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# Toxicity of Metals Associated with Sediments from the Columbia River to Early Life Stages of White Sturgeon

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## Abstract

In the Upper Columbia River (UCR) between Trail, BC, and Grand Coulee Dam, WA, it has been hypothesized that metals associated with sediments might be contributing to poor recruitment of white sturgeon. Accordingly, the present study employed laboratory flow-through tests to characterize chronic toxicity of UCR sediments to early life stages of white sturgeon (Acipenser transmontanus). Sediments were collected from areas of the UCR known to be sturgeon spawning- and/or nursing-grounds and contained a range of concentrations of metals of primary interest, including copper (Cu), cadmium (Cd), lead (Pb), and zinc (Zn). Early life stage white sturgeon were exposed for 60 days and survival and growth were related to concentrations of metals in bulk sediment, pore water, overlying water, and water at the sediment-water interface. Based on probable effect concentrations (PECs) and excess simultaneously extracted metals (SEMX), site sediments in the present study had the potential to elicit adverse effects to sediment-dwelling organisms. In addition, the Biotic Ligand Model (BLM) was used to allow for more explicit consideration of bioavailability of metals to white sturgeon. BLM predictions indicated that concentrations of Cu in pore water slightly exceeded the threshold for effects (up to a factor of 2.2) for two of the five site sediments, while concentrations of metals in overlying water and at the sediment-water interface were below the threshold for effects. No effects were observed, however, in survival or growth of white sturgeon exposed to site sediments that were relatable to concentrations of metals. Of the methods used to characterize potential effects due to exposure to metals associated with sediments, BLM predictions corresponded best with the observed results.

Keywords: Sturgeon; Columbia River; Metal toxicity; Sediment; Risk assessment

# Introduction

Populations of sturgeon are threatened throughout the world. Factors such as age to reproductive maturity make sturgeons particularly susceptible to alterations in the environment and their benthic lifestyle might result in exposure to contaminants associated with sediments of concern. Alteration of habitats, including pollution, has been hypothesized to be a contributing factor to their global decline [1-6]. Specifically, in some North American rivers it has been hypothesized that metals associated with sediments might be contributing to poor recruitment of white sturgeon (Acipenser transmontanus; [6-8]). One population of particular concern that has been experiencing poor annual recruitment for over forty years resides in the Upper Columbia River (UCR), between Grand Coulee Dam in the USA and Hugh L. Keenleyside Dam in southern BC, Canada [4,7,8]. In 2006, this population was listed as endangered by the Canadian government [9] and it is suspected that without a successful remedial program this population might face extinction within the next half century [4,7,8]. Although specific reasons for the observed decreases in the number of sturgeon are not fully understood, pollution has been hypothesized as one potential contributor to recruitment failure of white sturgeon in the UCR [8].

Municipal and industrial sources of pollution in the UCR include discharges from, among others, municipal wastewater treatment plants, the pulp and paper industry, and metallurgical operations [8]. In Trail, BC, Canada, a metal smelter has been operational for over one hundred years and historically released slag, a by-product of the refining process, into the UCR, but ceased to do so in 1995. Consequently, metals such as copper (Cu), lead (Pb), cadmium (Cd), and zinc (Zn), have been found at concentrations in sediments downstream of the facility that are greater than those in sediments from reference locations [10-13]. In 2006, a remedial investigation and feasibility study (RI/FS) was initiated in the UCR, under the oversight of the US EPA (www.ucr-rifs. com). One of the concerns to be addressed by the RI/FS was potential toxicity of chemicals associated with sediments to early life stages of white sturgeon, including metals. Early life stages of white sturgeon inhabit benthic habitats, on the surface of sediment or in interstitial space between stones, and at the yolksac fry stage tend to hide in refugia ([14-19]; personal observation in the laboratory).

Previous studies have calculated thresholds for effects of aqueous concentrations of metals to white sturgeon and have found early life stages to be equal or more sensitive to effects of certain metals, such as Cu, than early life stages of other sensitive fishes such as rainbow trout (*Oncorhynchus mykiss*; [20-23]. Other studies have investigated

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the release of elements from contaminated sediments in the Columbia River into pore water, overlying water, and supernatants of aggressively tumbled slurries, and found that under certain conditions, there might be exposure to concentrations of metals sufficient to cause adverse effects [24]. Therefore, the current study employed an experimental design with controlled, fluvial, laboratory exposure settings, and water characteristics comparable to conditions found within the UCR stretch of concern, in order to assess UCR sediment toxicity to early life stages of white sturgeon.

The present study was conducted under the oversight of the US EPA (www.ucr-rifs.com), and data obtained from this work will be used to supplement information in a baseline ecological risk assessment (BERA) and as part of a remedial investigation and feasibility study (RI/FS). Results from the present study are presented in two parallel articles. A review of the concentrations of metals in UCR sediments and their associated matrices is the subject of a companion paper [25]. The present article characterizes bioavailability and biological responses. Concentrations of metals in different matrices such as pore water, overlying water, and water at the sediment-water interface were sampled by use of various sampling techniques and quantified to assess bioavailability. Peepers [26], diffusive gradients in thin films (DGTs; [27]), and active sampling/suction techniques were employed throughout the experiment to characterize exposures to metals and chemical parameters in the different matrices. Various risk assessment approaches, including probable effect concentrations (PECs), excess simultaneously extracted metals (SEMX), and the biotic ligand model (BLM) were used to characterize risk to white sturgeon.

