

Toxicity of Metals to Early Life Stages of White Sturgeon (*Acipenser transmontanus*)

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ABSTRACT

Throughout North America populations of white sturgeon (*Acipenser transmontanus*) are threatened, in part due to poor annual recruitment. Definitive causes for this are not yet known, but the effects of contaminants are suspected to contribute. White sturgeon are not commonly studied in ecotoxicology and their vulnerability as a species to contaminants of environmental concern is not well defined. Specifically, little work has been conducted to characterize the impact of metals to white sturgeon. To date no chronic exposure studies have been conducted with embryos, fry, and/or juveniles, which are life stages often considered susceptible to pollutants. In addition, few projects have worked with this species under experimental conditions so no information exist outlining effective methods for conducting early life stage toxicity tests. Due to the decline of many sturgeon populations, and the potential contribution of metals, there is a need for information and tools that would help with the rehabilitation of the species.

The first part of this thesis presents a sub-chronic field study that was conducted on the Upper Columbia River investigating the effects of liquid effluent released by Teck American Incorporated Metals Ltd., a local metallurgical facility in Trail, BC, Canada, on early life stages of white sturgeon (Chapter 2). The primary objectives of this study were to develop the logistics for future definitive studies, gain information to design and establish an artificial exposure system for experimental testing of white sturgeon early life stages, and, if possible, to evaluate the potential impact of effluent on survival and growth of early life stage white sturgeon.

Based on the experience made during the conduct of these studies we successfully established a design for an exposure system (Appendix A) that was successfully used in future definitive studies (Chapter 3). These portable, self-contained, artificial flow-through exposure systems can be completely disassembled, easily transported, and set up directly in the field or in the laboratory to be used with a water source of choice (e.g. river-water, laboratory water, effluent mixtures, contaminant dilutions, etc.). The flow regime in the re-circulating exposure units can be adjusted such that it simulates fluvial conditions of interest. Each exposure chamber contains numerous screens, and inserts of varying size, allowing modifications to the exposure area and adjustment of water height. Valuable insight was also gained into white sturgeon culturing that enabled us to successfully rear early life stages under experimental conditions. Long term studies raising embryos to 60 days post hatch led to effective embryo incubation techniques and feeding regimes to transition larvae to exogenous food (Appendix B). The most effective feeding regime for transitioning larvae to exogenous food under experimental exposure conditions included 24 h feeding at approximately 2 h intervals. A combination of live brine shrimp, frozen bloodworms, and a semi-moist powder diet worked best. Furthermore, results from this study indicate that the effluent of Teck American Incorporated Metals Ltd.'s Trail facility is not toxic to white sturgeon early life stages at the dilutions studied. There were, however, limitations and deficiencies identified in the study that promoted fish mortalities and caution should be made when interpreting the results. In this study both fertilized eggs and 16 d old sturgeon larvae were successfully reared in the exposure system. The study setup permitted successful hatching of embryos with hatching rates > 80%. Fish exposed to the greatest concentration of effluent (25%) showed greater survival rates than any of the other treatments

and the fry gained weight more rapidly. It is hypothesized that this might have been due to the greater amount of nutrients or other essential trace elements available in the effluent. At termination of the experiments, percent mortalities relative to initial numbers of fish were 52%, 30%, 29%, and 20% in the controls, 1%, 5%, and 25% treatments, respectively.

The second part of this thesis investigated sensitivity of early life stages of white sturgeon to metals. A study is presented that established baseline toxicity data for the sub-chronic exposure of early life stages to copper, cadmium, and zinc that can be used in metal related risk assessments (Chapter 3). Embryos, larvae, and fry were exposed to increasing concentrations of dissolved copper, cadmium, and zinc for 66 d using laboratory based flow-through exposure systems. Hatching success was greater than 79% for all controls and there were no significant differences observed among treatment groups, or between treatments and controls. Chronic lethal concentrations at which 20% mortality occurred (LC20s) for Cd (1.5 µg/L), Cu (5.5 µg/L), and Zn (112 µg/L) obtained for white sturgeon in the present study were comparable to sensitive salmonid species. Based on LC50 and LC20 values for 19 and 58 d post hatch white sturgeon, the United States national ambient water quality criteria and the Canadian water quality guidelines for the protection of aquatic life that have been established for copper, cadmium, and zinc are protective of white sturgeon early life stages.

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
ASTM	American Society for Testing and Materials
BLM	Biotic Ligand Model
Cd	Cadmium
CSO III	Teck Combined Sewer Outfall 3
Cu	Copper
DOC	Dissolved Organic Carbon
DPH	Days Post Hatch
EC	Effect Concentration
ELS	Early life stage
EPA	United States Environmental Protection Agency
GAW	Gram Atomic Weight
HDPE	High Density Polyethylene
ICP-MS	Inductively Coupled Plasma Mass Spectrometry
LC	Lethal Concentration
LOAEC	Lowest Observed Adverse Effect Concentration
LOD	Limit of Detection
MS-222	Tricaine Methanesulfonate
NOAEC	No Observed Adverse Effect Concentration
PVC	Polyvinyl Chloride

ROW	Reverse Osmosis Water
SARA	Species At Risk Act
SD	Standard Deviation
Teck	Teck American Incorporated Metals Ltd.
TOC	Total Organic Carbon
UCR	Upper Columbia River
UCWSRI	Upper Columbia White Sturgeon Recovery Initiative
Zn	Zinc

CHAPTER 1

1.0 GENERAL INTRODUCTION

1.1 Introduction

The white sturgeon (*Acipenser transmontanus*) is the largest freshwater fish native to North America. It can live to be over 100 yr old, weigh more than 800 kg and reach lengths of 6 metres or more. It is also one of the most archaic fishes with their prehistoric ancestors dating back an estimated 175 million years (UCWSRI, 2002). Presently, however, sturgeon (Acipenseridae) populations are threatened throughout the world and over the past century have been decreasing in Northern Europe, Asia and North America (Birstein, 1993; Coutant, 2004; Gisbert and Williot, 2002). Factors that are hypothesized to have contributed to this decline are over-harvesting, habitat alteration and contamination (Birstein, 1993; Gisbert and Williot, 2002; Hu et al., 2009; Irvine et al., 2007; Luk'yanenko et al., 1999; Paragamian and Hansen, 2008). In North America, populations of white sturgeon have been reported to be declining in the north-western USA and British Columbia, Canada. Populations of white sturgeon have been listed as endangered in parts of Canada (COSEWIC, 2003) and in the USA (U.S. Fish and Wildlife Service, 2011). Decreases in sturgeon populations in the Columbia, Fraser, and Sacramento-San Joaquin rivers and their tributaries have been attributed primarily to poor annual recruitment (Coutant, 2004; NRWSRI, 2004; Scott and Crossman, 1973, 1998; UCWSRI, 2002). Some simulation models have predicted that without implementation of successful remedial efforts the white sturgeon will become extinct in these rivers within fifty years (Irvine et al., 2007; Paragamian et al., 2005; Paragamian and Hansen, 2008; UCWSRI, 2002).

Several hypotheses for recruitment failures of white sturgeon have been suggested. These include: habitat alteration, varying flow regimes, decreased water quality, including, among others, temperature, turbidity, total dissolved gases, poor nutrition, genetic bottlenecks or inbreeding depression, predation by introduced species such as walleye (*Sander vitreus*), inter-specific competition, pathogens, and pollution (Coutant, 2004; Kruse and Scarnecchia, 2002a; Kruse and Webb, 2006; UCWSRI, 2002). The white sturgeon is a relatively long-lived scavenger that preys on benthic species and spends much of its life closely associated with sediments. Therefore, in addition to exposure to pollutants in the water column, sturgeon can be exposed to sediment-bound contaminants (Feist et al., 2005; Kruse and Scarnecchia, 2002a). A limited number of studies have examined the effects of pollutants on white sturgeon and there is a general consensus that further investigations are needed to assess the relative sensitivity of this species to environmental contaminants (Foster et al., 2001a,b; Gundersen et al., 2008; Kruse and Scarnecchia, 2002a,b; Kruse and Webb, 2006; UCWSRI, 2002; Webb et al., 2006). To date, most studies involving sturgeon have been conducted with acute exposures and no studies of the effects of contaminants have been conducted with early life stages (ELS's) from embryo through juvenile. Fish are considered to be most sensitive to the exposure of contaminants during this period of development (Hutchinson et al., 1998), and chronic studies of the effects of contaminants on ELS's are needed to assess potential effects to sturgeon. The Columbia River is a model system for the study of white sturgeon as it contains various populations experiencing different degrees of recruitment failures. The lower, unimpounded section of the river contains the most viable, self-sustaining population of white sturgeon, while northern, impounded sections experience moderate to great recruitment failures (Coutant, 2004).

1.2 Contaminants and the Columbia River

The Columbia River is the largest river in western North America. Its headwaters flow northward from Columbia Lake in the Canadian Rockies up to the Kinbasket Reservoir, British Columbia. Once reaching the Big Bend region the Columbia abruptly turns south and flows back down through the Kootenay Mountains of British Columbia and crosses over the border into the eastern part of Washington State. The river continues to meander south until it meets the Washington-Oregon border where it curves west and eventually empties into the Pacific Ocean (Fig. 1.1).



Figure 1.1. Columbia River basin. (Image provided by K. Musser)

The Columbia's fast flow rate and its significant drop in elevation over a moderate distance make it an optimal river for hydroelectric power generation. Fourteen major dams are situated on the main stem of the river and another 400 dams are located within its tributaries; making it the largest hydroelectric power-producing river in North America and the most hydroelectrically developed river system in the world (CCRH, 2008).

The Columbia River is home to a variety of native fish species, some of which include Kokanee salmon (*Oncorhynchus nerka*), rainbow trout (*Oncorhynchus mykiss*), pike minnow (*Ptychocheilus oregonensis*) and white sturgeon. Introduced fishes include carp (*Cyprinus carpio*), brook trout (*Salvelinus fontinalis*), brown trout (*Salmo trutta*), walleye (*Sander vitreus*), largemouth bass (*Micropterus salmoides*), smallmouth bass (*Micropterus dolomieu*) and channel catfish (*Ictalurus punctatus*), among others (WDFW, 2006). In addition, several anadromous fishes, such as salmon (Salmonidae) and white sturgeon, use the river and its tributaries as spawning grounds. The white sturgeon population is an important fish species for many people, including the First Nations who have historically utilized sturgeon for both survival and cultural purposes (UCWSRI, 2002). Human activities, including damming and industrial effluents have impacted the Columbia River ecosystem, and concerns have been raised within the scientific community over the failure of this population of white sturgeon. Therefore, it has been deemed important to investigate possible causes for their decline. White sturgeon are an essential component of the Columbia River ecosystem but within the last century their population numbers have been drastically declining, to the point where in 2006 the federal Canadian government listed white sturgeon as endangered under the Species At Risk Act (SARA). Poor recruitment in the Upper Columbia River (UCR) between Grand Coulee Dam in the U.S. and the

Hugh L. Keenleyside Dam in southern British Columbia, Canada, has been documented since the 1970s (Hildebrand et al., 1999). Information on this population was gathered between Hugh L. Keenleyside Dam and the U.S.-Canada border (Fig. 1.2), and numbers were estimated at 1,120 individuals in 1995 and 1,157 individuals in 2004 (R.L., & L. Environmental Services, 1996; Irvine et al., 2007), but through the use of length-frequency distributions it is speculated that this population has suffered poor recruitment of juvenile white sturgeon over the past few decades (Hildebrand et al., 1999).

There is evidence that adult white sturgeon are spawning and depositing viable eggs in certain areas of the Canadian portion of the Columbia River, especially at Waneta Eddy located at the Pend'Oreille River-Columbia River confluence, just north of the U.S.-Canada border (Golder Associates Ltd., 2007; Howell and McLellan, 2006). Limited numbers of ELS white sturgeon have been observed in areas considered to be suitable habitats. In 2000, the Upper Columbia White Sturgeon Recovery Initiative (UCWSRI), a trans-boundary collaboration, was formed to help identify possible issues related to this recruitment failure and to help re-establish a self-sustaining population. As a result, the Upper Columbia River Sturgeon Conservation Hatchery Program was established whereby juvenile white sturgeon are raised in a hatchery and subsequently released into the Columbia River as yearlings. One-year old white sturgeon that are stocked into the river appear to be adapting well to the natural habitat and exhibit good survival, growth rates and body condition (UCWSRI, 2002).

