# THE EFFECTS OF METAL CONTAMINATION ON LARVAL WHITE STURGEON IN THE UPPER COLUMBIA RIVER

---

A Dissertation

presented to

the Faculty of the Graduate School

at the University of Missouri

---

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

---

by

HOLLY JANE PUGLIS

Dr. Edward E. Little, Dissertation Advisor

MAY 2018

The undersigned, appointed by the dean of the Graduate School,

have examined the dissertation entitled

THE EFFECTS OF METAL CONTAMINATION ON LARVAL WHITE STURGEON IN THE UPPER COLUMBIA RIVER

Presented by Holly J. Puglis,

a candidate for the degree of

Doctor of Philosophy,

and hereby certify that, in their opinion, it is worthy of acceptance.

_____

Professor Edward E. Little

_____

Professor Raymond D. Semlitsch

_____

Professor Johannes Schul

_____

Professor Craig P. Paukert

Dedicated to Thomas and Maxine Puglis.

# ACKNOWLEDGEMENTS

I have to start by thanking my advisor, Dr. Ed Little. Without Ed's encouragement, I would not have gone back to school. I learned a lot about behavioral toxicology under Ed's tutelage and he helped remind me to keep a work-life balance, which was critical during this process. I am also incredibly grateful for all of my colleagues at the Columbia Environmental Research Center, where I worked full time throughout my doctoral program. The entire crew there has been so incredibly supportive and were critical to completing this research. In particular, I have to thank the Conservation Ecology group: Robin Calfee, Bethany Kunz, Nick Fisher, Jon Davis, Sammi Tidwell, Taylor Tidwell, Erinn Scott, and Liz Brothers. Whether it was watching countless videos of fish swimming in circles, setting up diluters, or measuring individual worm segments, they were up for anything. I also need to thank the Toxicology Branch (Chris Ingersoll, Ning Wang, John Besser, Rebecca Dorman, Doug Hardesty, Eric Brunson, and Chris Ivey) for helping me keep the water on, the diluters running, and for answering countless questions. The Environmental Chemistry Branch at CERC is amazing and I am so thankful for their help in analyzing all those samples.

I am also thankful for everyone at the University of Missouri for their support. I am especially indebted to Ray Semlitsch who adopted me into his lab while I was on campus, always had time to answer my questions, and really helped me be a better scientist. I also need to thank Ray's students (Tom Anderson, Britt Ousterhout, Katie O'Donnell, Freya Rowland, Tom Luhring, Bill Peterman, Jake Burkhart, Arianne Messerman, Mark Mackey, Dana Drake, Grant Connette, and Rachelle Riegerix) who

welcomed me into the lab and were a constant source of support and friendship. Additionally, I need to thank my committee members (Ray, Craig Paukert, and Johannes Schul) for your thought provoking questions and guidance, which greatly improved my work. Many thanks to Nila Emerich who kept me on track, fed me on chocolate, and knew when I needed a hug.

Last but certainly not least, I need to thank my friends and family. Thank you to Monica, Jess, and Kevin for your friendship, which has kept me sane and reminded me to laugh. My siblings, Betsy, Pam, Katy, Sarah, Tom, and Alex, my Aunt MaryAnn and Uncle Jim, and my in-laws, Debby and Greg, thank you for your unwavering support and love. I also am incredibly thankful for my husband, Ryan. Thank you for your love, friendship, and support. Without you, this wouldn't have been possible. Thank you to my children, Arthur and Violet, for your sweet little hugs and reminding me of what is important. And finally, I need to thank my parents, Tom and Maxine, who were so very excited and proud that I was going back to school, for always being in my corner and encouraging me to never stop learning.

## TABLE OF CONTENTS

## LIST OF TABLES

**Chapter 2.**



**Chapter 3.**

## LIST OF FIGURES

**Chapter 2.**



**Chapter 3.**



**Chapter 4.**

THE EFFECTS OF METAL CONTAMINATION ON LARVAL WHITE STURGEON IN THE UPPER COLUMBIA RIVER


## Abstract

Sturgeon populations are imperiled worldwide. Declines are attributed to many factors including habitat degradation and alteration, overexploitation, and contamination. In North America, white sturgeon (*Acipenser transmontanus*) populations in the upper Columbia River (UCR) face many of the same factors. White sturgeon in this system have been declining for decades due to a lack of recruitment to the population, despite evidence of spawning and early larval survival in the wild. Early life stage white sturgeon are among the most sensitive of aquatic species to copper. The UCR has been contaminated with metal laden slag and liquid effluents from smelter and mining activities, resulting in UCR sediment metal concentrations, including copper, being elevated. The goal of my dissertation was to understand the potential role of metal contamination in the decline of white sturgeon in the UCR. This effort included toxicity tests with copper contaminated water as well as sediments collected from the URC, with a focus on the behavioral responses of exposed fish as a consequence of sublethal exposures.

In chapter 1, I exposed early life stage white sturgeon to sublethal concentrations of copper, one of the contaminants of concern in the UCR, to characterize and quantify the effects of copper on swimming and feeding behavior. I found that changes in larval sturgeon swimming behavior were apparent up to seven days earlier than mortality and that copper exposure reduced food consumption in juvenile sturgeon. Critical swimming

performance, however, was not affected by copper exposure. While not directly lethal, these alterations in behavior would impair a sturgeon's ability to locate, capture, and consume prey, thus impacting survival.

In chapter 2, I evaluated the toxicity of metal contaminated sediments from the UCR to larval white sturgeon. Sediment was collected from six sites in the transboundary reach of the UCR. All six sites had elevated metal concentrations above equilibrium partitioning sediment benchmarks, which would suggest the metals could pose a risk to benthic invertebrates. The overlying water metal concentrations were also above water quality criteria levels in three of the six sites. I found sediment at one site reduced survival and affected swimming behavior of larval sturgeon.

In chapter 3, I provide a synthesis evaluation of white sturgeon life history with what has been reported about metal contamination in the UCR, including documented concentrations from the river, and what we know about how metals affect larval sturgeon behavior and survival. Of particular concern are reported field observations of large numbers of sturgeon larvae with empty guts at a critical point in their life cycle. By putting all these pieces together, I found that metal contamination in the UCR could be reducing the prey base of larval sturgeon, altering swimming behavior to increase likelihood of starvation or predation, and evidence of an additional exposure route when larval sturgeon ingest sediment.

Thus, the most important findings from my dissertation include 1) low, environmentally relevant concentrations of copper alter swimming behavior of larval sturgeon indicative of sublethal injury, 2) sediments collected from the UCR can affect larval sturgeon survival and swimming behavior, and 3) metal concentrations measured at

some sites in the UCR are above effect concentrations from laboratory studies. These findings suggest that metal contamination could be a factor in the decline of the upper Columbia River white sturgeon, and should be considered in recovery efforts for the population.

# CHAPTER 1

## UPPER COLUMBIA RIVER WHITE STURGEON

Holly J. Puglis

## Introduction

Sturgeon populations are imperiled worldwide. Five of the nine sturgeon species in North America are classified as at least Vulnerable on the International Union for Conservation of Nature Redlist. Some White Sturgeon subpopulations, such as the upper Columbia River (UCR) and Kootenai River white sturgeon, are further threatened. The factors threatening all sturgeon populations in North America include habitat loss/degradation, overexploitation, and barriers restricting access to spawning areas. Sturgeon are long-lived fish that are slow to reach sexual maturity and do not typically spawn annually, increasing their vulnerability for recruitment failures. For some species of sturgeon, contamination also plays a role in their population declines (Haxton et al. 2016).

The UCR, the segment of the Columbia River above Grand Coulee Dam and below Hugh Keenleyside Dam, is home to a population of white sturgeon that has been declining due to a lack of natural recruitment for decades. There is substantial evidence of spawning and early larvae survival, but efforts to catch young of the year have so far failed, and no wild spawned sub-adults have been caught. To prevent a collapse of this economically and culturally important fishery, the population is currently being sustained with hatchery released fish (Hildebrand and Parsley 2013). As is often the case, multiple factors are likely impacting this population of fish, including contamination. Over the course of decades, more than 12 million tons of slag, a glassy material containing metals, and liquid effluents were discharged into the UCR from a zinc-lead smelting operation just above the international border. This slag contains a high concentration of heavy

metals, including copper, and can be released to the aquatic environment (Paulson et al. 2006, Paulson and Cox 2007), thus making the metals available to the biota.

In order to effectively manage the UCR white sturgeon population and its recovery, the causes of the decline must be well understood. Until recently, little was known about the sensitivity of white sturgeon to heavy metals. However, in the last five years researchers have demonstrated that white sturgeon are among the most sensitive of aquatic organisms to copper (Vardy et al. 2013, Calfee et al. 2014, Wang et al. 2014). These studies have well characterized the concentrations of copper that are lethal to white sturgeon at various ages, but sublethal effects of metals to white sturgeon remain poorly understood. Behavioral impairments arising from sublethal exposure to contaminants can result in reduced survival, viability, and reproduction. Thus behavioral changes can provide early indications of toxicity and injury. Evaluating such behavioral changes can provide resource managers with information needed to determine protective guidelines for restoring sturgeon habitat in the UCR.

The primary objectives of my dissertation were to i) characterize the behavioral effects of copper on white sturgeon larvae and ii) to understand whether metal contamination in the UCR could be a causal factor in the decline of the UCR white sturgeon population, and iii) enhance white sturgeon conservation efforts through toxicological and behavioral assessment of metals exposure. I also wanted to address the gaps in our knowledge on sublethal effects of copper on sturgeon behavior and understanding life history vulnerabilities of white sturgeon relative to field contamination. To meet these objectives, I conducted a series of laboratory exposures to copper and sediments using white sturgeon. I confirmed metal concentrations, measured

behavioral changes, and recorded mortality. I hypothesized that sublethal exposures to copper would cause deleterious behavioral changes in white sturgeon larvae. In Chapter 2, I sought to understand the impacts of sublethal copper exposure by characterizing and quantifying the effect of copper exposure on larval white sturgeon swimming and feeding activity. In Chapter 3, I investigated the toxicity of site collected UCR sediment to larval white sturgeon to verify the toxicity of sediment bound metals. In Chapter 4, I combined our current understanding of the effects of copper on white sturgeon with documented copper concentrations in the UCR and field observations of sturgeon larvae to explore how contamination could impact white sturgeon larvae in the UCR. These investigations will be used to define safe, "no-effect" concentrations for white sturgeon in the UCR, which will be used by federal and state regulatory and management agencies during restoration efforts.

## Literature Cited


Calfee, R. D., E. E. Little, H. J. Puglis, E. Scott, W. G. Brumbaugh, and C. A. Mebane. 2014. Acute sensitivity of white sturgeon (*Acipenser transmontanus*) and rainbow trout (*Oncorhynchus mykiss*) to copper, cadmium, or zinc in water-only laboratory exposures. Environmental Toxicology and Chemistry **33**:2259-2272.

Haxton, T. J., K. Sulak, and L. Hildebrand. 2016. Status of scientific knowledge of North American sturgeon. Journal of Applied Ichthyology **32**:5-10.

Hildebrand, L. R., and M. Parsley. 2013. Upper Columbia White Sturgeon Recovery Plan -2012 Revision. Prepared for the Upper Columbia White Sturgeon Recovery Initiative.

Paulson, A. J., and S. E. Cox. 2007. Release of elements to natural water from sediments of Lake Roosevelt, Washington, USA. Environmental Toxicology and Chemistry **26**:2550-2559.

Paulson, A. J., R. J. Wagner, R. F. Sanzolone, and S. E. Cox. 2006. Concentrations of elements in sediments and selective fractions of sediments, and in natural waters in contact with sediments from Lake Roosevelt, Washington, September 2004. 2331-1258.

Vardy, D. W., J. Oellers, J. A. Doering, H. Hollert, J. P. Giesy, and M. Hecker. 2013. Sensitivity of early life stages of white sturgeon, rainbow trout, and fathead minnow to copper. Ecotoxicology **22**:139-147.

Wang, N., C. G. Ingersoll, R. A. Dorman, W. G. Brumbaugh, C. A. Mebane, J. L. Kunz, and D. K. Hardesty. 2014. Chronic sensitivity of white strugeon (*Acipenser transmontanus*) and rainbow trout (*Oncorhynchus mykiss*) to cadmium, copper, lead, or zinc in laboratory water-only exposures. Environmental Toxicology and Chemistry **33**:2246-2258.

# CHAPTER 2

## BEHAVIORAL EFFECTS OF COPPER ON LARVAL WHITE STURGEON

Holly J. Puglis

**Abstract:** Early life stage white sturgeon are sensitive to copper with adverse behavioral impairments observed during previous studies. The objectives of this study were to quantify behavioral responses of white sturgeon after exposure to copper and determine when the behavioral changes occur. Larval sturgeon (1-2, 28 or 35 days post hatch) were exposed to copper (0.5-8 µg/L) for 4-14 days. Abnormal behavioral changes, including loss of equilibrium and immobilization, were observed within the first few days of exposure. Digital video tracking software was used to analyze spontaneous swimming activity and analyses showed distance moved, time spent swimming, and swimming velocity decreased with increasing copper concentration. Significant changes in behavior and mortality endpoints occurred at concentrations of copper between 1-8 µg/L. With the youngest fish tested during a 14 day exposure, behavioral changes were observed up to seven days before mortality occurred. Brief 4 day exposures with older fish caused similar effects with behavioral endpoints typically more sensitive than mortality. An additional assay with 1 day post hatch larvae designed to detect rapid changes in swimming behavior following exposure to copper revealed hyperactivity in larval sturgeon beginning 1 hour after exposure to 2-8 µg/L copper. In a feeding behavior assay, juvenile white sturgeon exposed to 12 µg/L copper consumed 37-60% less food than controls after 3 days of exposure. Our results indicate that behavioral endpoints were more sensitive than some standard toxicity test endpoints. Swimming behavior was impaired to the extent that survival in the field would likely be jeopardized. Such data would be useful to managers characterizing the risks of copper contamination to white sturgeon.

## Introduction

Copper occurs naturally in the environment and is spread through both natural and anthropogenic processes. Natural processes include forest fires, wind-blown dust and decaying vegetation while anthropogenic activities including combustion of fossil fuels, release from wastewater treatment plants, agricultural applications, anti-fouling paint used in marine applications, and mining activities also spread copper throughout the environment. Copper is an essential nutrient, necessary for the growth and survival of organisms, including fish. It is a key factor in many enzymes and plays an integral role in metabolism. In excess, however, copper can be toxic to aquatic organisms, causing a range of effects from acute mortality to alterations in metabolism and brain function.

Earlier studies have documented changes in swimming behavior when fish have been exposed to low levels of copper (Atchison et al. 1987, Little and Finger 1990, Scott and Sloman 2004). More recent studies determined early life-stage white sturgeon to be among the most sensitive aquatic organisms to copper (Little et al. 2012, Calfee et al. 2014). The behavioral changes observed in these studies progressed in severity from reduced swimming activity to a loss of equilibrium during the exposure. The magnitudes of these behavioral aberrations were such that similar effects in the wild would be sufficient to reduce long-term survival, and thus, have implications for recruitment failure (Little 2002).

In aquatic toxicity testing, behavior is often a more sensitive measure of toxicity than other more traditional endpoints such as mortality (Melvin and Wilson 2013). An organism's behavior is their link between physiology and ecology; thus, maladaptive behaviors could lead to reduced survival, growth, or reproduction—or even death. For

behavior to be a useful measure of toxicity in ecological risk assessment and injury investigations, the behavioral endpoint should be easy to measure and sensitive to contaminants (Rand 1984). Swimming ability is inherently important to many ecologically relevant events such as locating and obtaining prey items, avoiding predation, migration, and spawning are all events that could be affected by abnormal swimming behaviors; therefore, the assessment of swimming behaviors could be a valuable and informative addition to standard toxicity testing.

The objective of the current study was to conduct behavioral observations of water-only metals-exposed white sturgeon and quantify the observed changes in swimming activity and performance to determine time to effect during early-life-stage development. To address this objective, we exposed larval white sturgeon to a series of copper concentrations and quantified behavioral observations, including spontaneous swimming activity and swimming performance assays. We compared behavioral effect concentrations to water quality criteria to assess whether the criteria would be protective relative to these behavioral aberrations.

## Materials and Methods

*Acquisition and Culture of Test Fish*

White sturgeon were obtained from the Yakama Nations Fish Hatchery (Toppenish, WA) as newly fertilized eggs in June 2013 and June 2014. Eggs were received at the U.S. Geological Survey, Columbia Environmental Research Center (CERC) about 36 hours after fertilization. The embryos were placed in 6L MacDonald hatching jars (Aquatic EcoSystems, Apopka, FL) with flowing well water diluted with deionized water to a hardness of about 100 mg/L as $CaCO_3$. Sturgeon started hatching 8

days after fertilization and continued for a period of 2–4 more days. The date of hatch was established as the day when >50% of the eggs hatched (10–11 d post fertilization).

The 100-mg/L hardness culture and toxicity test water was prepared in two 7,000-L polypropylene tanks by diluting well water with a hardness of about 300 mg/L as $CaCO_3$ with deionized water to a hardness of about 100 mg/L as $CaCO_3$ (alkalinity of about 90 mg/L as $CaCO_3$, pH of about 8.0, and dissolved organic carbon [DOC] of about 0.4 mg /L). Water samples were collected weekly to measure water-quality characteristics of the culture water, including measures of dissolved oxygen (DO), temperature, conductivity, pH, alkalinity, hardness, and total ammonia. The average water-quality characteristics for culture water were DO (9.8 ± 0.47 mg/L), temperature (15.03 ± 0.20 ºC), conductivity (264.83 ± 18.1 µS/cm$^2$ at 25 ºC), pH (8.10 ± 0.08), alkalinity (95.53 ± 3.04 mg/L as $CaCO_3$), hardness (103.27 ± 3.13 mg/L as $CaCO_3$), and total ammonia (0.16± 0.08 mg/L as nitrogen). The sturgeon culture was maintained with a 16-h light, 8-h dark photoperiod with an average light intensity ranging from 280–300 lux. Smooth, flat stones with a diameter of 4 to 5 cm (Red chocolate pebbles, Geosubstrate, www.hagen.com) were added to culture tanks/aquaria during the hiding stage of the larvae.

After hatching, the yolk sac larvae were transferred to a 1,850-L flow-through fiberglass tank for holding until testing. Larvae were fed 1-d-old brine shrimp (*Artemia* sp.) nauplii (Brine Shrimp Direct, Ogden, UT) starting about 1 week (10 to 12 days post hatch (dph)) before the start of exogenous feeding, and at 18 dph, the larvae were transitioned to chopped and then whole, live oligochaetes (*Lumbriculus variegatus*, Eastern Aquatics, Lancaster, PA). Once larval sturgeon were feeding actively, Rangen

Sturgeon Diet (Rangen, Buhl, ID), a semi-moist commercial food, was provided every 3 h using an automated feeder. Sturgeon were fed a sufficient amount to result in a residual of uneaten food typically 2 to 3 h after each feeding.

*Water-Only Copper Exposures*

We conducted a series of 14-d and 4-d tests as outlined in Table 1. All research was done in accordance with guidelines outlined by the American Society of Ichthyologists and Herpetologists, the American Fisheries Society, and the American Institute of Fishery Research Biologists (Nickum et al. 2004) well as all CERC protocols for the humane treatment of test organisms during culture and experimentation. All exposures were conducted in a modified Mount and Brungs (Mount and Brungs 1967) diluter following guidelines outlined by ASTM International (International 2013c, a, b) ( Table 2). The water used in testing had a target hardness of 100 mg/L and contained DOC concentration of about 0.4 mg/L. Tests were performed at 15 °C. Two intermittent flow-through proportional diluters were used and provided a control and five concentrations of copper in a 50% dilution series (nominal concentrations of 0, 0.5, 1, 2, 4 and 8 µg/L). Metal salts were obtained from Sigma-Aldrich (St. Louis, MO). A stock solution of copper was prepared by adding the American Chemical Society reagent grade (>98% purity) copper II sulfate pentahydrate to deionized water. Test stock solutions were prepared 2 d before the start of exposures in volumetric flasks and wrapped with aluminum foil to reduce exposure to ambient light. Stock solutions were delivered to the diluters using a Hamilton® Syringe Dispenser (MicroLab® 600 Series, Hamilton Company, Reno, NV). The diluters were cycled for at least 2 d before starting the exposures to ensure stable copper concentrations. Four glass replicate chambers were

held in each of twelve 40-L rectangular glass aquaria in two temperature-controlled water baths. Each water bath was divided into 2 blocks such that every concentration was delivered to 4 replicate chambers per block, resulting in 8 replicate chambers per metal concentration per diluter. Each replicate test chamber (28x13.5x25 cm) had a hole (4-cm diameter) in the side covered with 30 mesh (0.5-mm opening) stainless steel screen and contained 7 L of water. An in-line 4-way flow splitter was attached to each delivery line to partition the water flow to each of 4 replicate chambers in the water bath (Brunson et al. 1998). Test solution flowed directly into test chambers and excess water overflowed to surrounding aquaria through the screen windows, so there was no exchange of test water among replicates. The diluter provided about 250 ml of water to each chamber every 30 minutes (resulting in about 2 volume additions per day to each replicate test chamber). The number of fish, number of replicates, and mass of fish in each exposure chamber were established in accordance with guidance provided in ASTM International (International 2013b), with the fish loading rate in test chambers less than the guidelines of 1 g/L of solution passing through a chamber each day at the end of the exposures or 10 g/L in the chamber at any time (at 17 °C or less).

The 14-d exposure was conducted in two diluters resulting in 4 blocks and 16 replicate chambers per concentration of copper tested. Each diluter was outfitted with overhead video cameras. The cameras provided simultaneous views of fish in 2 replicate exposure chambers in each treatment tank housing the exposure chambers. These cameras were used to document general behavior of the organisms daily during testing for qualitative analysis. Additionally, each treatment tank was outfitted with a second

overhead camera to provide a clear and continuous view of fish required for the digitized quantification of swimming activity behavior.

At the beginning of the 14-d exposure, 30 fish (1-dph) were randomly assigned to replicate chambers. One block was used for standard toxicity endpoints only (that is, mortality, growth, daily visual observations) and the other 3 blocks were used to collect additional behavioral measurements that required destructive subsampling of fish throughout the exposures. The replicate chambers used for standard toxicity endpoint testing were located at one end of one of the diluters. During the 14-d exposure, a behavioral checklist evaluation (Table 3) was made daily in the standard toxicity endpoint replicates. In addition to the behavioral checklist evaluation, a video sample of swimming activity was also made daily in the additional behavioral endpoints replicates following methods from Calfee et al. (Calfee et al. 2016). For the video sample of swimming activity, 1 fish was impartially selected from each of the four behavioral replicates at each concentration within each block and was placed in an observation arena, a polystyrene cylinder 9-cm diameter x 11.5-cm tall, filled with approximately 700 ml of test water within the treatment tank at the same exposure concentration. One hour after fish were transferred into observation arenas, swimming activity was recorded for 10 minutes with overhead surveillance cameras in each behavioral replicate simultaneously. After the swimming activity recording, fish in the observation arenas were euthanized. Swimming activity trials were conducted daily during the 14-d exposure starting on test day 3. Fish were fed during the 14-d exposures before the onset of exogenous feeding (about 12 dph) ad libitum twice daily starting with brine shrimp (*Artemia*) nauplii following procedures outlined by Wang et al. (Wang et al. 2014).

For each 4-d exposure, 20 fish (21–35 dph) were transferred impartially into each replicate chamber. Swimming activity trials were recorded daily starting on Day 1 using the same methods as the 14-d exposure. In addition to the behavioral checklist observations, mortality, and spontaneous swimming activity endpoints, critical swimming performance was measured on Day 1 and Day 4 during the 4-d exposures.

For the critical swimming performance trials, 1 fish from two replicates per concentration within each of the 3 behavioral endpoint blocks was tested on each day, resulting in 72 fish being measured per exposure. Each fish was placed in one of three 5-L swim tunnel respirometers (Loligo Systems). The fish was placed in the test section of the tunnel (30x7.5x7.5 cm) and acclimated at a velocity of 18 cm/sec for 5 minutes. After the acclimation period, the velocity was increased by 5 cm/sec every 3 minutes. The test concluded when the fish fatigued (that is, was swept downstream and impinged on the screen for 5 seconds). Following the trial, total length and wet weight for each fish were obtained and the fish were euthanized. Fish were not fed during the 4-d exposures.

For the 4d exposure of 1 dph larvae (4_1dph), one diluter water bath was used and four replicate polystyrene observational arenas were placed in each 40-L glass aquaria, resulting in eight replicates per concentration of copper tested. The same nominal concentrations of copper were tested and the test solutions were prepared as in the previous tests. This test was conducted under static conditions to determine the time to effect for the swimming activity responses. One 1-dph fish was transferred impartially into each replicate chamber. After a 30-minute acclimation period, spontaneous swimming activity was recorded with overhead security cameras for 10 minutes in each replicate arena simultaneously. Activity was recorded on the same fish hourly for 5 h.

After 17 h of exposure, activity was recorded hourly for 7 h. After 24 h of exposure, trials were recorded every 12 h until the end of the study. Water was renewed in each arena on Day 2. The test was concluded after 4 days and all surviving fish were removed from the observational arenas and euthanized.

*Feeding Assay*

A 4-d feeding response exposure was conducted with 58-dph white sturgeon (4d_58dph). One intermittent flow-through proportional diluter was used and provided 8 replicate glass chambers of a control and 5 concentrations of copper at nominal concentrations of 0, 1, 2, 4, 8 and 16 µg/L for a total of 48 chambers. The diluter provided about 250 ml of water to each chamber every 30 minutes (resulting in about 2 volume additions per day to each replicate test chamber). The test was conducted at 15 °C. At the start of the test, one 58-dph sturgeon was transferred impartially into each replicate chamber. After a 30-minute acclimation period, a feeding trial was conducted. To conduct a feeding trial, 20 approximately 1-cm-long segments of live oligochaetes (mean mass of 20 worm segments = 0.052 ± 0.007 g, n=12) were added to each chamber. Fifteen minutes after adding the worm segments, any remaining segments were counted and removed. A feeding trial was conducted daily until the end of the test. Fish were not fed other than during the feeding trials. Mortality was checked daily and dead fish were removed. The endpoint measured was the number of worm segments remaining at the end of a trial. After 96 h of exposure, all surviving fish were euthanized and wet weights and lengths were recorded for each fish.

