	102 (93 – 112)	1.6E-6	112 (99 – 128)	1.7E-6	340 (315 – 366)	5.2E-6	250 (226 – 277)	3.8E-6	87	1.3E-6

^a Mortality was adjusted for seeding density mortality for the 58 dph LC calculations. LCs are presented as measured concentrations and gram-atomic weight concentrations (GAW). The 95% confidence intervals are presented in parentheses.

^b Criteria Continuous Concentration for freshwater species adjusted to the average hardness of 70 mg CaCO_3/L observed during the experiments (National Recommended Water Quality Criteria, U.S. EPA; <http://www.epa.gov/waterscience/criteria/wqtable/>).

^c Site-specific guidelines—Freshwater criteria calculated using the biotic ligand model [32].

personal communication). Similarly, Siberian sturgeon experience difficulties during the transitional period under hatchery conditions [3]. The variability in hatchery survival during this transition period is in part attributed to the type of diet provided and whether the larvae readily accept it [25,26]. Some studies suggest that sturgeon undergo morphophysiological changes to the digestive system during or just before this transition phase, and proper timing and development, often affected by environmental conditions and dietary requirements, are necessary for larval survival [26–28]. Based on the life history strategy of white sturgeon, mortality of larvae at this stage of development might be natural and unavoidable. Because little is known about rates of survival of white sturgeon larvae in their natural environment, assessing what percentage of larval mortality is normal is difficult. White sturgeon are long-lived (> 80 years), and females can spawn up to one million eggs in a season [8]; therefore, even with low fertilization or hatching success, natural mortality of early life stages is likely inherent [2,8]. Based on these observations, transition to exogenous feeding represents a period during early development of white sturgeon that is characterized by natural mortality.

An additional factor that contributed to the mortality during the transition life stage was the seeding density of fish per treatment chamber. Because no guidelines exist for the conduct of chronic tests with white sturgeon early life stages, seeding rates were calculated such that they were always less than those recommended by ASTM for fish in general [20], and those outlined in the hatchery manual for white sturgeon [14]. A parallel field study that was being conducted with the same exposure systems and same batch of fish showed a significant and strong linear relationship between seeding rates and mortality (A. Tompsett et al., University of Saskatchewan, Saskatoon, SK, Canada). This relationship was unique to the period when fry transitioned to exogenous feeding and may be an indication that transition stages of white sturgeon are particularly sensitive to competition. As soon as sturgeon larvae successfully adapt to exogenous food, they seem to be more robust with regard to this factor. Limitation of food resources as a possible reason for the density-related increase in mortalities can be excluded because fish were fed ad libitum, and they did not consume all food provided. Furthermore, no differences were present in water quality, including dissolved oxygen, ammonia, nitrate, nitrite, pH, temperature, phosphate, dissolved organic carbon, and total organic carbon between treatment chambers and groups, which could have explained elevated mortalities in the exposure systems with greater fish densities.

Comparable mortalities during the life stage associated with transition to feeding were observed among treatments. This indicates that mortality during this period was largely independent of exposure to metals. The significant relationship between seeding density and mortality among treatment groups, then, raised the question as to what degree seeding density versus metal concentration contributed to the overall mortality during the four different life stages. Multivariate analyses indicated that during the yolk sac and swim-up life stages the concentration of metal was almost entirely responsible for mortality in the exposure groups, with complete mortality in the greatest concentrations. During transition to feeding, however, seeding density was driving Cu and Zn mortality, whereas both seeding density and concentration of Cd influenced mortality in the Cd treatment group. Based on these analyses, overall mortality of the reference group can be attributed to the initial mortalities after hatch (as would be expected under natural conditions); the transition to exogenous feeding, characterized by natural mortality, and the initial

seeding densities (white sturgeon do not fall in the stocking density range recommended by ASTM [20] for other test species). Consequently, overall reference and treatment group mortalities were adjusted for seeding density mortality.