The UCR white sturgeon population is experiencing a life-stage specific bottleneck, but no definitive cause for this phenomenon has been determined. A single cause or, more likely, a

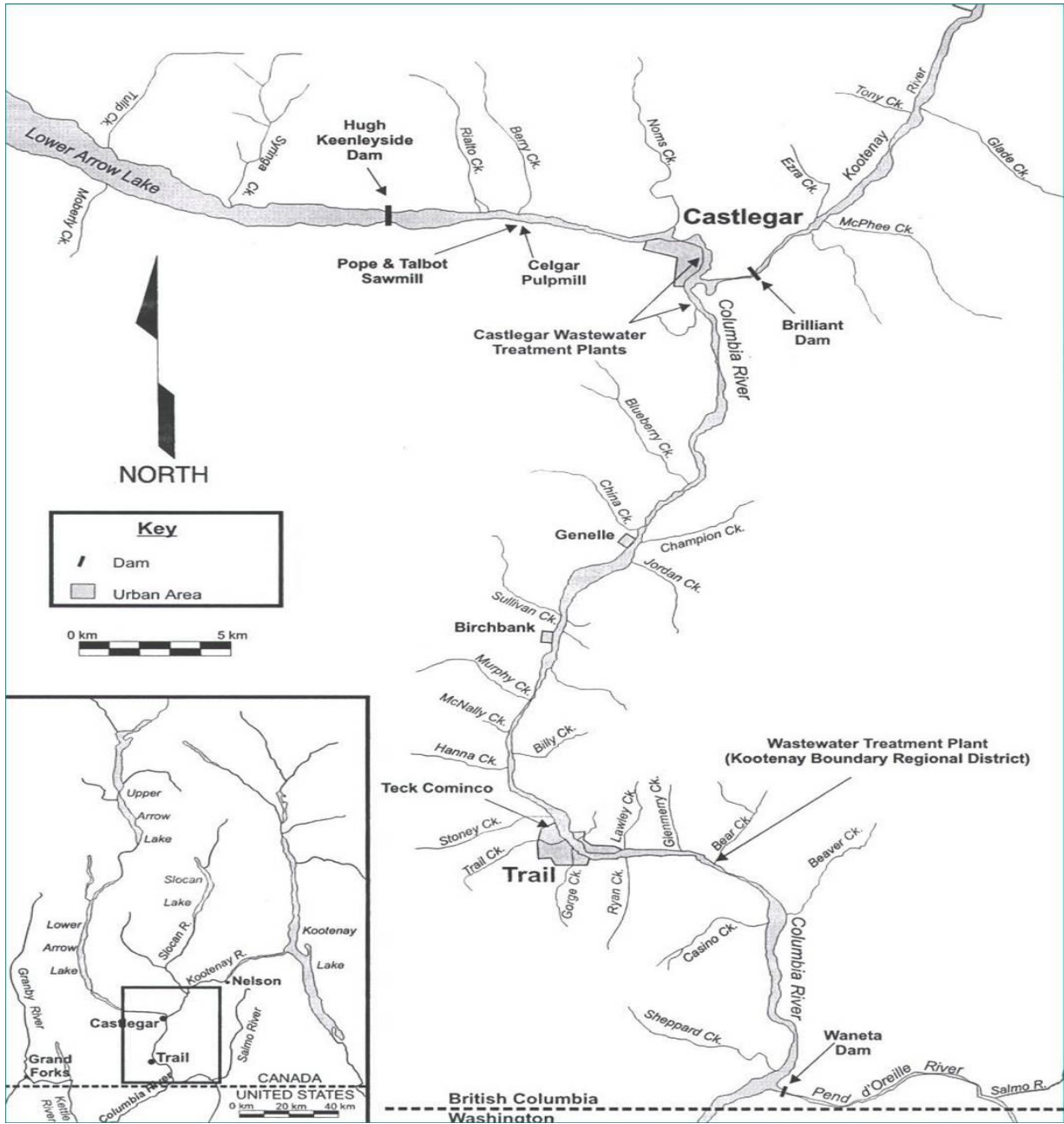


Figure 1.2. Canadian portion of the distribution range of a population of white sturgeon that resides between Hugh L. Keenleyside Dam, BC, Canada and the Grand Coulee Dam, WA, USA.

combination of several potential factors, including metal pollution, may be contributing to the recruitment failure. The potential for exposure to metals and other contaminants in the Columbia River is of concern due to past and present activities of mines, metallurgical facilities, pulp and paper mills, sewage treatment plants, as well as other industrial and municipal sources (UCR WPRIFS, 2008). Teck American Incorporated Metals Ltd. (Teck) is the largest lead-zinc smelter in the world located in the southern Kootenay Mountains (49°06.5' N; 117°42" W) and has been operating in Trail, BC, Canada since 1906. Historically, Teck released slag and processed effluent into the Columbia River. In 1995 the company ceased to release slag into the river but as of date continues to discharge liquid effluent. Slag is composed of ferrous granules that are relatively dense and tend to accumulate on the river bottom. There is concern over the toxicological properties, such as leaching of metals, from slag and the potential impact on white sturgeon. Effluent from Teck has also been of concern since it contains various contaminants, including trace amounts of zinc, mercury, copper, ammonia, arsenic, cadmium, chlorine, and lead. The main focus of this project was to specifically address the potential impact of exposure to metals on ELS white sturgeon.

1.3 Objectives

Little work has been conducted to characterize potential effects of metals or other contaminants on white sturgeon. In addition, few projects have worked with this species under experimental conditions and little to no information exist outlining effective methods for conducting ELS toxicity tests with white sturgeon. Therefore, a combination of controlled field

and laboratory studies were used to develop methodology for white sturgeon testing and to assess the impact of metals of concern (i.e. copper, cadmium, and zinc) to ELS's.

1.3.1 Objective # 1 – Teck Effluent Toxicity Experiment

An initial experiment was conducted to test currently available exposure methods (Hruska and Dubé, 2004) for their potential to assess Teck Combined Sewer Outfall 3 (CSO III) effluent toxicity to ELS white sturgeon. Teck discharges liquid effluent directly into the UCR from four outfalls. Teck CSO III effluent has been shown to contain the greatest concentrations of metals and, in turn, to be the most toxicologically relevant. Previous studies (Bruno, 2004) have demonstrated 100% mortality in juvenile white sturgeon when exposed to dilutions of 50% or greater of CSO III effluent. Due to the nature of this past study, however, calculations for threshold concentrations at which biological effects occur were not possible. This presented an opportunity to conduct studies of ELS white sturgeon under experimental conditions, gain insight into their biology, while refining the dosing regime of the previous study to gather information to characterize the effects of increasing concentrations of CSO III effluent on ELS white sturgeon.

Testable Null Hypotheses # 1:

H₀₁. There are no statistically significant and concentration dependent differences in hatchability, survival, growth and development of ELS white sturgeon exposed to control water versus dilutions of Teck CSO III liquid effluent.

1.3.2 Objective # 2 – Semi Chronic Metal Toxicity Experiment

The objective of this portion of the research was to utilize the developed methods and technologies and apply them to a definitive study characterizing white sturgeon sensitivity to metals of concern. Specifically, a baseline of toxicity was established for sub-chronic exposure of ELS white sturgeon to Cu, Cd, and Zn that can be used in assessments of the potential risks associated with environmental metal exposure to white sturgeon.

Testable Null Hypotheses # 2:

H₀₁. Sub-chronic exposure to increasing concentrations of selected metals under controlled conditions in the laboratory does not cause effects on hatchability, survival, growth and development of ELS white sturgeon.

CHAPTER 2

2.0 TOXICOLOGICAL ASSESSMENT OF WHITE STURGEON (*ACIPENSER TRANSMONTANUS*) EARLY LIFE STAGES EXPOSED TO LIQUID EFFLUENTS OF THE TECK AMERICAN INCORPORATED METALS LTD. TRAIL FACILITY

2.1 Abstract

White sturgeon (*Acipenser transmontanus*) have been experiencing poor annual recruitment for many decades in the Upper Columbia River (UCR) and a definitive cause for this is not yet known. Teck American Incorporated Metals Ltd. (Teck), a local metallurgical facility in Trail, BC, Canada, has been releasing liquid effluent for the past century and concerns over its toxic effect on white sturgeon and its contribution to the poor recruitment of this species have risen. To date, few experiments have been conducted with sturgeon and the primary purpose of this study was to gather information on white sturgeon biology in order to design an experimental exposure system specific to early life stages, and if possible, to address the potential impact of contaminants in the liquid effluent released by Teck into the UCR on white sturgeon. White sturgeon embryos and 16 day old larvae were exposed to increasing concentrations of Teck liquid effluent in order to characterize potential impacts on embryo hatchability and embryo development, as well as survival and growth of fry and juveniles. An effective experimental design was achieved, and a successful methodology for sturgeon rearing was produced. There were, however, limitations and deficiencies identified that promoted fish mortalities. Greater than 80% of the sturgeon embryos were successfully hatched, and based on the results of this study the effluent of Teck's Trail facility does not appear toxic at the dilutions

studied, and dilution in the river would further reduce the potential toxicity of the effluent to white sturgeon embryos, larvae, and juveniles.

2.2 Introduction

Poor recruitment of white sturgeon (*Acipenser transmontanus*) has been documented since the 1970s in the Upper Columbia River (UCR). There are many possible causes for this phenomenon, including contamination effects. One of the potential contributing factors suggested by some researchers for this life stage specific “gap” in recruitment of white sturgeon populations along the UCR is the release of liquid effluent by Teck American Incorporated Metals Ltd.’s (Teck) local metallurgical facility in Trail, BC, Canada. It has been demonstrated previously that effluent of Teck’s Trail facility is toxic to juvenile white sturgeon (exposed from ~ 12 to ~ 62 d post hatch [dph]) at concentrations of 100% and 50% (Bruno, 2004). However, the dosing regimen selected for these studies (0, 1, 50 and 100% effluent) was such that no dose-response relationships could be identified (0 or 100% mortality). This presented us with an unique opportunity to refine the overall experiment while gaining valuable insight into working with a species not commonly researched. We conducted a toxicity study using a refined experimental setting to help identify the threshold concentration of Teck’s Trail facility effluent at which significant toxicity in embryonic/juvenile sturgeon occurs. Studies were initiated at an earlier life stage compared to that investigated by Bruno (2004), using embryos to test for potential impacts of effluent on early development and hatchability. Both of these factors represent key aspects in early sturgeon development, and potential impacts of Teck effluent on these factors have not been researched to date.

During the experiments, white sturgeon embryos were exposed to increasing dilutions of Combined Sewer Outfall 3 (CSO III) effluent from Teck's Trail facility. This effluent has been shown to contain the greatest concentrations of metals such as Pb, Zn, Cu, and Cd while other effluent outfalls are not considered to be of any toxicological relevance. Exposures began after fertilization of eggs and for 16 dph larvae and continued through approximately 40 days. Exposures were conducted on site at Teck's Trail facility. The primary objectives of this study were: to develop the logistics for future definitive studies by interacting and coordinating efforts with local agencies and organizations involved in sturgeon work on the UCR, to establish a working relationship with the Kootenay Trout Hatchery to obtain sturgeon embryos and larvae, to achieve training in sturgeon early life stage (ELS) handling and culture, to gain information to design and establish an artificial exposure system that allows for experimental testing of white sturgeon ELS's, and, if permitted, evaluate the potential impact of Teck's CSO III effluent on survival and growth of sturgeon larvae.

2.3 Methods and Materials

2.3.1 Experimental Fish

Five day post fertilization embryos and 16 dph larvae were obtained from the Kootenay Trout Hatchery, Fort Steele, BC, Canada. Embryos and larvae were transported on separate occasions to the field based laboratory in Trail, BC, Canada, and were acclimated to temperature and effluent dilutions before being placed directly into their corresponding exposure chambers.

2.3.2 Exposure Design

Toxicity testing was conducted on Teck property at the Trail, BC, facility, in an outdoor experimental setup using modular exposure systems described by Hruska and Dubé (2004). Four polyethylene exposure systems were housed in an outdoor tent and were connected to a flow-through dilution system. One exposure chamber table was used per treatment (control, 1, 5, 25% effluent), each containing eight replicate exposure chambers. On day 0 of the study, embryos were received from the hatchery and 100 individuals allocated to each replicate exposure chamber. Shortly after, however, a facility wide power outage occurred that led to a substantial loss of fish. Consequently, the remaining fish in each treatment were combined into four exposure chambers. 16 dph white sturgeon were obtained from the Kootenay Trout Hatchery, and 100 individuals were reallocated to each of the four remaining replicate chambers per treatment group. As a result, embryos and 16 dph larvae were exposed to 1, 5, and 25% dilutions of Teck CSO III effluent (as well as filtered municipal water as a control). Effluent dilutions were made daily in head tanks and metered into their corresponding exposure tables, providing four full turnovers a day. The target temperature of 15 °C was achieved by placing chillers into the reservoirs of each exposure table. All fish were tested under natural light conditions (~ 18 h light/6 h dark).

Fish were fed live brine shrimp (*Artemia salina*) and frozen bloodworms (Hagen, San Francisco Bay Brand, Edmonton, AB, Canada), *ad libitum*, two to four times, respectively, throughout the day and into the evening. Exposure chambers were cleaned twice a day. Dead

fish were removed, measured, weighed and fixed in 10% buffered formalin. Measurement endpoints included percent hatched, percent survival, and body condition of juveniles.

2.3.3 Biological Parameters

At termination of the study fish were euthanized using tricaine methanesulfonate (MS-222, Sigma-Aldrich, Oakville, ON, Canada) and total body weight and caudal length were determined. Caudal length was defined as the straight line distance from the anterior most point of the head to the middle of the caudal fin. Due to the rapid growth of larvae, length/weight relationships that are typically used for the calculations of condition indices of adult fish do not meet the assumptions for the calculation of the condition indices for fry. Furthermore, the nature of the data set was such that growth parameters for the use in a condition calculation model could not be established. Therefore, evaluation of the health status of sturgeon at the end of the experiment was conducted based on length and weight measures.

2.3.4 Water Chemistry

Basic water quality parameters including, temperature, pH, dissolved oxygen (DO) and conductivity were recorded daily with symphony electrodes (VWR, Mississauga, ON, Canada). Hardness, alkalinity, ammonia, nitrate, nitrite, and chlorine were recorded weekly using LaMotte colorimetric and titrator test kits (Chestertown, MD, USA). Water samples collected for quantification of metals, were filtrated on 0.45 μm polycarbonate filters (Whatman, Clifton, NJ, USA) and acidified to 2% with HNO_3 . Samples were then analyzed by inductively coupled plasma mass spectrometry using an XSeries 2 ICP-MS spectrometer with PlasmaLab software

and collision cell (Thermo fisher Scientific, Mississauga, ON, Canada) following U.S. Environmental Protection Agency (EPA) method ILM05.2D (Creed et al., 1994). Indium was used as the internal standard. Limit of detection (LOD) was calculated using Equation 2.1.

$$[3 * \text{standard deviation of the blank (in count)} + \text{blank (in count)}] / \text{slope of calibration curve.} \quad (2.1)$$

2.3.5 Data Analyses

All data are expressed as the mean \pm 1 standard deviation (SD). Mortalities are expressed as cumulative numbers of dead fish as a function of exposure time. Prior to conducting statistical comparisons data were tested for normality using the Shapiro-Wilks test and tested for homogeneity of variance using the Levene's test. When data were normally distributed or approximated normal distribution, analysis of variance (ANOVA) and a Dunnett's post hoc test were used to detect significant differences between treatment and control groups. In cases where data were not normally distributed, statistical analysis was conducted using the Kruskal Wallis test followed by the Mann-Whitney U test. Comparisons between egg and juvenile groups were made using a Tukey post-hoc test. Statistical significance was accepted when $p < 0.05$. Systat statistical software (Systat Software Inc., Chicago, IL, USA) was used for all analyses.