*Water Quality and Chemistry*

Filtered water samples for the analysis of dissolved copper were collected from each block at all exposure concentrations 3 times (Days 0, 7, and 14) during the 14-d water-only exposure and twice (Day 0 and Day 4) during the 4-d exposures except for the 2013_Yak_4d_1dph exposure, when samples were collected twice; on Day 0 and immediately following water renewal on Day 2. Water samples were collected with a polypropylene syringe, filtered through a 0.45-µm pore size polyethersulfone membrane into polyethylene bottles, and stabilized within 24 h by adding concentrated nitric acid (16 molar) to each sample at a volume proportion of 1:100 (1 volume/volume %). These samples were then analyzed by inductively coupled plasma mass spectrometry according to U.S. Environmental Protection Agency (EPA) Method 6020a (Agency 2007b, Brumbaugh et al. 2007). Water samples for the analysis of DOC were collected from each control and medium treatment at the beginning and the end of the 14-d exposure and once (on Day 2) during the 4-d exposures. Water for DOC analyses (60–80 ml) was collected by oven-baked glass pipet into a certified DOC-free amber glass bottle that had been rinsed with 20 ml of sample water just before sampling. The samples were stored at 4 °C and subsequently filtered through a 0.45-µm pore size membrane within 96 h of collection, after which it was preserved with dilute sulfuric acid to a pH of <2.0. All DOC samples were analyzed according to EPA method 415.2 (Agency 1983). Water samples (filtered with a 0.45-µm pore size cartridge) for the analyses of major anions and cations were collected the same way as for metals during the 14-d and 4-d exposures. The cation samples were preserved the same way as the metals samples and were analyzed by inductively coupled plasma atomic emission spectroscopy according to EPA method

200.7 (Agency 1994). The anion samples were stored at 4 °C for as many as 28 d before analysis by ion chromatography according to EPA method 9056a (Agency 2007c).

Water temperature in the exposures was monitored daily. Water quality (DO, pH, conductivity, hardness, alkalinity, ammonia) in the exposures was measured in 0, 2, and 8 µg/L copper (Cu) treatments in all blocks at three times (Days 0, 7, and 14) during the 14-d exposure and twice (Day 0 and Day 4) during the 4-d exposures following standard methods (Eaton et al. 2005). Average starting wet weight and dry weight of fish were determined at the beginning and end of the 14-d exposure (using about 20 fish) and wet weight was determined only at the initiation of the 4-d exposures to ensure loading densities were within acceptable range. At the end of each exposure, surviving fish in each replicate chamber were counted and euthanized using tricaine methanesulfonate. Wet weight of surviving fish was determined by replicate by gently blotting fish on a dry paper towel and placing the fish in a tared aluminum pan. Dry weight of fish was determined in each replicate by drying the fish at about 60 °C to a constant weight.

*Statistical Analysis*

In all exposures, mortality and substantial changes in behavior (loss of equilibrium, change in fish location in aquaria, loss of feeding, inactivity, darkened coloration, or rapid respiration; (International 2013a)) were recorded with daily observations and qualitatively documented with overhead video of treatment replicates. Dead fish were removed daily from all exposure chambers. The primary standard toxicity endpoints measured daily in the exposures included mortality, the loss of equilibrium, and immobilization. The 50% lethal concentrations (LC50s) were calculated using mortality only, whereas the 50% effect concentrations (EC50s) were calculated using

mortality plus the loss of equilibrium and immobilization (effective mortality) following guidance provided in Stephan et al. (Stephan et al. 1985), EPA (Agency 2002), and ASTM International (International 2013b). Effect concentrations for survival and behavior were determined using Toxicity Relationship Analysis Program (TRAP, Version 1.22) (Erickson 2012). Death was defined as no movement of gills or appendages and no reaction to gentle prodding. Loss of equilibrium was defined as the inability of fish to maintain an upright position within the water column. Immobilization was defined as the inability to swim or move unless prodded.

Video analysis of the swimming activity trials was conducted using EthoVision® XT (Version 9.0, Noldus Information Technology, Wageningen, The Netherlands) digital imaging software. Endpoints calculated from the spontaneous swimming activity trials included total distance traveled, average velocity, and time spent moving. The EC50s on swimming activity were also calculated in the TRAP software using total distance traveled, average velocity, and time spent moving. Absolute critical swimming speeds ($U_{crit}$) of sturgeon were calculated using the following equation: $U_{crit} = u_i + (t_i/t_{ii}u_{ii})$, where $u_i$ is the highest velocity maintained for the prescribed period (cm/sec), $u$ii is the velocity increment (5.09 cm/s), $t_i$ is the time (minutes) fish swam at the "fatigue" velocity, and $t_{ii}$ is the prescribed period of swimming (3 minutes) (Brett 1964). Each absolute value of the $U_{crit}$ was converted to relative critical swimming speed in body lengths per second.

Repeated measures of swimming activity, behavioral observations, critical swimming performance, proportion of worms consumed, and mortality were collected on each unit of analysis over time. We tested for differences in swimming activity and

critical swimming performance endpoints among copper treatments over time for the water-only exposures by fitting linear mixed models to the clustered longitudinal data (West et al. 2015). The unit of analysis in each model was replicate chamber, which was nested within blocks (clusters). Swimming activity and critical swimming performance data were square root or log transformed prior to analysis. Mortality, proportion of worms consumed, and loss of equilibrium/immobility observational endpoints were analyzed using 2-way analysis of variance (ANOVA) with repeated measures on 1 factor (O'Rourke et al. 2005). Mortality and behavioral observation data were arc sine square root transformed prior to analysis to better meet the assumption of normality. Mean differences were determined using Fisher's LSD (least significant difference).

The bioavailability, and thus toxicity, of copper is modified by water chemistry parameters such as pH, DOC, Ca, Mg, Na, etc. (Agency 2007a). To account for this variation, EPA uses a biotic ligand model (BLM) to adjust water quality criteria to specific water characteristics to be protective of aquatic life (Agency 2007a). We calculated U.S. EPA acute and chronic water quality criteria for Cu in our exposure water using the Windward Environmental Cu BLM (Version 3.1.2.37). The mean of each water chemistry parameter was taken from each test for use in the model (data available in the associated data release; Puglis et al. 2018). Comparisons of our effect concentrations were then compared to the water quality criteria determined by the BLM.

## Results

### *Water-Only Copper Exposures*

Water-quality characteristics remained consistent throughout all exposures (DO 8.9 ± 0.25 mg/L, specific conductance 257 ± 1.07 µS/cm at 25 °C, pH 8.2 ± 0.05,

alkalinity 95 ± 1.15 mg/L as $CaCO_3$, hardness 104 ± 1.57 mg/L as $CaCO_3$, and total ammonia 0.11 ± 0. mg N/L, Puglis et al. 2018). All measured temperatures were between 14 and 16 °C. Major cations and anions were also consistent throughout exposures (Puglis et al. 2018). DOC values were 0.3 ± 0.07 mg/L (Puglis et al. 2018). Measured copper values were generally between 80% and 120% of nominal values (Table 4).

Mortality in the control treatments was below 10% for all exposures (Puglis et al. 2018). In the 14-d exposure, mortality was no consistent over time across concentrations (time-by-concentration interaction, 14d_1dph, $p<0.0001$). Significant mortality was observed at the 3.31 µg/L Cu concentration starting at 3 days of exposure (Figure 1, Puglis et al. 2018). The lowest observable effect concentration (LOEC) dropped on Day 14, when significant mortality was observed in the 0.88 µg/L Cu treatment. The most sensitive LOEC for mortality was on Day 14.

Effective mortality was not consistent over time across copper concentrations (time-by-concentration interaction) in the 14-d exposure (14d_1dph, $p<0.0001$). Significant increases in effective mortality in the 3.31 µg/L Cu treatment compared to the control treatment were observed on Day 4 (Figure 1, Puglis et al. 2018). There was some variability in the LOECs throughout the exposure, with LOECs fluctuating between several concentrations daily, particularly between Days 9 and 14. For the 14d_1dph exposure, the most sensitive LOEC for effective mortality was 0.88 µg/L Cu on Day 14.

Swimming activity endpoints were also not consistent over time across copper concentrations in the 14d exposure (time-by-concentration interactions; distance traveled, $p = 0.0088$; average velocity, $p = 0.0087$; time spent moving, $p = 0.0024$). There was a significant decrease in total distance traveled in the 7.12 µg/L Cu treatment on Day 3 of

the study and in all swimming activity responses in the 3.31 µg/L Cu treatment on Day 4 (Figure 1, Puglis et al. 2018). In general, the LOECs decreased over time until Days 8–10, after which the LOECs fluctuated between several concentrations of copper but generally increased (Figure. 2). The most sensitive LOEC for a swimming activity response in the 14-d exposure was 0.88 µg/L Cu, which was observed on Day 10.

In the 14-d exposure, swimming activity tended to be the most sensitive endpoint followed by effective mortality and finally mortality, which was the least sensitive response measured. In the 14d_1dph exposure, all responses reached a LOEC of 0.88 µg/L Cu; however, for the swimming activity responses, significant changes were observed days earlier than either effective mortality or mortality (Figure 1).

Effect concentrations were also calculated for all responses on Days 4 and 14 of the 14-d exposure. For the 14d_1dph exposure, as much as 14% of fish exposed at the highest copper concentration were either dead, displayed a loss of equilibrium, or were immobile after 4 days of exposure; therefore, 4-d LC50s and effective mortality EC50s were recorded as greater than the highest concentration (Table 5). The 4-d EC50s for swimming activity endpoints in the 14d_1dph exposure ranged from 3.38–6.52 µg/L Cu; however, these effect concentrations should be used with caution because the effect sizes were not very large, resulting in wide 95% confidence intervals (Table 5). After 14 days of exposure, the estimated 20% lethal concentration values was 4.36 µg/L Cu and the effective mortality 20% effect concentrations (EC20) was 2.13 µg/L Cu (Table 5). Swimming activity EC20s ranged from 1.30–1.50 µg/L Cu (Table 5). The BLM calculated water quality criteria was less than the effect concentrations for all endpoints

except 20% effect concentrations for effective mortality and the three swimming activity endpoints from Day 14 of the exposure.

Toxicity to copper also varied during the 4-d exposures stocked with fish between 28 and 35 dph with the mortality response being just as sensitive, if not more sensitive, than effective mortality, swimming activity, and critical swimming performance responses. Mortality was not consistent over time across copper concentrations in either 4d exposure (time-by-concentration interaction: 4d_28dph, $p<0.0001$; 4d_35dph, $p<0.0001$). A significant increase in mortality was first observed in the 3.55 µg/L and greater Cu treatments on Day 3 of the 4d_28dph exposure (Puglis et al. 2018) and in the 6.92 µg/L Cu treatment on Day 2 of the 4d_35dph exposure (Puglis et al. 2018). Effective mortality was also not consistent over time across concentration in the 4d exposures (time-by-concentration interaction: 4d_28dph, $p<0.0001$; 4d_35dph, $p = 0.0008$). A significant increase in effective mortality was observed in the 7.38 µg/L Cu treatment on Day 4 of the 4d_28dph exposure and in the 6.92 µg/L Cu treatment on Day 2 of the 4d_35dph exposure, which was the most sensitive LOEC for effective mortality between these exposures.

There was a significant effect of copper concentration on total distance traveled ($p=0.0361$) and average velocity ($p=0.0433$) in the 4d_28dph exposure, with fish in the 7.38 µg/L Cu treatment swimming slower and shorter distances than fish in the control treatment (Puglis et al. 2018). There were no significant effects on time spent moving in that exposure. None of the swimming activity responses were consistent over time across copper concentration in the 4d_35dph exposure (time-by-concentration interaction: total distance traveled, $p=0.0008$; average velocity, $p=0.0007$; time spent moving, $p=0.0023$).

In general, distance traveled, average velocity, and time spent moving decreased with increasing copper concentration and over time. The LOECs ranged from 3.25–6.92 µg/L Cu between Days 2 and 4 and was the most sensitive at 1.63 µg/L Cu on Day 3 (Puglis et al. 2018). There were no significant effects duration of exposure or copper concentration on the critical swimming performance of fish in the 4d_28dph or 4d_35dph exposures.

Lethal effect concentration values (LC50s) for the 4-d tests ranged from 11.91–15.00 µg/L Cu and effective mortality EC50s ranged from 8.71–8.88 µg/L Cu (Table 5). Swimming activity EC50s from the 4d_35dph exposure ranged from 4.26–4.78 µg/L Cu (Table 5). Swimming activity EC50s are reported as greater than the highest concentration of copper for the 4d_28dph exposure because swimming activity was not affected by Day 4. The BLM calculated water quality criteria was below all the effect concentrations for these 4d exposures.

There were no mortalities in the 4d_1dph exposure, but there was a significant interaction of exposure duration and concentration of copper on swimming activity ($p<0.0001$). Sturgeon in the higher copper concentrations (between 1.86 and 7.20 µg/L Cu) had significantly greater activity compared to the control fish between 1 and 5 hours of exposure followed by a significant decrease in activity compared to controls at ≥ 48 hours of exposure in the 7.25 µg/L Cu treatment (Figures 3-4, Puglis et al. 2018).

*Feeding Assay*

The feeding response of white sturgeon larvae was not consistent over time across copper concentrations (day by concentration interaction, $p=0.0018$), with fewer worms being consumed by sturgeon in the highest copper concentration treatment over time. By Day 3, there was a significant reduction of worms consumed by sturgeon in the highest copper treatment compared to the control (Table 6). Fish in this treatment consumed

approximately 49% of food offered compared to 86% of food consumed by fish in the control treatment (Table 6). This trend continued on Day 4 with 30% and 90% of food being consumed by fish in the high copper treatment and control treatment, respectively. Because the feeding response only changed in the highest concentration, no EC50 for feeding rate could be calculated.

## Discussion

Larval white sturgeon exhibited changes in swimming activity that were often detected earlier or in lower concentrations of copper than changes in mortality, loss of equilibrium, and/or immobilization. Endurance endpoints were less sensitive than swimming activity endpoints and, in some cases, less sensitive than mortality.

Species sensitivity distributions with previously reported values put white sturgeon larvae among the most sensitive species tested for copper toxicity (Vardy et al. 2011, Little et al. 2012, Calfee et al. 2014, Wang et al. 2014). This sensitivity to copper was reaffirmed in the present study with effect concentrations for mortality and effective mortality similar to previously reported values (Calfee et al. 2014), with the exception of the 4d EC50 for effective mortality. In the current study, EC50s for this endpoint ranged from 8.71–8.88 µg Cu/L for 1–35 dph white sturgeon larvae and Calfee et al. (Calfee et al. 2014) reported values of 2.67–6.31 µg Cu/L. The differences in sensitivity could be due to different populations of white sturgeon. Fish from the upper and middle Columbia Rivers were used in the current study, whereas Calfee et al. (Calfee et al. 2014) used fish exclusively from the UCR. No other studies with white sturgeon larvae have used effective mortality as an endpoint, so further comparisons are difficult. However, in a recent study with Chinese sturgeon, similar swimming aberrations indicative of a loss of

equilibrium such as fish swimming in corkscrews and losing their balance, were observed after 4-6 hours of exposure (Guangpeng et al. 2017).

Swimming activity was often the most sensitive response of sturgeon in these exposures, as reflected in lower EC50 and EC20 values and/or earlier detection of differences between copper exposed and control fish. For example, in the 14d_1dph exposure, the most sensitive LOEC for a swimming activity endpoint, 0.88 µg/L Cu, was recorded on Day 10. This same LOEC was observed for mortality and effective mortality but not until Day 14. This result was anticipated as changes in swimming activity are usually apparent earlier than mortality (Little and Finger 1990). The lack of response at lower concentrations of copper on Day 11 and continuing until the end of the test is due to a decrease in activity of fish in the control replicates and a relative increase in activity of fish in lower concentrations of copper. The relative increase in activity could be an indication of recovery with detoxification systems beginning to have an effect. The decrease of activity in the control fish may indicate an energy intensive point in development that could be delayed in the copper treatments. By the end of the 14-d exposure, fish in the control treatment had absorbed their yolk sacs, whereas fish in the highest concentration maintained yolk sacs until the end of the study (Holly Puglis, personal observation).

Our data indicate that between 0 and 3 days of exposure, sturgeon in 4 µg/L Cu or greater were hyperactive followed by a reduction in swimming activity, which continued for the duration of the exposure. Initial periods of hyperactivity followed by either reduction in activity or a return to normal activity levels following exposure to copper has been recorded with other fish species as well (Drummond et al. 1973, Scarfe et al. 1982,

Campbell et al. 2002) and may be an avoidance behavior (Scarfe et al. 1982). Campbell et al. (Campbell et al. 2002) reported decreased locomotor activity in rainbow trout after dietary exposure to copper and linked the reduction in swimming activity to increased metabolic costs for exposed trout. We did not measure any physiological endpoints but we did observe increased respiration rates as indicated by rapid opercula movement in white sturgeon in some of our copper exposures. This suggests higher oxygen consumption and thus increased metabolic costs for exposed sturgeon and offers a potential explanation for the reduction in activity in copper exposed fish.

Changes in swimming behavior after exposure to a contaminant are not limited to white sturgeon and copper. A recent review of the behavioral effects of environmentally relevant pesticide exposure on aquatic vertebrates found dozens of studies making hundreds of comparisons of swimming behavior endpoints with fish and amphibians (Shuman-Goodier and Propper 2016). Such changes in swimming activity have been directly linked to effects on survival, growth, and reproduction. For example, in response to a predator cue, unexposed juvenile Coho salmon reduced their swimming activity but copper exposed juveniles did not leading to much higher predation rates on copper exposed fish (McIntyre et al. 2012). Also, reduced swimming activity of fathead minnows exposed to esfenvalerate were preyed on at higher rates and were smaller than control fish (Floyd et al. 2008). These examples suggest that alterations in swimming activity can have direct consequences on survival via increased predation rates. In addition, decreased growth and delayed development can increase the susceptibility of larvae to predation. In the current study, time to effect for the swimming activity endpoints was up to seven days earlier than mortality and up to three days earlier than

effective mortality at the lower copper concentrations tested. Thus earlier time to effect of these behavioral changes could lengthen the window of vulnerability for larvae in their environment. Additionally, the chronic BLM-based water quality criteria was not protective of the behavioral effect concentrations (effective mortality and swimming activity endpoints) from Day 14 of the 14d exposure. Further evidence of the sensitivity of these endpoints.

Critical swimming performance of white sturgeon was the least sensitive response measured in this study with typically no significant effects at the highest concentrations tested. Previous studies have found swimming performance to be of variable sensitivity to copper. For example, Vieira et al. (Vieira et al. 2009) found the EC50 for swimming resistance (a measure of how long the fish swam against a current) of the common goby to be 17% of the 96-h copper LC50. Waiwood and Beamish (Waiwood and Beamish 1978) reported a wide range of critical swimming performance effect concentrations for rainbow trout exposed to copper in various treatments of water hardness and pH, with impairment evident at concentrations as low as 25% of LC50 values in low pH and low water hardness and nearing lethal concentrations at higher pH and hardness values. To date, the present study is the first to explore the sensitivity of critical swimming performance of white sturgeon after exposure to a contaminant. Based on our findings, it appears that critical swimming performance is not a sensitive measure of copper toxicity for this fish species or that the mechanism of toxicity for copper does not immediately result in decreased swimming performance.

The feeding response of sturgeon exposed to sublethal copper concentrations varied over the 96-h test. After 24 h of exposure to 0.69–10.25 µg Cu/L, fish ate

significantly more food (between 71 and 83% of food offered) compared to fish in the control treatment, which consumed 60% of food offered. The appetite of fish in the 10.65 µg Cu/L treatment was significantly reduced after 3 d of exposure, which is consistent with other studies that have also documented a rapid loss of appetite in copper-exposed fish with many species recovering appetites after several weeks of exposure (Drummond et al. 1973, Buckley et al. 1982, De Boeck et al. 1997). Abdel-Moneim et al. (Abdel-moneim et al. 2015) reported copper-induced feeding inhibition in zebrafish after 48 h of exposure with a calculated EC50 for feeding inhibition approximately two times lower than the LC50 for lethality. Several studies have noted increased food consumption in chronic studies of copper-exposed fish without increasing growth rates, suggesting greater metabolic demands as a result of copper exposure (Buckley et al. 1982, Collvin 1985, De Boeck et al. 1997, McGeer et al. 2000).

*Conclusions*

The behavioral measures of toxicity were consistently more sensitive than traditional acute measures of mortality and immobility, providing an early indication of injury with up to seven days earlier time to effect. Current testing supports previous conclusions that concentrations as low as 2 µg/L Cu cause significant, measurable effects in larval white sturgeon. These results are consistent with a number of previous studies that indicate the high sensitivity of early-life-stage white sturgeon, including those of populations from mid- and upper reaches of the Columbia River, as well as those of remote Kootenai River stocks. The behavioral studies serve as an additional line of evidence in the characterization of the toxicity of copper to white sturgeon and highlight the more sensitive behavioral effects copper has on this species.

**Acknowledgements:** I would like to thank the staff in the Ecology Branch, Toxicology Branch, and Environmental Chemistry Branch of the US Geological Survey (USGS), Columbia Environmental Research Center, for technical assistance. I thank C. Mebane and J. Besser for comments that improved the manuscript. Funding for the present study was provided by the National Park Service through the Department of the Interior Central HazMat Fund the USGS Contaminants Biology Program.

**Data availability:** The data underlying the findings reported here are freely available in the ScienceBase public repository (*https://doi.org/10.5066/P9QB60EV)*.

## Literature Cited


Abdel-moneim, A., M. Moreira-Santos, and R. Ribeiro. 2015. A short-term sublethal toxicity assay with zebra fish based on preying rate and its integration with mortality. Chemosphere **120**:568-574.

Agency, U. S. E. P. 1983. Method 415.2 Total organic carbon, in Methods for chemical analysis of water and wastes. EPA/600/4-79-020, U.S. Environmental Protection Agency, Washington, D.C.

Agency, U. S. E. P. 1994. Method 200.7—Determination of metals and trace metals in water and wastes by inductively coupled plasma-atomic emission spectrometry, revision 4.4. U.S. Environmental Protection Agency, Cincinnati, Ohio.

Agency, U. S. E. P. 2002. Short-term methods for estimating the chronic toxicity of effluents and receiving water to freshwater organisms (5th ed.). EPA/821/R-02/013, U.S. Environmental Protection Agency, Washington, D.C.

Agency, U. S. E. P. 2007a. Aquatic life ambient freshwater-quality criteria—Copper. EPA-822-R-07-001, U.S. Environmental Protection Agency, Office of Water, Washington, D.C.

Agency, U. S. E. P. 2007b. Method 6020A—Inductively coupled plasma-mass spectrometry. SW-846 Online—Test methods for evaluating solid waste, physical/chemical methods. U.S. Environmental Protection Agency, Washington, D.C.

Agency, U. S. E. P. 2007c. Method 9056—Determination of inorganic anions by ion chromatography. SW-846 Online—Test methods for evaluating solid waste, physical/chemical methods. U.S. Environmental Protection Agency, Washington, D.C.

Atchison, G. J., M. G. Henry, and M. B. Sandheinrich. 1987. Effects of metals on fish behavior: a review. Environmental Biology of Fishes **18**:11-25.

Brett, J. R. 1964. The Respiratory Metabolism and Swimming Performance of Young Sockeye Salmon. Journal of the Fisheries Research Board of Canada **21**:1183-1226.

Brumbaugh, W. G., T. W. May, J. M. Besser, A. L. Allert, and C. J. Schmitt. 2007. Assessment of elemental concentrations in streams of the New Lead Belt in southeastern Missouri, 2002-05. U. S. Geological Survey, Scientific Investigations Report 2007-5057, Reston, VA.

Brunson, E. L., T. J. Canfield, F. J. Dwyer, C. G. Ingersoll, and N. E. Kemble. 1998. Assessing the Bioaccumulation of Contaminants from Sediments of the Upper Mississippi River Using Field-Collected Oligochaetes and Laboratory-Exposed Lumbriculus variegatus. Archives of Environmental Contamination and Toxicology **35**:191-201.

Buckley, J. T., M. Roch, J. A. McCarter, C. A. Rendell, and A. T. Matheson. 1982. Chronic exposure of coho salmon to sublethal concentrations of copper—I. Effect on growth, on accumulation and distribution of copper, and on copper tolerance. Comparative Biochemistry and Physiology Part C: Comparative Pharmacology **72**:15-19.

Calfee, R. D., E. E. Little, H. J. Puglis, E. Scott, W. G. Brumbaugh, and C. A. Mebane. 2014. Acute sensitivity of white sturgeon (*Acipenser transmontanus*) and rainbow trout (*Oncorhynchus mykiss*) to copper, cadmium, or zinc in water-only laboratory exposures. Environmental Toxicology and Chemistry **33**:2259-2272.

Calfee, R. D., H. J. Puglis, E. E. Little, W. G. Brumbaugh, and C. A. Mebane. 2016. Quantifying Fish Swimming Behavior in Response to Acute Exposure of Aqueous Copper Using Computer Assisted Video and Digital Image Analysis. JoVE: Journal of Visualized Experiments:e53477.

Campbell, H. A., R. D. Handy, and D. W. Sims. 2002. Increased metabolic cost of swimming and consequent alterations to circadian activity in rainbow trout (Oncorhynchus mykiss) exposed to dietary copper. Canadian Journal of Fisheries and Aquatic Sciences **59**:768-777.

Collvin, L. 1985. The effect of copper on growth, food consumption and food conversion of perch Perca fluviatilis L. Offered maximal food rations. Aquatic Toxicology **6**:105-113.

De Boeck, G., A. Vlaeminck, and R. Blust. 1997. Effects of Sublethal Copper Exposure on Copper Accumulation, Food Consumption, Growth, Energy Stores, and Nucleic Acid Content in Common Carp. Archives of Environmental Contamination and Toxicology **33**:415-422.

Drummond, R. A., W. A. Spoor, and G. F. Olson. 1973. Some Short-term Indicators of Sublethal Effects of Copper on Brook Trout, Salvelinus fontinalis. Journal of the Fisheries Research Board of Canada **30**:698-701.