# Methods

# Study design

Exposure to and potential effects of UCR sediments to early life stages of white sturgeon were assessed by use of flow-through chambers within re-circulating systems at the University of Saskatchewan Aquatic Toxicology Research Facility (UofS ATRF), Saskatoon, SK, Canada. The experimental methods, including selection of sites from which sediments were collected, efforts, and study design have been described previously [25]. Early life stages of white sturgeon were exposed to UCR sediments in the laboratory from 1 day post hatch (dph) through 60 days to sediments collected from areas of the UCR known to be white sturgeon spawning- and/or nursing-grounds [28,29] and contained a range of concentrations of chemicals of potential concern (COPC) in sediments assumed to contain granulated slag [12,13]. Specifically, the primary COPCs were postulated to include Cd, Cu, Pb, and Zn [12,13]. Sediments were collected using a power VanVeen grab sampler at five locations in the UCR downstream of the metallurgical facility in Trail, BC, Canada: Deadman's Eddy (DE), Northport (NP), Little Dalles (LD), Upper Marcus Flats (UMF), and Lower Marcus Flats (LMF; [25] for map and details), collectively referred to as "site sediments". Sediments from two reference locations were collected from areas upstream of the metallurgical facility at Genelle (GE) and Lower Arrow Lakes (LALL; [25] for map and details). In addition, artificial sediment (Aquarium Substratum Item No. 12648, Rolf C. Hagen Inc., Baie d'Urfe, QC, Canada) and a water only exposure were included as negative controls (termed CTRL and H<sub>2</sub>0, respectively).

Water samples were collected by use of active and passive sampling techniques. Active sampling was conducted via suction by use of high density polyethylene (HDPE) syringes and pipettes in overlying water and at the sediment-water interface, respectively. Overlying water is defined as within the top 15 cm of the water column ( $\sim$  30 cm total

depth), and the sediment-water interface is defined as the boundary between sediment and the overlying water column within 1 cm above the sediment surface. In addition, peepers and DGTs were employed to simultaneously sample water at the sediment-water interface as well as in pore water, 1 cm below the sediment surface. At 2.5 cm below the sediment surface ceramic air-stones (RENA Micro Bubbler 6-in., Mars Inc. Hackettstown, NJ, USA) were distributed along the length of each exposure chamber for additional pore water sampling (Supplemental Materials [25]).

# Fish culture and exposure

Fertilized white sturgeon eggs were obtained from the Kootenay Trout Hatchery, Fort Steele, BC, Canada, and transported to the UofS ATRF where they were incubated until hatch. Transportation and incubation procedures followed the methods described by Vardy et al. [21]. Approximately one week prior to initiation of tests and placement of fish into the exposure systems, periphyton was allowed to grow on substrata to condition the water and provide a grazing environment for sturgeon transitioning to feeding. At approximately 7 dph, food was introduced to the exposure chambers to familiarize white sturgeon larvae with a food scent. Fish were fed a combination of live brine shrimp nauplii (Artemia salina), a semi-moist powder diet containing one part # 0 trout chow, three parts cyclopeze, two parts krill and one part tubifex (Argent Laboratories, Redmond, WA, USA) prior and during the transition to feed stage. In addition, frozen bloodworms (Hagen, San Francisco Bay Brand, Edmonton, AB, Canada) were fed as their primary diet throughout the experiment (Supplemental Materials for nutritional information). Fish were fed ad libitum four to eight times throughout the day and into the evening. Rates of feeding were increased when larvae were transitioning to feeding and fish were fed throughout the night, since this has been shown to be a critical period for survival [21]. Exposure chambers were cleaned twice daily by use of a modified pipette (Supplemental Materials [25]).

Endpoints included survival and growth of white sturgeon reared on site sediments versus reference sediments. During the course of the study, exposure chambers were visually inspected twice daily. If a sturgeon died, it was removed using a disinfected fish net, blotted dry, and wet biomass determined to the nearest 0.001 g. Total length, defined as distance from tip of tail to tip of snout, was measured with calipers to the nearest 0.01 mm, and preserved in 10 % formalin for 24 hrs. At the end of the 24 hr preservation period, the formalin was replaced with 70 % ethanol for long-term storage. At the end of the study, all remaining fish were sampled over a 2-day period, euthanized with Tricaine<sup>®</sup>-S (MS-222), and measured as above.

# **Risk characterization**

Concentrations of metals in sediment and the different matrices associated with sediments, such as pore water, overlying water, and the sediment water interface, were measured to characterize risk using different hazard assessment approaches. The full suite of chemical analyses conducted on sediment and water samples has been previously described [25]. To evaluate potential effects of concentrations of metals in site sediments, PECs, threshold effect concentrations (TECs), and mean probable effect concentration quotients (mPECQs) for metal mixtures were calculated following the methods outlined by MacDonald et al. [30]. In addition, acid volatile sulfide (AVS) and simultaneously extracted metals (SEM) were used to define excess SEM (SEMX; SEMX = SEM – AVS), and carbon-normalized excess SEM (SEMX, organic carbon [OC]; SEMX, OC = SEMX/ fraction of organic carbon in sediment [fOC]; [31,32]). Based on these estimates of bioavailable