2.4 Results and Discussion

A modular exposure system was successfully established on site (Teck's Trail facility) that allowed for testing of the potential impacts of different dilutions of Teck's CSO III effluent to ELS white sturgeon. In this study both fertilized eggs and 16 d old sturgeon larvae were

successfully reared in the exposure system. The study setup permitted successful hatching of embryos with hatching rates > 80%. Given the nature of the studies as well as restrictions in time and space, complete effluent exposure experiments were only conducted with larvae and not embryos. Exposure conditions for the embryo experiments were sub-optimal for the reasons stated below, and therefore, these studies were terminated prematurely with no interpretable toxicological data collected.

2.4.1 Study Design Related Findings

Fish exposed to the greatest concentration of effluent (25%) showed greater survival rates than any of the other treatments and the fry gained weight more rapidly. It is hypothesized that this might have been due to the greater amount of nutrients or other essential trace elements available in the effluent. The 25% effluent treatment resulted in greater growth of algae compared to the controls or 1% treatment group. This is likely a consequence of removing components such as algae spores and other compounds by carbon filtering the reference water used in the controls and to dilute the effluent. The greatest treatment group likely contained such components as it received 25% of non-filtered effluent. This hypothesis was confirmed by removing the carbon filtration step for the reference water, which resulted in an increased growth of algae in the other treatment groups. It was decided to remove the carbon filtering process of water from the protocols after water testing insured that only very low amounts of chlorine were present in the tap water that were not expected to have any impact on the fish. This measure improved the survival of larvae in the low and control treatment groups.

There were a series of issues encountered during the initiation phase of the experiment that are typical for these type of studies but that required re-defining of some of the experimental conditions, and that may have consequences for the interpretability of some of the data generated. One of the major issues encountered was the exceptionally high air temperatures in the study area during the conduct of the experiments ($> 40^{\circ}\text{C}$ for extended times). Due to the fact that the studies had to be conducted in a tent outside on a concrete surface under full sunlight it was not always possible to maintain the temperature of $14\text{-}16^{\circ}\text{C}$ preferred by white sturgeon even when using chillers. To cope with the extreme temperature a second set of chillers was ordered and installed in the exposure tables. In addition, two 12,000 BTU air conditioners were installed inside the experiment tent. However, even with these measures it was not possible to maintain the desired temperature, and during the hot times of some days the temperature in the exposure chambers would increase to maximum values of 19°C .

Initial access to water/effluent supply was also an issue. An access tap to the Combined Sewer Outfall 3 (CSO III) prior to the initiation of the exposure studies for the purpose of drawing 24 h composite effluent samples was not available due to mechanical reasons. The timing of this was important as the exposure systems require to be run with the test solution for at least 48 h prior to the addition of organisms. Furthermore, fertilized eggs of the life stage of interest are only available for a limited period of time. As a result, initiation of the exposure studies with fertilized eggs had to be postponed by approximately one week, resulting in the exposure of older embryos. This limited the opportunity to test for possible effects on early embryonic development and hatchability due to reduced exposure time of the embryos.

Electrical failures were a major issue that led to fish mortality. An emergency alarm system in case of power failure prior to the initiation of the studies that would notify us immediately of any black outs was supposed to be installed. Such a system, however, was not installed until several weeks into the studies, for logistical reasons. Due to the lack of such an emergency system, the studies were subjected to several power failures that occurred during the nights, and thus, went unnoticed. As a consequence, the pumps operating the system shut down, and the water temperature increased and the dissolved oxygen levels decreased such that a large percentage of the larvae died. As a result, the initial experiment was prematurely terminated due to the fact that the great mortalities observed as a function of this issue rendered it impossible to detect any treatment-related effects. A fresh batch of slightly older larvae (16 dph) was obtained from the hatchery, and a series of new experiments were initiated. Due to time restrictions, however, these experiments were conducted for an abbreviated time period of 42 d.

2.4.2 Water Quality and Exposure

With the exception of some temperature spikes, water quality parameters were consistent throughout the duration of the experiment and there were no treatment related differences with the exception of ammonium (NH_4^+), which appeared to be slightly greater during some periods when compared to the other treatment groups (Fig. 2.1). Ammonia was greatest in the 25% effluent treatment group which was in accordance with the increased amount of bio-materials such as algae in this treatment group. However, none of the ammonia concentrations measured exceeded water quality protection criteria for aquatic life published for British Columbia (chronic criterion for water temperatures between 15 and 20 °C is between 3 and 4 mg NH_4/L ;

Gov. BC, 2001). NO₃ and NO₂ were not detectable in any of the samples analyzed. pH was within the normal range and concentrations of DO represented very good conditions.

To verify exposure concentrations a total of 25 metals were determined at three time-points during the exposure experiment (at initiation and near termination of the studies). Concentrations of Be, B, and Se were typically less than the method detection limit of the applied analytical methods. Here only concentrations of a selection of elements that were deemed of greatest relevance for this study are reported. No time courses for metal concentrations over the duration of the experiments could be calculated due to the limited number of samples taken. Prior to the initiation of the experiments, a series of filtered and unfiltered supply-water samples were analyzed to evaluate possible contaminations of the control treatment groups. These analyses indicated some contamination of the water with certain metals, especially lead (Fig. 2.2). The pattern of metals present in the control water was typical for that found in the dust of the metallurgical facility. The major problem assumed here is the location of the studies being outside on the smelter property. The key metal of concern was lead, which was present in the controls up to 4.6 µg/L. Due to the nature of the exposure scenario – location at a lead smelter – there was no immediate or effective method to deal with this contamination.

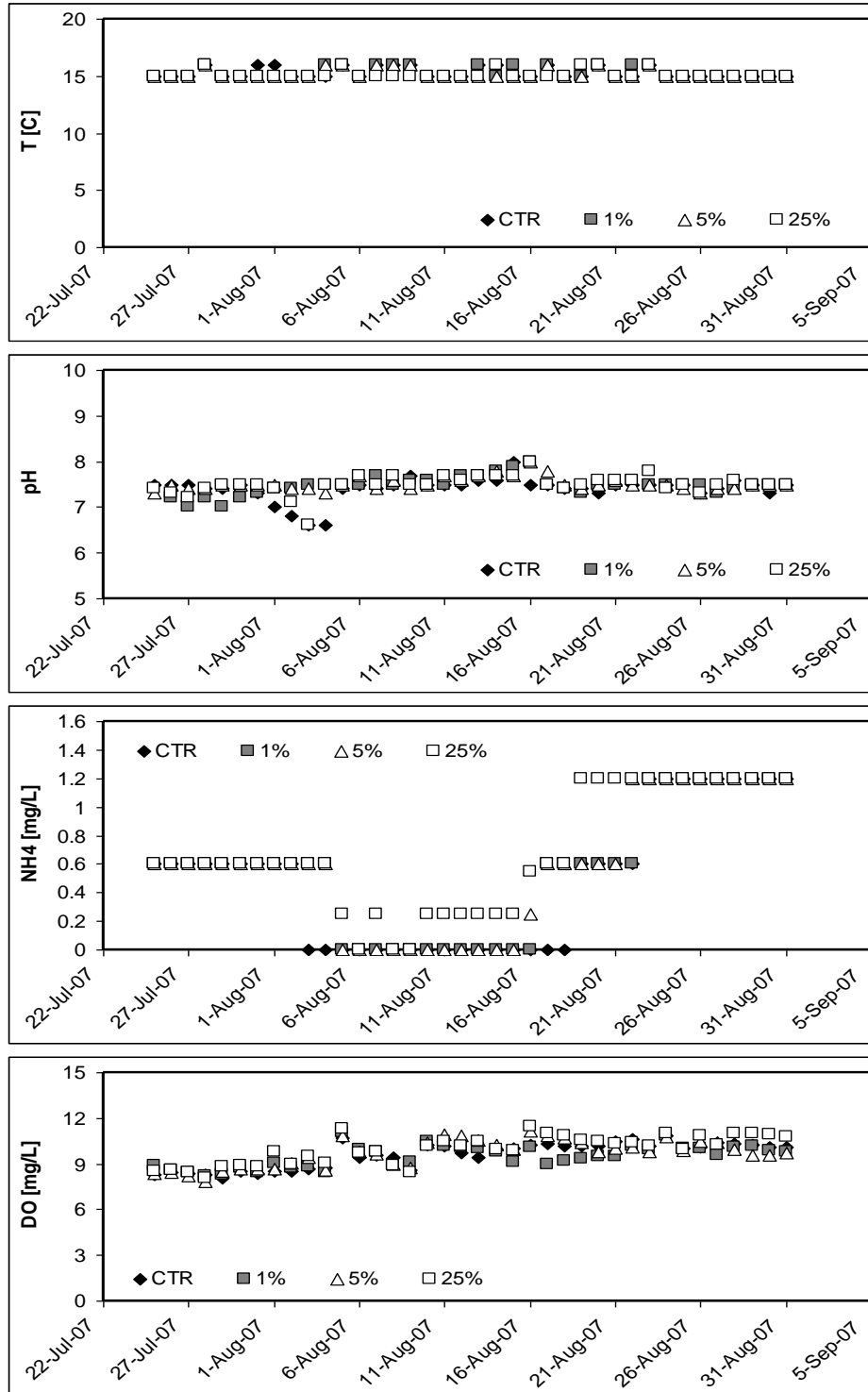


Figure 2.1. Water quality profiles in the controls and different effluent dilution exposure groups.

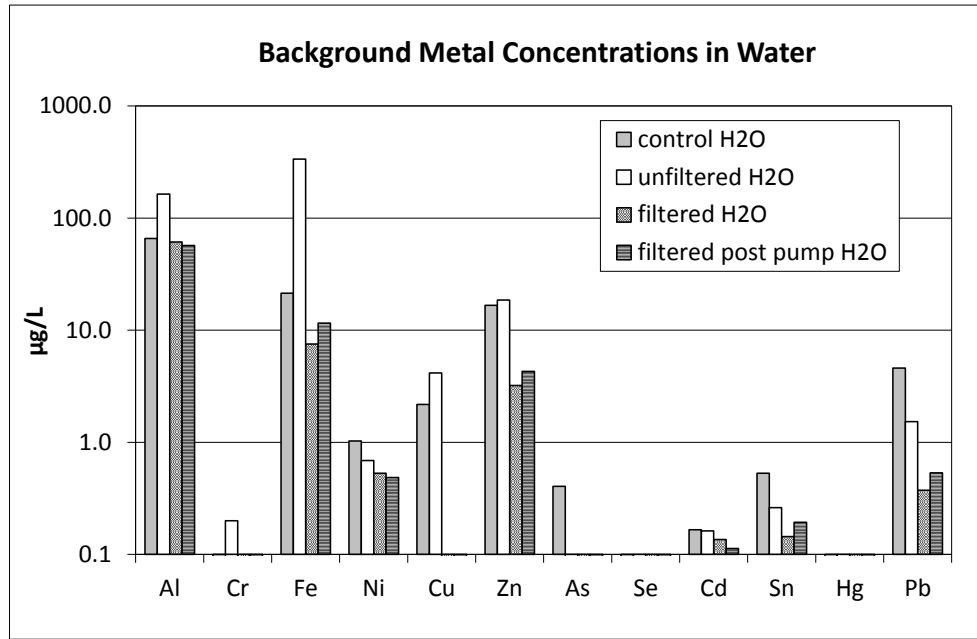
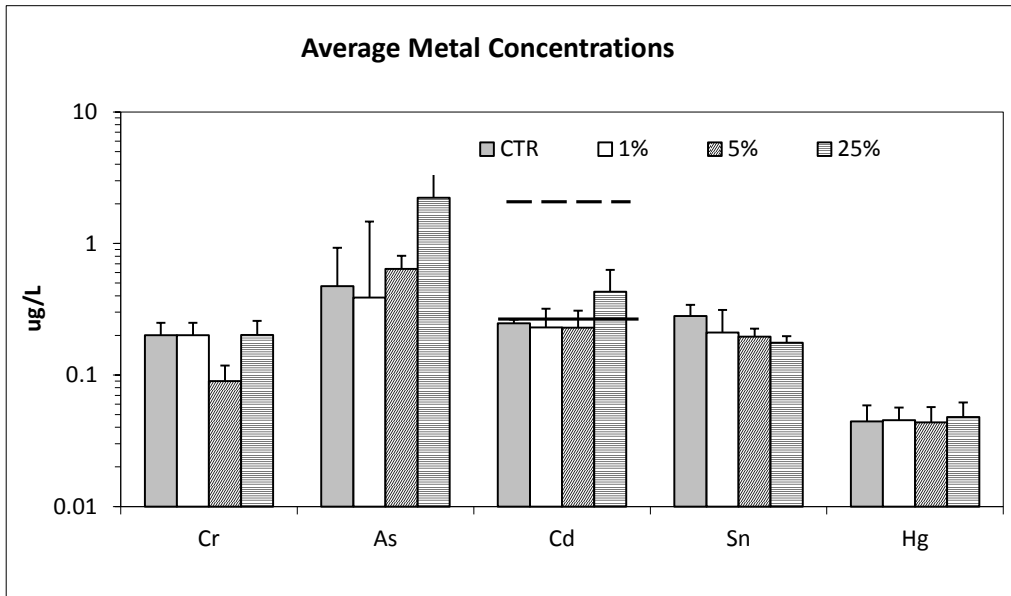


Figure 2.2. Background metal concentrations in water used for the effluent exposure studies at Teck’s Trail facility. Missing bars indicate concentrations < LOD. LOD = limit of detection. control H₂O = control exposure chamber water; unfiltered H₂O = tap water on site; filtered H₂O = water that has been filtered at 0.45 µm to remove metals bound to particles; filtered post pump H₂O = water that has been pumped from storage container into mixing tanks.

There were no significant differences in average metal concentrations among treatment groups (Fig. 2.3). Of the elements reported here, As and Cr were below the LOD in two control and one 5% treatment groups. Concentrations of As and Cd were approximately 2-fold greater in the 25% effluent dilution compared to all other treatment groups. Pb was approximately 2-fold less in the two greatest exposure groups than in the controls and the exposure chambers receiving 1% effluent. None of the elements exceeded the United States Environmental Protection Agency’s (EPA) chronic water quality criteria for metals with the exception of Al, Cd and Pb, and all of the elements analyzed were less than the acute water quality criteria (EPA,

2009). The only element that exceeded the chronic water quality criteria and that could be associated with increasing effluent concentration was Cd. Cd in the 25% effluent treatment group was approximately 2-fold greater than the chronic water quality criterion for this element. It is assumed that the elevated Pb concentrations are due to the contamination with dust particles at the site, and not to water contamination because the two least effluent dilutions had the least Pb concentrations. Average Al concentrations were below the chronic water quality criteria but individual values exceeded these.