Eaton, A. D., L. S. Clesceri, E. W. Rice, and A. E. Greenberg, editors. 2005. Standard methods for the examination of water and wastewater. 21st edition. American Public Health Association, Water Environment Federation, American Water Works Association, Washington, D.C.

Erickson, R. J. 2012. Toxicity Relationship Analysis Program (TRAP), version 1.21. EPA/600/C-11/002, U.S. Environmental Protection Agency, Washington, D.C.

Floyd, E. Y., J. P. Geist, and I. Werner. 2008. Acute, sublethal exposure to a pyrethroid insecticide alters behavior, growth, and predation risk in larvae of the fathead minnow (Pimephales promelas). Environmental Toxicology and Chemistry **27**:1780-1787.

Guangpeng, F., Y. Zhifeng, S. Xiaotao, Z. Longzhen, and Z. Ping. 2017. Copper and Zinc Toxicity Estimates for Juvenile Chinese Sturgeon Acipenser sinensis in the Yangtze River Estuary. International Journal of Agriculture & Biology **19**:539-544.

International, A. 2013a. Standard guide for measurement of behavior during fish toxicity tests, E1711–95 (2008). Annual Book of ASTM Standards, v. 11.06. ASTM International, West Conshohocken, Pennsylvania.

International, A. 2013b. Standard guide for performing acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians, E729–96 (2007). Annual Book of ASTM Standards, v. 11.06. ASTM International, West Conshohocken, Pennsylvania.

International, A. 2013c. Standard guide for performing early life-sage toxicity tests with fishes, E1241–05. Annual Book of ASTM Standards, v. 11.06. ASTM International, West Conshohocken, Pennsylvania.

Little, E. E. 2002. Behavioral measures of environmental stressors in fish. Pages 431-472 *in* S. M. Adams, editor. Biological indicators of aquatic ecosystem stress. American Fisheries Society.

Little, E. E., R. D. Calfee, and G. Linder. 2012. Toxicity of Copper to Early-life Stage Kootenai River White Sturgeon, Columbia River White Sturgeon, and Rainbow Trout. Archives of Environmental Contamination and Toxicology **63**:400-408.

Little, E. E., and S. E. Finger. 1990. Swimming behavior as an indicator of sublethal toxicity in fish. Environmental Toxicology and Chemistry **9**:13-19.

McGeer, J. C., C. Szebedinszky, D. G. McDonald, and C. M. Wood. 2000. Effects of chronic sublethal exposure to waterborne Cu, Cd or Zn in rainbow trout. 1: Iono-regulatory disturbance and metabolic costs. Aquatic Toxicology **50**:231-243.

McIntyre, J. K., D. H. Baldwin, D. A. Beauchamp, and N. L. Scholz. 2012. Low-level copper exposures increase visibility and vulnerability of juvenile coho salmon to cutthroat trout predators. Ecological Applications **22**:1460-1471.

Melvin, S. D., and S. P. Wilson. 2013. The utility of behavioral studies for aquatic toxicology testing: A meta-analysis. Chemosphere **93**:2217-2223.

Mount, D. I., and W. A. Brungs. 1967. A simplified dosing apparatus for fish toxicology studies. Water Research **1**:21-29.

Nickum, J., H. Bart Jr, P. Bowser, I. Greer, C. Hubbs, J. Jenkins, J. MacMillan, J. Rachlin, J. Rose, and P. Sorensen. 2004. Guidelines for the use of fishes in research. American Fisheries Society, Bethesda, MD.

O'Rourke, N., L. Hatcher, and E. J. Stepanski. 2005. A step-by-step approach to using SAS for univariate and multivariate statistics. 2d ed. edition. SAS Institute Inc., Cary, North Carolina.

Puglis, H.J., R.D. Calfee, and E.E. Little. 2018. Behavioral effects of copper on larval white sturgeon. U.S. Geological Survey Data Release. *https://doi.org/10.5066/P9QB60EV*

Rand, G. M. 1984. The use of behavioral measurements to assess toxicant induced stress in marine organisms. Pages 431-456 *in* G. Persoone, E. Jaspers, and C. Claus, editors. Ecotoxicological testing for the marine environment, vol. 2. Institute for Marine Scientific Research, Bredene, Belgium.

Scarfe, A. D., K. A. Jones, C. W. Steele, H. Kleerekoper, and M. Corbett. 1982. Locomotor behavior of four marine teleosts in response to sublethal copper exposure. Aquatic Toxicology **2**:335-353.

Scott, G. R., and K. A. Sloman. 2004. The effects of environmental pollutants on complex fish behaviour: integrating behavioural and physiological indicators of toxicity. Aquatic Toxicology **68**:369-392.

Shuman-Goodier, M. E., and C. R. Propper. 2016. A meta-analysis synthesizing the effects of pesticides on swim speed and activity of aquatic vertebrates. Science of The Total Environment **565**:758-766.

Stephan, C. E., D. I. Mount, D. J. Hansen, J. H. Gentile, G. A. Chapman, and W. A. Brungs. 1985. Guidelines for deriving numerical national water-quality criteria for the protection of aquatic organisms and their uses. EPA 822-R-85-100, U.S. Environmental Protection Agency, Duluth, Narragansett, and Corvallis.

Vardy, D. W., A. R. Tompsett, J. L. Sigurdson, J. A. Doering, X. W. Zhang, J. P. Giesy, and M. Hecker. 2011. Effects of subchronic exposure of early life stages of white sturgeon (*Acipenser transmontanus*) to copper, cadmium, and zinc. Environmental Toxicology and Chemistry **30**:2497-2505.

Vieira, L. R., C. Gravato, A. M. V. M. Soares, F. Morgado, and L. Guilhermino. 2009. Acute effects of copper and mercury on the estuarine fish Pomatoschistus microps: Linking biomarkers to behaviour. Chemosphere **76**:1416-1427.

Waiwood, K. G., and F. W. H. Beamish. 1978. Effects of copper, pH and hardness on the critical swimming performance of rainbow trout (Salmo gairdneri Richardson). Water Research **12**:611-619.

Wang, N., C. G. Ingersoll, R. A. Dorman, W. G. Brumbaugh, C. A. Mebane, J. L. Kunz, and D. K. Hardesty. 2014. Chronic sensitivity of white strugeon (*Acipenser transmontanus*) and rainbow trout (*Oncorhynchus mykiss*) to cadmium, copper, lead, or zinc in laboratory water-only exposures. Environmental Toxicology and Chemistry **33**:2246-2258.

West, B. T., K. B. Welch, and A. T. Galecki. 2015. Linear mixed models: A practical guide using statistical software. 2d ed. edition. CRC Press, Boca Raton, Florida.

Table 1. Summary of study information including age of fish at test, duration of test, unique test code for each study conducted, and endpoints measured in each test. Dph = days post hatch; d = day, e. mortality = effective mortality (mortality + loss of equilibrium + immobility), s. activity = spontaneous swimming activity (total distance moved, time spent moving, average velocity), s. performance = critical swimming performance.

| Age of Fish Tested | Test Duration | Study Code | Endpoints |
|---|---|---|---|
| **1-dph** | 4-d | 4d_1dph | Mortality, E. Mortality, S. Activity |
| **1-dph** | 14-d | 14d_1dph | Mortality, E. Mortality, S. Activity |
| **28-dph** | 4-d | 4d_28dph | Mortality, E. Mortality, S. Activity, S. Performance |
| **35-dph** | 4-d | 4d_35dph | Mortality, E. Mortality, S. Activity, S. Performance |
| **58-dph** | 4-d | 4d_58dph | Mortality, Feeding |

Table 2. Conditions for conducting water-only toxicity tests with white sturgeon (adapted and modified for fish from ASTM 2013a, b, c).

| Parameter | Conditions |
|---|---|
| **1. Species** | White sturgeon (*Acipenser transmontanus*) |
| **2. Chemicals** | (1) 14-d exposure & (2, 3, 4) 4-d exposures: $CuSO_4$ |
| **3. Test type:** | (1) 14-d exposure & (2, 4) 4-d exposures: Water-only exposures in intermittent proportional diluters. (3) 4-d exposure: Water-only in static waterbath |
| **4. Temperature:** | 15 °C |
| **5. Light quality:** | Ambient laboratory light |
| **6. Light intensity:** | About 50-100 (lux) |
| **7. Photoperiod (light:dark):** | 16:08 |
| **8. Test chamber size:** | (1, 2, 4) 9.5 L (3) 730 ml |
| **9. Test solution volume:** | (1) 14-d exposure & (2, 4) 4-d exposures: 7 L; (3) 4-d exposure: 700 ml |
| **10. Water renewal:** | (1) 14-d exposure & (2, 4) 4-d exposures: 0.25 L/chamber/30 min (1.7 volume additions/d). (3) 4-d exposure: static with water renewal on test day 2.<br>Water addition may be increased or number of fish may be reduced in a chamber to accommodate increased mass of fish during the exposures (see Item #12 below on "Loading") |
| **11. Organisms/chamber:** | (1) 14-d exposure: 30<br>(2) 4-d exposures by life stage: 20<br>(3,4): 4-d exposures: 1 |
| **12. Loading:** | <0.5 g fish/L of test solution passing through the chamber over 24 hours and <5 g fish/L in chamber at any given time |
| **13. Replicates:** | (1,2) 14-d and 4-d exposures: 4 replicate chambers/treatment/spatial block; 4 blocks (1 block undisturbed and used for toxicity endpoints only)<br>(3) 4-d exposure: 4 replicate arenas/treatment/spatial block; 2 blocks<br>(4) 4-d exposure: 4 replicate chambers/treatment/spatial block, 2 blocks |
| **14. Duration:** | (1) 14-d exposure: 14 days<br>(2,3,4) 4-d exposures: 4 days |
| **15. Age of test organisms:** | (1) 14-d & (3) 4-d exposures: 1 dph<br>(2) 4-d exposures: 28 and 35 dph<br>(4) 4-d exposure: 58 dph |
| **16. Feeding:** | (1) 14-d exposure: Ad libitum twice daily starting with brine shrimp (Artemia) at 12 dph and then transitioning to live oligochaetes (*Lumbriculus variegatus*) monitoring daily food consumption<br>(2, 3) 4-d exposures: No feeding<br>(4) 4-d exposure: Fed live oligochaetes daily |
| **17. Chamber cleaning:** | Once daily to remove dead fish and if screens became clogged during a test, gently brushed the outside of the screen |
| **18. Test water:** | Well water diluted with deionized water: about 100 mg/L hardness as $CaCO_3$, pH 8.2, DOC 0.4 mg/L µg Cu/L |
| **19. Dilution series:** | (1,2,3) 14-d and 4-d exposures: 0, 0.50, 1.0, 2.0, 4.0, 8.0 µg Cu/L |

| | |
|---|---|
| | (4) 4-d exposure: 0, 1, 2, 4, 8, 16 |
| **20. Chemical residues:** | 20-ml filtered water sample (0.45 µm) for Cu analysis was collected at all exposure concentrations at (1) 14-d exposure: Days 0, 7, and 14; (2,4) 4-d exposures: Days 0 and 4; (3) 4-d exposure: Day 0. |
| **21. Water quality:** | Dissolved oxygen, pH, conductivity, hardness, alkalinity, ammonia were determined at control, medium, and high concentrations in the (1) 14-d exposure on Days 0, 7, and 14 and on Days 0 and 4 during the (2, 3, 4) 4-d exposures.<br>40-ml filtered water samples (0.45-µm) for DOC analysis were collected from the control and medium concentrations on Days 0 and 14 during the (1) 14-d exposure and on Day 2 during (2, 3) 4-d exposures.<br>Filtered water samples (0.45 µm) for the analyses of major anions (40 ml) and cations (40 ml) were collected from the control and medium concentrations on Days 0 and 14 during the (1) 14-d exposure and on Day 2 during (2) 4-d exposures. The cation samples were stabilized within 24 hours by adding concentrated nitric acid (16 M) to each sample at a volume proportion of 1:100 (1% volume/volume). |
| **22. Aeration:** | None unless dissolved oxygen <4 mg/L |
| **23. Endpoints:** | Mortality and visual observations in behavior (loss of equilibrium, immobilization, change in fish location in aquaria, feeding, activity, coloration, or respiration, lack of hiding ability; ASTM 2013c) were recorded daily in the (1) 14-d and (2, 3) 4-d exposures. Swimming activity assays were conducted and total distance traveled (cm), average velocity (cm/sec), and time spent moving (%) were calculated daily during (1) 14-d exposures beginning on Day 3-4 and daily in (2, 3) 4-d exposures. Critical swimming performance (body length/second) was measured on Days 1 and 4 of (2) 4-d exposures. Critical swimming performance trials were conducted on Day 4 of (2) 4-day exposures and critical swimming speeds were calculated. |
| **24. Test acceptability:** | 80% survival in controls |

Table 3. Checklist for daily observations for swimming abnormalities in acute exposures (based on ASTM International 2013b).

| Code | Observation |
|---|---|
| A | Fish exhibit immobilization[1] or hyperactivity |
| B | Loss of equilibrium[1] (inability to maintain upright position in water column, fish are upside down or on their sides) |
| C | Position in the water (on bottom, at surface, ambient) |
| D | Respiration (fast or slow) |
| E | Pigmentation (light or dark) |
| F | Other (swimming in circles, spasms, tremors, coughing, swollen abdomens, curved spines) |

[1]Defined as effective mortality.

Table 4. Mean measured copper concentration (± standard deviation) measured on day 0, day 7 and day 14 during each 14 day exposure and day 0 and day 4 during each 4 day exposure with white sturgeon (*Acipenser transmontanus*).

| | Exposure | | | | |
|---|---|---|---|---|---|
| Treatment | 14d_1dph | 4d_28dph | 4d_35 dph | 4d_1dph[1] | 4d_58dph |
| Control | 0.15 (±0.05) | 0.19 (±0.02) | 0.15 (±0.06) | 0.13 | 0.20 (±0.01) |
| 1 | 0.50 (±0.05) | 0.60 (±0.06) | 0.48 (±0.06) | 0.24 | 0.69 (±0.06) |
| 2 | 0.88 (±0.08) | 0.98 (±0.05) | 0.84 (±0.06) | 0.52 | 1.31 (±0.06) |
| 3 | 1.67 (±0.11) | 1.87 (±0.10) | 1.63 (±0.04) | 1.69 | 2.80 (±0.14) |
| 4 | 3.31 (±0.13) | 3.55 (±0.13) | 3.25 (±0.07) | 1.72 | 5.10 (±0.10) |
| 5 | 7.12 (±0.25) | 7.38 (±0.23) | 6.92 (±0.23) | 7.25 | 10.65 (±0.33) |

[1]Copper concentration only measured on Day 0

Table 5. Effect concentrations for mortality, effective mortality (mortality plus loss of equilibrium and immobilization), and swimming activity endpoints (total distance traveled, average velocity, and time spent moving) (n = 12) of white sturgeon (Acipenser transmontanus) in 14 and 4-day tests. 95-percent confidence intervals (CI) are presented for each endpoint. The BLM calculated water quality criteria (WQC) based on the water chemistry in each test is compared to the effect concentrations. For 50% effect concentrations, the Criterion Maximum Concentrations are used for comparison. For 20% effect concentrations, the Criterion Continuous Concentration is used. Gray shading indicates the effect concentration is less than the BLM water quality criteria. LC50, lethal concentration at which 50 percent of population would be effected; EC50, effect concentration at which 50 percent of population would be effected. NE = not estimated because the data did not meet requirements of probit analysis or logistic regression.



**Survival Endpoints**

| | | Mortality (%) | | | | Effective Mortality (%) | | | |
|---|---|---|---|---|---|---|---|---|---|
| Exposure | Days of Exposure | Effect Concentration | | 95% CI | BLM WQC | Effect Concentration | | 95% CI | BLM WQC |
| 14d_1dph | 4 | LC50 | >7.12 | NE | 3.59 | EC50 | >7.12 | NE | 3.59 |
| | 14 | LC20 | 4.36 | (3.75-5.06) | 2.23 | EC20 | 2.13 | (1.89-2.40) | 2.23 |
| 4d_28dph | 4 | LC50 | 11.91 | (7.50-18.91) | 3.38 | EC50 | 8.71 | (6.78-11.20) | 3.38 |
| 4d_35dph | 4 | LC50 | 15.00 | (7.27-30.92) | 2.53 | EC50 | 8.88 | (6.58-11.98) | 2.53 |

## Swimming Activity Endpoints

| Exposure | Days of Exposure | Total Distance Traveled (cm) | | | | Average Velocity (cm/sec) | | | | Time Spent Moving (sec) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Effect Concentration | | 95% CI | BLM WQC | Effect Concentration | | 95% CI | BLM WQC | Effect Concentration | | 95% CI | BLM WQC |
| 14d_1dph | 4 | EC50 | 6.49 | (1.05-40.02) | 3.59 | EC50 | 6.52 | (1.25-34.12) | 3.59 | EC50 | 3.38 | (0.58-19.54) | 3.59 |
| | 14 | EC20 | 1.50 | (0.62-3.64) | 2.23 | EC20 | 1.45 | (0.62-3.64) | 2.23 | EC20 | 1.30 | (0.81-2.09) | 2.23 |
| 4d_28dph | 4 | EC50 | >7.38 | NE | 3.38 | EC50 | >7.38 | NE | 3.38 | EC50 | >7.38 | NE | 3.38 |
| 4d_35dph | 4 | EC50 | 4.26 | (2.07-8.76) | 2.53 | EC50 | 4.28 | (2.08-8.77) | 2.53 | EC50 | 4.78 | (2.37-9.64) | 2.53 |

Table 6. Percent of worms uneaten by juvenile white sturgeon (58 dph) exposure to copper. Effect concentrations with 95-percent confidence limits (CL) are presented for each endpoint as well as no observed effect concentrations (NOEC) and lowest observed effect concentrations. Gray shading indicates significant reduction from the control (Fishers LSD; $p<0.05$). NE = not estimated because data do not meet the conditions of a probit analysis or logistic regression.

| Toxicant | Measured Conc. (µg/L) | Percent of Worms Uneaten | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Day 0 | | Day 1 | | Day 2 | | Day 3 | | Day 4 | |
| | | Mean | SD (n=8) | Mean | SD (n=8) | Mean | SD (n=8) | Mean | SD (n=8) | Mean | SD (n=8) |
| Cu | 0.20 | 24 | 18 | 40 | 32 | 16 | 20 | 14 | 15 | 10 | 9 |
| | 0.69 | 28 | 24 | 29 | 27 | 18 | 14 | 23 | 19 | 16 | 17 |
| | 1.31 | 33 | 27 | 19 | 18 | 15 | 35 | 13 | 15 | 19 | 33 |
| | 2.80 | 23 | 19 | 21 | 21 | 11 | 16 | 13 | 15 | 21 | 27 |
| | 5.10 | 31 | 31 | 18 | 20 | 27 | 26 | 20 | 12 | 29 | 18 |
| | 10.65 | 54 | 34 | 29 | 14 | 22 | 17 | 51 | 22 | 70 | 19 |
| NOEC | | 10.65 | | 0.2 | | 10.65 | | 5.1 | | 5.1 | |
| LOEC | | >10.65 | | 0.69 | | >10.65 | | 10.65 | | 10.65 | |
| EC10 (CL) | | | | | | | | | | NE | |
| EC20 (CL) | | | | | | | | | | NE | |
| EC50 (CL) | | | | | | | | | | NE | |

## Figure Legends

Figure 1: Time to reach the lowest observed effect concentrations (days to first response) for three endpoints in a 14-day copper exposure with larval white sturgeon (14d_1dph). Effective mortality includes mortality plus organisms that displayed a loss of equilibrium or immobility.

Figure 2: Total distance traveled during a 10-minute activity trial of larval white sturgeon exposed to copper. Activity was measured daily, starting on Day 3, throughout the 14-day test started with 1 day-post-hatch sturgeon (14d_1dph). Asterisks indicate the lowest concentration with a significant change in activity from fish in control treatment (lowest observable effect concentration). Error bars represent +/- standard error.

Figure 3: Total distance traveled during 10-minute recordings of larval white sturgeon exposed to different concentrations of copper for a 96-hour time period. Error bars represent +/- standard error.

Figure 4: Total distance traveled during 10-minute recordings of larval white sturgeon exposed to difference concentrations of copper for a 96-hour time period. The control, lowest copper concentration that was significantly different from the control, and the highest concentration of copper tested are displayed with the other intermediate concentrations removed to clearly show the trends described in the text. Asterisks represent a significant change in distance traveled compared to control treatment.

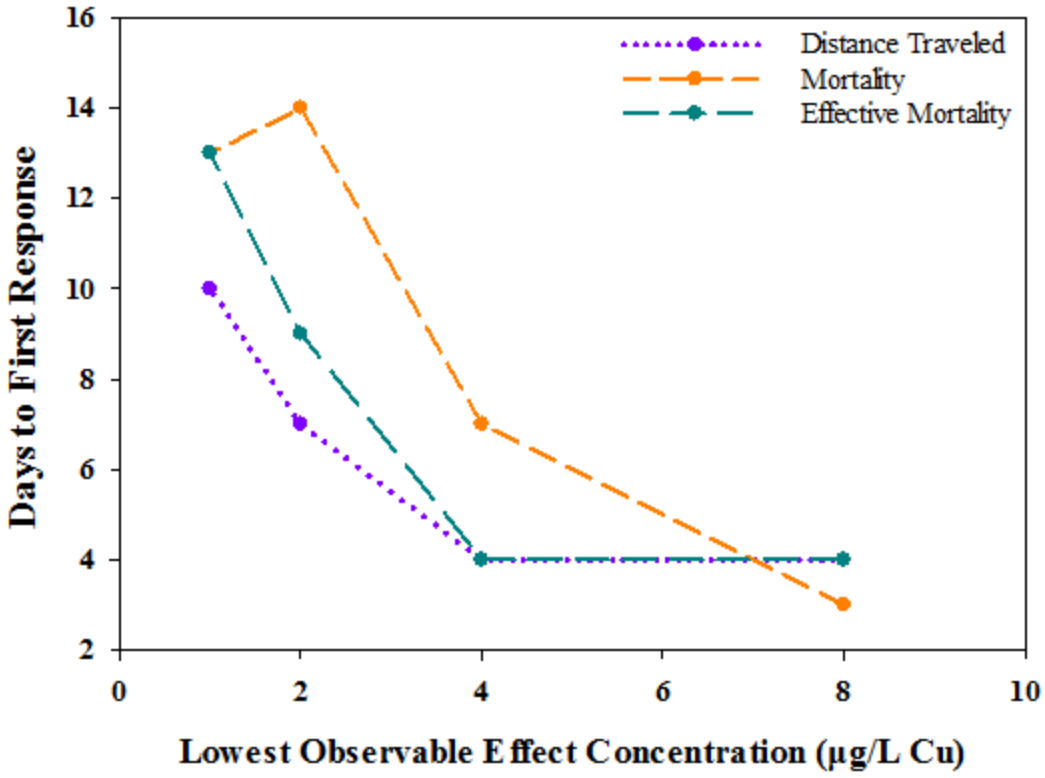

Distance Traveled
Mortality
Effective Mortality
Days to First Response
16
14
12
10
8
6
4
2
0
2
4
6
8
10
Lowest Observable Effect Concentration (µg/L Cu)

Figure 1.

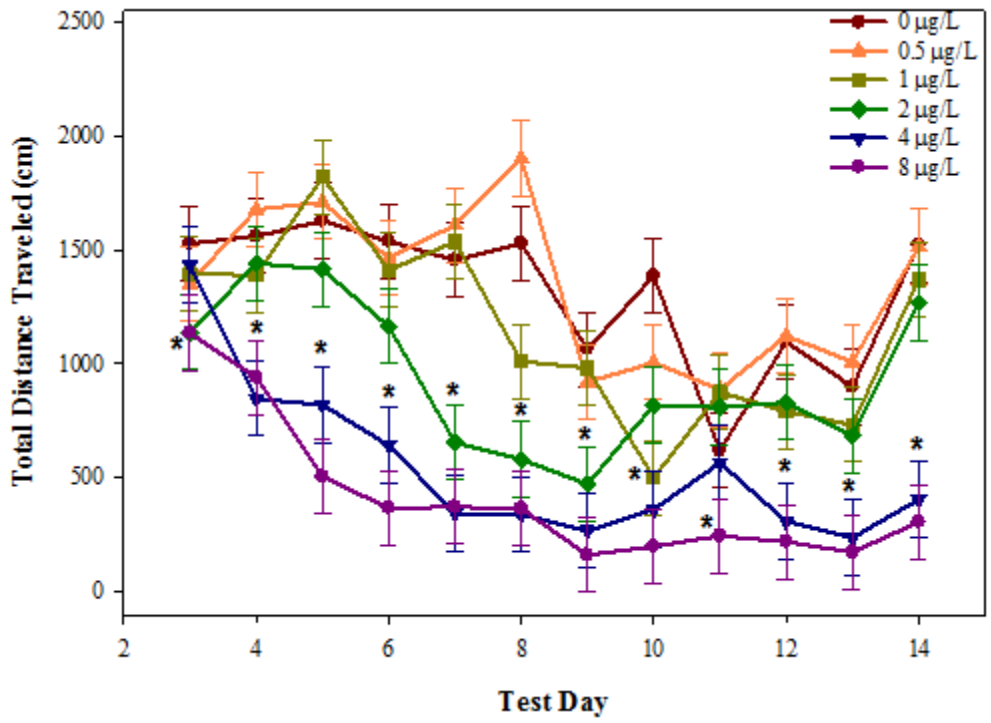

Total Distance Traveled (cm)
Test Day
0 µg/L
0.5 µg/L
1 µg/L
2 µg/L
4 µg/L
8 µg/L
2500
2000
1500
1000
500
0
2
4
6
8
10
12
14

Figure 2.