metal, benthic organisms should be adequately protected in sediments if SEM does not exceed AVS (SEMX  $\leq$  0) when AVS  $\geq$  0.1 µmol/g. On the basis of SEMX, it has been shown that sediments with SEMX < 1.7 µmol/g pose low risk of adverse biological effects, whereas sediments with SEMX > 120 µmol/g might be expected to cause adverse biological effects [32]. For SEMX between 1.7 and 120 µmol/g, the potential for toxicity is uncertain. Sediments with lesser carbon-normalized SEMX (< 130 µmol/gOC) should pose little risk of adverse biological effects due to SEMs. For sediments with greater carbon-normalized (SEMX > 3000 µmol/gOC) adverse biological effects due to SEMs might be expected. For sediments with intermediate carbon-normalized SEMX (> 130 µmol/gOC and < 3000 µmol/gOC) there is uncertainty about whether effects are expected [32].

# Application of biotic ligand model

The biotic ligand model (BLM; [33-36]) was used to characterize exposure to metals associated with UCR sediments to early life stages of white sturgeon. Input files for application of the BLM were prepared using the analytical dataset from the present study [25], with model simulations utilizing conservative survival-based parameter files as developed from previous studies with early life stages of white sturgeon [21,22]. Predicted effect concentrations resulting from the BLM for Cd, Cu, Pb and Zn were then evaluated against dissolved metal concentrations measured during the study. In a limited number of instances and where appropriate, assumptions and mean values were used to represent input data to the BLM model for a given sample within an exposure chamber. For instance, if concentrations of sulfate and chloride were unavailable within a given sample type as collected within that exposure chamber were used.

A significant number of measured dissolved organic carbon (DOC) concentrations were qualified as estimated due to field duplicate imprecision [25]. Therefore, prior to being used and incorporated as a BLM input file, measured concentrations of DOC were "blank-corrected" to account for this imprecision. Specifically, given that the mean DOC concentration recorded in quality assurance and quality control (QA/QC) samples, such as measurement blanks and laboratory controls (H<sub>2</sub>O) was 1.93 mg/L, DOC concentrations for all exposure chambers and sample types were "blank-corrected" by subtracting 1.93 mg/L from the measured DOC concentrations. To ensure that measured DOC concentrations were not "over-corrected" (e.g., a negative value), "blank-corrected" concentration for UofS ATRF testing waters of 1.50 mg/L.

Survival predicted with the BLM was based on measured survival in white sturgeon toxicity tests [25]. Calibration of these survival endpoints was accomplished by adjusting the critical accumulation of metal at the biotic ligand (BL). To provide the most conservative evaluation, when multiple measures of survival were available, calibration of the BLM was based on the most sensitive observed endpoint (LC<sub>20</sub>). Using this approach, resulting critical accumulation values at the BL for Cd, Cu, Pb and Zn were 2.5 nmol/gw, 0.0042 nmol/ gw, 0.028 nmol/gw, and 1.2 nmol/gw, respectively. Predictions from the BLM model were compared with observed concentrations for each metal in overlying water, water from sediment-water interface, and pore water (1 and 2.5 cm). To aid this comparison, toxic units (TU) were calculated by dividing respective measured metal concentrations by the BLM-predicted effects concentration. For a given exposure chamber, replicate, and type of matrix, the geometric mean of measured concentrations was calculated or estimated by use of the maximum likelihood estimate (MLE) procedure (described previously [25]) and Page 3 of 13

used to calculate TUs. If more than 80 % of measured concentrations were qualified (e.g., being below detection limit [BDL] or estimated) the calculated geometric mean was concomitantly flagged as a "<" value. As detailed in Supplemental Materials, this approach provides an upper bound of the exposure concentration, because the actual value will be less than this estimate.

## Survival and growth of early life stages of white sturgeon

Survival and growth were analyzed to determine if white sturgeon were adversely affected by exposure to UCR sediments, relative to fish exposed to reference sediments. Survival was analyzed to provide estimates of cumulative survival of individual white sturgeon at the end of the study as well as during the course of exposure. Survival analysis considers and accounts for all fish introduced to exposure chambers, including those fish culled or "missing". Size data for fish surviving to test termination were used to quantify the effect of exposure to site sediments on fish size.

#### Survival analyses

The Kaplan-Meier method was used to estimate survival during the course of the study. The Kaplan-Meier method allows for a transparent and consistent treatment of data for all exposure chambers, regardless of whether or not censoring due to lost fish occurred (see Section 3.3 for explanation of lost fish in the present study). The Kaplan-Meier method operates on the number of fish at risk at any given time point, and if a fish is removed for any reason (other than death), it is not counted as a mortality event, but rather, it decreases the number of fish at risk of dying at subsequent time points. Mathematically, the Kaplan-Meier method is a product limit function (Equation 1):

$$\hat{S}(t_{(f)}) = \prod_{i=1}^{J} \hat{\Pr}[T > t_{(i)} / T \ge t_{(i)}]$$
(1)

Where:

 $\hat{S}(t_{(j)})$  = estimated survival probability at time j,

I = index of multiplication,

T = survival time, and

t = time=point of interest.

In practice,  $\hat{S}(t_{(j)})$  can be determined directly from the survival data (Equation 2):

$$\hat{S}(t_{(f)}) = \prod_{i=1}^{j} \left( \frac{n_i - d_i}{n_i} \right)$$
(2)

Where:

 $d_i =$  number of deaths at time *I*,

 $n_i$  = number of organisms at risk of dying at time *i*.