The large adjustment required for the seeding density correction does, however, add some uncertainty to the resulting dose–response relationships, because fully elucidating seeding density effects and metal effects (or possibly interactive effects) on mortality remains difficult. For this reason, toxicity values were presented for 19 dph fish before the transition to feeding die-off and associated seeding density normalization, as well as at the termination of the study (58 dph). The LC50 and LC20s expressed as gram-atomic weight concentrations (Table 4) showed that early life stages of white sturgeon were most sensitive to Cd, followed by Cu then Zn. Most metal-related mortalities occurred early during exposure, and the general patterns of toxicity are in accordance with previous studies of Cu, Cd, and Zn exposure to fish. The greater mortalities near the end of the period during which sturgeon were exposed to Cd could be attributed in part to differences in Cd's toxic mode of action. Hypocalcaemia, attributable to Cd inhibition of Ca^{2+} uptake across the gills, is the key mechanism of acute Cd toxicity. In chronic exposures, Cd has been shown to accumulate in internal organs such as liver and kidneys, in addition to the gills [17]. Cadmium's effect on internal organs likely contributed to the later mortalities and poor body condition of fish in the greatest remaining Cd exposure, which resulted in the lesser LC values observed at the later life stage (58 dph). Because of the similar response of white sturgeon to the exposure to Cu, Cd, and Zn observed in the present study, white sturgeon possibly respond to these metals in a similar manner to other fish species at these concentrations. In general, the gill is the primary site of action in acute toxicity caused by metals, and even at small concentrations these waterborne metals primarily affect ion regulation [29]. However, a more detailed examination of the white sturgeon gill, including protein expression after exposure to metals, is needed to confirm similar patterns of toxicity.

Early life stages of white sturgeon appear to be relatively sensitive to Cu, Cd, and Zn relative to other aquatic species. The most sensitive LC20s determined in the present study were compared with chronic values outlined in the EPA's chronic toxicity to freshwater organisms dataset in each metal's ambient water quality criteria document [30–32]. Chronic values are typically calculated by taking the geometric mean of the greatest no observed adverse effect concentration and the lowest observed adverse effect concentration, or, if possible, by regression or probit analysis to estimate effect concentrations for 20% reduction in survival [32,33]. The LC20s from the present study, adjusted to comparable hardness, were almost always within a factor of two of the chronic value of the most sensitive fish species. Studies conducted with rainbow trout (*Oncorhynchus mykiss*) and sculpins (*Cottus* spp.), two species often considered to be sensitive to metal exposure, demonstrated comparable if not lesser sensitivity to Cu, Cd, and Zn compared with early life stages of white sturgeon [18,34,35]. Chronic LC20 values for Cd (1.5 $\mu\text{g/L}$) and Cu (5.5 $\mu\text{g/L}$) obtained for white sturgeon were comparable to those previously reported for Chinook salmon (Cd = 1.6 $\mu\text{g/L}$ and Cu = 5.9 $\mu\text{g/L}$) [36], which was the most sensitive species compared with several other freshwater teleosts such as rainbow trout, fathead minnow, bluegill, and others [31,32]. The LC50 values obtained for white sturgeon after exposure to Zn (58 dph = 250 $\mu\text{g/L}$, 19 dph = 340 $\mu\text{g/L}$) compared well with those reported by

Mebane et al. [37] for rainbow trout (LC50 = 387 µg/L). Hardness in the present study was approximately two times greater than that reported by Mebane et al. [37].

Based on the LC values, the United States national recommended water quality criteria for aquatic life [30–32] (National Recommended Water Quality Criteria, U.S. EPA; <http://www.epa.gov/waterscience/criteria/wqctable/>) are protective of white sturgeon early life stages for Cd, Zn, and Cu (Table 4). However, the guidelines for Cu are site-specific, and freshwater criteria are calculated using the Biotic Ligand Model [32]. The Canadian water quality guidelines for the protection of aquatic life for Cu, Cd, and Zn (2.8 µg/L, 0.017 µg/L, and 30 µg/L, respectively), adjusted to comparable hardness levels, are less than LC20 values obtained in the present study for early life stages of white sturgeon (Canadian Council of Ministers of the Environment guidelines for the protection of aquatic life, Environment Canada; <http://www.waterquality.ec.gc.ca/waterqualityweb/guidelines.aspx?catId=1>).

Some sturgeon species (family Acipenseridae) may be relatively sensitive to certain chemicals based on acute studies [24,38,39]. However, no information on chronic toxicity was available prior to this study that included all of the early life stages, from embryo through juvenile, which are often assumed to be the most sensitive [15]. Therefore, the present subchronic study can help characterize the risks associated with waterborne exposure of Cu, Cd, or Zn to early life stages of white sturgeon under environmentally relevant conditions. Further investigation is needed, however, to examine other potential routes of metal exposure, such as uptake through sediment. White sturgeon are benthic scavengers that feed on prey closely associated with sediment, and bioaccumulation through the food web may be an issue. Ongoing research is focusing on the effects of contaminated sediment on early life stages of white sturgeon.

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