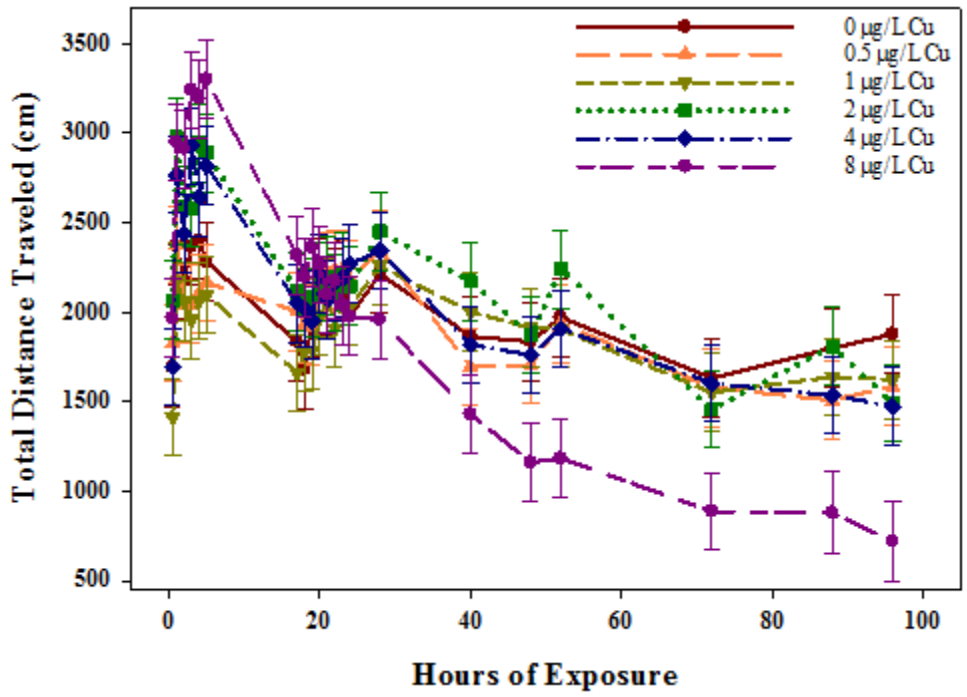

Total Distance Traveled (cm)
3500
3000
2500
2000
1500
1000
500
0
20
40
60
80
100
Hours of Exposure
0 µg/L Cu
0.5 µg/L Cu
1 µg/L Cu
2 µg/L Cu
4 µg/L Cu
8 µg/L Cu

Figure 3

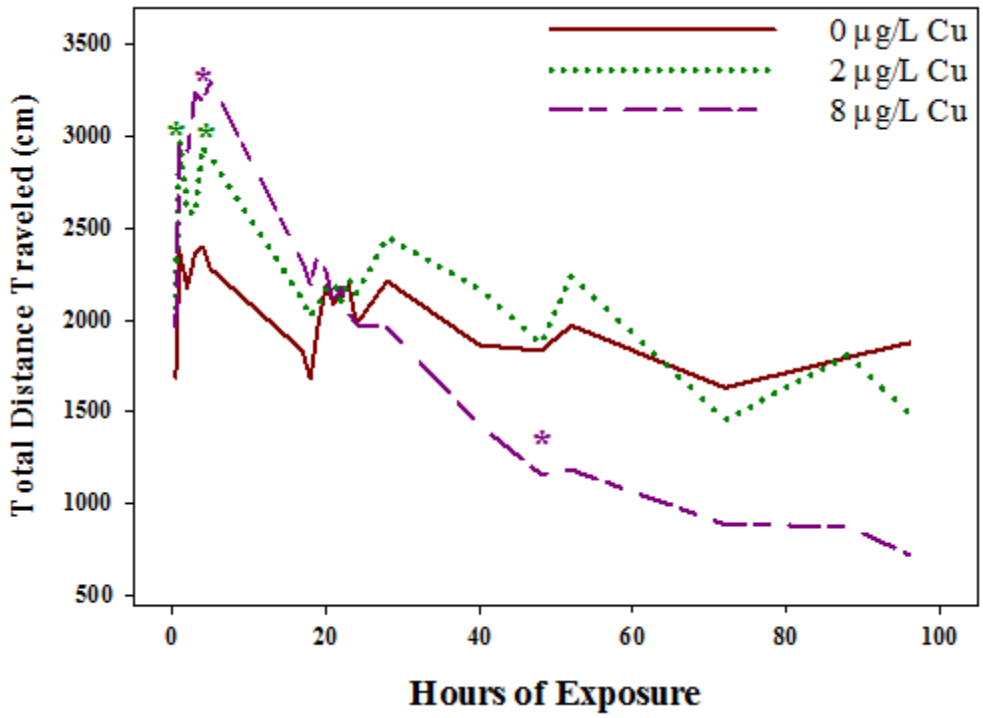

0 µg/L Cu
2 µg/L Cu
8 µg/L Cu
Total Distance Traveled (cm)
3500
3000
2500
2000
1500
1000
500
0
20
40
60
80
100
Hours of Exposure

Figure 4.

# CHAPTER 3

## TOXICITY OF METAL CONTAMINATED SEDIMENTS FROM THE UPPER COLUMBIA RIVER TO EARLY LIFE STAGE WHITE STURGEON

Holly J. Puglis

**Abstract:** The upper Columbia River (UCR) has been contaminated with metals from effluents resulting from smelter and mining activities, resulting in some UCR sediment metal concentrations being elevated above biological criteria concentrations in some sediments. In the transboundary reach of the UCR, White Sturgeon population declines have been associated with little to no natural recruitment for decades. Copper, which is present in significant concentrations in slag, can be mobilized from slag containing sediments and is highly toxic to early life-stage (ELS, <30 days post hatch [dph]) White Sturgeon. Our objective was to evaluate toxicity of slag-contaminated sediments to ELS White Sturgeon and to assess responses of various behavioral endpoints during exposure using field-collected sediments from the affected reaches of the UCR. We exposed ELS White Sturgeon to sediments from sites in the UCR downstream from the Canadian border. Sediments were selected for testing based on slag characteristics, copper concentration, and the potential for use as habitat by ELS sturgeon. Sturgeon were exposed to sediments for 14 days, starting with 3 dph fish. Mortality was recorded and swimming activity endpoints were measured at the end of the exposure. In the overlying water, cumulative hardness-based chronic toxic units for the metal mixture of copper, cadmium, lead, nickel, and zinc were >1.0 for three of the six sediment treatments tested. Chronic toxic units for copper estimated with a biotic ligand model were also >1.0 in three of the six sediment treatments with both measures of toxic units >1.0 in two of those three treatments. Sturgeon exposed to sediment collected near the city of Northport, WA, which had toxic units >1.0 for both measures, had significant increases in mortality and significant decreases in swimming activity, compared to sturgeon in the control treatment, which were exposed to clean quartz sand. No effects were observed

in the other sediment treatments. This study sheds light on the relationships between exposure to metals associated with slag and adverse effects on the White Sturgeon population.

## Introduction

White sturgeon populations in North America have been declining for decades. In the transboundary reach of the upper Columbia River (UCR), the declines have been associated with little to no natural recruitment in the population since the late 1960s (Hildebrand and Parsley 2013). Poor recruitment in this system may be due to a number of factors including habitat degradation (Beamesderfer and Farr 1997, McAdam et al. 2005, McAdam 2011), hydrologic changes (Parsley and Beckman 1994, Paragamian et al. 2009), and predation by introduced species (Gadomski and Parsley 2005).

The UCR has also been contaminated by metal-loaded effluents resulting from smelter and mining activities (Johnson et al. 1988, Johnson et al. 1990, Bortleson et al. 2001, Paulson and Cox 2007). Approximately 10 million metric tons of slag, as much as 360 tons per day, in addition to liquid effluent were released from a lead-zinc smelter in Trail, British Columbia, from 1947 until 1995, resulting in significant metal loads being released to the river. Bortleson et al. (2001) determined that concentrations of metals in UCR sediments were elevated above biological criteria concentrations. Nursery areas for early-life-stage sturgeon have been found in the area between the Little Dalles and Dead Man's Eddy (Howell and McLellan 2009), in the upper reaches of the Columbia River, where there are also significant accumulations of slag. Recent studies demonstrated that copper is toxic to

early-life-stage white sturgeon (Little et al. 2012, Vardy et al. 2013, Calfee et al. 2014, Puglis 2018). Significant concentrations of copper are present in slag and can be mobilized from slag contaminated sediments (Paulson et al. 2006, Paulson and Cox 2007).

While the chemistry would suggest that metal concentrations from these sediments are of concern for ELS white sturgeon, whole sediment toxicity studies with UCR sediment have not been conducted with white sturgeon. Thus, the objective of the current study was to evaluate the toxicity of slag-contaminated sediments collected at various sites in the UCR to early-life-stage white sturgeon, and to assess various behavioral endpoints of early-life-stage sturgeon exposed to these slag-contaminated and reference sediments.

## Materials and Methods

The sediment toxicity test was conducted in basic accordance with standard methods for conducting short-term whole-sediment toxicity tests with freshwater invertebrates (ASTM 2013a) and applied to sturgeon. This research complied with animal use guidelines described by the American Society of Ichthyologists and Herpetologists, the American Fisheries Society, and the American Institute of Fishery Research Biologists (Nickum et al. 2004), in addition to all USGS, Columbia Environmental Research Center's protocols for the humane treatment of test organisms during culture and experimentation. Sediments from sites in the upper reaches of the Columbia River were targeted for testing based on slag characteristics, copper concentration, and the potential for habitat use by very early-life-stage sturgeon. Sediment was collected by the UCR natural resources trustees in September 2013 from six sites within the first reach UCR, just downstream from the international border with Canada (Figure 1), and a tributary, which was included as a reference site. Shallow water

sites were identified and sediment was collected using a PVC scoop from the top 7.5 cm of bed sediment. After collection, the sediment samples were sieved to exclude particles larger than 2mm. Sediments were shipped to the Columbia Environmental Research Center and stored at 4°C until testing.

In addition to the reference site, in-house quartz sand control sediment was also tested. The sediments were intended to simulate the benthic habitat that very early-life-stage white sturgeon may encounter in the upper reaches of the UCR during spawning and while entering into the drift and hiding phases. About 1 week before the start of the sediment toxicity test, each sediment sample was homogenized, 1 L of sediment was placed into glass exposure chambers resulting in nearly 3 cm sediment substrate to which 5.5 L of overlying water (hardness of 100 mg/L and DOC of about 0.4 mg/L) was added. A total of 5 pieces of smooth flat stone (4- to 5-cm diameter) were placed on the surface of the substrate in each replicate chamber to provide shelter for fish through the hiding stage (Wang et al. 2014). One proportional diluter was used in the study. Three replicate chambers of each sediment were randomly placed in each spatial block of the diluter, resulting in 6 replicate chambers per sediment. Before the start of the exposure, the sediments were held under static conditions to allow the sediment samples to equilibrate with oxygenated overlying water (Ingersoll et al. 2008, Wang et al. 2013). Water delivery to the exposure chambers began four days before animals were added to the system. The diluter system provided about 250 ml of water to each replicate chamber every 4 hours (about 2 volume replacements/d). This test was conducted at 15 °C. At the start of the exposure, 30 white sturgeon (3 dph) were randomly transferred to each replicate chamber.

We intended to utilize a behavioral checklist to identify the onset of behavioral impairment (loss of equilibrium, change in fish location in aquaria, loss of feeding, inactivity, darkened coloration, or rapid respiration; (ASTM 2013b)) associated with sturgeon contact with the sediment; however, visibility varied among replicates after the addition of sturgeon due to fine sediments being suspended in the water column from sturgeon swimming activity. In some cases, visibility was very low and made it difficult or impossible to record observations from the behavioral checklist. Fish were fed brine shrimp ad libitum twice daily starting on Day 9, before the onset of exogenous feeding. When visibility allowed, dead fish were removed daily.

On Day 14, spontaneous swimming activity trials were conducted by randomly selecting one surviving individual from each of 6 replicates and placing each fish in an observation arena, a polystyrene cylinder 9-cm diameter x 11.5-cm tall. Each observation arena contained 450 mls of control water, rather than test water, to increase visibility. Once each observation arena was stocked with fish, the fish were allowed to acclimate for 60 minutes in the absence of personnel activity near the test system. Following the acclimation period, spontaneous swimming activity for all fish in the observation arenas was recorded, simultaneously, for 10 minutes using overhead security cameras. This process was repeated with a second fish from each replicate such that swimming activity of two fish from each replicate chamber was recorded. All fish used in the swimming activity assay, as well as the remaining survivors from the sediment chambers, were euthanized at the end of the exposure.

Both physical and chemical characterization of sediment samples occurred in conjunction with a different study in the fall of 2013 (Besser et al. 2017). Each sediment

sample was homogenized and a subsample was sent to University of Missouri soil characterization laboratory (NRCS 2014) for particle size and total organic carbon analysis. Sediment samples were also analyzed for total recoverable metals (As, Cd, Cr, Cu, Pb, Ni, and Zn), acid volatile sulfide (AVS), and simultaneously extracted metals (SEM; Cd, Cu, Pb, Ni, Zn) according to Besser et al. (2017). Briefly, sediments for total recoverable analyses were microwave digested in concentrated acid (similar to U.S. EPA 3050B), which was intended to solubilize metals that could become environmentally or biologically available. Sulfide was acid extracted, trapped in an antioxidant buffer, and quantified using a sulfide-selective electrode (Brumbaugh et al. 2011). Total recoverable and SEM metals were quantified by inductively coupled plasma-mass spectrometry (ICP-MS; similar to U.S. EPA 6020B).

The fraction of slag present in samples of UCR sediment was determined by comparison of the elemental proportion of individual sediment grains. The proportion of calcium, iron, and silica in slag typical found in the UCR differs substantially from the proportions found in common rock forming minerals present in Columbia River sediments. Physical and elemental characteristics of slag from the UCR obtained from samples of granulated slag collected from Black Sand Beach (2009) and from near the abandoned Northport smelter site (2015). Elemental composition of individual sediment grains was determined using a scanning electron microscope interfaced with a computer-controlled energy dispersive X-ray spectrometer at the USGS Denver Microbeam Laboratory in Denver, Colorado. The fraction of slag present in a sediment sample was based on analysis of approximately 1000 individual sediment grain per sediment sample. Polished grain mounts

were examined using a JEOL 5800LV scanning electron microscope, operated at 20 keV and approximately 1 nA current, interfaced with a NORAN™ System 7 X-ray microanalysis system. Procedures followed those described by (Keulen et al. 2008). To differentiate between slag and non-slag particles ternary diagrams were prepared using normalized element weight percent values for Si, Ca, and Fe. Individual spectra were also reviewed for the indications of the presence of copper and zinc which are characteristic of UCR slag.

Water samples for major cations and metals analysis were taken from overlying water just above the sediment layer on Days -1, 7 and 14 of the exposure. Samples were collected using a syringe fitted with a pre-cleaned polypropylene straw and then filtered through a 0.45 µm polyethersulfone membrane for analysis of metals (Cd, Cu, Pb, Ni, and Zn), major ions (Ca, Mg, Na, Sr, K, Fr, Mn, F, Cl, $NO_2$, $NO_3$, Br, $SO_4$, and $PO_4$), and dissolved organic carbon (DOC). Metals and cation samples were acidified to 1-2 % (v/v) with house-distilled nitric acid for preservation and analysis. Anion samples were refrigerated for up to 30 days prior to analysis, and DOC samples were acidified with 9 N sulfuric acid to 0.5 % (v/v) and then refrigerated for up to 28 days prior to analysis. Metals and cations were measured by ICP-MS (similar to U.S. EPA 6020B), and anions were measured using an ion chromatography system equipped with a conductivity detector (similar to U.S. EPA 9056A (U.S. EPA 2007a)). The DOC was measured as non-purgeable organic carbon using a total carbon analyzer (similar to U.S. EPA 415.3 (Potter and Wimsatt 2005)).

We tested for differences in mortality and swimming activity endpoints among sites using linear mixed models with the spatial blocks as a random factor and mortality or the activity endpoints as fixed effects. The unit of analysis in each model was replicate chamber.

Swimming activity was log transformed prior to analysis and mortality was arc sine square root transformed prior to analysis to better meet the assumption of normality. Mean differences were determined using Fisher's LSD (least significant difference).

We calculated several metrics to compare the potential metal-induced toxicity of the UCR sediments. Acid volatile sulfide binds to cationic metals and forms insoluble sulfide complexes that are generally not bioavailable, thus AVS is a key component controlling the potential metal toxicity of sediments (Di Toro et al. 1990, Di Toro et al. 1992). We calculated excess SEM values for each sediment using the following equation: ∑SEM-AVS. Sediments with no excess SEM (∑SEM ≤ AVS) are generally considered safe for benthic invertebrates exposed to sediments contaminated with metals (U.S. EPA 2005). Organic carbon can also bind metals, removing them from the bioavailable fraction of metals; therefore, we also calculated organic carbon normalized excess SEM using the equation (∑SEM-AVS)/$f_{OC}$, where $f_{OC}$ is the fraction of organic carbon in sediment (U.S. EPA 2005). Adverse effects due to metals would be predicted at concentrations of excess carbon normalized SEM greater than 3000 µmol/$g_{OC}$ (U.S. EPA 2005). We also calculated cumulative metal toxic units in overlying water using measured metal data. To determine the cumulative hardness-based toxic unit of the metal mixture, consisting of cadmium, copper, lead, nickel and zinc, at each site, chronic water-quality criteria were calculated for each metal (U.S. EPA 2009) using average water hardness from overlying water in each treatment. Chronic toxic units were calculated for each metal on each sampling date and then summed to determine the cumulative chronic toxic unit of the metal mixture from the overlying water of each sediment treatment on each sampling date (U.S. EPA 2005). Because copper is the

primary metal of concern at the site, and because the biotic ligand model (BLM) may be a better estimate of copper toxicity, we calculated copper toxic units in the overlying water from each sediment treatment using average water-quality characteristics from each site, based on EPA (U.S. EPA 2007b) water-quality criteria determined by Windward Environmental Cu BLM (Version 3.1.2.37).

## Results

Percent sand ranged from 93-98%, with fines (silt + clay) ranging from 2-7% at all sites (Table 1). Site sediments had a range of 5%–38% slag content and the reference site had no slag (Table 1). Total organic carbon ranged from 0.05-0.24% at all sites (Table 1). Total recoverable metals are provided in the associated data release (Puglis et al. 2018). Simultaneously extracted metals are summarized in Table 2. AVS ranged from 3.74-32 µmol/g in the site sediments and the reference site had AVS of 0.27 µmol/g (Table 2). All sites, except for the reference site, had an excess of SEM with values (∑SEM-AVS) ranging from 15-199 µmol/g (reference was -0.22 µmol/g) (Table 2). All sites, except for the reference, also had an excess carbon-normalized SEM value ([∑SEM-AVS]/$f_{OC}$) greater than 3000 µmol/g with values ranging from 3000-39800 µmol/g among sites (reference was -44 µmol/g) (Table 2).

Overlying water-quality characteristics were similar among sediment treatments (DO [mg/L] 7.4 ± 1.07, conductivity [µs/cm, 25°C] 273 ± 9.97, pH 7.9 ± 0.07, alkalinity as $CaCO_3$ [mg/L] 97 ± 3.06, hardness as $CaCO_3$ [mg/L] 106 ± 3.62, and total ammonia [mg N/L] 0.06 ± 0.05; Table 4). DOC values in overlying water varied by sediment treatment and over time during the exposure, with values ranging from 0.32–1.18 mg/L (Table 4). Anions and cations in overlying water were similar among sediment treatments (Tables 5–6). Measured metals

from overlying water are summarized in Table 7. Briefly, nickel concentrations ranged from <0.05 to 0.48 µg/L, copper values ranged from 0.17 to 11.90 µg/L, zinc values ranged from 0.86 to 59.50 µg/L, cadmium values ranged from <0.02 to 0.43 µg/L, and lead values ranged from <0.02 to 0.69 µg/L.

Inputs used to calculate hardness-based cumulative toxic units are summarized in the associated data release (Puglis et al. 2018). Average hardness-based cumulative toxic units ranged from 0.13–1.79, and overlying waters of sediments from CERC 04, CERC 06, and CERC 07 exceeded 1.0 toxic unit (Table 8). Inputs used to calculate BLM normalized chronic copper toxic units are summarized in the associated data release (Puglis et al. 2018). Average BLM-based copper toxic units ranged from 0.10–2.24 and overlying waters of sediment from CERC 04, CERC 05, and CERC 06 exceeded 1.0 toxic units (Table 8). Average cumulative hardness-based chronic toxic units with the BLM-normalized chronic copper toxic units substituted for the hardness-based copper values ranged from 0.21-2.70 and CERC 02, CERC 04, CERC 05, CERC 06, and CERC 07 had TUs > 1 (Table 8).

There was a significant effect of site on mortality ($p<0.0001$). Mortality ranged from 2.8–6.1% for all sediment treatments, including the reference sediment and the quartz sand control, except for sturgeon exposed to sediment from CERC 06. This treatment had an average mortality of 27% by Day 14, significantly greater mortality than the reference site (Table 9). There was also a significant effect of site on swimming activity endpoints (distance traveled $p=0.039$; average velocity $p=0.035$; time spent moving $p=0.027$) with a reduction in all swimming activity endpoints for fish exposed to sediments from CERC 06 compared to the

reference site, with a 42–50% decrease in distance traveled, velocity, and time spent moving (Table 9).

## Discussion

Toxicity tests conducted with aquatic sediments collected from sites in the UCR provided an opportunity to test the hypothesis that such exposure would induce injury similar to that observed during aqueous copper exposures. Little et al. (2014) determined that leachates derived from slag-contaminated sediments collected from dry gravel bars induced mortality in white sturgeon and Paulson and Cox (2007) noted that metals such as copper increased markedly in water that had been incubated over these sediments for up to 39 d. Freshwater invertebrates also showed signs of toxicity due to exposure to slag-contaminated sediments; for example, amphipods failed to colonize slag-contaminated sediments, potentially because of toxicity, avoidance of metals, and/or avoidance of angular slag particles (Fairchild et al. 2012). In contrast, some white sturgeon life stages did not display aversive behaviors in their epibenthic association with the sediments (Little et al. 2014), a circumstance that would maximize exposure.

In the present study, a 14-day exposure of larval white sturgeon to sediment from CERC 06 induced both significant mortality and significant reductions in swimming behavior. CERC 06 is approximately 0.4 km upstream from river mile 735 and approximately 30 km from the smelter. It is located near the city of Northport and corresponds to site LR7 in Besser et al. (2008). CERC 06 is about 2 river miles downstream from the Deadman's Eddy site previously investigated by Fairchild et al. (2012) and Little et al. (2014). Sediment from this site is characterized as being dominated (>70%) by medium

and coarse sand and is just downstream from a large gravel bar. Exposure to sediments from the other sites did not induce mortality during the 14-d exposures. It remains uncertain if other behavior such as position in the water column, loss of equilibrium, immobility, etc., was affected by exposure since observation was obscured by turbidity. Turbidity is noteworthy; however, in that it was a result of fish attempting to seek cover in the sediments.

In previous studies, Little et al. (2014) conducted toxicity tests with sediment samples from gravel bars and determined toxicity to white sturgeon at several sites, as well as elevated concentrations of metals, including copper, similar to those observed during the present study. The samples collected at gravel bars were likely quite different than the aquatic sediments tested in the present study because the gravel bars were subjected to repeated intervals of submerged and exposed conditions that would likely change the oxidative state of the metals as well as other changes in water chemistry. This in turn would influence metal dissociation from slag particles and alter availability and toxicity to fish.

Environmental variables also vary along the course of the river, which could influence the availability and toxicity of metals. Different chemical environments could influence dissociation of metals from slag particles. Water-quality variables including water hardness, pH, DOC, anions, cations, and other chemical variables could significantly increase or decrease the availability and toxicity of copper and other metals (U.S. EPA 2007b). In the present study, anions, cations, water hardness, and pH were similar among sites (Tables 3, 5, 6). DOC was generally higher at the beginning of the study and stabilized by Day 7 sampling, with DOC ranging from 0.32–1.18 mg/L (Table 4). Excess carbon-normalized SEM values were calculated to normalize the sediment chemistry and toxic units were

calculated to normalize overlying water chemistries to a common effect level. Adverse biological outcomes were predicted according to the excess carbon-normalized SEM values at all sites except for the reference; however, toxicity was only observed at CERC 06. The theory behind these calculations is based on data using benthic invertebrates, many of which burrow into the sediment, thus such values may not be good predictors of toxicity for benthic fishes such as white sturgeon. The hardness-based and BLM-normalized toxic units are calculated by comparing metal concentrations to the appropriate ambient water quality criteria, thus values > 1 indicate concentrations of the metals are greater than the water quality criteria and suggest potential toxicity. Of the overlying water of each site with toxic units >1, only sediment from CERC 06 induced toxicity to larval white sturgeon in this study. CERC 06 did not have the highest average cumulative hardness-based toxic unit, but did have the highest average BLM-normalized copper toxic unit and average cumulative hardness-based toxic unit with the BLM-normalized Cu toxic unit, suggesting that copper was driving the toxicity at this site. Additionally, copper in the overlying water in the CERC 06 sediment treatments were consistently elevated throughout the exposure compared to other sediment treatments (Table 7).

In water only toxicity studies, significant effects on mortality and behavior have been documented below acute and chronic water quality criteria values for copper (Vardy et al. 2013, Calfee et al. 2014, Puglis 2018). In the present study with sediment, copper values were above the BLM normalized chronic WQC in CERC 04, CERC 05, and CERC 06 (i.e. BLM normalized copper TU > 1; Table 8). Predicting the toxicity of mixtures is critical to accurately assessing the risk of contaminated environments as contaminants are often present

in mixtures. Our current approach in calculating the toxic units of the overlying water, assumes the toxicity of the mixture is additive. This approach is commonly used for mixtures, but in reviews of metal mixture data, greater than 40% of metal mixtures in the literature had less than additive toxicity (Norwood et al. 2003, Meyer et al. 2015). There is currently no consensus on how to model multi-metal mixtures (Nys et al. 2018). The lack of effect in the current study suggests that the mixture of metals in the overlying water are antagonistic, or that the presence of sediment somehow reduces the toxicity of the overlying water. Differences between water only and sediment toxicity tests have been documented previously. For example, Besser and Leib (2007) found that in a system affected by acid mine drainage, the toxicity of impacted stream water to amphipods was significantly reduced in the presence of sediments. The authors speculated that the sediment was sorbing metals from the overlying water, thus reducing the bioavailable concentration of metals in the water. Conversely, amphipods exposed to sediments contaminated by polycyclic aromatic hydrocarbons and polycyclic chlorinated biphenyls, resulted in greater toxicity than amphipods exposed to only the overlying water without sediment (Ingersoll et al. 2000). It is unclear why the presence of sediment in the current study would reduce the toxicity of the overlying water.