The effect of censoring due to lost fish can be incorporated in Equation 2 by defining  $n_i = n_{i.1} - c_{i.1} - d_{i.1}$ , such that the number of organisms censored (*c*) or died (*d*) at a previous time, time *i*-1, are removed from the risk set of interest, at time *i*. The ability to consider censored observations in the survival probability function provides a direct means of accounting for loss of test specimens, while allowing lost fish to contribute to estimates of survival up to the time at which they were lost from the exposure chamber.

The primary objective of this study was to determine if survival and growth of white sturgeon were adversely affected when fish were reared on site sediments versus reference sediments. As a result, laboratory controls (H<sub>2</sub>O and CTRL exposure chambers) were excluded from statistical analyses of survival. End-of-test (EOT) estimates of survival were then statistically compared to test the null hypothesis of no difference in survivals at EOT among treatment groups. This statistical comparison was accomplished by conducting an analysis of variance (ANOVA with  $\alpha = 0.05$ ). Because of a violation of parametric assumptions, a similar, but non-parametric test was conducted (Kruskal-Wallis with  $\alpha$  = 0.05). Statistical comparisons were conducted on two different permutations of the dataset. In one set of comparisons, GE and LALL sediments (references) were included in the statistical test individually or pooled. In a separate set of comparisons, an extreme value for decreased EOT survival in a UMF replicate (see survival analyses section in results) was either considered or omitted during statistical comparisons between UMF sediments and reference sediments. All data analysis procedures were conducted with R version 2.9.1 [37].

## Length and mass

To evaluate if growth was adversely affected at termination of the study length and mass of white sturgeon reared on site sediments were compared with those of fish reared on reference sediments. As with the survival analyses discussed in the methods section, statistical procedures were conducted with the exclusion of laboratory controls. Length and mass of sturgeon surviving to test termination were observed to vary as a function of number of fish (density) remaining at EOT. Consequently, an analysis of covariance (ANCOVA) was performed to assess the effect of site sediments on growth endpoints. An ANCOVA ( $\alpha = 0.05$ ) was conducted with each reference site separately and with pooled references. Additional analyses were conducted with greater specificity to the secondary hypotheses, which was to determine whether fish exposed to site sediments were smaller than fish exposed to reference sediments. The approach initially taken was to conduct a nested ANOVA followed by a Dunnett's test to determine which values were significantly smaller than reference values. Results from the various statistical analyses were effectively the same, so only the ANCOVA results are described herein.

Physical and chemical characteristics of sediments were examined to explore potential causal explanations for the statistically smaller fish observed in UMF exposure chambers (see length and mass section in results). Single variable correlation analyses were performed to identify relationships between size of fish and chemical characteristics of sediments. A multiple factor analysis [38] was conducted in an attempt to identify discriminators, such as sediment particle size, clay and sand content of sediment, and water quality characteristics in exposure chambers, that could categorize fish by size.

# **Results and Discussion**

Total concentrations of acid-extractable metals of concern, Cu, Cd, Pb and Zn from site sediments spanned the spectrum of concentrations observed to date within the site, and often exceeded the 90<sup>th</sup> centile of previously reported data [12,13], whereas concentrations within reference sediments were statistically less (p < 0.001), generally less than the 10<sup>th</sup> centile of site sediments [25].

## **Toxicity of sediments**

There are several significant limitations to determining toxicity of metals in sediments and to date, there is no single, definitive method for deriving sediment quality guidelines (SQGs) or assessing risks posed by metals associated with sediments that is without limitations [39]. Empirical approaches utilize large co-occurrence databases to

statistically compare chemical concentrations and biological effects under field and laboratory conditions, whereas mechanistic approaches are theoretically based and designed to predict sediment toxicity based on an understanding of the chemical and variables that influence toxicity [39]. Consensus based approaches combine previously established guidelines that used different methods of derivations but produced similar results and generate new threshold values from their central tendencies [39]. One major uncertainty in assessing potential effects of metals is bioavailability [40]. In the present study, maximum acidextractable concentrations of metals in site sediments ranged from 712 - 3180 mg Cu/Kg DW, 1.18 - 3.56 mg Cd/kg DW, 5060 - 25600 mg Zn/ kg DW, and 254 - 3410 mg Pb/kg DW. Following the consensus-based approach outlined by MacDonald et al. [30], concentrations of metals in site sediments consistently exceeded respective TECs of 31.6 mg Cu/ Kg DW, 0.99 mg Cd/kg DW, 121 mg Zn/kg DW, and 35.8 mg Pb/kg DW, and PECs of 149 mg Cu/kg DW, 4.98 mg Cd/kg DW, 459 mg Zn/kg DW,



Figure 1: Mean probable effect concentration quotients (mPECQs) calculated for the four primary metals of interest, copper, cadmium, lead, and zinc (top panel), and for the eight metals commonly calculated to express potential effects for mixtures of metals in sediments, arsenic, cadmium, chromium, copper, lead, mercury, nickel, and zinc (bottom panel; [30]). Treatments included artificial sediment (CTRL), reference sediments from Lower Arrow Lake (LALL) and Genelle (GE), and site sediments from Deadman's Eddy (DE), Northport (NP), Little Dallas (LD), Upper Marcus Flats (LMF) and Lower Marcus Flats (LMF).