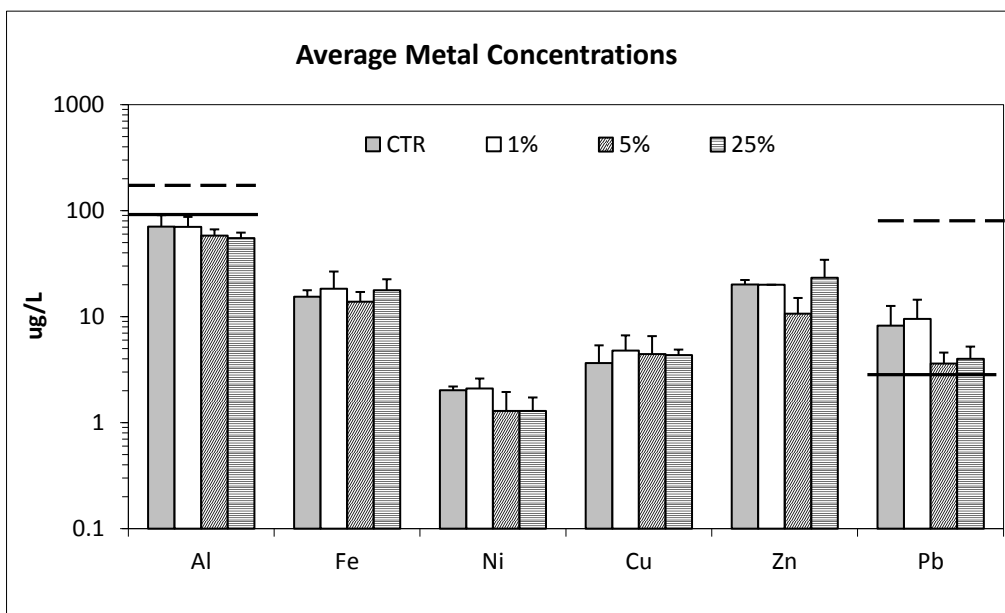
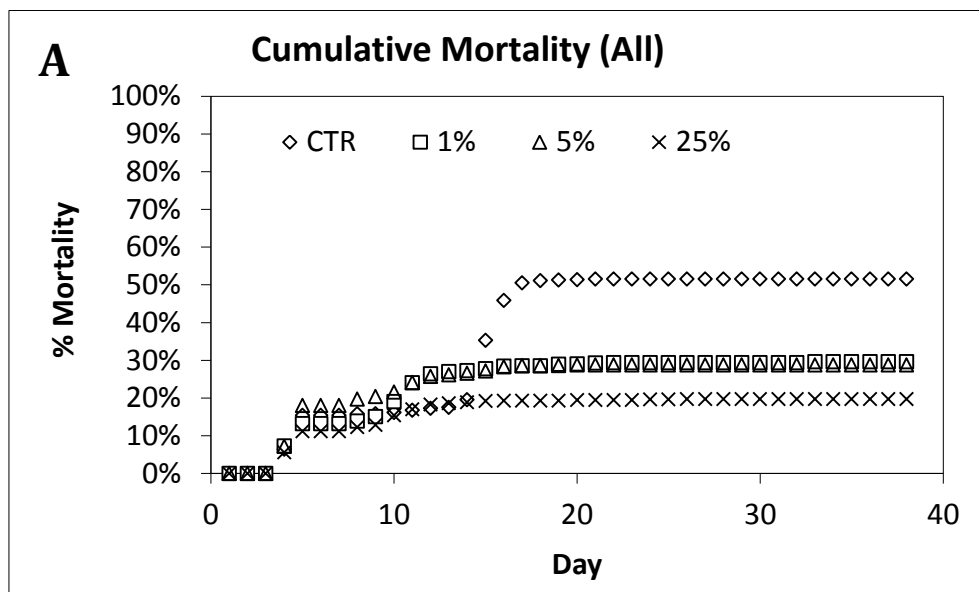


Figure 2.3. Average concentrations of metals in the controls and different effluent dilution exposure groups. Error bars = 1 x standard deviation. Asterisks indicate significant differences from the controls, * p<0.05; ** p<0.01; *** p<0.001. Solid and dashed lines indicate the chronic and acute water criteria, respectively (EPA, 2009). Concentrations that were < the LOD were reported as ½ * LOD.

2.4.3 Biological Responses

As discussed previously (section 2.4.1), fish in the effluent treatment groups gained weight faster and experienced significantly less mortalities than those in the control exposure chambers. These differences in mortalities were apparent up to day 15 with the least death in the greatest effluent exposure group and the greatest mortalities in the controls (Fig. 2.4). At termination of the experiments, mortalities relative to initial numbers of fish were 52%, 30%, 29%, and 20% in the controls, 1%, 5%, and 25% treatments, respectively. After experimental day 15 mortalities did not change among treatment groups. This coincided with the removal of the carbon filter from the fresh water supply and increases in algae growth in the control streams,

indicating that carbon filtering process removed components from the water that are essential for sturgeon early life stages. Furthermore, larvae raised from eggs at the site had greater survival rates than those transferred post hatch (Fig. 2.5). This difference, however, was only statistically significant for the 1% effluent treatment group. It is assumed that this difference might be due to the stress invoked through the transport of hatched larvae to a different environment, while fish raised from eggs did not have to cope with such changes. In addition, the hatchery had some issues regarding the transition of larvae to the artificial food it was required to use due to a change in feed suppliers (R. Ek, Kootenay Trout Hatchery, Fort Steele, BC, Canada). The larvae obtained from the hatchery post hatch were already subjected to this food prior to their transfer to the experimental site where their diet was changed to a live food mixture as recommended by the hatchery. In contrast, larvae that were hatched on site were fed directly with a mixture of live foods.



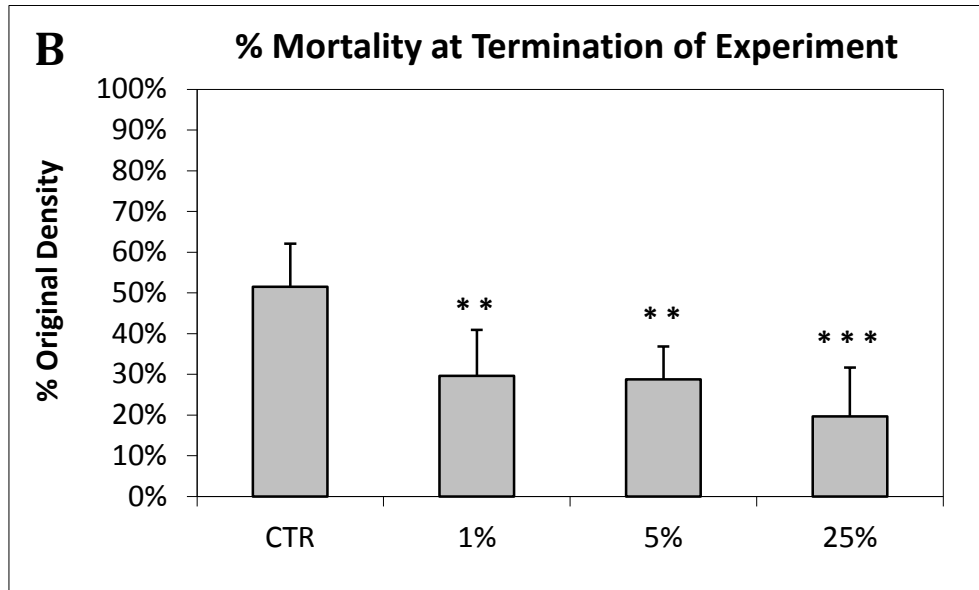


Figure 2.4. Cumulative mortalities of white sturgeon larvae exposed to 1, 5, and 25% effluent, and in the controls over the duration of the experiment (A) and at termination of the studies (B). Asterisks indicate significant differences from the controls, ** $p < 0.01$; *** $p < 0.001$.

Density of fish in the exposure chamber may have also led to fish mortality. Fish were seeded at a density of 100 per chamber. During the initial weeks after the initiation of feeding relative great mortalities were observed. After fish reached average densities of 30 – 60 individuals per experimental replicate unit mortalities rates dropped down significantly, and dead larvae were only observed occasionally. Although densities of fish per treatment group did not exceed ASTM loading rates for fresh water fish (ASTM, 1996) it was deemed in future studies that ELS white sturgeon fall below these guidelines (chapter 5, section 5.3.5.1)

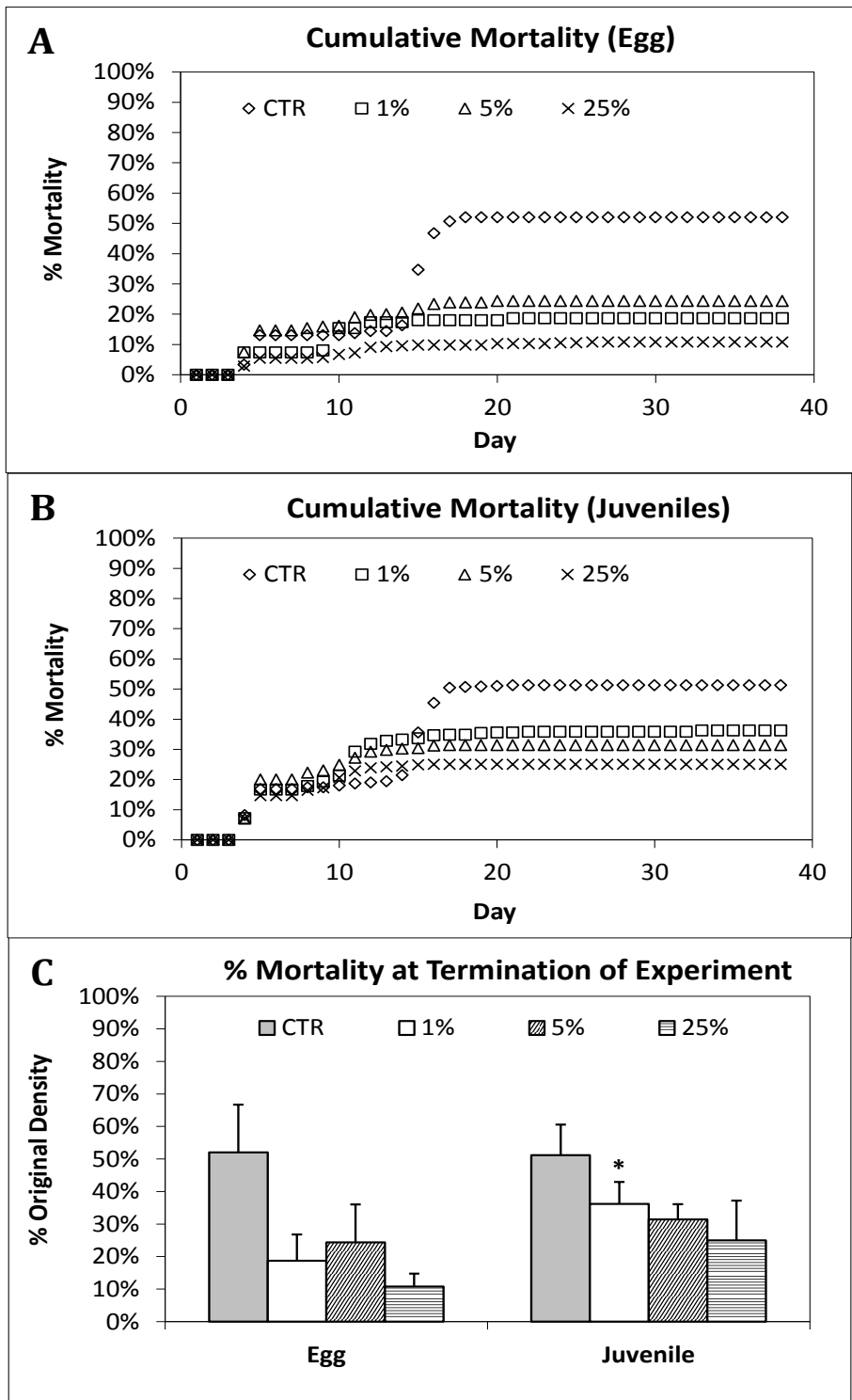


Figure 2.5. Cumulative mortalities of white sturgeon larvae raised from egg at the site (A) and obtained from the hatchery (B) exposed to

1, 5, and 25% effluent, and in the controls over the duration of the experiment (**A**, **B**) and at termination of the studies (**C**). Asterisks indicate significant differences from the larvae hatched at site, * $p < 0.05$.

Although small, there were statistically significant differences in lengths of fry between the effluent treatment groups and the controls at the end of the study with fish in the controls being slightly longer than those in the effluent groups (Fig. 2.6). No differences among treatment groups were observed for weight. The maximum difference in lengths was observed between the controls and the 25% effluent treatment group (6%). It is unclear what the reasons for these differences in length were. However, the magnitude of the observed differences was such that no further biological consequences are expected.

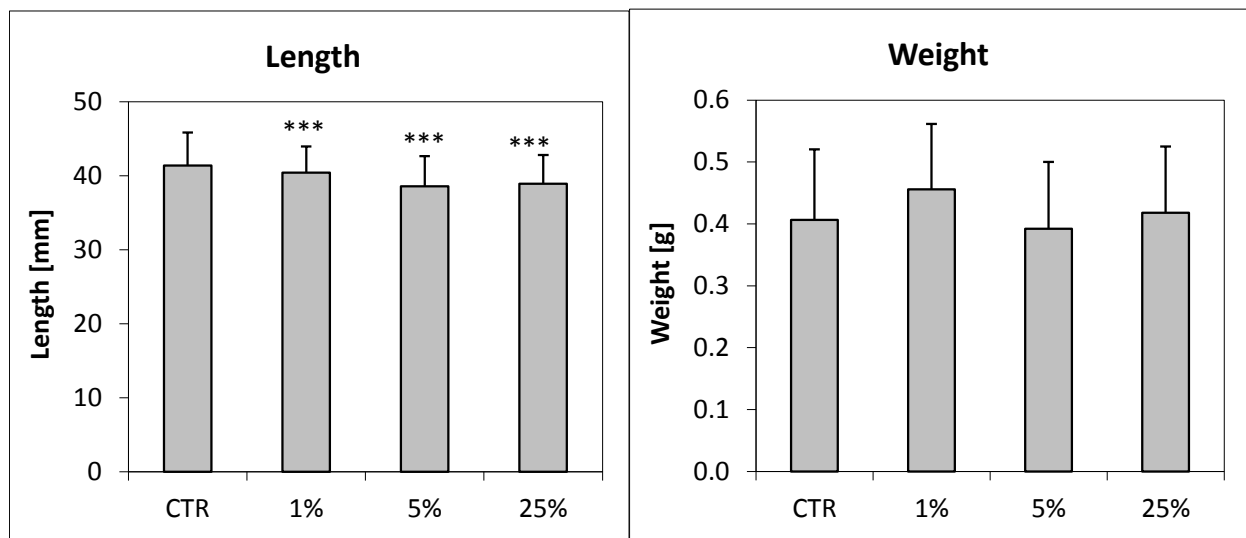


Figure 2.6. Standard length (mm) and weight (g) of white sturgeon larvae exposed to 1%, 5% and 25% effluent, and in the controls. Asterisks indicate significant differences from the controls, ** $p < 0.01$, *** $p < 0.001$.