In the UCR, white sturgeon are able to successfully spawn, but no natural recruitment to the population has been detected in decades with no wild juveniles or subadults being captured in the system in decades (Hildebrand and Parsley 2013). Efforts to assist in the recovery of the population have historically centered on catching wild adults to spawn in hatcheries, rear the larvae in hatcheries, and then release the juveniles back into the system.

This method yields great success with hatchery-reared sub-adults being successfully recruited into the population. More recent efforts have documented that tens of thousands of wild larvae are drifting from spawning locations, but still, no wild juveniles have been documented later in the season (Jason McLellan, personal communication). This would corroborate the findings of the present study- that white sturgeon are able to hatch and survive for a couple of weeks in the UCR. It may also indicate that our study was not long enough to determine whether slag plays a role in the white sturgeon population declines documented in the UCR.

**Acknowledgements:** I thank the staff in the Ecology Branch, Toxicology Branch, and Environmental Chemistry Branch of the US Geological Survey (USGS), Columbia Environmental Research Center, for technical assistance. I thank C. Mebane and J. Besser for comments that improved the manuscript. Funding for the present study was provided by the National Park Service through the Department of the Interior Central HazMat Fund the USGS Contaminants Biology Program.

**Data availability:** The data underlying the findings reported here are freely available in the ScienceBase public repository (*https://doi.org/10.5066/P95TNP8G)*.

## Literature Cited


ASTM International. 2013a. Standard test method for measuring the toxicity of sediment-associated contaminants with freshwater invertebrates, E1706-05(2010). Annual Book of ASTM Standards, v. 11.06. ASTM International, West Conshohocken, Pennsylvania.

ASTM International. 2013b. Standard guide for measurement of behavior during fish toxicity tests, E1711–95 (2008). Annual Book of ASTM Standards, v. 11.06. ASTM International, West Conshohocken, Pennsylvania.

Beamesderfer, R. C. P., and R. A. Farr. 1997. Alternatives for the protection and restoration of sturgeons and their habitat. Environmental Biology of Fishes **48**:407-417.

Besser, J. M., W. G. Brumbaugh, C. D. Ivey, C. G. Ingersoll, and P. W. Moran. 2008. Biological and chemical characterization of metal bioavailability in sediments from Lake Roosevelt, Columbia River, Washington, USA. Archives of Environmental Contamination and Toxicology **54**:557-570.

Besser, J. M., and K. J. Leib. 2007. Toxicity of metals in water and sediment to aquatic biota. Integr Investig Environ Eff Hist Min Animas River Watershed S Juan Cty Colo.

Besser, J.M., J.L. Kunz, S.E. Cox, C.G. Ingersoll, and W.G. Brumbaugh. 2017. Physical and chemical data from an evaluation of metal-contaminated sediments and pore water from the Upper Columbia River, Washington, 2013-2014. U.S. Geological Survey Data Release. https://doi.org/10.5066/F72F7KPC.

Bortleson, G. C., S. Cox, M. Munn, R. Schumaker, E. Block, L. Bucy, and R. Cornelius. 2001. Sediment-quality assessment of Franklin D. Roosevelt Lake and the upstream reach of the Columbia River, Washington, 1992. 2331-1258, US Geological Survey; USGS Earth Science Information Center [distributor].

Brumbaugh, W. G., C. R. Hammerschmidt, L. Zanella, E. Rogevich, G. Salata, and R. Bolek. 2011. Interlaboratory comparison of measurements of acid-volatile sulfide and simultaneously extracted nickel in spiked sediments. Environmental Toxicology and Chemistry **30**:1306-1309.

Calfee, R. D., E. E. Little, H. J. Puglis, E. Scott, W. G. Brumbaugh, and C. A. Mebane. 2014. Acute sensitivity of white sturgeon (*Acipenser transmontanus*) and rainbow trout (*Oncorhynchus mykiss*) to copper, cadmium, or zinc in water-only laboratory exposures. Environmental Toxicology and Chemistry **33**:2259-2272.

Di Toro, D. M., J. D. Mahony, D. J. Hansen, K. J. Scott, A. R. Carlson, and G. T. Ankley. 1992. Acid volatile sulfide predicts the acute toxicity of cadmium and nickel in sediments. Environmental Science & Technology **26**:96-101.

Di Toro, D. M., J. D. Mahony, D. J. Hansen, K. J. Scott, M. B. Hicks, S. M. Mayr, and M. S. Redmond. 1990. Toxicity of cadmium in sediments: the role of acid volatile sulfide. Environmental Toxicology and Chemistry **9**:1487-1502.

Fairchild, J., N. Kemble, A. Allert, W. Brumbaugh, C. Ingersoll, B. Dowling, C. Gruenenfelder, and J. Roland. 2012. Laboratory toxicity and benthic invertebrate field colonization of upper Columbia River sediments: finding adverse effects using multiple lines of evidence. Archives of Environmental Contamination and Toxicology **63**:54-68.

Gadomski, D. M., and M. J. Parsley. 2005. Laboratory studies on the vulnerability of young white sturgeon to predation. North American Journal of Fisheries Management **25**:667-674.

Hildebrand, L. R., and M. Parsley. 2013. Upper Columbia White Sturgeon Recovery Plan - 2012 Revision. Prepared for the Upper Columbia White Sturgeon Recovery Initiative.

Howell, M. D., and J. G. McLellan. 2009. Lake Roosevelt White Sturgeon Recovery Project: Annual Progress Report, January 2003–March 2004. Bonneville Power Administration (BPA), Portland, OR (United States).

Ingersoll, C., J. Besser, W. Brumbaugh, C. Ivey, N. Kemble, J. Kunz, T. May, N. Wang, D. MacDonald, and D. Smorong. 2008. Sediment chemistry, toxicity, and bioaccumulation data report for the US Environmental Protection Agency–Department of the Interior sampling of metal-contaminated sediment in the Tri-state Mining District in Missouri, Oklahoma, and Kansas. Report prepared by MacDonald Environmental Sciences Ltd and USGS Columbia MO for USEPA, Kansas City, MO and Dallas, TX and for USFWS, Columbia, MO.

Ingersoll, C. G., C. D. Ivey, E. L. Brunson, D. K. Hardesty, and N. E. Kemble. 2000. Evaluation of toxicity: Whole-sediment versus overlying-water exposures with amphipod Hyalella azteca. Environmental Toxicology and Chemistry **19**:2906-2910.

Johnson, A., D. Norton, B. Yake, and S. Twiss. 1990. Transboundary metal pollution of the Columbia River (Franklin D. Roosevelt Lake). Bulletin of Environmental Contamination and Toxicology **45**:703-710.

Johnson, A., D. E. Norton, B. Yake, and S. Twiss. 1988. An assessment of metals contamination in Lake Roosevelt. Washington State Department of Ecology, Water Qualtity Investigations Section.

Keulen, N., D. Frei, S. Bernstein, M. T. Hutchison, C. Knudsen, and L. Jensen. 2008. Fully automated analysis of grain chemistry, size and morphology by CCSEM: examples from cement production and diamond exploration. Geological Survey of Denmark and Greenland Bulletin **15**:93-96.

Little, E. E., R. D. Calfee, and G. Linder. 2012. Toxicity of Copper to Early-life Stage Kootenai River White Sturgeon, Columbia River White Sturgeon, and Rainbow Trout. Archives of Environmental Contamination and Toxicology **63**:400-408.

Little, E. E., R. D. Calfee, and G. Linder. 2014. Toxicity of smelter slag-contaminated sediments from Upper Lake Roosevelt and associated metals to early life stage White Sturgeon (Acipenser transmontanus Richardson, 1836). Journal of Applied Ichthyology **30**:1497-1507.

McAdam, S. O. 2011. Effects of substrate condition on habitat use and survival by white sturgeon (Acipenser transmontanus) larvae and potential implications for recruitment. Canadian Journal of Fisheries and Aquatic Sciences **68**:812-822.

McAdam, S. O., C. J. Walters, and C. Nistor. 2005. Linkages between white sturgeon recruitment and altered bed substrates in the Nechako River, Canada. Transactions of the American Fisheries Society **134**:1448-1456.

Meyer, J. S., K. J. Farley, and E. R. Garman. 2015. Metal mixtures modeling evaluation project: 1. Background. Environmental Toxicology and Chemistry **34**:726-740.

Nickum, J., H. Bart Jr, P. Bowser, I. Greer, C. Hubbs, J. Jenkins, J. MacMillan, J. Rachlin, J. Rose, and P. Sorensen. 2004. Guidelines for the use of fishes in research. American Fisheries Society, Bethesda, MD.

Norwood, W., U. Borgmann, D. Dixon, and A. Wallace. 2003. Effects of metal mixtures on aquatic biota: a review of observations and methods. Human and Ecological Risk Assessment **9**:795-811.

NRCS. 2014. Kellogg soil survey laboratory methods manual, Soil Survey Investigations. Report No. 42, Version 5.0 USDA, Natural Resources Conservation Service, Lincoln, Nebraska.

Nys, C., T. Van Regenmortel, C. R. Janssen, K. Oorts, E. Smolders, and K. A. De Schamphelaere. 2018. A framework for ecological risk assessment of metal mixtures in aquatic systems. Environmental Toxicology and Chemistry **37**:623-642.

Paragamian, V. L., R. McDonald, G. J. Nelson, and G. Barton. 2009. Kootenai River velocities, depth, and white sturgeon spawning site selection - a mystery unraveled? Journal of Applied Ichthyology **25**:640-646.

Parsley, M. J., and L. G. Beckman. 1994. White sturgeon spawning and rearing habitat in the lower Columbia River. North American Journal of Fisheries Management **14**:812-827.

Paulson, A. J., and S. E. Cox. 2007. Release of elements to natural water from sediments of Lake Roosevelt, Washington, USA. Environmental Toxicology and Chemistry **26**:2550-2559.

Paulson, A. J., R. J. Wagner, R. F. Sanzolone, and S. E. Cox. 2006. Concentrations of elements in sediments and selective fractions of sediments, and in natural waters in contact with sediments from Lake Roosevelt, Washington, September 2004. 2331-1258.

Potter, B., and J. Wimsatt. 2005. METHOD 415.3—Measurement of total organic carbon, dissolved organic carbon and specific UV absorbance at 254 nm in source water and drinking water. US Environmental Protection Agency, Washington, DC Google Scholar.

Puglis, H.J. 2018. Behavioral effects of copper on larval White Sturgeon.

Puglis, H.J., R.D. Calfee, and E.E. Little. 2018. Toxicity of metal contaminated sediments from the upper columbia river to early life stage white sturgeon. U.S. Geological Survey Data Release. *https://doi.org/10.5066/P95TNP8G*

U.S. EPA. 2005. Procedures for the derivation of equilibrium partitioning sediment benchmarks (ESBs) for the protection of benthic organisms: Metal mixtures (cadmium copper, lead nickel, silver, and zinc). EPA-600-R-Q2-011, U.S. Environmental Protection Agency, Office of Research and Development, Washington DC.

U.S. EPA. 2007a. Method 9056—Determination of inorganic anions by ion chromatography. SW-846 Online—Test methods for evaluating solid waste, physical/chemical methods. U.S. Environmental Protection Agency, Washington, D.C.

U.S. EPA. 2007b. Aquatic life ambient freshwater-quality criteria—Copper. EPA-822-R-07-001, U.S. Environmental Protection Agency, Office of Water, Washington, D.C.

U.S. EPA. 2009. National recommended water quality criteria. U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology. http://water.epa.gov/scitech/swguidance/standards/criteria/current/index.cfm#altable

Vardy, D. W., J. Oellers, J. A. Doering, H. Hollert, J. P. Giesy, and M. Hecker. 2013. Sensitivity of early life stages of white sturgeon, rainbow trout, and fathead minnow to copper. Ecotoxicology **22**:139-147.

Wang, N., C. G. Ingersoll, R. A. Dorman, W. G. Brumbaugh, C. A. Mebane, J. L. Kunz, and D. K. Hardesty. 2014. Chronic sensitivity of white strugeon (*Acipenser transmontanus*) and rainbow trout (*Oncorhynchus mykiss*) to cadmium, copper, lead,

or zinc in laboratory water-only exposures. Environmental Toxicology and Chemistry **33**:2246-2258.

Wang, N., C. G. Ingersoll, J. L. Kunz, W. G. Brumbaugh, C. M. Kane, R. B. Evans, S. Alexander, C. Walker, and S. Bakaletz. 2013. Toxicity of sediments potentially contaminated by coal mining and natural gas extraction to unionid mussels and commonly tested benthic invertebrates. Environmental Toxicology and Chemistry **32**:207-221.

Table 1. Physical characterization of site sediments collected from the upper Columbia River.

| Lab Sample ID | Total Organic Carbon (%) | Clay (%) | Silt, total (%) | Silt, coarse (%) | Silt, fine (%) | Fines (silt+clay) (%) | Sand, total (%) | Sand, very coarse (%) | Sand, coarse (%) | Sand, medium (%) | Sand, fine (%) | Sand, very fine (%) | Slag (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CERC 01 | 0.24 | 0.5 | 6.1 | 3.5 | 2.6 | 6.6 | 93.4 | 0 | 1.2 | 27.8 | 52.6 | 11.8 | 9.6 |
| CERC 02 | 0.1 | 0.1 | 4 | 3.6 | 0.4 | 4.1 | 95.9 | 0.1 | 0.3 | 32.3 | 56.2 | 7 | 5.1 |
| CERC 04 | 0.09 | 0.1 | 2.5 | 1.8 | 0.7 | 2.6 | 97.4 | 0.1 | 15.8 | 52.8 | 24.4 | 4.3 | 38.2 |
| CERC 05 | 0.05 | 0.1 | 2.2 | 2.2 | 0 | 2.3 | 97.7 | 0.2 | 17.5 | 60.5 | 17.4 | 2.1 | 50.4 |
| CERC 06 | 0.12 | 0.1 | 2.5 | 2.5 | 0 | 2.6 | 97.4 | 1.9 | 26.8 | 45.5 | 21.1 | 2.1 | 33.7 |
| CERC 07 | 0.12 | 0.1 | 3.4 | 2.7 | 0.7 | 3.5 | 96.5 | 0.5 | 10.4 | 48.3 | 34.9 | 2.4 | 18.0 |
| CERC 09 | 0.16 | 0.1 | 2.3 | 1.6 | 0.7 | 2.4 | 97.6 | 8.8 | 23.1 | 35.4 | 26.4 | 3.9 | 0.0 |

Table 2. Chemical characterization of site sediments collected from the upper Columbia River.

| Simultaneously Extracted Metals and Acid Volatile Sulfides (µmol/g) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Lab Sample ID** | **AVS** | **Arsenic** | **Cadmium** | **Copper** | **Lead** | **Nickel** | **Zinc** | **Total SEM** | **ΣSEM-AVS** | **(ΣSEM-AVS)/$f_{OC}$ [1]** |
| CERC 01 | 12.7 | 0.0251 | 0.00538 | 1.64 | 0.353 | 0.086 | 32.3 | 34.4 | 21.7 | 4340 |
| CERC 02 | 3.74 | 0.039 | 0.00258 | 1.54 | 0.316 | 0.0499 | 16.8 | 18.7 | 15 | 3000 |
| CERC 04 | 11 | 0.0771 | 0.00627 | 10.5 | 0.813 | 0.141 | 136 | 147 | 136 | 27200 |
| CERC 05 | 10.6 | 0.117 | 0.0109 | 16.4 | 1.14 | 0.13 | 192 | 209 | 199 | 39800 |
| CERC 06 | 9.3 | 0.127 | 0.00685 | 13.5 | 2.11 | 0.122 | 166 | 182 | 172 | 34400 |
| CERC 07 | 32 | 0.0541 | 0.017 | 2.04 | 3.28 | 0.0649 | 101 | 106 | 73.9 | 14800 |
| CERC 09 | 0.265 | 0.00334 | 0.000089 | 0.00929 | 0.00652 | 0.0145 | 0.0153 | 0.0457 | -0.22 | -44 |

[1](ΣSEM-AVS)/$f_{OC}$ was calculated by dividing (ΣSEM-AVS) by the fraction of organic carbon ($f_{OC}$) in the sediment, where $f_{OC}$ is the percent of organic carbon in a sediment sample divided by 100. A minimum threshold of 0.5% total organic carbon was used in these calculations.

Table 3. Mean water quality characteristics (n=15 for temperature and dissolved oxygen, n=3 for all other measurements) for 14-d UCR sediment exposure with white sturgeon (*Acipenser transmontanus*). SD = standard deviation.

| | Temperature (°C) | | Dissolved Oxygen (mg/L) | | Conductivity (µs/cm, 25 ºC) | | pH | | Alkalinity as $CaCO_3$ (mg/L) | | Hardness as $CaCO_3$ (mg/L) | | Total ammonia (mg N/L) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Site** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** | **Mean** | **SD** |
| CERC 01 | 15.2 | 0.17 | 7.01 | 0.85 | 283 | 17.98 | 7.9 | 0.04 | 99 | 3.06 | 104 | 2.00 | 0.11 | 0.12 |
| CERC 02 | 15.2 | 0.16 | 7.33 | 0.92 | 286 | 11.26 | 7.9 | 0.07 | 100 | 2.00 | 109 | 3.06 | 0.04 | 0.03 |
| CERC 04 | 15.2 | 0.17 | 6.94 | 1.08 | 266 | 2.53 | 8.0 | 0.09 | 95 | 3.06 | 106 | 5.29 | 0.03 | 0.01 |
| CERC 05 | 15.3 | 0.18 | 7.51 | 1.27 | 272 | 6.83 | 7.9 | 0.08 | 99 | 1.91 | 108 | 2.83 | 0.04 | 0.01 |
| CERC 06 | 15.3 | 0.12 | 7.79 | 1.08 | 272 | 1.98 | 7.9 | 0.05 | 98 | 2.00 | 107 | 2.28 | 0.06 | 0.01 |
| CERC 07 | 15.2 | 0.19 | 7.47 | 1.11 | 274 | 0.76 | 7.9 | 0.08 | 99 | 1.15 | 109 | 1.15 | 0.06 | 0.05 |
| CERC 09 | 15.2 | 0.14 | 7.19 | 1.01 | 264 | 3.27 | 7.9 | 0.10 | 93 | 1.15 | 103 | 5.03 | 0.07 | 0.05 |
| Quartz Sand | 15.2 | 0.18 | 7.90 | 1.05 | 264 | 0.76 | 7.9 | 0.10 | 94 | 0.00 | 101 | 1.15 | 0.10 | 0.04 |

Table 4. Dissolved organic carbon (DOC) concentrations measured from overlying water during 14-d exposure of upper Columbia River sediments with white sturgeon (*Acipenser transmontanus*). SD = standard deviation.

| | DOC (mg/L) | | | | |
|---|---|---|---|---|---|
| **Site** | **Day -1** | **Day 7** | **Day 14** | **Average** | **SD** |
| CERC 01 | 1.07 | 0.43 | 0.45 | | |
| CERC 01-duplicate | 1.02 | 0.43 | 0.55 | 0.66 | 0.30 |
| CERC 02 | 0.78 | 0.44 | 0.40 | 0.54 | 0.21 |
| CERC 04 | 1.18 | 0.45 | 0.51 | 0.71 | 0.41 |
| CERC 05 | 0.61 | 0.36 | 0.38 | 0.45 | 0.14 |
| CERC 06 | 0.74 | 0.43 | 0.45 | 0.54 | 0.17 |
| CERC 07 | 0.64 | 0.43 | 0.47 | | |
| CERC 07-duplicate | 0.59 | 0.41 | 0.43 | 0.50 | 0.10 |
| CERC 09 | 0.80 | 0.46 | 0.50 | 0.59 | 0.19 |
| Quartz Sand | 0.34 | 0.32 | 0.39 | 0.35 | 0.04 |

Table 5. Major cation concentrations measured from overlying water during 14-d exposure of upper Columbia River sediments with white sturgeon (*Acipenser transmontanus*). SD = standard deviation. < = less than and indicates the detection limit. N/A = not applicable as the concentration was below the detection limit of the method.

| | **Ca (mg/L)** | | | | | **Mg (mg/L)** | | | | | **Na (mg/L)** | | | | | **K (mg/L)** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Test Day** | | | | | **Test Day** | | | | | **Test Day** | | | | | **Test Day** | | | | |
| **Site** | **-1** | **7** | **14** | **Mean** | **SD** | **-1** | **7** | **14** | **Mean** | **SD** | **-1** | **7** | **14** | **Mean** | **SD** | **-1** | **7** | **14** | **Mean** | **SD** |
| CERC 01 | 30.9 | 28.8 | 29.5 | 29.7 | 1.1 | 8.3 | 8.6 | 8.9 | 8.6 | 0.3 | 8.4 | 8.7 | 9.2 | 8.8 | 0.4 | 1.8 | 1.3 | 1.3 | 1.5 | 0.3 |
| CERC 02 | 30.7 | 29.2 | 31.6 | 30.5 | 1.2 | 9.4 | 9.0 | 9.7 | 9.4 | 0.3 | 9.3 | 9.0 | 10.0 | 9.4 | 0.5 | 1.6 | 1.2 | 1.3 | 1.3 | 0.2 |
| CERC 04 | 30.3 | 28.8 | 30.5 | 29.9 | 0.9 | 6.8 | 8.5 | 8.9 | 8.1 | 1.1 | 9.6 | 9.5 | 10.1 | 9.7 | 0.3 | 2.3 | 1.7 | 1.6 | 1.8 | 0.4 |
| CERC 05 | 30.1 | 28.5 | 30.6 | 29.7 | 1.1 | 8.5 | 9.0 | 9.5 | 9.0 | 0.5 | 7.9 | 8.5 | 9.8 | 8.7 | 1.0 | 1.2 | 1.1 | 1.2 | 1.2 | 0.1 |
| CERC 06 | 30.7 | 29.7 | 28.9 | 29.8 | 0.9 | 8.7 | 9.2 | 9.4 | 9.1 | 0.4 | 8.1 | 9.1 | 9.5 | 8.9 | 0.7 | 1.2 | 1.2 | 1.1 | 1.2 | 0.1 |
| CERC 07 | 31.1 | 31.0 | 30.2 | 30.8 | 0.5 | 8.6 | 9.3 | 9.5 | 9.1 | 0.5 | 9.5 | 9.7 | 10.0 | 9.7 | 0.3 | 1.5 | 1.2 | 1.2 | 1.3 | 0.2 |
| CERC 09 | 28.3 | 28.9 | 27.7 | 28.3 | 0.6 | 8.6 | 9.5 | 9.1 | 9.1 | 0.4 | 8.2 | 9.3 | 9.2 | 8.9 | 0.6 | 1.3 | 1.1 | 1.1 | 1.1 | 0.2 |
| Quartz Sand | 28.8 | 28.0 | 27.3 | 28.0 | 0.8 | 8.7 | 9.1 | 9.4 | 9.1 | 0.4 | 8.5 | 9.1 | 9.2 | 8.9 | 0.4 | 1.2 | 1.0 | 1.0 | 1.0 | 0.1 |
| | Overall Mean | | | **29.6** | **1.0** | Overall Mean | | | **8.9** | **0.4** | Overall Mean | | | **9.1** | **0.4** | Overall Mean | | | **1.3** | **0.2** |

Table 5 continued.

| Site | Site | | | | | Site | | | | | Site | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Test Day | | | | | Test Day | | | | | Test Day | | | | |
| | -1 | 7 | 14 | Mean | SD | -1 | 7 | 14 | Mean | SD | -1 | 7 | 14 | Mean | SD |
| CERC 01 | 0.13 | 0.14 | 0.14 | 0.14 | 0.00 | < 0.05 | < 0.05 | < 0.05 | N/A | N/A | < 0.01 | < 0.01 | < 0.01 | N/A | N/A |
| CERC 02 | 0.14 | 0.14 | 0.15 | 0.14 | 0.01 | < 0.05 | < 0.05 | < 0.05 | N/A | N/A | < 0.01 | < 0.01 | < 0.01 | N/A | N/A |
| CERC 04 | 0.12 | 0.13 | 0.14 | 0.13 | 0.01 | < 0.05 | < 0.05 | < 0.05 | N/A | N/A | < 0.01 | < 0.01 | < 0.01 | N/A | N/A |
| CERC 05 | 0.12 | 0.13 | 0.14 | 0.13 | 0.01 | < 0.05 | < 0.05 | 0.07 | N/A | N/A | < 0.01 | < 0.01 | < 0.01 | N/A | N/A |
| CERC 06 | 0.13 | 0.14 | 0.14 | 0.14 | 0.01 | < 0.05 | < 0.05 | < 0.05 | N/A | N/A | 0.03 | < 0.01 | < 0.01 | N/A | N/A |
| CERC 07 | 0.12 | 0.13 | 0.13 | 0.13 | 0.01 | < 0.05 | < 0.05 | < 0.05 | N/A | N/A | < 0.01 | < 0.01 | < 0.01 | N/A | N/A |
| CERC 09 | 0.12 | 0.13 | 0.13 | 0.13 | 0.01 | < 0.05 | < 0.05 | < 0.05 | N/A | N/A | 0.03 | < 0.01 | < 0.01 | N/A | N/A |
| Quartz Sand | 0.12 | 0.13 | 0.13 | 0.13 | 0.01 | < 0.05 | < 0.05 | < 0.05 | N/A | N/A | 0.01 | < 0.01 | < 0.01 | N/A | N/A |
| | Overall Mean | | | **0.13** | **0.01** | Overall Mean | | | **< 0.05** | | Overall Mean | | | **<0.01** | |

Table 6. Major anion concentrations measured overlying water during 14-d exposure of upper Columbia River sediments with white sturgeon (*Acipenser transmontanus*). SD = standard deviation. < = less than and indicates the detection limit. N/A = not applicable as the concentration was below the detection limit of the method.