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and 128 mg Pb/kg DW, for the four primary metals of concern, except Cd, where most of the concentrations in site sediments ranged between the threshold and probable effect concentrations (Figure 1) [25]. Based on comparisons of measured concentrations of acid-extractable metals to the TECs, exposure to site sediments from the studied region of the UCR was predicted to result in adverse effects. However, these PECs were developed to classify sediments of potential toxicity to infaunal organisms, especially benthic invertebrates and especially insects [30], and are not necessarily developed to evaluate potential effects on demersal fishes such as white sturgeon. Furthermore, calculations are typically based on total concentrations of acid-extractable contaminants in sediment [41] and variations in speciation and bioavailability are not normally incorporated and instead are only assumed to be accounted for through the use of large and diverse sample sets. Hence, for the present study, TEC and PEC concentrations are included to qualitatively assess

the range and gradient of potential effects.

Similarly, to qualitatively assess potential effects of mixtures of metals at each sampling area, a mean PEC quotient (mPECQ) was calculated for each exposure chamber for the four primary metals of interest, and for the eight metals, arsenic (As), Cd, chromium (Cr), Cu, Pb, mercury (Hg), nickel (Ni), and Zn commonly calculated to express potential effects for mixtures of metals in sediments [30]. The arithmetic mean mPECQ was then calculated for each sampling area from the respective replicate exposure chambers. For the four study-specific primary metals of interest mean mPECQ for site locations ranged from a minimum of 4 to a maximum of 17, whereas reference locations and controls had mPECQ values of approximately 0.04 (Figure 1). Considering all eight metals, mean mPECQ values for site locations ranged from a minimum of 2 to a maximum of 9, whereas reference locations and controls were approximately 0.04 (Figure 1). Following EPA methods of evaluation,



benchmark approach [32]. Based on these estimates of bioavailable metal, benthic organisms should be adequately protected in sediments if SEM does not exceed AVS (SEMX  $\leq$  0) when AVS  $\geq$  0.1 µmol/g (denoted by a horizontal solid line in top panel). On the basis of SEMX, it has been shown that sediments with SEMX < 1.7 µmol/g (denoted by a horizontal dashed line in bottom panel) pose low risk of adverse biological effects, whereas sediments with SEMX > 120 µmol/g (denoted by a horizontal solid line in bottom panel) pose low risk of adverse biological effects, whereas sediments with SEMX > 120 µmol/g (denoted by a horizontal solid line in bottom panel) pose low risk of adverse biological effects (SEMX between 1.7 and 120 µmol/g, the potential for toxicity is uncertain. Treatments included artificial sediment (CTRL), reference sediments from Lower Arrow Lake (LALL) and Genelle (GE), and site sediments from Deadman's Eddy (DE), Northport (NP), Little Dallas (LD), Upper Marcus Flats (UMF) and Lower Marcus Flats (LMF).

based on mPECQs [42], adverse effects would be predicted for benthicdwelling organisms following exposure to site sediments. However, caution should be taken when interpreting these predictions because they might not be fully applicable to benthic fish such as sturgeon. In addition, the mPECQ method utilizes previously derived empirical sediment quality guidelines to calculate consensus-based probable effect concentrations, and inherently incorporates their limitations [39]. Issues with bioavailability, potential effects of co-occurring contaminants on individual effect concentrations, and the possibility of statistically diluting the effects of dominant toxicants when calculating mean quotients, are limitations worth considering [39]. In the present study, mPECQ values for the four primary metals were greater than values calculated for all eight metals because PECQ's for As, Cr, Hg and Ni were typically smaller than for Zn, Cu, Pb and Cd and due to the calculation where sums of PECQs were divided by eight rather than four.

Mechanistic approaches to assessing risk of contaminants associated with sediments consider differences in bioavailability through equilibrium partitioning (EqP) in the interstitial water [41]. In the present study, AVS and SEM were measured to characterize the potential toxicity of sediments contaminated with metals as part of the equilibrium sediment partitioning benchmark approach [32].

AVS and SEM are used to define (SEMX and (SEMX, and OC; (see risk characterization section in methods for details of calculations and interpretations). AVS concentrations for site sediments associated with the present study were > 0.1  $\mu$ mol/g, while AVS concentrations for reference and control sediments were < 0.1  $\mu$ mol/g (Figure 2). When SEMX was calculated for site sediments from the present study values were found to be within the range of uncertainty (SEMX between 1.7 and 120  $\mu$ mol/gd; Figure 2). As a result, these samples were further evaluated by incorporating carbon-normalization (Figure 3). Site sediments evaluated in the present study had relatively great carbon-

normalized excess SEM (SEMX > 3000  $\mu$ mol/gOC for all locations). Deadman's Eddy was found to have the greatest mean SEMX, OC value (~ 50,000  $\mu$ mol/g), while Lower Marcus Flats had the least (~ 6,000  $\mu$ mol/g). Based on concentrations of SEMX and OC, all site sediment areas would be expected to elicit adverse effects on sediment-dwelling organisms. However, the SEM-AVS approach also has limitations [39]. The dynamic nature of sediment influences redox status and achievement of thermodynamic equilibrium between metals and pore water, which is a major assumption of the EqP approach, and its applicability to accurate assessment of bioavailability for certain metals, such as Cu, has been questioned [39,43-45]. As with all environmental assessments, the deficiencies of the methods should be evaluated and considered during risk characterization and considered in a multiple lines of evidence approach.