2.5 Conclusion

The primary purpose of this study was to investigate designs for future development of an exposure system that allows evaluation of the potential impact of contaminants on ELS sturgeon, to gain insight into white sturgeon culturing by way of establishing relationships with experts in the field, and if possible, to evaluate the potential impact of Teck's CSO III effluent on survival and growth of sturgeon larvae. Based on the experience made during the conduct of these studies we successfully established a design for an exposure system (Chapter 3) that was successfully used in future definitive studies (Chapter 5). In addition, we gained valuable insight into white sturgeon culturing that eventually enabled us to successfully rear ELS's under experimental conditions (Chapter 4). As previously discussed, however, this study only provided limited information regarding the toxicological assessment of ELS white sturgeon exposed to Teck's liquid effluent. There were a series of limitations and deficiencies encountered during the initiation of the experiment that may have consequences for the reliability of some of the data generated and caution should be made when interpreting the results. Despite these uncertainties, it does appear from this study that the effluent of Teck's Trail facility is not toxic at the dilutions studied. Furthermore, dilution of the effluent in the UCR would further reduce the potential toxicity to white sturgeon embryos, larvae, and juveniles.

CHAPTER 3

3.0 EFFECTS OF SUB-CHRONIC EXPOSURE OF EARLY LIFE STAGES OF WHITE STURGEON (*ACIPENSER TRANSMONTANUS*) TO COPPER, CADMIUM AND ZINC

3.1 Abstract

Populations of sturgeon (Acipenseridae) are declining in many places in the world because of a number of potential factors, including over-harvesting, habitat alteration and pollution. In North America, populations of the white sturgeon (*Acipenser transmontanus*) have been experiencing poor annual recruitment in major river systems such as the Columbia, Fraser, and Sacramento-San Joaquin, for over three decades. Metal pollution has been hypothesized as a potential contributing factor to the poor recruitment of this species in many of the water bodies. In general, little is known about the chronic toxicity of metals such as copper (Cu), cadmium (Cd) and zinc (Zn) to white sturgeon and their potential influence on survival of embryos and/or juveniles. A study was conducted to establish baseline toxicity data for the sub-chronic exposure of early life stages of white sturgeon to Cu, Cd, and Zn that can be used in assessments of the potential effects of metals to white sturgeon. Embryos, larvae and fry were exposed to increasing concentrations of dissolved Cu, Cd, and Zn for 66 d using laboratory based flow-through exposure systems. Hatching success was greater than 79% for all controls and there were no significant differences observed among treatment groups, or between treatments and controls. Concentrations at which 20% mortality occurred (LC20s) for Cd (1.5 µg/L), Cu (5.5 µg/L), and Zn (112 µg/L) during chronic exposures obtained for white sturgeon in the present study were comparable to sensitive salmonid species. Based on LC50 and LC20 values for 19

and 58 d post-hatch white sturgeon, the United States national ambient water quality criteria and the Canadian water quality guidelines for the protection of aquatic life that have been established for Cd, Cu, and Zn are protective of white sturgeon early life stages.

3.2 Introduction

Sturgeon (Acipenseridae) populations are threatened throughout the world and have been decreasing over the past century in Northern Europe, Asia and North America (Birstein, 1993; Coutant, 2004; Gisbert and Williot, 2002). Factors that are hypothesized to contribute to this decline are over-harvesting, habitat alteration and contamination (Birstein, 1993; Gisbert and Williot, 2002; Hu et al., 2009; Irvine et al., 2007; Luk'yanenko et al., 1999; Paragamian and Hansen, 2008). In North America, populations of white sturgeon (*Acipenser transmontanus*) have been reported to be declining in the north-western USA and British Columbia, Canada. Populations of white sturgeon have been listed as endangered in parts of Canada (COSEWIC, 2003) and in the USA (U.S. Fish and Wildlife Service, 2011). Decreases in sturgeon populations in the Columbia, Fraser, and Sacramento-San Joaquin rivers and their tributaries have been attributed primarily to poor annual recruitment (Coutant, 2004; NRWSRI, 2004; Scott and Crossman, 1973, 1998; UCWSRI, 2002). Some simulation models have predicted that without implementation of successful remedial efforts the white sturgeon will become virtually extinct in these rivers within fifty years (Irvine et al., 2007; Paragamian et al., 2005; Paragamian and Hansen, 2008; UCWSRI, 2002).

Several hypotheses for recruitment failures of white sturgeon have been suggested. These include habitat alteration, varying flow regime, decreased water quality (temperature,

turbidity, total dissolved gases), poor nutrition, genetic bottlenecks or inbreeding depression, predation by introduced species such as walleye (*Sander vitreus*), inter-specific competition, pathogens, and pollution (Coutant, 2004; Kruse and Scarnecchia, 2002a; Kruse and Webb, 2006; UCWSRI, 2002). A limited number of studies have examined the effects of pollutants on white sturgeon and there is a general consensus that further investigations are needed to assess the relative sensitivity of this species to environmental contaminants (Foster et al., 2001a,b; Gundersen et al., 2008; Kruse and Scarnecchia, 2002a,b; Kruse and Webb, 2006; UCWSRI, 2002; Webb et al., 2006). To date, most studies involving sturgeon have been conducted with acute exposures and no studies of the effects of contaminants have been conducted with early life stages (ELS's) from egg through juvenile. Fish are considered to be most sensitive to the exposure with contaminants such as metals during this period of development (Hutchinson et al., 1998), and chronic studies of the effects of contaminants on ELS's are needed to assess sturgeon sensitivity.

The potential for exposure to metals and other contaminants in the Columbia River is of concern due to past and present activities of mines, metallurgical facilities, pulp and paper mills, as well as other industrial and municipal sources (UCR WPRIFS, 2008). Aquatic ecological risk assessment of metals is affected by the fact that toxicity largely depends on each organism's ability to regulate and/or store the metal, as well as the toxicokinetics and toxicodynamics of specific metals in particular environments (Fairbrother et al., 2007; McGeer et al., 2000). In general, little is known about the potential toxicity of metals such as copper (Cu), cadmium (Cd) and zinc (Zn) to white sturgeon, or the tolerance of white sturgeon in comparison to other fish species. Previous studies have shown that some standard test species, such as rainbow trout

(*Oncorhynchus mykiss*), are relatively sensitive to certain metals, whereas others, such as fathead minnows (*Pimephales promelas*), are more tolerant (Besser et al., 2007; Taylor et al., 2000). Species-specific chronic dose-response relationships are needed to establish metal toxicity threshold values for white sturgeon that then can be used in environmental risk assessments. Therefore, the objective of the present study was to determine the chronic toxicity of Cu, Cd, and Zn to white sturgeon under laboratory conditions. Embryos and ELS white sturgeon were exposed for 66 d to increasing concentrations of dissolved Cu, Cd, and Zn that bracketed respective concentrations observed in the Columbia River.

3.3 Methods and Materials

3.3.1 Experimental Fish

Fertilized white sturgeon eggs were obtained from the Kootenay Trout Hatchery, Fort Steele, BC, Canada. Eggs were collected from four breeding pairs of adult white sturgeon caught in the Columbia River near Waneta, BC, Canada. Fertilization of the eggs was harmonized in the hatchery by injecting adult sturgeon with a gonadotropin analog (LHRH analog, Syndel International Inc, Qualicum Beach, BC, Canada) on two subsequent days. Embryos were transported in oxygenated bags and received at the exposure facility within 6 h of fertilization. Embryos were gradually acclimated to their solution waters for 1 h before being randomly assigned to McDonald-type hatching jars (Aquatic Ecosystems, Apopka, FL, USA). Embryos were incubated under a low flow velocity (~ 3 L/min) for 72 h until neurulation occurred. After 72 h, the flow velocity was increased to gently agitate the embryos and prevent the development of fungus. Hatching began approximately 5-7 d post fertilization. Towards the

end of the hatching period all remaining eggs and yolk sac larvae from each replicate were enumerated and separated into two experimental chambers.

3.3.2 Test chemicals

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

3.3.3 Exposure Design

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

The exposure design consisted of a continuous flow-through system constructed to provide 17 treatments: five concentrations of Cu, Cd, and Zn, plus two references containing only laboratory water and ROW (1:1 ratio). Nominal exposure concentrations (Table 3.1) were prepared separately in individual 1000 L holding tanks, and in turn were metered into

corresponding 100 L reservoirs. Each treatment was split into two true replicates (two reservoirs), which in turn were fed into two replicate exposure chambers, for a total of four replicate exposure chambers per metal concentration. Exposure chambers contained 40 L of exposure water with 10 L/h exposure water replacement, providing four volume replacements per day.

Table 3.1. Nominal, mean \pm standard deviation (SD; numbers in brackets) measured exposure concentrations and gram-atomic weight concentrations (GAW) for Cu, Cd and Zn during the 66 d flow-through exposure experiments with white sturgeon (*A. transmontanus*).

Treatment	Cu concentration			Cd concentration			Zn concentration		
	Nominal ($\mu\text{g/L}$)	Measured ($\mu\text{g/L}$)	GAW (mol/L)	Nominal ($\mu\text{g/L}$)	Measured ($\mu\text{g/L}$)	GAW (mol/L)	Nominal ($\mu\text{g/L}$)	Measured ($\mu\text{g/L}$)	GAW (mol/L)
Reference	0	0.6 (\pm 0.1)	9.4E-9	0	0.05 (\pm 0.02)	4.4E-10	0	7.2 (\pm 1.8)	1.1E-7
1	0.1	0.7 (\pm 0.1)	1.1E-8	0.02	0.06 (\pm 0.01)	5.3E-10	1	5.8 (\pm 0.6)	8.9E-8
2	0.6	1.8 (\pm 0.4)	2.8E-8	0.16	0.17 (\pm 0.02)	1.5E-9	6	9.3 (\pm 2.9)	1.4E-7
3	3.6	5.9 (\pm 0.1)	9.3E-8	1.28	1.1 (\pm 0.02)	9.8E-9	36	34 (\pm 3.0)	5.2E-7
4	21.6	36 (\pm 1.4)	5.7E-7	10.24	8.3 (\pm 0.11)	7.4E-8	216	198 (\pm 5.3)	3.0E-6
5	129.6	217 (\pm 42.0)	3.4E-6	81.92	69 (\pm 2.02)	6.1E-7	1296	1214 (\pm 39.0)	1.9E-5

At day 0 of the study, each true replicate system for every metal concentration contained an egg hatching jar with 800 embryos, loaded within 8 h of fertilization to maximize exposure during embryo development. Post hatch, larvae were divided into the corresponding two replicate chambers for each treatment, 10 individuals at a time to provide a random distribution. The exposure period consisted of 66 d and at 38 d post hatch (dph) the fish were culled to 115 individuals per exposure chamber to ensure that the loading density was less than 0.5 g fish/L per American Society for Testing and Materials (ASTM, 2005). Fish that were removed from

chambers were euthanized using tricaine methanesulfonate (MS-222, Sigma-Aldrich, Oakville, ON, Canada), measured, weighed, and then fixed in 10% buffered formalin (Sigma-Aldrich, Oakville, ON, Canada). At 7 dph, food was introduced to the chambers to familiarize the larvae with a food scent. Fish were fed a combination of live brine shrimp (*Artemia salina*) and frozen bloodworms (Hagen, San Francisco Bay Brand, Edmonton, AB, Canada), *ad libitum* four to eight times throughout the day and into the evening. Feeding rates were increased when larvae were transitioning to feeding since this has been shown to be a critical period for survival (Conte et al. 1988). Exposure tanks were cleaned twice a day. Dead fish, if any, were removed, measured, weighed and fixed in 10% buffered formalin. Measurement endpoints included percent hatched, percent survival, body condition (mass/length), and morphology of larvae and juveniles. Although not used as an assessment endpoint, behaviour (mobility, swimming performance) was also observed and noted during times of cleaning and feeding.

3.3.4 Water chemistry

Basic water quality parameters (temperature, pH, dissolved oxygen and conductivity) were recorded daily with symphony electrodes (VWR, Mississauga, ON, Canada, Cat no. 11388-328). Hardness, alkalinity, ammonia, nitrate, nitrite, chlorine, sulfate, sulfide, and phosphate were recorded weekly using LaMotte colorimetric and titrator test kits (Chestertown, MD, USA); except during the first two wk post hatch when measurements were performed twice a week while the fish were transitioning from yolk sac to free feeding. Water samples were collected weekly from each chamber by use of acid-cleaned polyethylene bottles, filtered through a 0.45 µm polycarbonate filter (Whatman Inc. Florham Park, NJ, USA, Cat no. 26592) using nalgene[®]

filter holders and receivers and acidified with ultrapure nitric acid to pH < 2. Metal analyses were performed weekly by inductively coupled plasma mass spectrometry (ICP-MS) following U.S. Environmental Protection Agency (EPA) method ILM05.2D (Creed et al., 1994). Cation and anion analyses were performed weekly based on EPA method 300.1 by Dionex ICS-3000 dual Ion Chromatography system (Dionex, Sunnyvale, CA, USA) equipped with dual pump, eluent generation, dual ion exchange column, dual conductivity detector, dual cation/anion self regenerating suppressor and X-Y autosampler. The software Chromeleon 6.80 (Dionex, Sunnyvale, CA, USA) was used to simultaneously quantify cations and anions. DOC analysis was performed weekly using a TOC analyzer (TOC-5050A, Shimadzu, Mandel Scientific, Guelph, ON, Canada).