| | **F (mg/L)** | | | | | **Cl (mg/L)** | | | | | **$NO_2$ + $NO_3$ (mg/L)** | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Test Day** | | | | | **Test Day** | | | | | **Test Day** | | | | |
| **Site** | **-1** | **7** | **14** | **Mean** | **SD** | **-1** | **7** | **14** | **Mean** | **SD** | **-1** | **7** | **14** | **Mean** | **SD** |
| CERC 01 | 0.6 | 0.6 | <0.4 | 0.5 | 0.1 | 9.2 | 9.8 | 10.3 | 9.8 | 0.5 | <0.4 | <0.4 | <0.4 | <0.4 | N/A |
| CERC 02 | 0.6 | 0.6 | <0.4 | 0.5 | 0.1 | 9.2 | 9.9 | 10.2 | 9.8 | 0.5 | <0.4 | 3.6 | 1.1 | 2.3 | 1.7 |
| CERC 04 | 0.6 | 0.6 | <0.4 | 0.5 | 0.1 | 9.5 | 10.0 | 11.4 | 10.3 | 1.0 | 3.7 | 3.7 | 1.0 | 2.8 | 1.6 |
| CERC 05 | 0.6 | 0.6 | <0.4 | 0.5 | 0.1 | 9.4 | 9.9 | 10.5 | 9.9 | 0.5 | <0.4 | 3.5 | 1.5 | 2.5 | 1.4 |
| CERC 06 | 0.6 | 0.6 | <0.4 | 0.5 | 0.1 | 9.3 | 10.0 | 10.5 | 9.9 | 0.6 | 3.6 | <0.4 | <0.4 | 1.5 | 1.9 |
| CERC 07 | 0.6 | 0.6 | <0.4 | 0.5 | 0.1 | 9.5 | 10.1 | 10.5 | 10.0 | 0.5 | <0.4 | <0.4 | <0.4 | <0.4 | N/A |
| CERC 09 | 0.5 | 0.6 | <0.4 | 0.5 | 0.1 | 9.3 | 9.9 | 10.5 | 9.9 | 0.6 | 3.8 | 3.8 | 1.1 | 2.9 | 1.5 |
| Quartz Sand | 0.5 | 0.6 | <0.4 | 0.5 | 0.1 | 9.9 | 10.0 | 10.5 | 10.1 | 0.3 | <0.4 | <0.4 | <0.4 | <0.4 | N/A |
| | Overall Mean | | | **0.5** | **0.0** | Overall Mean | | | **10.0** | **0.2** | Overall Mean | | | **1.6** | **1.1** |

Table 6 continued.

| | Br (mg/L) | | | | | $SO_4$ (mg/L) | | | | | $PO_4$ (mg/L) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Test Day | | | | | Test Day | | | | | Test Day | | | | |
| **Site** | **-1** | **7** | **14** | **Mean** | **SD** | **-1** | **7** | **14** | **Mean** | **SD** | **-1** | **7** | **14** | **Mean** | **SD** |
| CERC 01 | <0.4 | <0.4 | <0.4 | <0.4 | N/A | 22.0 | 22.7 | 21.1 | 21.9 | 0.8 | <0.4 | <0.4 | <0.4 | <0.4 | N/A |
| CERC 02 | <0.4 | <0.4 | <0.4 | <0.4 | N/A | 24.1 | 22.5 | 20.8 | 22.5 | 1.6 | <0.4 | <0.4 | <0.4 | <0.4 | N/A |
| CERC 04 | <0.4 | <0.4 | <0.4 | <0.4 | N/A | 23.7 | 23.0 | 20.8 | 22.5 | 1.5 | <0.4 | <0.4 | <0.4 | <0.4 | N/A |
| CERC 05 | <0.4 | <0.4 | <0.4 | <0.4 | N/A | 23.0 | 22.2 | 20.4 | 21.8 | 1.3 | <0.4 | <0.4 | <0.4 | <0.4 | N/A |
| CERC 06 | <0.4 | <0.4 | <0.4 | <0.4 | N/A | 22.5 | 22.2 | 20.3 | 21.7 | 1.2 | <0.4 | <0.4 | <0.4 | <0.4 | N/A |
| CERC 07 | <0.4 | <0.4 | <0.4 | <0.4 | N/A | 24.2 | 23.6 | 21.9 | 23.2 | 1.2 | <0.4 | <0.4 | <0.4 | <0.4 | N/A |
| CERC 09 | <0.4 | <0.4 | <0.4 | <0.4 | N/A | 21.7 | 21.6 | 20.0 | 21.1 | 1.0 | <0.4 | <0.4 | <0.4 | <0.4 | N/A |
| Quartz Sand | <0.4 | <0.4 | <0.4 | <0.4 | N/A | 22.3 | 21.5 | 20.0 | 21.3 | 1.2 | <0.4 | <0.4 | <0.4 | <0.4 | N/A |
| | | | Overall Mean | **<0.4** | | | | Overall Mean | **22.0** | **0.7** | | | Overall Mean | **<0.4** | |

Table 7. Dissolved metal concentrations measured from overlying water during 14-d exposure of upper Columbia River sediments with white sturgeon (*Acipenser transmontanus*). Rep=replicate, Avg=average, SD= standard deviation.

| | | Ni (µg/L) | | | | | Cu (µg/L) | | | | | Zn (µg/L) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Site** | **Rep** | **Day 0** | **Day 7** | **Day 14** | **Avg** | **SD** | **Day 0** | **Day 7** | **Day 14** | **Avg** | **SD** | **Day 0** | **Day 7** | **Day 14** | **Avg** | **SD** |
| CERC 01 | 2 | 0.05 | 0.05 | 0.14 | | | 2.33 | 0.85 | 1.17 | | | 11.60 | 33.50 | 28.20 | | |
| CERC 01 | 5 | 0.05 | 0.05 | 0.05 | | | 2.01 | 0.52 | 0.97 | | | 10.60 | 17.30 | 29.90 | | |
| CERC 01 | 5 | 0.05 | 0.07 | 0.12 | 0.07 | 0.03 | 1.99 | 0.47 | 0.95 | 1.25 | 0.69 | 11.20 | 17.60 | 29.40 | 21.03 | 9.19 |
| CERC 02 | 1 | 0.06 | 0.05 | 0.08 | 0.06 | 0.02 | 1.35 | 1.03 | 1.61 | 1.33 | 0.29 | 7.48 | 15.10 | 18.30 | 13.63 | 5.56 |
| CERC 04 | 4 | 0.09 | 0.06 | 0.14 | 0.09 | 0.04 | 11.90 | 2.89 | 3.05 | 5.95 | 5.16 | 13.00 | 23.30 | 16.20 | 17.50 | 5.27 |
| CERC 05 | 6 | 0.06 | 0.06 | 0.19 | 0.10 | 0.08 | 2.10 | 2.85 | 4.21 | 3.05 | 1.07 | 8.72 | 20.50 | 18.80 | 16.01 | 6.37 |
| CERC 06 | 1 | - | 0.11 | 0.05 | | | - | 4.37 | 4.74 | | | - | 24.50 | 20.30 | | |
| CERC 06 | 2 | 0.11 | 0.12 | 0.05 | | | 6.34 | 5.21 | 5.18 | | | 11.00 | 25.10 | 16.90 | | |
| CERC 06 | 4 | - | - | 0.23 | 0.11 | 0.07 | - | - | 4.86 | 5.12 | 0.67 | - | - | 24.50 | 20.38 | 5.59 |
| CERC 07 | 3 | 0.11 | 0.12 | 0.20 | | | 1.59 | 1.43 | 1.84 | | | 21.10 | 54.70 | 40.20 | | |
| CERC 07 | 3 | 0.16 | 0.06 | 0.17 | | | 1.70 | 1.40 | 1.82 | | | 21.60 | 53.60 | 40.70 | | |
| CERC 07 | 6 | 0.16 | 0.06 | 0.17 | 0.13 | 0.05 | 1.88 | 1.42 | 1.94 | 1.67 | 0.21 | 33.20 | 59.50 | 42.50 | 40.79 | 13.80 |
| CERC 09 | 5 | < 0.05 | < 0.05 | < 0.05 | <0.5 | 0.00 | 0.24 | 0.20 | 0.27 | 0.24 | 0.04 | 3.23 | 6.48 | 0.86 | 3.52 | 2.82 |
| Quartz Sand | 4 | 0.48 | 0.37 | 0.09 | 0.31 | 0.20 | 0.22 | 0.17 | 0.39 | 0.26 | 0.12 | 28.00 | 11.10 | 5.97 | 15.02 | 11.53 |

Table 7 continued.

| | Cd (µg/L) | | | | | Pb (µg/L) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Site** | **Day 0** | **Day 7** | **Day 14** | **Avg** | **SD** | **Day 0** | **Day 7** | **Day 14** | **Avg** | **SD** |
| CERC 01 | 0.08 | 0.06 | 0.09 | | | 0.19 | 0.08 | 0.31 | | |
| CERC 01 | 0.07 | 0.02 | 0.11 | | | 0.16 | 0.09 | 0.12 | | |
| CERC 01 | 0.07 | 0.02 | 0.07 | 0.06 | 0.03 | 0.19 | 0.07 | 0.13 | 0.15 | 0.07 |
| CERC 02 | 0.03 | 0.08 | 0.11 | 0.07 | 0.04 | 0.10 | 0.10 | 0.17 | 0.12 | 0.04 |
| CERC 04 | 0.02 | 0.05 | 0.07 | 0.04 | 0.02 | 0.12 | 0.09 | 0.15 | 0.12 | 0.03 |
| CERC 05 | 0.08 | 0.09 | 0.11 | 0.09 | 0.02 | 0.05 | 0.22 | 0.69 | 0.32 | 0.33 |
| CERC 06 | - | 0.08 | 0.06 | | | - | 0.26 | 0.40 | | |
| CERC 06 | 0.02 | 0.09 | 0.06 | | | 0.20 | 0.44 | 0.37 | | |
| CERC 06 | - | - | 0.02 | 0.06 | 0.03 | - | - | 0.40 | 0.35 | 0.09 |
| CERC 07 | 0.18 | 0.39 | 0.31 | | | 0.12 | 0.29 | 0.41 | | |
| CERC 07 | 0.18 | 0.37 | 0.32 | | | 0.13 | 0.27 | 0.45 | | |
| CERC 07 | 0.26 | 0.43 | 0.33 | 0.31 | 0.09 | 0.31 | 0.20 | 0.52 | 0.30 | 0.14 |
| CERC 09 | 0.02 | 0.02 | 0.02 | 0.02 | 0.00 | 0.02 | 0.03 | 0.03 | 0.03 | 0.01 |
| Quartz Sand | < 0.02 | < 0.02 | < 0.02 | <0.2 | 0.00 | 0.02 | 0.02 | 0.02 | 0.02 | 0.00 |

Table 8. Average (n=3) cumulative hardness-based chronic toxic units (TU) for the metal mixture, biotic ligand model (BLM) normalized copper only chronic TUs, and cumulative hardness-based with BLM normalized copper chronic TUs in the overlying water of each upper Columbia River sediment treatment in a 14-d exposure with white sturgeon (*Acipenser transmontanus*). Shading represents TU>1.

| | Cumulative Hardness-Based TU | | BLM-Normalized Cu TU | | Cumulative Hardness-Based TU with BLM Cu TU | |
|---|---|---|---|---|---|---|
| Site | Mean | SD | Mean | SD | Mean | SD |
| CERC 01 | 0.61 | 0.17 | 0.44 | 0.10 | 0.92 | 0.22 |
| CERC 02 | 0.58 | 0.23 | 0.64 | 0.31 | 1.08 | 0.51 |
| CERC 04 | 1.02 | 0.48 | 1.68 | 0.42 | 2.04 | 0.31 |
| CERC 05 | 0.92 | 0.32 | 1.70 | 0.94 | 2.30 | 1.16 |
| CERC 06 | 1.03 | 0.13 | 2.24 | 0.26 | 2.70 | 0.45 |
| CERC 07 | 1.79 | 0.49 | 0.76 | 0.20 | 2.38 | 0.61 |
| CERC 09 | 0.13 | 0.04 | 0.10 | 0.04 | 0.21 | 0.04 |
| Quartz Sand | 0.25 | 0.09 | 0.16 | 0.07 | 0.38 | 0.07 |

Table 9. Table 10. Mortality and swimming activity of white sturgeon (Acipenser transmontanus) exposed to whole sediment from the upper Columbia River for 14 days. Gray shading indicates significant reduction from the reference site (CERC 09; Fishers LSD; $p<0.05$). SD= standard deviation.

| | Mortality (%) | | Distance Traveled (cm) | | Time Spent Moving (sec) | | Average Velocity (cm/sec) | |
|---|---|---|---|---|---|---|---|---|
| Site | Mean | SD (n=6) | Mean | SD (n=6) | Mean | SD (n=6) | Mean | SD (n=6) |
| CERC 01 | 4.44 | 6.55 | 2820.99 | 1258.87 | 3.46 | 1.70 | 528.66 | 230.50 |
| CERC 02 | 3.89 | 3.90 | 2560.98 | 1359.64 | 2.95 | 1.51 | 484.60 | 220.29 |
| CERC 04 | 6.11 | 3.90 | 2447.92 | 1476.39 | 2.88 | 1.55 | 468.71 | 262.46 |
| CERC 05 | 3.33 | 2.11 | 3421.57 | 1819.96 | 3.87 | 2.06 | 592.03 | 299.54 |
| CERC 06 | 26.67 | 12.29 | 1446.37 | 1500.42 | 1.64 | 1.69 | 239.89 | 256.32 |
| CERC 07 | 4.44 | 5.02 | 3029.97 | 1262.26 | 3.60 | 1.56 | 559.45 | 197.59 |
| CERC 09 | 2.78 | 3.28 | 2488.56 | 1217.62 | 2.87 | 1.43 | 478.77 | 243.59 |
| Quartz Sand | 3.33 | 3.65 | 2830.16 | 1082.32 | 3.25 | 1.27 | 556.72 | 162.49 |

## Figure Legends

Figure 1. Overview of the upper Columbia River study area, Washington, USA. "• 01" on the inset map indicates sediment sampling sites CERC 01, and so on.

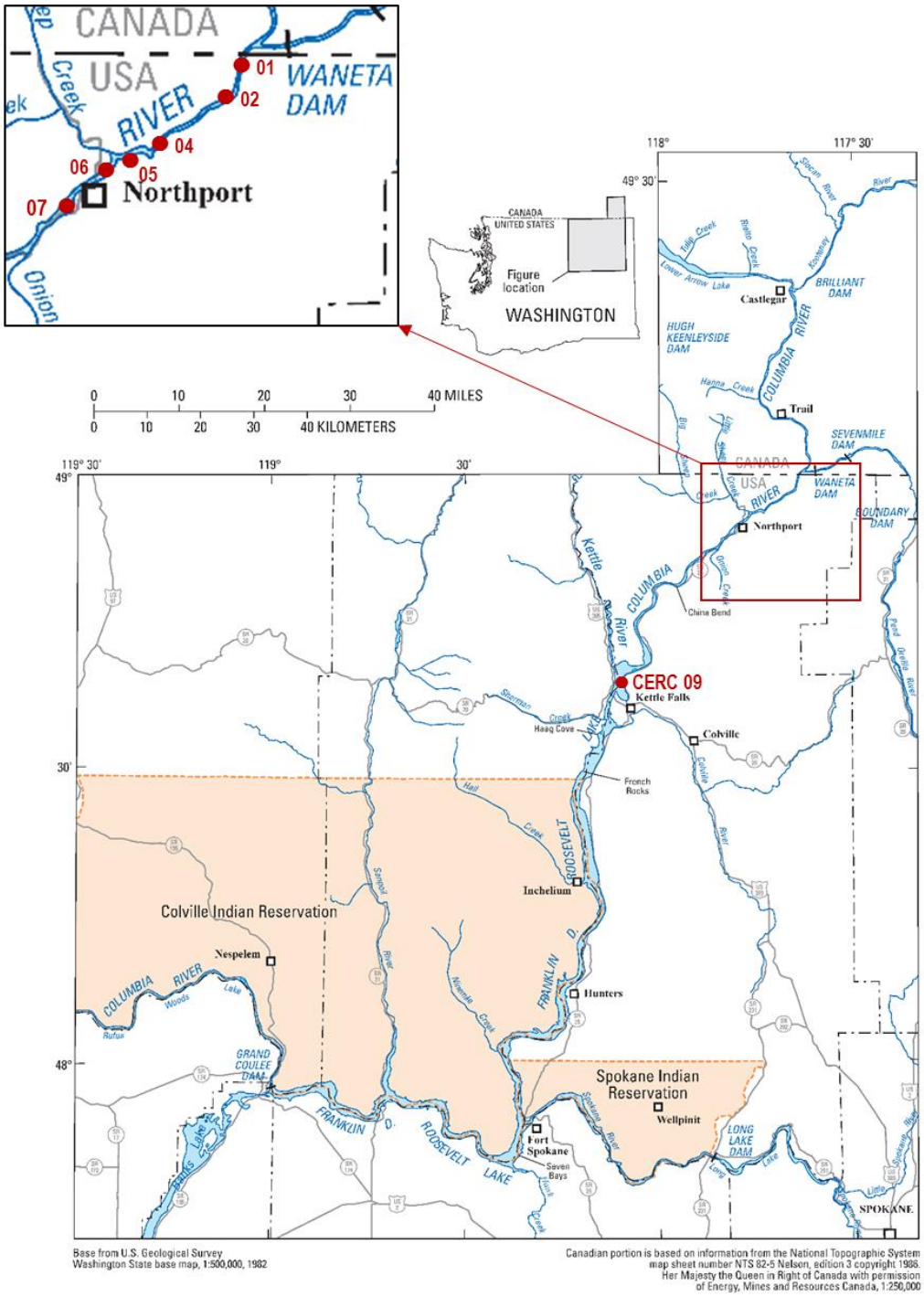

CANADA
USA
RIVER
WANETA DAM
01
02
04
05
06
07
Northport
Onion Creek
CANADA
UNITED STATES
Figure location
WASHINGTON
0 10 20 30 40 MILES
0 10 20 30 40 KILOMETERS
118°
117° 30'
49° 30'
Castlegar
BRILLIANT DAM
HUGH KEENLEYSIDE DAM
COLUMBIA RIVER
Trail
SEVENMILE DAM
BOUNDARY DAM
China Bend
119° 30'
119°
30'
49°
30'
48°
Kettle River
CERC 09
Kettle Falls
Colville
French Rocks
ROOSEVELT
Inchelium
Colville Indian Reservation
Nespelem
FRANKLIN D.
Hunters
GRAND COULEE DAM
Spokane Indian Reservation
Wellpinit
Fort Spokane
LONG LAKE DAM
SPOKANE
Base from U.S. Geological Survey
Washington State base map, 1:500,000, 1982
Canadian portion is based on information from the National Topographic System map sheet number NTS 82-5 Nelson, edition 3 copyright 1986. Her Majesty the Queen in Right of Canada with permission of Energy, Mines and Resources Canada, 1:250,000

Figure 1.

# CHAPTER 4

## WHITE STURGEON IN THE UPPER COLUMBIA RIVER: LIFE HISTORY AND THEIR POTENTIAL EXPOSURE TO TRACE METALS

Holly J. Puglis

**Abstract:** Currently there is little natural recruitment of White Sturgeon (*Acipenser transmontanus*) in the upper Columbia River located in British Columbia and Washington, USA. This review of life history, physiology and behavior of White Sturgeon along with data from recent toxicological studies suggest that trace metals present in the area may affect survival and behavior of the early lifestage fish. During early lifestages that include free embryos, first feeding embryos, and mixed feeding embryos; sturgeon utilize interstitial spaces between gravel. While concentrations of copper in the water column of the upper Columbia River may be low, samples collected immediately at the sediment-water interface were as high as 24 µg Cu/L and exceed water quality criteria defined to protect aquatic resources in the United States. Toxicological studies reviewed here demonstrate mortality and effective mortality (a measurement that includes immobility observations) at concentrations of 1.5 – < 16 µg Cu/L. Behavioral hyperactivity has been documented at 1.9 to 7.2 µg Cu/L. In addition to potential direct mortality and behavioral changes from water exposure, contaminated invertebrates and slag particles provide another route of exposure for White Sturgeon, including indirect effects from starvation due to potential lack of prey items during critical early feeding lifestages, and ingestion of contaminated prey or slag particles. The lack of food in stomachs during these critical early life stages may coincide with a threshold/"point of no return" where sturgeon will be unable to survive even if food becomes available following that early timeframe. These findings become especially important as work progresses to enhance White Sturgeon recruitment in the Upper Columbia River. Neglecting to consider the toxic effects of trace metals in the system could jeopardize the restoration efforts.

## Introduction

White Sturgeon (*Acipenser transmontanus*) populations have been declining in the upper Columbia River (UCR) (Figure 1), located in British Columbia, Canada and Washington, USA with little to no natural recruitment to the population since the 1970s (Hildebrand et al. 1999; Irvine et al. 2007). Without intervention, the wild population in the UCR is projected to decline to fewer than 500 fish in the next 50 years (Hildebrand and Parsley 2013). While evidence of spawning has been detected with eggs and larvae being collected during sampling events in the 1990's to 2011, sampling efforts to collect wild young of the year have failed (Golder 2007b, 2009; Howell and McLellan 2011, 2013, 2014, 2015). Thus, the population is being sustained by a hatchery program where wild fish are spawned and the larvae are reared in hatcheries until their release in the UCR after the larvae reach 5 to 10 months of age (Hildebrand and Parsley 2013). More recently, work is being done to collect naturally produced larvae to rear in hatcheries for later release back into the system as juveniles (Howell and McLellan 2014, 2015).

One debated aspect of the decline of White Sturgeon in the upper Columbia River is whether contaminants from smelting operations in the town of Trail, BC have played a role. Smelting operations began by 1896 and developed into one of world's largest metals smelters. Until 1995, smelter slag, a black, glassy substance with high concentrations of copper, lead and zinc, was discharged directly into the Columbia River. The volumes of slag released was about 12 million tons released from 1940 to 1995 (Parrish 2005) in addition to liquid effluent. The legacy smelter practices have resulted in persistent sediment contamination in the downstream river and in the Lake Roosevelt impoundment further downstream (Johnson et al. 1990). Metals present in the slag can be released into

the overlying water (Paulson and Cox 2007). McAdam (2015) reviewed various factors for decline in the upper Columbia River sturgeon populations and concluded that substrate alterations, particularly the increase in fine sediments associated with upstream dam construction and flow regulation, was a leading factor for decline. Contaminant effects were discounted as a factor for decline based on the timing of the sturgeon declines which in some locations began coincident with upstream dam construction. Secondly, research testing the chemistry and toxicity of river water to White Sturgeon showed that the water column was low in metals and non-toxic to sturgeon larvae (McAdam 2015; Tompsett et al. 2014). Here, we review the life history of White Sturgeon in the context of further research on substrate contamination in the upper Columbia River that were not available to McAdam (2015)

## Life History of White Sturgeon

White Sturgeon typically spawn in high flow areas with high turbidity, perhaps to reduce predation on eggs and larvae (Hildebrand et al. 1999). In the UCR, evidence of spawning has been found in the area just below the Waneta Dam at the confluence of the Pend Oreille and Columbia rivers, near Northport, WA, and near China Bend (river km 1,174) every year the locations were surveyed (1993-2011 for Waneta Dam, 2005-2008 for Northport, and 2007-2008 for China Bend; Golder 2007b; Howell and McLellan 2011, 2013). White Sturgeon eggs are negatively buoyant thus they sink and adhere to substrate near spawning locations. Depending on temperature, the eggs hatch between 4-14 days after spawning (Conte 1988). After hatch, White Sturgeon larvae immediately hide in small interstitial spaces if appropriate substrate, such as gravel, is found, but will swim up and disperse if substrate consists of either sand or cobble with very large

interstitial spaces (Bennett et al. 2007; McAdam 2011) (Figure 2). Once larvae begin exogenously feeding (between 8 – 14 days post hatch - dph; (Conte 1988)), animals swim up and disperse downstream (Parsley et al. 2002). Larval surveys in the UCR, downstream of the Northport spawning locations corroborate this timeline. A pattern of larvae collection typically follows a bimodal distribution with a small peak of early stage free embryos soon after spawning and a much larger peak of early larvae (animals that are preparing to transition or have recently transitioned to exogenous feeding) about a week later (Howell and McLellan 2011, 2013, 2014, 2015). Drifting larvae are at great risk of predation (McAdam 2011) and any alteration in these typical early-life stage behaviors, such as changes in activity levels, may influence the timing and duration of drift. Therefore, changes in behavior could influence survival of larval White Sturgeon.

## Potential Role of Metal Contamination

White Sturgeon free embryos, fish that have hatched and are using endogenous food resources, are hiding in the interstitial spaces of the substrate at the bottom of the river. During this lifestage, sturgeon are approximately 1 to 10 dph and their sensitivity to copper as indicated by concentrations that are lethal to 50 percent of the experimental population in 96 hours (96h LC50s) range from 5 to >16 μg Cu/L (Calfee et al. 2014; Vardy et al. 2013; Wang et al. 2014; Table 1). However, effective mortality, an endpoint that includes mortality, loss of equilibrium and immobility in its calculation, for the same lifestage, is the most sensitive acute endpoint for copper tested to date with a 96h effect concentration (96h EC50) of 1.5 μg Cu/L for 2 dph White Sturgeon (Calfee et al. 2014).

In addition to the directly lethal effects of metal exposure for fish in this lifestage, behavioral effects were also measured. Within an hour of exposure to copper

concentrations between 1.9 and 7.2 µg Cu/L, 1 dph White Sturgeon were hyperactive compared to fish in the control treatment, swimming 25% more than control fish (Puglis 2018). This effect continued for at least five hours of exposure. Increases in activity, when the fish is typically hiding in interstitial spaces, could attract predators and reduce survival, similar to observations with copper exposed juvenile salmon (McIntyre et al. 2012). After 2 days of exposure to copper, significant decreases in swimming activity were observed in the 7 µg/L Cu treatment compared to the activity of fish in the control treatment (Puglis 2018). This reduction in activity continued to the end of the 14d test, with the severity of effect increasing over time and peaking at 10 days of exposure. After 10 days of exposure, White Sturgeon in the 0.88 - 7 µg/L Cu treatments swam between 40 and 86% less than fish in the control treatment (Puglis 2018). Such a deviation from the behavior of fish in the control treatment may affect survival in the wild as a decrease in activity level could prevent a fish from seeking suitable habitat for hiding. In fact, Wang et al. (2014) documented an increased number of fish displaying a lack of hiding in 2-10 dph White Sturgeon free embryos after 4 and 8 days of exposure to 1.7 to 6.9 µg/L Cu compared to fish in control treatments. Decreases in swimming activity could also prevent sturgeon from leaving the hiding phase to enter the drift phase around 10 dph, which could be considered a developmental delay and may result in depletion of yolk sac nutrition prior to animals reaching nursery habitats, and increase the likelihood of starvation at the onset of exogenous feeding, especially when they reach the point of no return.