# Application of biotic ligand model

Application of the BLM resulted in 5,632 different BLM predictions for each primary metal of interest namely Cd, Cu, Pb and Zn. Toxic units were calculated for each of these primary metals of interest in overlying water, water at the sediment water interface, and pore water at 1 and 2 cm depths. Cu was the only metal that had calculated TUs greater than 1.0, in pore waters for DE substrata and LD sediments (Figures 4-7). TUs for Cu in pore water collected at 2.5 cm from DE exposure chambers ranged from 1.9 to 2.2. However, DE substrata were collected above the high water line [25], and differences in concentrations of metals between materials collected within the water versus above is not known. Exposure chambers containing LD sediments also had calculated TUs close to and in some replicates slightly in excess of 1.0 for Cu in pore waters at depths of 1 and 2.5 cm.

In the present study, TUs represent a ratio between measured concentrations of metals in a given sample and the concentration predicted by the BLM. Metal-specific BLM-predicted effect



Figure 5: Carbon-hormalized excess simulateously extracted (SEMX) metals for the write sturgeon sediment toxicity tests. A sediment with ow carbon-hormalized SEMX < 130 µmol/gOC (denoted by horizontal dashed line) should pose low risk of adverse biological effects due to SEMs (i.e., cadmium, copper, lead, or zinc). For sediments with high carbon-normalized SEMX >3000 µmol/gOC (denoted by a horizontal solid line), adverse biological effects due to SEMs (i.e., cadmium, copper, lead, or zinc). For sediments with intermediate carbon-normalized SEMX >130 µmol/gOC and <3000 µmol/gOC there is considerable uncertainty about whether effects are expected [32]. Treatments included artificial sediment (CTRL), reference sediments from Lower Arrow Lake (LALL) and Genelle (GE), and site sediments from Deadman's Eddy (DE), Northport (NP), Little Dallas (LD), Upper Marcus Flats (UMF) and Lower Marcus Flats (LMF).

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**Overlying Water** 10<sup>0</sup> 10-10<sup>-2</sup> 10-3 NP UMF H<sub>2</sub>O CTRL LALL GE DE LD LMF Sediment - Water Interface 10<sup>0</sup> 10<sup>-1</sup> 10<sup>-2</sup> 10<sup>-3</sup> H2O CTRL LALL GE DE NP LD UMF LMF Porewater (1 cm) 10<sup>0</sup> 10<sup>-1</sup> 10<sup>-2</sup> 10<sup>-3</sup> H2O CTRL LALL GE DE NP LD UMF LMF Porewater (2.5 cm) 10<sup>0</sup> 10 10<sup>-2</sup>  $10^{-3}$ UMF H2O CTRL GE DE NP LD LMF LALL

**Figure 4:** Geometric mean toxic units (TUs) for dissolved copper as a function of treatment and sample type. If  $\geq$  80% of measure concentrations were qualified (i.e., censored) maximum likelihood estimate procedures [25] were used to derive geometric mean TUs. These calculations are identified with a less than symbol ("<") positioned above respective columns. Sample types (e.g., porewater) are identified in the top left-hand corner of each plot, with replicate exposure chambers illustrated with colored bars. The dashed line represents a TU of 1. Treatments included negative controls with water only (H2O) and artificial sediment (CTRL), reference sediments from Lower Arrow Lake (LALL) and Genelle (GE), and site sediments from Deadman's Eddy (DE), Northport (NP), Little Dallas (LD), Upper Marcus Flats (UMF) and Lower Marcus Flats (LMF).

concentrations were developed using the least observed effect concentration  $(LC_{20})$  for survival of white sturgeon, and were based on concentrations of each metal of interest associated with a 20 % reduction in survival. Given this ratio and approach, if the calculated toxic unit is > 1.0, a 20 % decrease in survival might be expected. However, interpretation of single point-estimates of pore water TUs over the duration of the exposure is not as straightforward because for Cu, Cd, and Zn the BLM was calibrated with threshold values derived from chronic studies to estimate continuous concentrations associated with a 20 % reduction in survival. Therefore, these calculations likely

represent a worst-case scenario. Nevertheless, this method provides a means of assessing risk of exposure to metals associated with UCR sediments that is more specific and applicable to early life stages of white sturgeon compared to the previous methods, such as PECs and SEMs that typically focus on effects on sediment dwelling invertebrates.

# Survival of white sturgeon

The target seeding density was 100 white sturgeon fry per exposure chamber. However, due to estimating the number of lost/escaping sturgeon observed at the beginning of the study, there was variability

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**Figure 5**: Geometric mean toxic units (TUs) for dissolved zinc as a function of treatment and sample type. If  $\ge$  80% of measure concentrations were qualified (i.e., censored) maximum likelihood estimate procedures [25] were used to derive geometric mean TUs. These calculations are identified with a less than symbol ("<") positioned above respective columns. Sample types (e.g., porewater) are identified in the top left-hand corner of each plot, with replicate exposure chambers illustrated with colored bars. The dashed line represents a TU of 1. Treatments included negative controls with water only (H2O) and artificial sediment (CTRL), reference sediments from Lower Arrow Lake (LALL) and Genelle (GE), and site sediments from Deadman's Eddy (DE), Northport (NP), Little Dallas (LD), Upper Marcus Flats (LMF).