3.3.5 Data analyses

3.3.5.1 Mortality Data

Mortality in the present study was dependent upon initial seeding density. This was determined based upon results from a concurrent field experiment conducted in 2008 (Tompsett et al., University of Saskatchewan, Saskatoon, SK, Canada) and reference data from the present laboratory experiment. A least squares linear regression was performed with initial seeding density as the independent variable and total number of fish that died during the experiment as the dependent variable. The relationship between these two parameters was statistically significant ($R^2=0.985$; $p<0.001$). This function (Equation 3.1) was then applied to all treatments at 58 dph in the present experiment to account for seeding density mortality.

$$Y = 0.9138x - 68.97 \quad (3.1)$$

Prior to calculation of lethal concentrations (LCs) from bioassays, it is customary to correct for control, or natural, mortality in order to minimize biased estimations (EPA, 2002; Hoekstra, 1987; Morgan, 1992). Accordingly, all data were normalized for reference mortality using the Abbott's formula (Equation 3.2; Abbott, 1925).

$$\% \text{ Corrected Mortality} = 1 - [(\% \text{ Survival Reference} - \% \text{ Survival Treatment}) / \% \text{ Survival Reference} \times 100] \quad (3.2)$$

Effective concentrations at which 50% and 20% mortality occurred (LC50 and LC20) at critical time periods (19 and 58 dph) were compared to assess potential confounding of metal induced mortalities by seeding densities. In addition, multivariate analysis was performed using the statistical program R (CRAN, TU Wien, Austria) to investigate the interactions between chemical concentration and seeding density on fish mortality, in relation to the critical time periods during white sturgeon ELS development.

3.3.5.2 General Data Analysis

All data were expressed as the mean \pm 1 standard deviation (SD). Fish mortality was analyzed by comparing the proportion of fish dead in each of the four exposure chambers of a given metal concentration to that of the reference. Percent mortality was adjusted for fish that were removed during culling. Hatchability was analyzed by comparing percent of hatched eggs per egg-hatching jar. Prior to conducting statistical comparisons data were tested for normality using the Shapiro-Wilks test and tested for homogeneity of variance using the Levene's test. When data were normally distributed or approximated normal distribution, analysis of variance

(ANOVA) and a Dunnett's post hoc test were used to detect significant differences between treatment and reference groups. In cases where data were not normally distributed, statistical analysis was conducted using the Kruskal Wallis test followed by the Mann–Whitney U test. Systat statistical software (Systat Software Inc., Chicago, IL, USA) was used for these analyses. LC50 and LC20s were estimated using TOXSTAT[®] software (Western EcoSystems Technology, 1996). A body condition index was calculated for each surviving fish at the termination of the experiment (Equation 3.3). Differences in body conditions between treatment groups and references were analyzed using the same statistical methods as those for mortalities.

$$\text{Condition Factor} = [(body\ weight\ (g) / length\ (mm)^3)] \times 100,000 \quad (3.3)$$

Statistical significance was accepted when $p < 0.05$.

3.4 Results

3.4.1 Exposure Verification

Measured concentrations of all target metals were comparable to nominal concentrations (Table 3.1). However, measured concentrations of Cu, Cd, and Zn in the references were greater than zero, and the actual concentrations measured in the test vessels were slightly greater than the target nominal concentrations in the two lowest doses of each metal (Table 3.1). As a consequence, the control is considered and referred to as the reference exposure for each metal. Concentrations within the exposure chambers remained constant throughout the exposure period. All calculations and reported values pertaining to metal concentrations are based on measured

concentrations. In addition, gram-atomic weight concentrations are presented in order to compare relative potencies of Cu, Cd, and Zn (Table 3.1).

3.4.2 Water Quality

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

3.4.3 Hatchability

No significant differences ($p > 0.05$) in hatching success were observed among treatment groups or between any of the treatments and the reference exposure ($> 79\%$ hatchability). All exposure chambers, including the reference group, contained a few deformed fish (between 1 to 3% of the original seeding density per exposure chamber) that exhibited kyphosis; an axial defect characterized by ventral spinal curvature around the yolk sac. These deformities were randomly distributed and could in part be due to transportation of the embryos from the hatchery to the laboratory via air. All deformed fish were identified, removed and euthanized within the first week post hatch.

3.4.4 Mortality and Body Condition

3.4.4.1 Reference exposure (control) and seeding density mortality

There was relatively great overall mortality within the reference group during the transition to feeding stage. It was later determined that this mortality was largely dependent upon seeding density (see section 3.3.5.1; Tompsett et al., University of Saskatchewan, Saskatoon, SK, Canada). Between 21 and 34 dph, when the fish were transitioning from yolksac to exogenous feed, 43% mortality occurred (of the total number of fish seeded in the reference group; Fig. 3.1). When the mortality for the reference group and treatment groups is stratified into four life stages (yolksac, swim up, transition to feed and juvenile), the mortality in the transition to exogenous feeding stage was near equivalent among treatments (Fig. 3.2). The greatest remaining concentration of Cu (5.9 µg/L), however, did not result in greater mortality during the transition life stage but this can be attributed to the fact that a large proportion of fry from this treatment group ($28\% \pm 3.2$) died during the previous swim up life stage, leaving a relatively small number of fish in this group. In addition, the greatest remaining concentration of Cd appears to have slightly delayed mortality, with the greatest percentage of deaths in the following juvenile life stage. Furthermore, a significant positive relationship between seeding density and mortality was observed for all groups regardless of treatment during the transition of larvae to exogenous feeding (Fig. 3.3). When compared to other life stages, no significant relationships exist in the yolksac and swim up stages. A significant positive relationship between seeding density and mortality was observed during the juvenile life stage, but this is driven by Cd 8.3 µg/L treatment which exhibited delayed mortality effects, as previously mentioned.

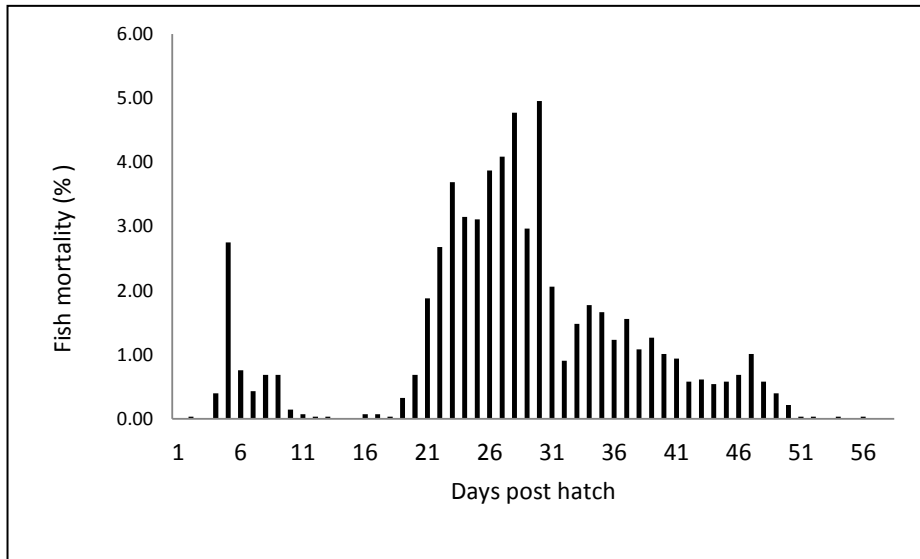
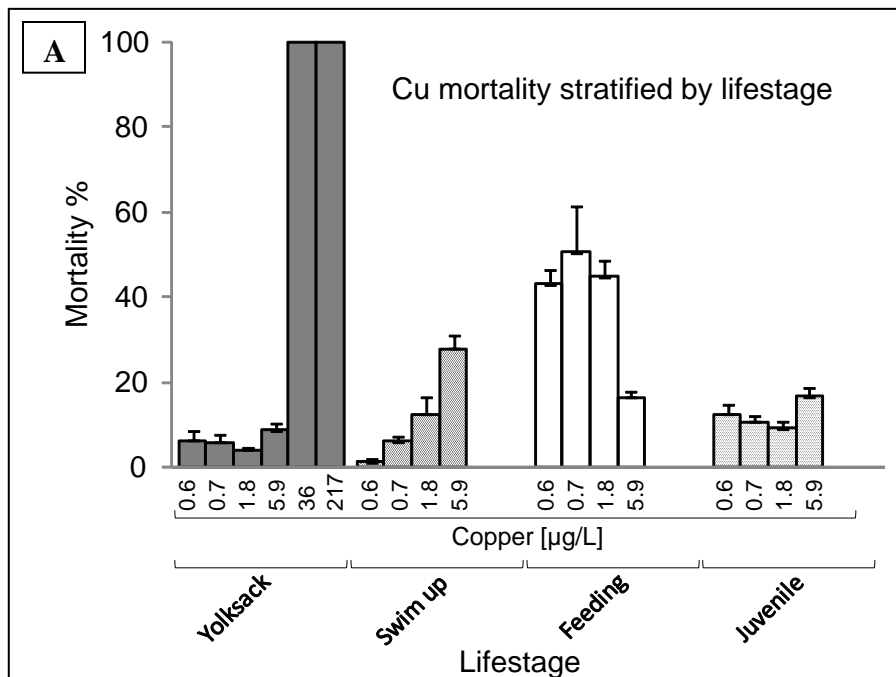


Figure 3.1. Mean reference group mortality throughout the exposure period. Expressed as average % mortality of fish seeded in reference group per day.



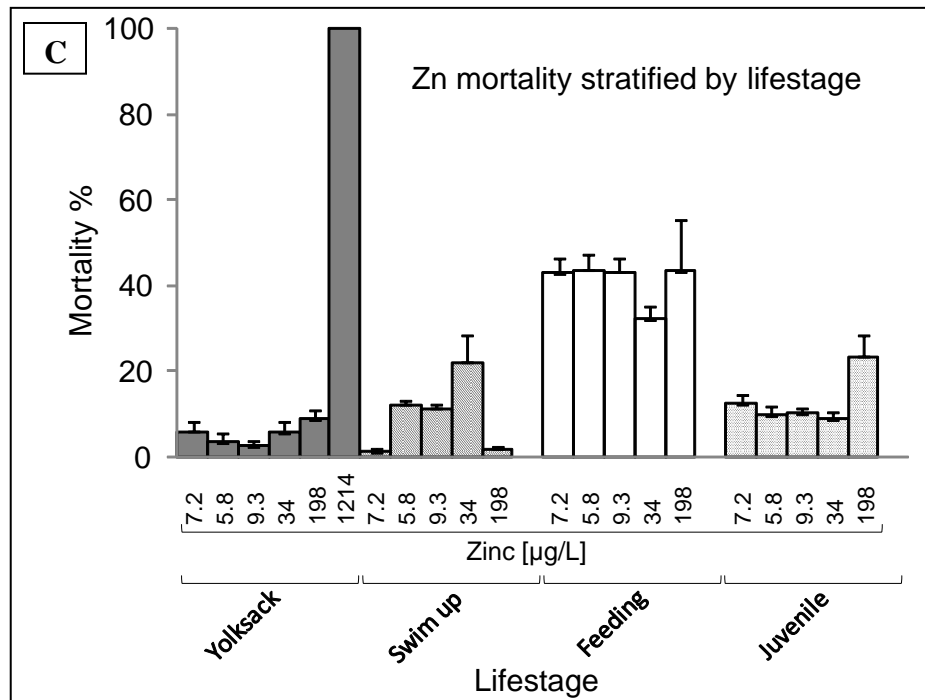
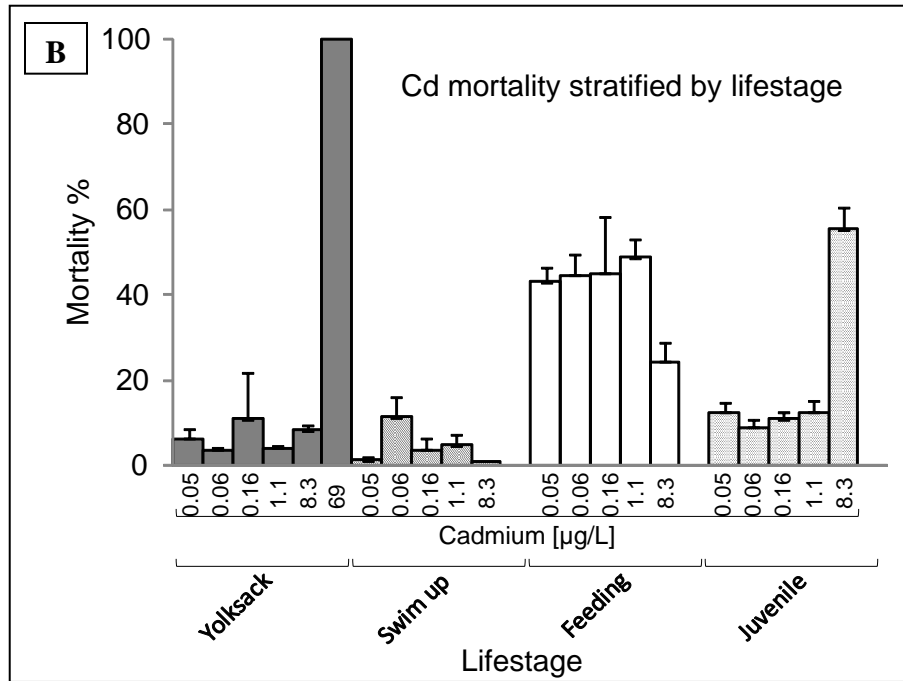


Figure 3.2. Mean mortality stratified into four lifestages (yolksack, swim up, transition to feeding and juvenile) for Cu (A), Cd (B) and Z

(C). Mortality bars for each lifestage represent the different concentrations of the individual metals, with reference group on the left and increasing concentrations to the right. Lifestages after yolk sac contain some mortality bars with 0 mortality because all of the fish in those particular concentrations had already died.