Just before transitioning to exogenous feeding, White Sturgeon leave their hiding places and drift to appropriate nursery habitat. While transitioning from a free embryo to

a first feeding larvae, White Sturgeon are utilizing a mixed feeding strategy, where they are still digesting yolk, the endogenous food resource, and actively consuming prey items. During this lifestage, sturgeon are approximately 10-16 dph with a 96h LC50 sensitivity between 2.9 and 4.4 μg/L Cu (Calfee et al. 2014; Vardy et al. 2013; Wang et al. 2014; Table 1) and a 96h EC50 for effective mortality of 2.6 μg/L Cu (Calfee et al. 2014; Table 1). With typical behavior patterns, White Sturgeon during this time could be up in the water column, drifting, or down in the substrate hiding or seeking prey. These fish are in a metabolically active and vulnerable state, and likely move into gravel and interstitial spaces to both hide from predators and search for food. Thus, they may be repeatedly exposed to any contaminants in groundwater upwelling and hyporheic flow through bed sediments as well as contaminants associated with feeding. If they drift and inhabit areas where slag has a significant presence in the gravel and cobble, coincidental ingestion of slag along with any trace metals bound to and within aquatic invertebrates while feeding may be likely.

After completing the transition to exogenous feeding, White Sturgeon larvae are actively foraging for food. During this lifestage, sturgeon are approximately 16-45 dph with a 96h LC50 between 2.2 and 11.8 μg/L Cu and one estimate of >34.1 for 44 dph larvae (Calfee et al. 2014; Little et al. 2012; Vardy et al. 2013; Wang et al. 2014; Table 1). The EC50 for effective mortality for 30 dph White Sturgeon larvae is 4.2 μg/L Cu and for 44 dph White Sturgeon larvae it is >34 μg/L Cu (Calfee et al. 2014; Table 1), indicating that as White Sturgeon larvae metamorphose into juveniles around 45 dph (Deng et al. 2002), sensitivity to copper decreases compared to the sensitivity of earlier life stages. Decreases in swimming activity levels were detected in White Sturgeon first

feeding larvae with a 14d EC20 for swimming activity of 1.5 µg/L Cu. After 14 days of exposure to copper, swimming activity in the 3 and 7 µg/L Cu treatments was 73 and 80% less than fish in the control, respectively. Swimming activity levels were also detected in *actively* feeding White Sturgeon larvae with a 96h EC50 for swimming activity of 4.3 µg/L Cu for 35 dph fish. White Sturgeon larvae swam 40 and 75% less in 3.3 and 6.9 µg/L Cu treatments compared to the swimming activity of control fish, respectively (Puglis 2018. With such decreases in activity, the likelihood of predation is increased and encountering prey items in the wild would be reduced.

Juvenile White Sturgeon have the appearance of a mature sturgeon, with all fins and scutes, but are not sexually mature. White Sturgeon larvae metamorphose into juveniles around 45 dph, and typically do not transition to sub-adults for several years, which occurs when they reach over a meter in fork length (Beamesderfer et al. 1995). Juveniles in their first year of life are referred to as age-0 (Figure 2). Juvenile sturgeon between 61-100 dph have an LC50 between 16.1 and 62.9 µg/L Cu an (Calfee et al. 2014; Vardy et al. 2013; Table 1). During this life stage, young juvenile White Sturgeon would be near the river bottom foraging for food and they primarily eat benthic invertebrates (Parsley et al. 2010). In addition to trace metal exposure from upwelling of porewater, incidental ingestion of sediment and slag is likely resulting in another route of exposure to trace metals for White Sturgeon larvae and juveniles.

## Metal Concentrations in the Upper Columbia River

Previous studies have shown that recent water column concentrations of metals in the upper Columbia River are low relative to environmental quality standards and to experimental effects to White Sturgeon. Copper is of particular concern relative to other

metals because copper can be released by upper Columbia River sediments into the overlying water (Paulson and Cox 2007), the survival of newly hatched sturgeon is dependent on their being able to shelter within the interstices of coarse gravel and cobble sediments (McAdam 2011), and newly hatched sturgeon may be extremely sensitive to copper, as explained more in later text. Tompsett et al. (2014) reported that copper concentrations in the upper Columbia River ranged from 0.26 to 1.3 µg/L, below environmental quality guidelines, and other metals concentrations were generally much lower than environmental quality standards. Paulson and Cox (2007) showed that sediment cores pulled from the Columbia River, taken to the laboratory and incubated with river water in laboratory, resulted in increased metals concentrations in the overlying waters. However, how sediment cores incubated in a laboratory setting relates to the ambient substrate conditions that larval sturgeon would actually experience in the Columbia River remains uncertain.

To obtain realistic representations of water conditions near the sediment water interface, Cox et al. (2016) used a weighted (85 kg) pore-water profile sampler to collect a suite of four water samples from above, at, and below the sediment-water interface from depths up to 20m at 29 sampling locations in the upper Columbia River. Copper concentrations in the water column were small, ranging only from <0.5 to 1 µg/L, consistent with the results of Tompsett et al. (2014). In contrast, copper concentrations in water samples collected immediately at the sediment-water interface were as large as 24 µg/L (Figure 4). Median copper concentrations in shallow porewaters (4.5 cm below the substrate surface) were similar to the copper 96-hour EC50 for effective mortality to 2 dph larval White Sturgeon obtained by Calfee et al. (2014) (Table 1). At some locations,

copper concentrations at the sediment-water interface and in shallow porewater were well above that EC50, suggesting that even relatively short duration occupancy of the sediment-water interface or shallow interstitial spaces in the substrate could result in reduced survival rates of larval sturgeon.

## Potential Role of Starvation

In addition to risk of direct toxicity to sturgeon from metals in slag contaminated porewater, the contaminated metals can be toxic to benthic organisms and are prey for White Sturgeon, particularly in the riverine reaches of the upper Columbia River (Besser et al. 2008). Recent testing showed that metal contaminated sediments in the river upstream of the Kettle River are often toxic to amphipods, whereas sediments were toxic to midges in both the riverine and reservoir portions of the U.S. portions of the upper Columbia River (Besser et al. 2017). This extensive toxicity of sediments to benthic organisms suggests potential effects to the prey base of feeding larval or juvenile sturgeon.

White Sturgeon are opportunistic feeders, with larval sturgeon eating a variety of benthic organisms. During passive sampling surveys in the Roosevelt Reach of the UCR between 2005 and 2008, first feeding larvae that had prey items in their guts had consumed mostly dipteran larvae (primarily chironomids) and copepods (Howell and McLellan 2014). However, between 74 - 100% of larvae that had depleted their yolk reserves, had empty guts (Howell and McLellan 2013). Further investigations of the feeding ecology of White Sturgeon in Lake Roosevelt involved sampling stomach contents of sturgeon larvae at nine locations in 2015. Prey items were only found in the stomachs of 9 of 590 larvae analyzed, suggesting that prey scarcity could be limiting white

sturgeon recruitment in Lake Roosevelt (Reihart 2016). These values are in stark contrast to 1.4% of similar age larvae collected with empty stomachs in the lower Columbia River by Muir et al. (2000). However, Muir et al. (2000) used active fished gear such that animals would be collected from nets within an hour of being caught while Howell and McLellan (2013) used more passively fished gear and animals could have spent longer time periods in the net prior to being collected. These technical differences may account for some of the disparity in numbers of feeding larvae with empty guts between the two efforts. Regardless, the presence of empty guts could prove difficult for larvae as they swim and search for food.

Starvation has both behavioral and physiological effects on sturgeon. With Sterlet Sturgeon (*Acipenser ruthenus*), swimming capability decreased when juvenile sturgeon went without food for greater than one week (Cai et al. 2017). Prolonged swimming in unfed White Sturgeon decreased gut blood flow, and indicated a diversion of energy from digestive activity when food is scarce and swimming activity is great (Farrell et al. 2001).

Though fat stores are available at the initial onset of exogenous feeding (Gisbert et al. 1998) and may protect somewhat against early starvation, losses from viscera during continued starvation can occur. In fact, starvation losses from viscera are initially greater than those from muscle, and any lipid stores seem to be the initial target (Hung et al. 1997). Additionally, tissue degeneration and necrosis (cell death) was documented at 10 -15 days of starvation (30 dph) for Green Sturgeon (*Acipenser medirostris*) in the laboratory (Gisbert and Doroshov 2003). It is important to note that the relatively protected environment of the laboratory would likely keep sturgeon alive longer than what might be observed in the field.

Onset of exogenous feeding documented at 9 dph in Siberian Sturgeon (*Acipenser baerii*) (Gisbert et al. 1998) corresponded with observations that the yolk sac was not completely depleted and the authors suggest that mixed feeding (from yolk sac and prey, also termed “mixed nutrition") occurs at this time. Mixed feeding appears to continue 9 - 12 dph. This observation implies that sturgeon would continue to occupy habitat among the gravel while still partially maintaining yolk sac nutrition. The “point of no return” is when 50% of larvae do not feed when food is reintroduced following starvation (Gerking 1994). In Chinese Sturgeon (*Acipenser sinensis*), point of no return has been documented at 14 dph (Chai et al. 2011).

If the same ecological physiology is true for White Sturgeon, there is not a lot of evidence of mixed feeding in early feeding larvae. Rather, the data would suggest these animals could be starving and even nearing the point of no return. In this weakened state, the White Sturgeon larvae would also come into contact with slag deposited on river bottoms.

## Ingestion and Potential Physiologic Changes

Early feeding White Sturgeon larvae and juveniles are coincidentally ingesting metals from slag and biota when they do feed in the Columbia River. These two routes of exposure: water in interstitial spaces and ingestion could limit the size class distribution and population of White Sturgeon in the Columbia River. Slag has been documented in the gut of a first feeding larvae (Figure 3) (Howell and McLellan 2011) and was present in the guts of 76% of 1 to 4 year old juvenile White Sturgeon collected in the UCR (Parsley et al. 2010). Gawlicka et al. (1995) observed a high degree of specialization along the gut once transition to exogenous feeding was complete. At this

time, the alimentary canal and digestive capacity are similar to adult White Sturgeon (Buddington and Doroshov 1986). Because it takes digestate approximately 24 - 32 h to pass entirely through the gut of sturgeon (Venero et al. 2015) the opportunity exists for trace metals to make contact with the gut lining and elsewhere as a result of slag ingestion. Trace metals bound in slag may be released along sections of the gut during the digestive process. We are unaware of research on whether the angular characteristics and other properties of slag could affect normal digestion in sturgeon.

Modes of action of trace metals, including Cu, can affect multiple biochemical and physiological processes in fish. Farag et al. (1995) noted intracytoplasmic granules that stained positive for Cu in livers of resident fish collected from a mining site. Trace metals from this site were also associated with elevated lipid peroxidation (oxidative stress), metallothionein increase, and tissue lesions. Lipid peroxidation products appear as a result of damage to polyunsaturated fatty-acid side chains of cell membranes (Halliwell and Gutteridge 1985). Fluid balance in cells are dependent on the unimpaired structure of the cell membranes, and upset to this balance may result in cell death and ultimately tissue damage. Farag et al. (1995) also noted nuclear vacuolation of cells in the livers of fish with elevated lipid peroxidation products that suggested irreversible cell destruction. Tang et al. (2016) documented elevated lipid peroxidation products in 15 dph White Sturgeon exposed to 10 µg Cu/L in background water similar to the Columbia River.

While there is debate on the meaning of elevated metallothionein and whether it is only indicative of exposure, previous researchers concluded that concomitant observations of elevated metallothionein and reduced growth indicated that there exists a

metabolic cost of acclimation to Cu (Dixon and Sprague 1981). Doering et al. (2015) observed a downregulation of metallothionein in livers of White Sturgeon exposed to Cu, but no concentrations were defined by the authors. They suggest that this downregulation, (hence less concentrations of metallothionein would be produced in wild White Sturgeon exposed to Cu) is indicative of greater sensitivity of White Sturgeon to Cu toxicity. Regardless of differences in these two interpretations, the presence of Cu does affect the production of metallothionein and the induction of metallothionein regulation.

## A Habitat of Disproportionate Importance: Waneta Eddy

The confluence of the Pend Oreille and Columbia River, about 500m upstream of the U.S./Canadian border (Figure 1), has morphological and circulation features that make it a uniquely important habitat feature for White Sturgeon in the upper Columbia River (Hildebrand et al. 1999; Hildebrand and Parsley 2013). The releases from the Waneta Dam on the Pend Oreille River produce a jet-like high-speed outflow for sturgeon spawning, with spawning observed in all 16 years of monitoring as of 2012 (Hildebrand and Parsley 2013). Newly hatched sturgeon are carried from the high velocity spawning sites to the deep water, low-velocity Waneta Eddy which provides a refuge for sturgeon rearing and feeding (Fissel and Jiang 2008; Hildebrand and Parsley 2013). Unfortunately, while the eddy provides a flow refugia, its depth and low velocities also collected slag, creating a severely contaminated refugia. A survey in 2003 found that 90% of the substrate consisted of slag in the Waneta Eddy sampling site. Overall abundance of benthic macroinvertebrates at the Waneta Eddy sampling was only 25% that of reference sites upstream of the Trail smelter. Not found at Waneta Eddy were

mayflies, enchytraeid oligochaetes, unionid mussels, isopods, or amphipods. These taxa were found in the majority of reference sites. Toxicity testing of Waneta Eddy substrates with the aquatic midge *Chironomus dilutus* with 20-day tests resulted in a 67% reduction in survival and a 81% reduction growth at Waneta Eddy relative to mean of control sites (Golder 2007a). The severity of these effects suggest that larval sturgeon taking refuge in this disproportionately important habitat would encounter reduced prey availability and directly toxic conditions in the slag dominated substrate.

## Summary

In his broad review of potential factors for decline of the upper Columbia River White Sturgeon population McAdam (2015) implicated increased fine substrates at spawning sites as the most plausible explanation for chronic recruitment failure. The additional factors we considered complement that perspective by showing scenarios wherein contamination within the substrates could contribute to the poor survival of newly hatched White Sturgeon. The life history and habit of the White Sturgeon make it likely that they will encounter exposure to trace metal contamination in the upper Columbia River. This finding is based on known White Sturgeon developmental physiology and behavior along with experimental documentation of this species sensitivity to trace metal, especially Cu, toxicity. It is likely that White Sturgeon will be subjected to contaminated substrates during sensitive early lifestages, such as during the transition to endogenous feeding, and that the toxicity of exposure could affect recruitment of White Sturgeon populations in the upper Columbia River. Furthermore, ingestion of slag and contaminated aquatic invertebrates while studied to a lesser degree in this system could provide an additional route of exposure and effects. Observations of

empty guts in early lifestage White Sturgeon in the upper reaches where slag is most prevalent compared to lower reaches where it is found to a lesser extent suggest a role of starvation in recruitment failure. This can be detrimental to the population because recovery from food deprivation during critical early life stages may render sturgeon unable to survive past a "point of no return" even if food is reintroduced. These findings that White Sturgeon may be exposed to trace metals during sensitive the early lifestage is especially important as clean-up and restoration efforts continue in the Upper Columbia River. While managers cannot ignore the contribution of fine substrates, they also may need to consider the contribution of contaminants to the recovery of White Sturgeon. New research is defining ways for ecological restoration efforts to incorporate risk of contaminant exposure into the design (Farag et al. 2015). Kaputska et al. (2015) presents an ecological planning framework whereby an ecological risk assessment is used to inform decisions for attaining the desired ecological end state, in this case, recovery of the White Sturgeon population. Farag et al. (2017) provides examples where Adaptive Management models that include contaminant questions were useful during restoration efforts. White Sturgeon recruitment could benefit from consideration of these approaches. Neglecting to consider the direct and indirect toxic effects of trace metals in the system could jeopardize the restoration efforts.

**Acknowledgements**

I wish to thank E. Little, C. Paukert, C. Mebane and A. Farag for comments that greatly improved this manuscript.

## Literature Cited


Beamesderfer, R.C.P., T.A. Rien, and A.A. Nigro. 1995. Differences in the Dynamics and Potential Production of Impounded and Unimpounded White Sturgeon Populations in the Lower Columbia River. *Transactions of the American Fisheries Society*. 124(6): 857-872. http://dx.doi.org/doi:10.1577/1548-8659(1995)124<0857:DITDAP>2.3.CO;2

Bennett, W.R., G. Edmondson, K. Williamson, and J. Gelley. 2007. An investigation of the substrate preference of white sturgeon (*Acipenser transmontanu*s) eleutheroembryos. *Journal of Applied Ichthyology*. 23(5): 539-542. http://dx.doi.org/10.1111/j.1439-0426.2007.01003.x

Besser, J.M., W.G. Brumbaugh, C.D. Ivey, C.G. Ingersoll, and P.W. Moran. 2008. Biological and chemical characterization of metal bioavailability in sediments from Lake Roosevelt, Columbia River, Washington, USA. *Archives of Environmental Contamination and Toxicology*. 54(4): 557–570. http://dx.doi.org/10.1007/s00244-007-9074-5

Besser, J.M., J.L. Kunz, S.E. Cox, C.G. Ingersoll, and W.G. Brumbaugh. 2017. Physical and chemical data from an evaluation of metal-contaminated sediments and pore water from the Upper Columbia River, Washington, 2013-2014. U.S. Geological Survey Data Release. https://doi.org/10.5066/F72F7KPC.

Buddington, R.K. and S.I. Doroshov. 1986. Structural and functional relations of the white sturgeon alimentary canal (*Acipenser transmontanus*). *Journal of Morphology*. 190(2): 201-213. http://dx.doi.org/10.1002/jmor.1051900205

Cai, L., D. Johnson, M. Fang, P. Mandal, Z. Tu, and Y. Huang. 2017. Effects of feeding, digestion and fasting on the respiration and swimming capability of juvenile sterlet sturgeon (Acipenser ruthenus, Linnaeus 1758). *Fish Physiology and Biochemistry*. 43(1): 279-286. http://dx.doi.org/10.1007/s10695-016-0285-4

Calfee, R.D., E.E. Little, H.J. Puglis, E. Scott, W.G. Brumbaugh, and C.A. Mebane. 2014. Acute sensitivity of white sturgeon (*Acipenser transmontanus*) and rainbow trout (*Oncorhynchus mykiss*) to copper, cadmium, or zinc in water-only laboratory exposures. *Environmental Toxicology and Chemistry*. 33(10): 2259–2272. http://dx.doi.org/10.1002/etc.2684

Chai, Y., C. Xie, and Q.W. Wei. 2011. Yolk-sac absorption and point of no return in Chinese sturgeon Acipenser sinensis larvae. *Journal of Applied Ichthyology*. 27(2): 687-689. http://dx.doi.org/10.1111/j.1439-0426.2010.01631.x

Conte, F.S. 1988. Hatchery manual for the White Sturgeon (*Acipenser transmontanus* Richardson): with application to other North American Acipenseridae. University of California Agriculture and Natural Resources. 104 pp.

Cox, S.E., W.G. Brumbaugh, L.S. Balistrieri, R.E. Wolf, M. Adams, A.J. Sanjer, and T.D. Olsen, 2016, Trace elements concentrations in pore water and surface water near the sediment-water interface in the Upper Columbia River, Washington (2015): U.S. Geological Survey Data Release, http://dx.doi.org/10.5066/F73N21GJ

Deng, X., J. Van Eenennaam, and S. Doroshov. 2002. Comparison of early life stages and growth of green and white sturgeon. American Fisheries Society Symposium.

Dixon, D.G. and J.B. Sprague. 1981. Acclimation to copper by rainbow trout (*Salmo gairdneri*) - a modifying factor in toxicity. *Canadian Journal of Fisheries and Aquatic Sciences*. 38(8): 880-888

Farag, A.M., M.A. Stansbury, H.L. Bergman, C. Hogstrand, and E. MacConnell. 1995. The physiological impairment of free-ranging brown trout exposed to metals in the Clark Fork River, Montana. *Canadian Journal of Fisheries and Aquatic Sciences*. 52(9): 2038-2050. http://dx.doi.org/10.1139/f95-795

Farag, A.M., R.N. Hull, W.H. Clements, S.G. Lomb, D.L. Larson, R. Stahl, J. Stauber. 2015. Restoration of Impaired Ecosystems: An Ounce of Prevention or a Pound of Cure? Introduction, Overview, and Key Messages from a SETAC-SER Workshop. *Integrated Environmental Assessment and Management*. 12: 247–252

Farag, A.M. , D.L. Larson, J. Stauber, R. Stahl, J. Isanhart, K. McAbee, and C.J. Walsh. 2015. Restoration of contaminated ecosystems: adaptive management in a changing climate. *Restoration Ecology*. 25(6): 884–893

Farrell, A.P., H. Thorarensen, M. Axelsson, C.E. Crocker, A.K. Gamperl, and J.J. Cech. 2001. Gut blood flow in fish during exercise and severe hypercapnia. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*. 128(3): 549-561. http://dx.doi.org/https://doi.org/10.1016/S1095-6433(00)00335-4

Fissel, D.B. and J. Jiang. 2008. 3 D Numerical Modeling of Flows at the Confluence the Columbia and Pend d'Oreille Rivers. 10th International Conference on Estuarine and Coastal Modeling. 15 pp. http://dx.doi.org/40990(324)50.

Gawlicka, A., S.J. Teh, S.S.O. Hung, D.E. Hinton, and J. de la Noüe. 1995. Histological and histochemical changes in the digestive tract of white sturgeon larvae during ontogeny. *Fish Physiology and Biochemistry*. 14(5): 357-371. http://dx.doi.org/10.1007/bf00003374

Gerking, S.D. 1994. Feeding Ecology of Fish. Academic Press. 416 pp

Gisbert, E. and S.I. Doroshov. 2003. Histology of the developing digestive system and the effect of food deprivation in larval green sturgeon (*Acipenser medirostris*). *Aquatic Living Resources*. 16(2): 77-89. http://dx.doi.org/10.1016/S0990-7440(03)00029-9

Gisbert, E., A. Rodriguez, F. Castelló-Orvay, and P. Williot. 1998. A histological study of the development of the digestive tract of Siberian sturgeon (Acipenser baeri) during early ontogeny. *Aquaculture*. 167(3): 195-209. http://dx.doi.org/https://doi.org/10.1016/S0044-8486(98)00312-3

Golder. 2007a. Report on sediment quality triad assessment of the effects of the Teck Cominco smelter in the Columbia River. Prepared by Golder Associates Ltd., Calgary, Alberta for Teck Cominco Metals, Trail, British Columbia. 86 pp.

Golder. 2007b. Upper Columbia River juvenile White Sturgeon monitoring: phase 4 investigations, 2005 – 2006. Prepared by Golder Associates, Castlegar, BC. Prepared for BC Hydro, Revelstoke, BC. 201 pp.

Golder. 2009. Upper Columbia River juvenile white sturgeon monitoring: Phase 5 investigations, November 2006., Report prepared by Golder Associates Ltd. for BC Hydro, Revelstoke, B.C. Golder Report No. 06-1480-049F. 203 pp.

Halliwell, D.B. and J.M.C. Gutteridge, editors. 1985. *Free Radials in Biology and Medicine*. Clarendon Press, Oxford, 139–170 pp

Hildebrand, L., C. McLeod, and S. McKenzie. 1999. Status and management of white sturgeon in the Columbia River in British Columbia, Canada: an overview. *Journal of Applied Ichthyology*. 15(4-5): 164-172. http://dx.doi.org/10.1111/j.1439-0426.1999.tb00227.x

Hildebrand, L. and M.J. Parsley. 2013. Upper Columbia River White Sturgeon Recovery Plan - 2012 Revision. Upper Columbia River White Sturgeon Recovery Team. 129 pp. https://www.nwcouncil.org/media/6836165/uppercolsturgeonplandec2012.pdf.

Howell, M.D. and J.G. McLellan. 2011. Lake Roosevelt white sturgeon recovery project, annual progress report, April 2007 – March 2008. Washington Department of Fish and Wildlife. Report the Bonneville Power Adminstration, Environment, Fish and Wildlife, Portland, OR. 126 pp. https://www.cbfish.org.

Howell, M.D. and J.G. McLellan. 2013. Lake Roosevelt white sturgeon recovery project, annual progress report, April 2008 – March 2009. Washington Department of Fish and Wildlife. Report the Bonneville Power Adminstration, Environment, Fish and Wildlife, Portland, OR. 128 pp. https://www.cbfish.org.

Howell, M.D. and J.G. McLellan. 2014. Lake Roosevelt white sturgeon recovery project, annual progress report, April 2010 – March 2011. Washington Department of Fish and Wildlife. Report the Bonneville Power Adminstration, Environment, Fish and Wildlife, Portland, OR. 104 pp. https://www.cbfish.org.

Howell, M.D. and J.G. McLellan. 2015. Lake Roosevelt white sturgeon enhancement project, 12/1/2012 - 11/30/2013 Annual Report, 2008-116-00. Colville

Confederated Tribes of Washington. Report the Bonneville Power Adminstration, Environment, Fish and Wildlife, Portland, OR. 104 pp. https://www.cbfish.org.