in actual seeding densities (Supplemental Material). During the initial period of exposure, sturgeon fry were observed to escape through small cracks in seals located near the outflow of exposure chambers. Within 48 hrs of test initiation, the seals were fixed and lost/escaping sturgeon replaced to the desired initial density of stocking. Obtaining an accurate tally of lost/escaping fish, however, was difficult due to an uncertainty in the number of sturgeon fry completely flushed from exposure chambers (beyond the posterior chamber; refer to Supplemental Material in [25] for a description of exposure chamber design). It was acknowledged that the re-seeding efforts might have resulted in differences in starting densities, but that these differences could be accounted for at the end of the study. As a result this did not adversely affect data quality or study

objectives. Seeding densities were calculated by summing the number of mortalities recorded for the duration of the study, the estimated number of lost/escaping fish, and the number of surviving fish at the end of the study.

Routine cleaning operations of exposure chambers UMF-D (Day 22) and CTRL-D (Day 23) resulted in a significant loss of sturgeon fry due to breaking of seals at the outflow (see validation assessment -overall data quality section in methods in [25]). As a result, these chambers were subsequently designated as "chemistry only" and were therefore not considered in the following analysis of biological data.



**Figure 6:** Geometric mean toxic units (TUs) for dissolved cadmium as a function of treatment and sample type. If  $\geq$  80% of measure concentrations were qualified (i.e., censored) maximum likelihood estimate procedures [25] were used to derive geometric mean TUs. These calculations are identified with a less than symbol ("<") positioned above respective columns. Sample types (e.g., porewater) are identified in the top left-hand corner of each plot, with replicate exposure chambers illustrated with colored bars. The dashed line represents a TU of 1. Treatments included negative controls with water only (H2O) and artificial sediment (CTRL), reference sediments from Lower Arrow Lake (LALL) and Genelle (GE), and site sediments from Deadman's Eddy (DE), Northport (NP), Little Dallas (LD), Upper Marcus Flats (UMF) and Lower Marcus Flats (LMF).

# Survival analyses

To minimize effects of differences in stocking densities among exposure chambers, the Kaplan-Meier method of survival analyses see survival and growth of early life stages of white sturgeon section in methods produced an EOT survival estimate that accounted for all fish introduced into exposure chambers at the onset of the study (Figure 8) [46]. Survival curves were consistent among replicate exposure chambers with the greatest mortalities occurring within a narrow window between 18 and 24-dph. This window coincides with the transitioning of fish to exogenous feeding and is recognized as a sensitive period of white sturgeon early life stage development [21,46]. Despite somewhat greater mortality during a certain window of time, overall rates of survival among exposure chambers were greater than 80%, which is in accordance with ASTM guidelines for chronic toxicity tests with early life stage fish [47]. Exceptions were limited to two exposure chambers, a laboratory control and a site sediment. Although these two exposure treatments are identified as having the poorest overall rates of survival (CTRL 68% survival and UMF 72%), in both instances, this was attributable to a single replicate exposure chamber (CTRL-A 17% survival and UMF-F 45% survival; see Supplemental Materials). In addition to the overall survival estimates, a complete

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Figure 7: Geometric mean toxic units (TUs) for dissolved lead as a function of treatment and sample type. If  $\geq$  80% of measure concentrations were qualified (i.e., censored) maximum likelihood estimate procedures [25] were used to derive geometric mean TUs. These calculations are identified with a less than symbol ("<") positioned above respective columns. Sample types (e.g., porewater) are identified in the top left-hand corner of each plot, with replicate exposure chambers illustrated with colored bars. The dashed line represents a TU of 1. Treatments included negative controls with water only (H2O) and artificial sediment (CTRL), reference sediments from Lower Arrow Lake (LALL) and Genelle (GE), and site sediments from Deadman's Eddy (DE), Northport (NP), Little Dallas (LD), Upper Marcus Flats (UMF) and Lower Marcus Flats (LMF).



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presentation of survival curves for each respective exposure chamber is provided within Supplemental Materials.

No statistically significant differences in survival between any of the site or reference sediment exposures were observed in any of the permutations of the statistical analysis (see survival and growth of early life stages of white sturgeon section in methods), with either ANOVA or Kruskal-Wallis (Supplemental Material). This conclusion is robust since it is supported by both the parametric as well as the less powerful nonparametric statistical tests. Therefore, in the present study, survival of white sturgeon was not adversely affected when reared on site sediments versus reference sediments.

## Length and mass

In addition to survival, more chronic sub-lethal effects on length and mass of white sturgeon were summarized on the basis of sediment type and exposure tank using box-and-whisker plots (Supplemental Material). The overall mean mass and length of white sturgeon at EOT was approximately 0.5 g and 48 mm across all exposure chambers. Sizes in some treatments were very consistent among replicate exposure chambers, including H<sub>2</sub>O and LALL, with greater variability in others, such as GE, LD, and UMF. Consistent with results based on survival, a laboratory control was again observed as the poorest performer. However, unlike the survival analyses, where only one replicate exposure chamber performed poorly, each replicate exposure chambers for the H<sub>2</sub>O treatment consistently had the smallest fish with a mean length and mass of 46 mm of 0.48 g, respectively. This trend might have occurred because in laboratory exposure systems fish might perform better when housed in exposure chambers that contain natural substrata containing greater amounts of nutrients and or organics such as periphyton upon which to feed. This has been reported previously for white sturgeon exposed to industrial effluents [46].