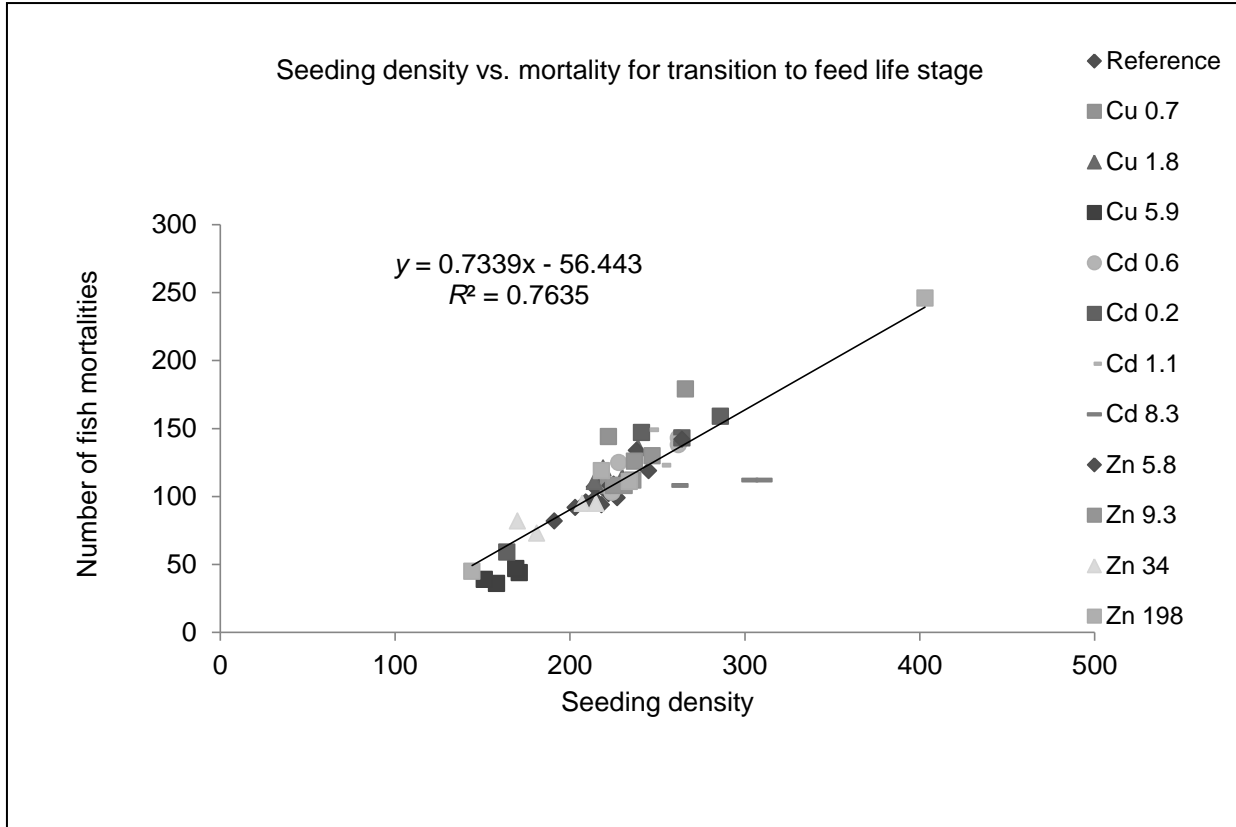


Figure 3.3. Seeding density regression for all metal treatments and reference group. Linear regression comparing seeding density at the beginning of the transition to feed life-stage to the number of fish surviving at the end of the life-stage. Numbers associated with the individual metals in the legend represent concentrations ($\mu\text{g/L}$).

The overall mean mortality of the reference group was 63%, and when seeding density mortality was accounted for the overall mean mortality was adjusted to 4% (Table 3.2). There were no noticeable changes in physical appearance or behaviour among the reference group fish during the exposure period.

Table 3.2. Mean \pm standard deviation (SD; numbers in brackets) percent mortality of early life stage white sturgeon (*A. transmontanus*) through 66 d of sub-chronic exposure to Cu, Cd, and Zn. Asterisk indicates significant mortality relative to reference group (Dunnett’s test; $p < 0.05$ *, $p < 0.01$ **, $p < 0.001$ ***). Averages and SDs are based on twelve and four replicate chambers per treatment for the reference and metal exposure groups, respectively.

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

3.4.4.1.1 Multivariate analysis

Concentration of metals and seeding density influenced fish mortality to varying degrees during the different ELS’s of white sturgeon development. For each metal, significant mortalities were observed early in the exposure at the greatest concentration, while significant positive relationships between seeding density and mortality were observed during the transition of larvae to exogenous feeding. Results of multivariate analyses indicated significant interactions between concentrations of metals and seeding density on fish mortality, during critical life stages (Table 3.3). Concentration of metals significantly ($p < 0.001$) influenced fish mortality during the yolk sac life stage for all metals; while seeding density affected Cu mortality

during this life stage ($p < 0.01$), but to a lesser degree. In the swim up life stage, concentration of metal influenced mortality for Cu and Cd ($p < 0.001$, $p < 0.01$, respectively), but not Zn. There was also no interaction between main effects of Zn. Seeding density significantly ($p < 0.001$) influenced fish mortality during the transition to feeding life stage for Cu and Zn, while for Cd there was a significant interaction ($p < 0.01$) between seeding density and chemical concentration that influenced fish mortality. In the juvenile life stage, results varied from no significant influences for Cu, a slight significant interaction between seeding density and chemical concentration on fish mortality for Cd ($p < 0.05$), and significant influences of chemical concentration ($p < 0.001$) and seeding density ($p < 0.05$) on Zn fish mortality.

Table 3.3. Multivariate analyses between seeding density, metal concentration and mortality during the yolksac, swim up, transition to feed and juvenile life stages. Asterisk indicates significant mortality relative to reference group ($p = 0.06$ #; $p < 0.05$ *; $p < 0.01$ **; $p < 0.001$ ***). SD = Seeding Density; Conc. = Metal Concentration; SD x Conc. = Combinatory Effect.

Metal	Life stage											
	Yolksac			Swim up			Transition to feed			Juvenile		
	SD	Conc.	SD x Conc.	SD	Conc.	SD x Conc.	SD	Conc.	SD x Conc.	SD	Conc.	SD x Conc.
Cu	**	***			***		***				#	
Cd		***			**			**	**			*
Zn		***					***			*	***	

3.4.4.2 Copper

Exposure of early white sturgeon life stages to Cu resulted in concentration dependent mortalities. Statistically significant greater mortalities relative to the reference group occurred at the two greatest concentrations of Cu (Table 3.2). Statistically significant increases in mortalities (100% mortality; $p < 0.001$) relative to the reference group occurred within the first 14 dph at the two greatest concentrations of Cu. Surviving juvenile white sturgeon in all of the

Cu exposures had no significant differences ($p > 0.05$) in body condition index at termination when compared to the reference group. There were no changes in physical appearance or behaviour in any of the Cu exposures.

3.4.4.3 Cadmium

Exposure of early white sturgeon life stages to Cd resulted in concentration dependent mortalities. Statistically significant greater mortalities relative to the reference group occurred at the two greatest concentrations of Cd (Table 3.2). Results from one of the 8.3 $\mu\text{g Cd/L}$ replicates were excluded due to loss of fish (72% of the original number seeded) through equipment failure. Statistically significant increases in mortalities (100% mortality; $p < 0.001$) relative to the reference group occurred within the first 14 dph at the greatest exposure concentration of Cd. Exposure to 8.3 $\mu\text{g Cd/L}$ caused 65% mortality ($p < 0.01$) over the duration of the experiment with most mortalities observed later (33 to 43 dph) in the experiment (juvenile stage). Body condition indices of surviving juvenile white sturgeon exposed to 8.3 $\mu\text{g Cd/L}$, the greatest Cd exposure with surviving fish at termination, were significantly less ($p < 0.001$) at termination than were those in the reference group. Most fish in this treatment lost their pigmentation and became almost translucent by the end of the exposure period. In addition, they were lethargic and appeared to eat less throughout the exposure.

3.4.4.4 Zinc

Exposure of early white sturgeon life stages to Zn resulted in concentration dependent mortalities. Statistically significant greater mortalities relative to the reference group occurred at

the two greatest concentrations of Zn (Table 3.2). One hundred percent mortality occurred within the first 14 dph at the greatest exposure concentration of Zn. Exposure to 198 µg Zn/L caused 40% mortality ($p < 0.01$). Surviving juvenile white sturgeon exposed to 198 µg Zn/L, the greatest Zn exposure with surviving fish at termination, had significantly reduced ($p < 0.001$) body condition indexes at termination when compared to the reference group. Changes in physical appearance and behaviour were not noted in any of the Zn exposures.

3.4.5 Effective concentrations

Significant differences in effective concentrations were observed among development periods (19 and 58 dph) for Cd, but not Cu and Zn exposures (Table 3.4). LC50 and LC20 values for Cu exposure at 19 dph (9.9 µg/L and 3.4 µg/L, respectively) and 58 dph (12.4 µg/L and 5.5 µg/L, respectively) were not significantly different. LC50 and LC20 values for Cd exposure at 19 dph (21.4 µg/L and 8.7 µg/L, respectively) were greater when compared to 58 dph (5.6 µg/L and 1.5 µg/L, respectively). LC50 and LC20 values for Zn exposure at 19 dph (340 µg/L and 102 µg/L, respectively) were comparable when compared to 58 dph (250 µg/L and 112 µg/L, respectively). In addition, LC50 and LC20 values for Cu, Cd, and Zn were expressed as gram-atomic weight concentrations to compare the relative potencies of each metal to ELS's of white sturgeon (Table 3.4). At 19 dph Cu and Cd appear to be similar in potency whereas Zn appears to be 20-30-fold less potent. At 58 dph Cd appears to be approximately 5-fold more potent than Cu and approximately 75-130-fold more potent than Zn, while Cu appears to be 19-fold more potent than Zn.

Table 3.4. Lethal Concentrations for Cu, Cd, and Zn at which 20 (LC20) and 50% (LC50) of white sturgeon early life stages died prior (19 dph) to transition to feed at the end of the exposure experiment (58 dph). Mortality was adjusted for seeding density mortality for the 58 dph LC calculations. LC's are presented as measured concentrations and gram-atomic weight concentrations (GAW). 95% confidence intervals are presented in brackets.

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

^a CCC refers to the Criteria Continuous Concentration for fresh water species adjusted to the average hardness of 70 mg CaCO₃/L observed during the experiments (EPA, 2009).

^b Site specific guidelines - Freshwater criteria calculated using the Biotic Ligand Model (BLM, 2007).

3.5 Discussion

Sub-chronic laboratory toxicity data were generated to characterize sturgeon sensitivity to selected metals. Toxicity threshold values for Cu, Cd, and Zn were developed for ELS white sturgeon. A limited number of studies have worked with ELS white sturgeon and it was demonstrated that this species was amenable for use in chronic toxicity tests. There was, however, a relatively great percentage of fish that died during transition of larvae to exogenous feeding. As a consequence the reference exposure mortality was greater than would be expected when compared to a toxicity test conducted with a standard test organism (ASTM, 2005). Relatively great larval mortality at transition to exogenous feeding is likely to be an inherent

characteristic of white sturgeon biology, rendering it necessary to develop independent standard ASTM and EPA guidelines for white sturgeon toxicity tests.

Mortality observed in the reference group is consistent with the expected mortality of sturgeon during this period when the larvae are transitioning from yolk sac to exogenous feed (Bennett and Farrell, 1998; Conte et al., 1988; Gisbert and Williot, 1997; Mohler et al., 2000). A cumulative assessment revealed mortality rates that were heterogeneously distributed over the course of the experiment with the greatest number of individuals dying between 21 and 34 dph coinciding with the transition to feeding on exogenous food. Groups that routinely spawn and breed white sturgeon such as the Kootenay Trout Hatchery, Canada, the Columbia Basin Hatchery, USA, and the University of California, USA, also experience die offs during this transition phase; sometimes in excess of 70% (R. Ek, Kootenay Trout Hatchery, Fort Steele, BC, Canada; B. Lyon, Columbian Basin Hatchery, Moses Lake, WA, USA; J. Van Eenennaam, University of California, Davis, CA, USA). Similarly, Siberian sturgeon experience difficulties during the transitional period under hatchery conditions (Gisbert and Williot, 2002). The variability in hatchery survival during this transition period is in part attributed to the type of diet provided and whether or not the larvae readily accept it (Bardi et al., 1998; Bennett and Farrell, 1998; Lutes et al., 1990). Some studies suggest that sturgeon undergo changes in morphology and physiology to the digestive system during and/or just prior to this transition phase and proper timing and development, often affected by environmental conditions and dietary requirements, are necessary for larval survival (Bardi et al., 1998; Buddington, 1991; Buddington and Christofferson, 1985). Based upon the life history strategy of white sturgeon, high larval mortality may be natural and unavoidable. Since little is known about white sturgeon larval

survival rates in their natural environment, it is difficult to assess what percentage of larval mortality is normal. White sturgeon are extremely long-lived (> 80 yrs) and females can spawn up to a million eggs in a season (UCWSRI, 2002) so even with low fertilization and/or hatching success, natural mortality of ELS's is likely inherent (Coutant, 2004). Based on these observations, transition to exogenous feeding represents a period during the early development of white sturgeon that is characterized by natural mortality.

An additional factor that contributed to the mortality during the transition life stage was the seeding density of fish per treatment chamber. Since no guidelines exist for the conduct of chronic white sturgeon ELS tests, seeding rates were calculated such that they were always less than those recommended by ASTM for fish in general (ASTM, 2005) and those outlined in the hatchery manual for white sturgeon (Conte et al., 1988). A parallel field study that was being conducted with the same exposure systems and same batch of fish revealed a significant and strong linear relationship between seeding rates and mortality (Tompsett et al., University of Saskatchewan, Saskatoon, SK, Canada). This relationship was unique to the period when fry transitioned to exogenous feeding and may be an indication that transition stages of white sturgeon are particularly sensitive to competition. As soon as sturgeon larvae successfully adapt to exogenous food, they seem to be more robust with regard to this factor. Limitation of food resources as a possible reason for the density-related increase in mortalities can be excluded because fish were fed *ad libidum*, and did not consume all food provided. Furthermore, there were no differences in water quality including dissolved oxygen, ammonia, nitrate, nitrite, pH, temperature, phosphate, DOC, and TOC between treatment chambers and groups, which could have explained elevated mortalities in the exposure systems with greater fish densities.