Hung, S.S.O., W. Liu, H. Li, T. Storebakken, and Y. Cui. 1997. Effect of starvation on some morphological and biochemical parameters in white sturgeon, *Acipenser transmontanus*. *Aquaculture*. 151(1): 357-363. http://dx.doi.org/https://doi.org/10.1016/S0044-8486(96)01506-2

Irvine, R.L., D.C. Schmidt, and L.R. Hildebrand. 2007. Population Status of White Sturgeon in the Lower Columbia River within Canada. *Transactions of the American Fisheries Society*. 136(6): 1472-1479. http://dx.doi.org/10.1577/T06-190.1

Johnson, A., D. Norton, B. Yake, and S. Twiss. 1990. Transboundary metal pollution of the Columbia River (Franklin D. Roosevelt Lake). *Bulletin of Environmental Contamination and Toxicology*. 45(5): 703-710. http://dx.doi.org/10.1007/bf01700989

Kaputska, L.A., K. Bowers, J. Isanhart, C. Martinez-Garzak, S. Finger, R.G. Stahl Jr., and J. Stauber. 2015. Coordinating Ecological Restoration Options Analysis and Risk Assessment to Improve Environmental Outcomes. *Integrated Environmental Assessment and Management*. 12(2):253-63

Little, E.E., R.D. Calfee, and G. Linder. 2012. Toxicity of copper to early-life stage Kootenai River white sturgeon, Columbia River white sturgeon, and rainbow Trout. *Archives of Environmental Contamination and Toxicology*. http://dx.doi.org/10.1007/s00244-012-9782-3

Little, E.E., R.D. Calfee, and G. Linder. 2014. Toxicity of smelter slag-contaminated sediments from Upper Lake Roosevelt and associated metals to early life stage White Sturgeon (*Acipenser transmontanus* Richardson, 1836). *Journal of Applied Ichthyology*. 30(6): 1497-1507. http://dx.doi.org/10.1111/jai.12565

McAdam, D.S.O. 2015. Retrospective weight-of-evidence analysis identifies substrate change as the apparent cause of recruitment failure in the upper Columbia River white sturgeon (*Acipenser transmontanus*). *Canadian Journal of Fisheries and Aquatic Sciences*. 72(8): 1208-1220. http://dx.doi.org/10.1139/cjfas-2014-0423

McAdam, S.O. 2011. Effects of substrate condition on habitat use and survival by white sturgeon (*Acipenser transmontanus*) larvae and potential implications for recruitment. *Canadian Journal of Fisheries and Aquatic Sciences*. 68(5): 812-822. http://dx.doi.org/10.1139/f2011-021

McIntyre, J.K., D.H. Baldwin, D.A. Beauchamp, and N.L. Scholz. 2012. Low-level copper exposures increase visibility and vulnerability of juvenile coho salmon to cutthroat trout predators. *Ecological Applications*. 22(5): 1460–1471. http://dx.doi.org/10.1890/11-2001.1

Muir, W.D., G.T.J. McCabe, M.J. Parsley, and S.A. Hinton. 2000. Diet of first-feeding larval and young-of-the-year white sturgeon in the lower Columbia River. *Northwest Science*. 74(1): 25-33

Parrish, A.L. 2005. Trail Smelter Déjà Vu: Extraterritoriality, International Environmental Law and the Search for Solutions to Canadian-U.S. Transboundary Water Pollution Disputes. *Boston University Law Review*. 363-429

Parsley, M.J., B.K. van der Leeuw, and D.G. Elliott. 2010. Characterization of the Contents and Histology of the Gastrointestinal Tracts of White Sturgeon (*Acipenser transmontanus*) Captured from Upper Lake Roosevelt, Washington, October 2008. U.S. Geological Survey Open-File Report 2010-1193. 32 pp. https://pubs.er.usgs.gov/publication/ofr20101193.

Paulson, A.J. and S.E. Cox. 2007. Release of elements to natural water from sediments of Lake Roosevelt, Washington, USA. *Environmental Toxicology and Chemistry*. 26(12): 2550-2559. http://dx.doi.org/10.1897/07-052.1

Puglis, H.J. 2018. Behavioral effects of copper on larval White Sturgeon.

Reihart, R.W. 2016. Invertebrate drift in the upper Columbia River with implications for young-of-the-year white sturgeon. 64 pp., EWU Masters Thesis Collection, 403. Eastern Washington University, Ellensburg, WA.

Santore, R.C. and K. Croteau. 2015. Biotic Ligand Model Windows Interface, Research Version 3.1.2.37 (October 2015): User's Guide and Reference Manual. Windward Environmental, LLC, Syracuse, NY. 46 pp. http://www.windwardenv.com/biotic-ligand-model

Tang, S., J.A. Doering, J. Sun, S.C. Beitel, K. Shekh, S. Patterson, S. Crawford, J.P. Giesy, S.B. Wiseman, and M. Hecker. 2016. Linking oxidative stress and magnitude of compensatory responses with life-stage specific differences in sensitivity of White Sturgeon (*Acipenser transmontanus*) to copper or cadmium. *Environmental Science & Technology*. 50(17): 9717-9726. http://dx.doi.org/10.1021/acs.est.6b03301

Tompsett, A.R., D.W. Vardy, E. Higley, J.A. Doering, M. Allan, K. Liber, J.P. Giesy, and M. Hecker. 2014. Effects of Columbia River water on early life-stages of white sturgeon (*Acipenser transmontanus*). *Ecotoxicology and Environmental Safety*. 101(0): 23-30. http://dx.doi.org/10.1016/j.ecoenv.2013.12.004

Vardy, D.W., J. Oellers, J.A. Doering, H. Hollert, J.P. Giesy, and M. Hecker. 2013. Sensitivity of early life stages of white sturgeon, rainbow trout, and fathead minnow to copper. *Ecotoxicology*. 22(1): 139-147 http://dx.doi.org/10.1007/s10646-012-1010-4

Vardy, D.W., R.C. Santore, A.C. Ryan, J.P. Giesy, and M. Hecker. 2014. Acute toxicity of copper, lead, cadmium, and zinc to early life stages of white sturgeon (*Acipenser transmontanus*) in laboratory and Columbia River water. *Environmental Science and Pollution Research*. 1-12. http://dx.doi.org/10.1007/s11356-014-2754-6

Venero, J.A., R.D. Miles, and F.A. Chapman. 2015. Food Transit Time and Site of Absorption of Nutrients in Gulf of Mexico Sturgeon. *North American Journal of Aquaculture*. 77(3): 275-280. http://dx.doi.org/10.1080/15222055.2015.1017129

Wang, N., C.G. Ingersoll, R.A. Dorman, W.G. Brumbaugh, C.A. Mebane, J.L. Kunz, and D.K. Hardesty. 2014. Chronic sensitivity of White sturgeon (*Acipenser transmontanus*) and rainbow trout (*Oncorhynchus mykiss*) to cadmium, copper, lead, or zinc in laboratory water-only exposures. *Environmental Toxicology and Chemistry*. 33(10): 2246–2258. http://dx.doi.org/10.1002/etc.2641

Table 1. Summary of acute toxicity defined in experiments conducted with White Sturgeon. LC50 = concentration that resulted in 50% reduction in survival compared to control. EC50 = concentration that resulted in occurrence of a sublethal parameter (e.g. swimming activity) in 50% of the experimental population exposed to that concentration. BLM = Biotic Ligand Model; a model that was used to normalize reported toxicity in different waters to a common water type.

| Life Stage | Age (dph) | Original 96 h LC50 (µg Cu/L) | BLM adjusted LC50 (µg Cu/L) | Reported EC50 | *BLM adjusted EC50 | Source |
|---|---|---|---|---|---|---|
| Free embryos (Yolk sac) | 1 | >7.1 | >4.4 | >7.1 | | (Puglis 2018) |
| | 2 | >23.6 | >16.2 | | 1.5 | (Calfee et al. 2014) |
| | 2 | 8.1 | 4.9 | | | (Wang et al. 2014) |
| | 8 | 22 | 5.3 | | | (Vardy et al. 2013) |
| | 8 | 22 | | | | (Vardy et al. 2014) |
| | 8 | 25 | | | | (Vardy et al. 2014) |
| First feeding larvae (mixed feeding) | 15 | 10 | 2.9 | | | (Vardy et al. 2013) |
| | 15 | 4.7 | | | | (Tang et al. 2016) |
| | 16 | 7.1 | 4.4 | | 2.6 | (Calfee et al. 2014) |
| | 26 | 4.5 | 2.2 | | | (Little et al. 2012) |
| | 27 | 6.8 | 8.2 | | | (Little et al. 2012) |
| | 28 | 11.9 | 7.8 | 8.7 | | (Puglis 2018) |
| | 30 | 16.4 | 11.8 | | 4.2 | (Calfee et al. 2014) |
| | 30 | 7.7 | | | | (Little et al. 2014) |
| | 35 | 15 | 10.5 | 8.9 | | (Puglis 2018) |
| | 38 | 4.1 | 2.3 | | | (Little et al. 2012) |
| | 40 | 9 | 2.4 | | | (Vardy et al. 2013) |
| | 40 | 9 | 4 | | | (Vardy et al. 2014) |
| | 40 | 4.7 | 5.5 | | | (Little et al. 2012) |
| | 44 | >50 | >34.1 | | >34.1 | (Calfee et al. 2014) |
| | 45 | 17 | 6.8 | | | (Vardy et al. 2013) |
| Juveniles (Young of the year - age-0) | 48 | >20 | | | | (Tang et al. 2016) |
| | 61 | <90 | <56 | | 22 | (Calfee et al. 2014) |
| | 72 | 74 | 51.3 | | 59 | (Calfee et al. 2014) |
| | 89 | 90 | 62.9 | | 17.3 | (Calfee et al. 2014) |
| | 100 | 54 | 16.1 | | | (Vardy et al. 2013) |
| | 139 | 66.5 | | | | (Tang et al. 2016) |

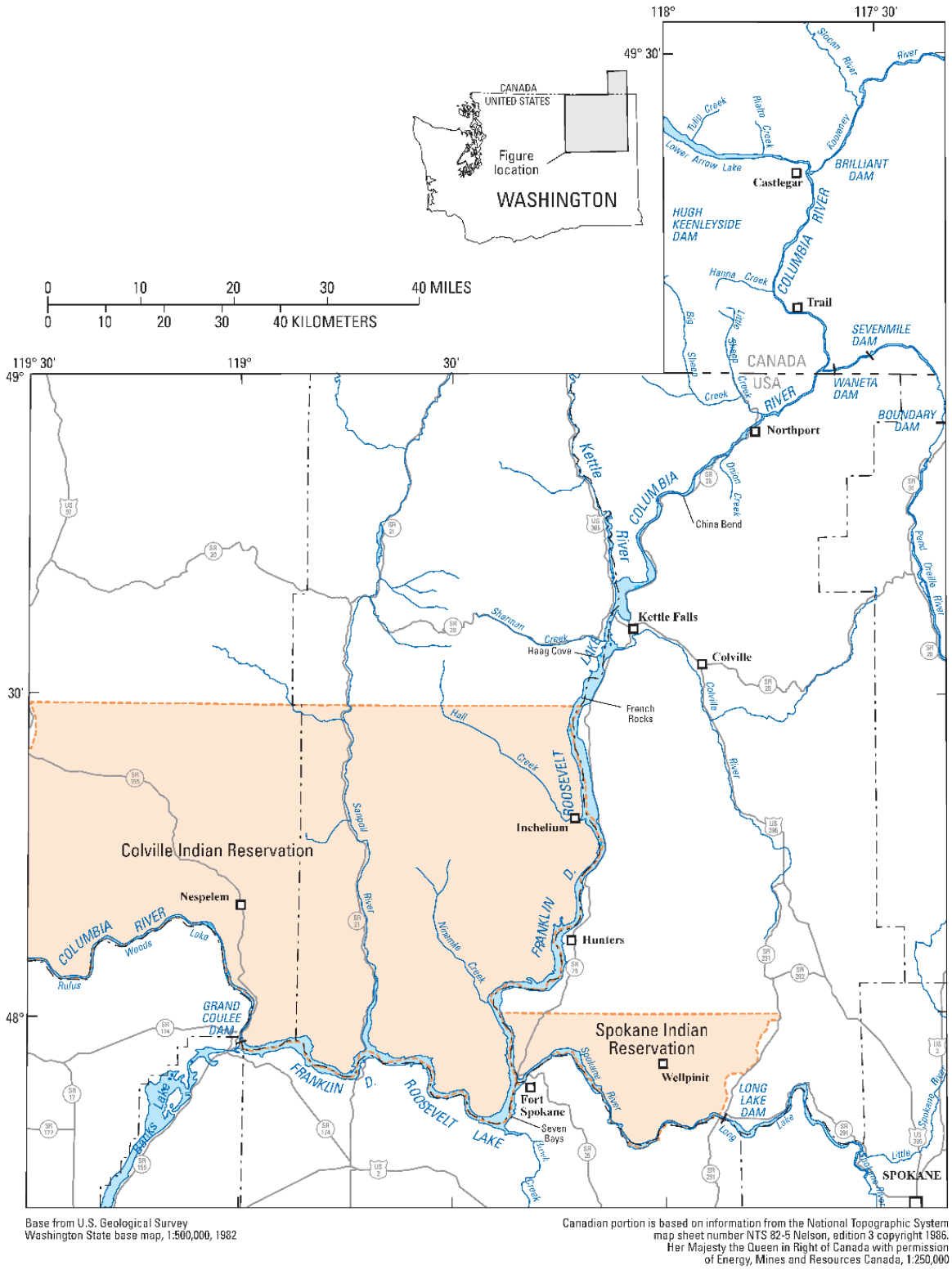
**Figure Legends**

Figure 1. Map of study area in the Upper Columbia River. Three reservoir reaches: Upper, Middle, & Lower are depicted along with major tributaries of the Spokane, Kettle, Colville, and Sanpoil rivers. Lake Roosevelt is the impoundment of the Columbia River behind Grand Coulee Dam.

Figure 2. Life history characteristics of White Sturgeon. (A) White Sturgeon from egg to juvenile with drifting and onset of feeding depicted. (B) Potential exposure routes available in the Upper Columbia River where early stages are in contact with groundwater upwelling, delayed feeding may affect normal development and survival, and slag is consumed during feeding.

Figure 3. The presence of slag material and associated trace metals during development of White Sturgeon in the Upper Columbia River. Panel A is an example of slag particles {dark colored "sand") among cobbles and gravels in the riverbed of the upper Columbia River near Northport, WA. Photo by Steve Cox, USGS ; Panel B photo left, bottom, and right define embryo embedded with slag material, benthic macroinvertebrate with entrained slag particle, and early lifestage sturgeon with ingested slag particle, All three photos are from Howell and McLellan 2011 ; Panel C depicts sturgeon early lifestage (-8-day old) inhabiting crevices between cobbles, presumably to avoid predators or being washed downstream; Panel D pictures older sturgeon (15-20 days after hatching) that now alternate time among the rocks with swimming to the surface, and start to eat live food, such as the worms visible on the bottom of this tank. Photos taken by USGS Columbia Environmental Research Center staff.

Figure 4. Copper concentrations measured in surface water collected 7.5 cm above the substrate (SW), directly at the sediment-water interface (SWI), from shallow porewater 4.5 cm below the substrate surface, and from deep porewater collected 14.5 cm below the substrate surface. Boxes show the upper 75th percentile, median, and lower 25th percentile of the data and the symbols show actual values. Data are from Cox et al (2016). The dashed line show the concentration causing 50% adverse effects to 2-day post hatch white sturgeon in a 96h exposure (Calfee et al. 2014), adjusted to the mean water quality characteristics of the SPW samples. Copper values less than the reporting limit of 0.5 µg/L are plotted as 0.25 µg/L, which was the minimum Cu value reported in a study of the UCR that achieved lower reporting limits (Tompsett et al. 2014). Adjustment of Calfee et al.'s (2014) EC50 for of 2.67 µg/L that resulted from copper exposures in laboratory water (e.g., pH 8.3 dissolved organic carbon (DOC) 0.4 mg/L, and hardness 105 mg/L) to a value of 2.33 µg/L for the mean characteristics of the shallow porewater (e.g., pH 7.9, DOC 1.1 mg/L, and hardness 68 mg/L) was made using the biotic ligand model software of Santore and Croteau (2015) following the procedure of EPA (2007).




Base from U.S. Geological Survey
Washington State base map, 1:500,000, 1982

Canadian portion is based on information from the National Topographic System map sheet number NTS 82-5 Nelson, edition 3 copyright 1986. Her Majesty the Queen in Right of Canada with permission of Energy, Mines and Resources Canada, 1:250,000

Figure 1.

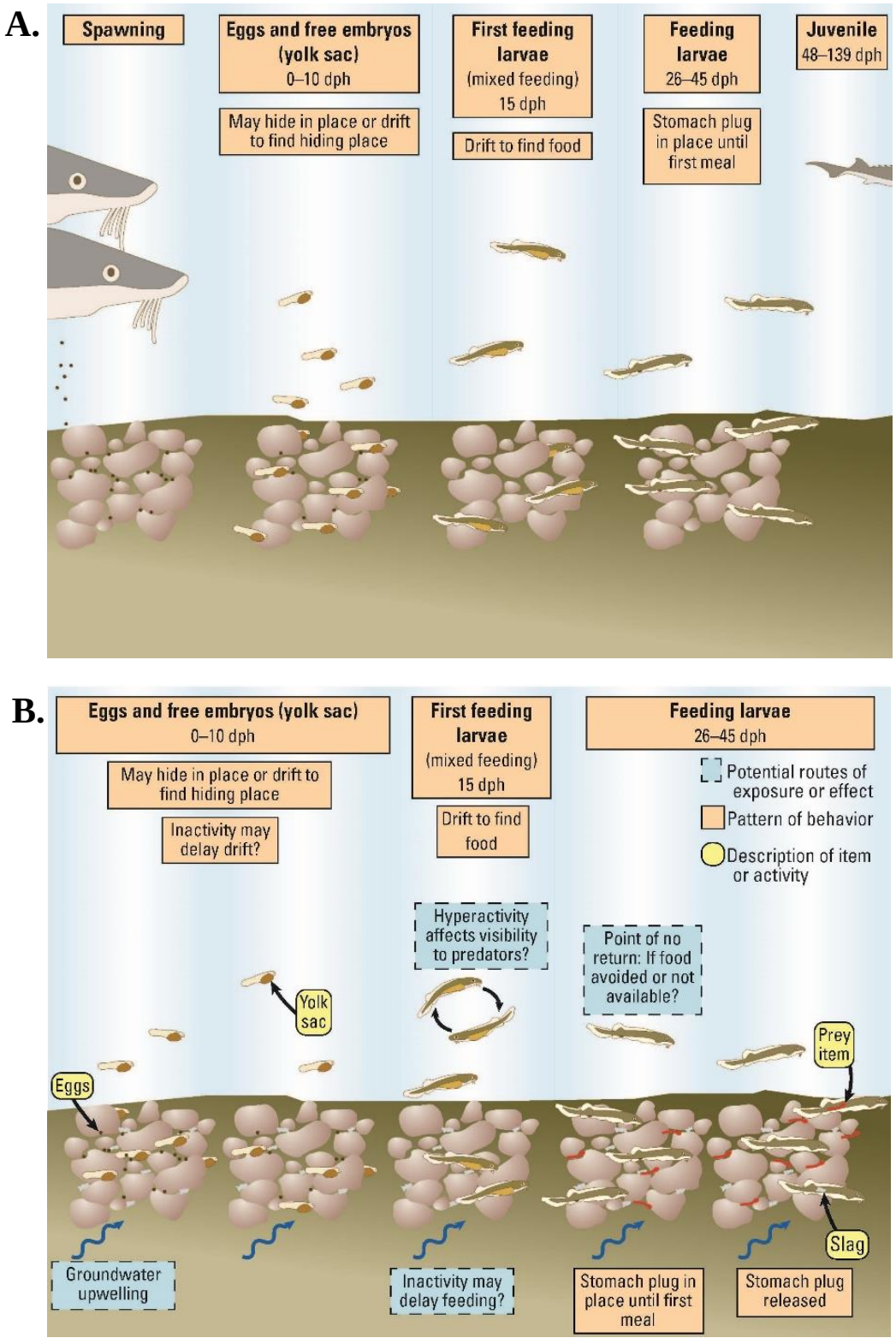
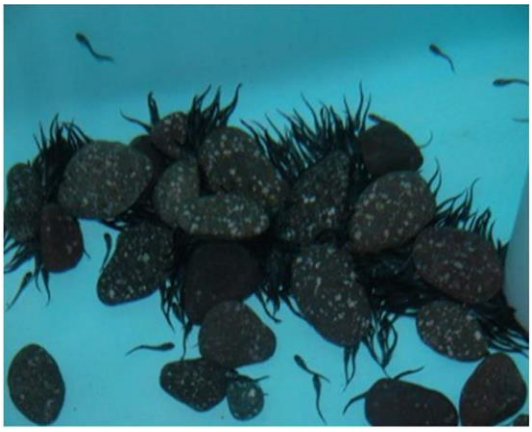

A.
Spawning
Eggs and free embryos (yolk sac)
0–10 dph
May hide in place or drift to find hiding place
First feeding larvae
(mixed feeding)
15 dph
Drift to find food
Feeding larvae
26–45 dph
Stomach plug in place until first meal
Juvenile
48–139 dph
B.
Eggs and free embryos (yolk sac)
0–10 dph
May hide in place or drift to find hiding place
Inactivity may delay drift?
First feeding larvae
(mixed feeding)
15 dph
Drift to find food
Feeding larvae
26–45 dph
Potential routes of exposure or effect
Pattern of behavior
Description of item or activity
Hyperactivity affects visibility to predators?
Point of no return: If food avoided or not available?
Yolk sac
Eggs
Prey item
Slag
Groundwater upwelling
Inactivity may delay feeding?
Stomach plug in place until first meal
Stomach plug released

Figure 2.

**A.**

**B.**


**C.**



**D.**

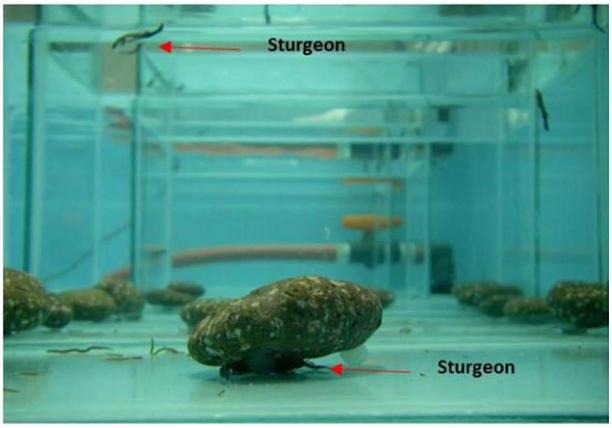



Figure 3.

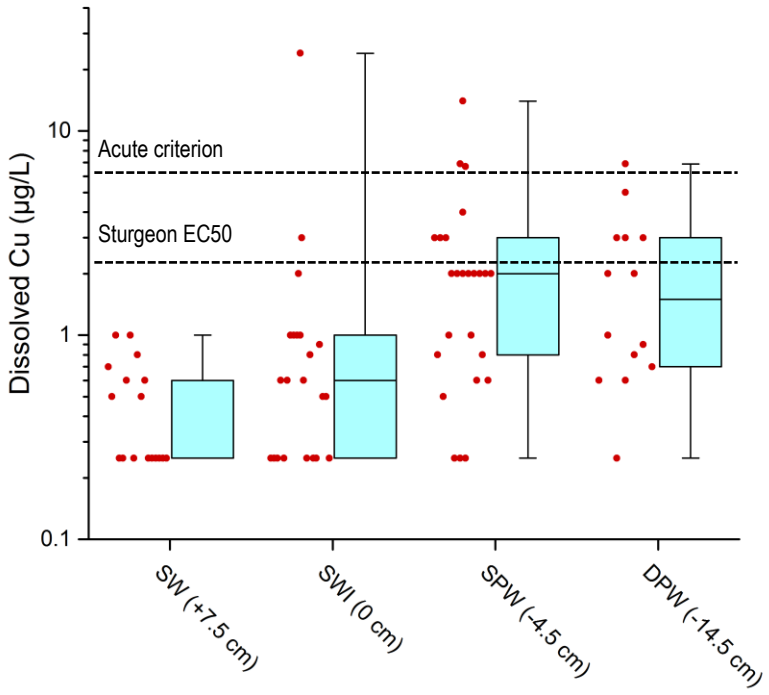

Dissolved Cu (µg/L)
10
1
0.1
Acute criterion
Sturgeon EC50
SW (+7.5 cm)
SWI (0 cm)
SPW (-4.5 cm)
DPW (-14.5 cm)

Figure 4.

# CHAPTER 5

CONCLUSIONS

Holly J. Puglis

## Summary

The factors surrounding population declines are often myriad and complex. For the white sturgeon in the upper Columbia River, it is critical that the role of contamination in their decline is well characterized and accounted for in any regulatory or restoration efforts. Behavior is often a sensitive measure of toxicity with effect concentrations below those that cause mortality. The effects of toxicity exist on a continuum, with some endpoints becoming apparent long before others. These early indications of toxicity can be useful to managers and conservationists at contaminated sites in helping set clean up criteria to protect the most sensitive of species at a site. My dissertation indicates that metal contamination in the UCR can affect larval white sturgeon at low, environmentally relevant concentrations and needs to be accounted for in any restoration efforts at the site. The following sections provide a summary of the key findings and conclusions of each dissertation chapter.

*Chapter 2 – Behavioral effects of copper on larval white sturgeon*

- Swimming activity of larval white sturgeon reduced in concentrations of copper as low as 2 µg Cu/L
- Hyperactivity was observed immediately after exposure to copper, but was short lived
- Older sturgeon larvae are not as sensitive as recently hatched white sturgeon larvae
- Juvenile white sturgeon food consumption was reduced in the presence of copper

*Chapter 3 – Toxicity of metal contaminated sediments from the upper Columbia River to early life stage white sturgeon*

- Found evidence that UCR sediments can affect survival and swimming behavior of larval sturgeon
- Sediment chemistry was not a good predictor of toxicity to white sturgeon larvae
- Overlying water was a better predictor of toxicity to white sturgeon larvae

*Chapter 4* – White sturgeon in the upper Columbia River: life history and their potential exposure to trace metals

- Modeled measured copper concentrations to the UCR and compared those values to laboratory effect concentrations to identify overlap indicating behavior effects seen in the lab could be occur in the field
- Found evidence of potential larval white sturgeon starvation in the UCR and pieced together disparate pieces of knowledge about the site to understand how copper could play a role

# VITA

Holly Jane Puglis was born on December 3, 1985 in Cleveland, Ohio to Thomas and Maxine Puglis. She grew up in Mentor, Ohio and graduated from Mentor High School in 2004 and went on to earn a B.A. in Zoology and a B.S. in Adolescent/Young Adult Life Science Education from Miami University in 2008. Holly then earned her M.S. in Zoology with Michelle Boone, studying the role of buffer zones in public green spaces and how they might bolster amphibian communities. In 2010, Holly began working at the USGS Columbia Environmental Research Center in Columbia, Missouri. She married Ryan G. Krankowski in July of 2012 and began her Ph.D. program in the fall of 2012. Her dissertation research focused on the sublethal effects of copper exposure to larval white sturgeon and the role these effects might have on the decline of white sturgeon in the upper Columbia River. Holly completed her Ph.D. in May 2018 and will continue to work at the USGS after graduation.