Length and mass of white sturgeon surviving until termination of the test varied as a function of fish density remaining at EOT. ANCOVA was performed to assess the effect of sediment type on length and mass of white sturgeon. For the ANCOVA, it was assumed that the slope of the relationship between length or mass and the number of fish surviving at EOT was equivalent for each sediment type. This assumption was unavoidable because of the imbalance in the number of replicate exposure chambers; with only two-replicate NP exposure chambers the slope would have been strongly positive when a reasonable slope is either zero or negative. This version of an ANCOVA used one overall slope, with an intercept for each source of sediment. Statistical comparison was based on a comparison of intercepts with a control or reference condition (Supplemental Material). This relationship evaluates the assumption that size of white sturgeon in exposure chambers was density dependent. With only one exception, number of white sturgeon within exposure chambers explained all of the variation observed in size (length and mass). The intercepts from the relationship of either length or mass relative to number of fish surviving were not significantly different than the intercept for reference locations. The exception was a small but statistically significant smaller size of white sturgeon that could not be completely attributed to number of fish in the UMF treatment (ANCOVA p-values of 0.005 and 0.0039 for mass and length, respectively).

Potential causal explanations for the statistically smaller white sturgeon observed in UMF exposure chambers were explored by examining physical and chemical characteristics of sediments. In an attempt to identify relationships between size of white sturgeon and various chemical characteristics of sediments, single variable correlation analyses were performed. No strong positive correlations between length or mass of white sturgeon and concentrations of individual metals were observed. In addition, a multiple factor analysis was conducted to identify discriminators that could categorize white sturgeon fry as small, medium, or large. The multi-component discriminant analysis failed to provide descriptors that were able to predict size categories associated with the various sediments examined in this study. The difficulty in attempting to identify sediment-specific chemical or physical characteristics that can explain differences observed in size of white sturgeon might be due to the relatively small magnitude of differences in size of fish ( $\leq 1$  mm). A relationship between masses of white sturgeon at test termination and number of fish at test termination explained 57 % of the variability. In the case of length, 53 % of the variability in size of white sturgeon was described by number of fish surviving.

# Conclusions

A lines of evidence (LoE) approach applied several theoretical methods to predict empirical methods and measure effects of sediments from the UCR on survival and growth of early life stages of white sturgeon. Sediments used in the present study covered a range of concentrations of targeted COPC that were representative and consistent of the range of concentrations observed in site sediments from the UCR, and captured the upper concentration range of previously reported data. Based on acid-extractable concentrations of metals, the calculated probably effect concentrations, and mean probable effect concentration quotients for metal mixtures, exposure to site sediments in the present study were predicted to have potential to result in adverse effects to benthic dwelling organisms. Similarly, based on concentrations of acidextractable metals with principles of bioavailability, such as excess SEM, site sediments in the present study were predicted to have potential to elicit adverse biological effects due to SEMs, Cu, Cd, Pb or Zn. However, these sediment toxicity assessments, which classify sediments based on potential toxicity to infaunal organisms in sediment, such as invertebrates, and might not be directly applicable when assessing risk to a benthic fish such as white sturgeon. As a result, concentrations of metals in pore water and overlying water within exposure chambers were analyzed using the BLM to allow for more explicit consideration of bioavailability to white sturgeon, based on metal accumulated at the gill (the biotic ligand) as the basis for predicting biological effects. On the basis of calculated individual TUs, under the laboratory conditions evaluated for this study, Cu was the only metal for which TUs were > 1.0 for a limited number of site sediments and replicate exposure chambers.

There was, however, a lack of concordance between the predicted potential for adverse effects and measured effects on survival and growth of white sturgeon. Survival of white sturgeon was not adversely affected by exposure to site sediments. The lack of observed effect may indicate that sturgeon were not exposed to sediment pore water. Concentrations of all metals in the overlying water and at the sediment water interface were less than BLM predicted threshold values for effects, and these matrices were more likely to accurately characterize exposure of sturgeon to metals. This result would also be consistent with changes in concentrations of metals in pore water with depth, since elevated concentrations of metal observed at 2.5 cm depth were greater than those observed at 1 cm depth, and it is unlikely that a benthic dwelling fish such as sturgeon would be exposed to pore waters from sediments well below the sediment-water interface. There were statistically significant differences in growth of white sturgeon; however, these differences were largely explained by numbers of white sturgeon fry in the chamber. The smallest recorded length and mass of white sturgeon

were consistently in a laboratory negative control ( $H_2O$ ). Differences in length and mass were not associated to any of the measured chemical characteristics including concentrations of individual metals.

The sturgeon-bioassay presented in the current study avoided many of the limitations of the theoretical approaches based on both equilibrium partitioning and comparison to global sediment quality criteria. The bioassay approach considers all of the potential toxicants, including those that might not have been identified and controls for the biologically available fraction. Thus, greater weight should be given to the bioassay results than the predicted effects. In conclusion, while some of the theoretical methods indicated the potential for effects, these adverse effects were not realized in the dynamic, flow-through system applied here. Based on the result of this study, it is unlikely that exposure to UCR sediments is directly affecting survival or growth of white sturgeon.

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