Comparable mortalities during the transition to feed life stage observed across all treatment groups indicated that mortality during this period was largely independent of metal exposure. The significant relationship between seeding density and mortality among treatment groups then raised the question as to what degree seeding density versus metal concentration contributed to the overall mortality during the four different life stages. Multivariate analyses indicated that during the yolk sac and swim up life stages the concentration of metal was almost entirely responsible for fish mortality in the exposure groups, with complete mortality in the greatest concentrations. During the transition to feed life stage, however, seeding density was driving Cu and Zn mortality, while both seeding density and concentration of Cd influenced mortality in the Cd treatment group.

Based on these analyses, overall mortality of the reference group can be attributed to the initial mortalities following hatch (as would be expected under natural conditions), the transition to exogenous feeding, characterized by naturally high mortality rates, and the initial seeding densities (white sturgeon do not fall in the stocking density range recommended by ASTM [2005] for other test species). Consequently, overall reference and treatment group mortalities were adjusted for seeding density mortality.

The large adjustment required for the seeding density correction does, however, add a certain degree of uncertainty to the resulting dose-response relationships as it is difficult to fully tease apart seeding density effects and metal effects (or possibly interactive effects) on mortality. For this reason, toxicity values were presented for 19 dph fish prior to the transition to feed die off (and associated seeding density normalization) as well as at the termination of the study (58

dph). LC50 and LC20s expressed as gram-atomic weight concentrations (Table 3.4) revealed that ELS white sturgeon were most sensitive to Cd, followed by Cu then Zn. Most metal-related mortality occurred early during exposure and the general patterns of toxicity are in accordance with previous studies of Cu, Cd, and Zn exposure to fish. The greater mortalities near the end of the exposure period in sturgeon exposed to Cd at 8.28 µg/L could in part be attributed to differences in Cd's toxic mode of action. Hypocalcaemia, due to Cd inhibition of Ca²⁺ uptake across the gills, is the key mechanism in acute Cd toxicity. In chronic exposures, Cd has been shown to accumulate in internal organs such as liver and kidneys, in addition to the gills (Cearly and Coleman, 1974; Hollis et al., 1999; McGeer et al., 2000; Verbost et al., 1987, 1988). Cadmium's effect on internal organs likely contributed to the later mortalities and poor body condition of fish in the greatest remaining Cd exposure, resulting in the lower LC values observed at the later life stage (58 dph). Due to the similar response of white sturgeon to Cu, Cd, and Zn exposure observed in the present study, it is possible that white sturgeon respond to these metals in a similar manner as other fish species at these concentrations. In general, the gill is the primary site of action in acute metal toxicity, and even at low concentrations these waterborne metals primarily affect ion-regulation (Playle et al., 1993; Wood, 2001). However, a more detailed examination of the white sturgeon gill, including protein expression post metal exposure, is needed to confirm similar patterns of toxicity.

ELS white sturgeon appear to be relatively sensitive to Cu, Cd, and Zn when compared to other aquatic species. The most sensitive LC20s determined in the present study were compared to chronic values outlined in EPA's chronic toxicity to freshwater organisms dataset in each metal's ambient water quality criteria document (EPA, 1987, 2001, 2007). Chronic values are

typically calculated by taking the geometric mean of the greatest no observed adverse effect concentration (NOAEC) and the lowest observed adverse effect concentration (LOAEC), or, if possible, using regression or probit analysis to estimate effect concentrations for 20 percent reduction (EC20; EPA, 1985, 2007). LC20s from the present study, adjusted to comparable hardness levels, were almost always within a factor of two of the chronic value of the most sensitive fish species. Studies conducted with rainbow trout (*Oncorhynchus mykiss*) and sculpins (*Cottus* spp.), species often considered to be sensitive to metal exposure, demonstrated comparable if not lesser sensitivity to Cu, Cd, and Zn compared to ELS white sturgeon (Besser et al., 2005, 2007; Hansen et al., 2002). Chronic LC20 values for Cd (1.5 µg/L) and Cu (5.5 µg/L) obtained for white sturgeon were comparable to those previously reported for Chinook salmon (Cd = 1.6 µg/L and Cu = 5.9 µg/L; Chapman, 1978), which was the most sensitive species in comparison to a number of other freshwater teleosts such as rainbow trout, fathead minnow, bluegill, etc. (EPA, 2001, 2007). LC50 values obtained for white sturgeon after exposure to Zn (58 dph = 250 µg/L, 19 dph = 340 µg/L) compared well overall with those reported by Mebane et al., (2007) for rainbow trout (LC50 = 387 µg/L). It should be acknowledged that hardness in our study was approximately two-times greater than that reported by Mebane et al. (2007).

Based on the LC values, the United States national recommended water quality criteria for aquatic life (EPA, 1987, 2001, 2007, 2009) are protective of ELS white sturgeon for Cd, Zn, and Cu (Table 5.4). It should be noted, however, that the guidelines for Cu are site-specific and freshwater criteria are calculated using the Biotic Ligand Model (BLM, 2007). The Canadian water quality guidelines (CWQGs) for the protection of aquatic life for Cu, Cd, and Zn (2.8 µg/L, 0.017 µg/L and 30 µg/L, respectively), adjusted to comparable hardness levels, are less

than the most sensitive LC values obtained in the present study for ELS white sturgeon (CCME, 2003).

It has been hypothesized that some sturgeon species (family Acipenseridae) are relatively sensitive to certain chemicals, based on acute studies (Bennett and Farrell, 1998; Dwyer et al., 2005; Hamlin, 2006). However, to date, no information on chronic toxicity was available that included all of the ELS's, from embryo through juvenile, which are often assumed to be the most sensitive (Hutchinson et al., 1998). The present sub-chronic study can help characterize the risks associated with water borne exposure of Cu, Cd, and Zn to ELS white sturgeon under environmentally relevant conditions.

CHAPTER 4

4.0 DISCUSSION and CONCLUSIONS

Within the last fifty years there has been increased attention towards white sturgeon biology. Initially due to interest of hatchery managers, and predominantly related to farming and caviar production, research efforts have recently begun to focus on conservation. As the sizes of populations decline there is a growing need to better understand this species in hopes of preserving it. White sturgeon are exposed to a range of contaminants as they tend to inhabit industrialized river systems such as the Columbia and Fraser. Few studies have examined the effects of pollutants on white sturgeon and further investigations are needed to assess the relative sensitivity of this species. Exposure to metals has been hypothesized as a potential contributing factor to poor recruitment in many of the water bodies. In general, little is known about the chronic toxicity of metals to white sturgeon and their potential influence on survival of embryos and/or juveniles. No information exists, however, regarding proper techniques for conducting white sturgeon experiments. In fact, only limited information is available for effective culturing. Individual organizations have developed their own procedures but there is a lack of public protocols. In addition, all protocols to date are designed for hatcheries and are not always applicable to experimental conditions. The information presented within this thesis helps to characterize white sturgeon ELS sensitivity to metals and presents effective methodology for conducting ELS sturgeon studies.

The initial field study characterizing Teck effluent toxicity provided an opportunity to work with ELS white sturgeon first hand and interact with experts familiar with sturgeon biology

in the UCR. During the experiment, several trips were made to the Kootenay Trout Hatchery to assist in brood stock spawning and egg harvesting. Working with adult sturgeon, collecting eggs and milt, and fertilizing/incubating embryos was instrumental in providing guidance to effectively conduct ELS toxicity tests. In addition, examining culture systems at the hatchery provided insight into creating the design of our exposure chambers, while conducting the semi-chronic effluent exposure with the modular exposure chambers was valuable in developing adaptations for overall system design. The combination of the two experiences allowed for the successful development of an exposure system that allows evaluation of the potential impact of contaminants on ELS sturgeon under chronic, fluvial conditions. In addition, we gained insight into white sturgeon culturing through expert advice, hands on practice, and trial and error learning that allowed us to optimize and harmonize culture/rearing conditions to enable standardized toxicity tests with ELS white sturgeon.

Once the exposure systems and experimental protocols were established, these technologies were applied to assess toxicity thresholds and sensitivity of ELS white sturgeon to metals of concern. A sub-chronic study characterizing the risks associated with water borne exposure of Cu, Cd, and Zn to ELS white sturgeon under environmentally relevant conditions was effectively conducted (Vardy et al., 2011). Based on our findings, ELS white sturgeon are relatively sensitive to the metals of interest, compared to some standard test species such as rainbow trout. Studies conducted with rainbow trout (*Onchorynchus mykiss*) and sculpins (*Cottus* spp.), two species often considered to be sensitive to metal exposure, demonstrated comparable if not lesser sensitivity to Cu, Cd, and Zn compared to ELS WS (Table 4.1). The most sensitive toxicity threshold values for ELS white sturgeon from the present study were

compared to chronic values outlined in EPA’s water quality criteria documents, and were found to be almost always within a factor of two of the chronic value of the most sensitive fish species. When compared to water quality guidelines for the protection of aquatic life in the USA and Canada, both sets of guidelines remain protective for ELS white sturgeon (Table 4.2). As a result, if water quality guidelines are met, water borne exposure to the individual metals of interest would not be expected to be a significant contributing factor to white sturgeon ELS mortality.

Table 4.1. Sensitivity of white sturgeon (*Acipenser transmontanus*), rainbow trout (*Onchorhynchus mykiss*), and mottled sculpins (*Cottus bairdi*) to copper, cadmium, and zinc.

	Early Life Stage Fish Species Sensitivity Comparison			
	White sturgeon (<i>Acipenser transmontanus</i>)	Rainbow trout (<i>Onchorhynchus mykiss</i>)	Rainbow trout (<i>Onchorhynchus mykiss</i>)	Mottled sculpins (<i>Cottus bairdi</i>)
Life stage test initiated	Larvae	Larvae	Swim-up fry	Swim-up fry
Test duration	60 days	58 days	28 days	28 days
Water hardness (CaCO ₃)	70 mg/L	170 mg/L	103 mg/L	103 mg/L
Cu chronic Value	5.5µg/L	16µg/L	40µg/L	13µg/L
Cd chronic Value	1.5µg/L		1.9µg/L	1.3µg/L
Zn chronic Value	112µg/L		219µg/L	128µg/L
Reference	Vardy et al. 2011	Besser et al. 2005	Besser et al. 2007	Besser et al. 2007

Table 4.2. Water quality guidelines for the protection of aquatic life in the USA (CCC) and Canada (CWQG) compared to the most sensitive toxicity threshold values for early life stage white sturgeon from the present study.

Metal	LC20	LC50	CCC ^a	CWQG ^b
	Measured Conc. (µg/L)	Measured Conc. (µg/L)	(µg/L)	(µg/L)
Cu	3.4	9.9	c	2.8
Cd	1.5	5.6	0.19	0.017
Zn	102	250	87	30

^a CCC refers to the Criteria Continuous Concentration for fresh water species adjusted to the average hardness of 70 mg CaCO₃/L observed during the experiments (EPA, 2009)

^b CWQG refers to the Canadian Water Quality Guidelines for the protection of aquatic life adjusted to the average hardness of 70 mg CaCO₃/L observed during the experiments (CCME, 2003)

^c Site specific guidelines - Freshwater criteria calculated using the Biotic Ligand Model (BLM)

Further investigation is needed, however, to examine other potential routes of metal exposure, such as uptake through sediment. White sturgeon are benthic scavengers that feed on prey closely associated with sediment and bioaccumulation through the food web may be an issue. Given the epibenthic nature of white sturgeon, there is the potential for increased exposure to contaminated sediments within the UCR. Specifically, there are concerns about the potential toxicity of contaminants of concerns associated with granulated slag to ELS white sturgeon, including the early hiding stage where fry are in proximity to sediments. Exposure pathways may include pore and surface water at the sediment-water interface. Water may move through the sediments either from upwelling from deeper sediments, downward movement from surface water, or lateral fluvial flow. Studies investigating contaminated sediments in habitats

considered suitable for ELS white sturgeon in the UCR are needed when considering possible causes for recruitment failures. In addition, ELS sturgeon inhabit benthic habitats on the surface of the bottom or in interstitial space between stones. Thus, juvenile white sturgeon could be exposed chronically to lesser concentrations of metals or for shorter periods of time to higher concentrations of metals in the surrounding water. For this reason future studies are needed to determine acute toxicity of metals to ELS white sturgeon.

Little is known about the mechanisms that sturgeon employ to deal with heavy metal exposure. Biochemical responses tend to be the first quantitative changes in aquatic organisms following toxicant exposure. These biological responses, or biomarkers, are useful tools as first indicators of stress in aquatic animals exposed to environmental pollutants. Metallothioneins, for example, are low-molecular weight proteins that have a high affinity to bind and detoxify metals (King, 1995; Shariati et al., 2010). Metallothioneins have been found to have maintained similar amino acid sequencing across various species, both aquatic and terrestrial, and from an evolutionary standpoint may have maintained their heavy metal detoxifying properties. Investigation into metallothionein characterization, especially in such an archaic species, is merited. In addition, a suitable and sensitive biomarker for the assessment of metal exposure in this species will aid in white sturgeon risk assessment.

Predictive and preventative tools to aid in white sturgeon risk assessment and population recovery efforts are also of great value. Metal speciation models such as the Biotic Ligand Model (BLM) are commonly used to predict toxicity to various aquatic organisms depending on ambient water quality parameters. With the development of dose response relationships and toxicity thresholds for ELS white sturgeon, models such as the BLM can be manipulated

specifically to characterize white sturgeon sensitivity under varying environmental conditions. This would greatly aid in risk assessment and regulation not only in the UCR, but in any water body where white sturgeon vulnerability is of concern. White sturgeon have been listed as endangered in parts of Canada and the USA, and collaborative efforts between both countries are essential in hopes of restoring healthy, self-sustaining populations. Groups such as the White Sturgeon Recovery Initiative play crucial roles in maintaining and aiding current white sturgeon populations, and research efforts have increased in the past few years in attempts to better understand their decline. More work and research is still needed, however, if we hope to save this unique and ancient fish from extinction.

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APPENDIX A

DEVELOPMENT OF EXPERIMENTAL EXPOSURE SYSTEMS FOR FIELD AND LABORATORY BASED STUDIES OF EARLY LIFE STAGES OF WHITE STURGEON UNDER FLUVIAL CONDITIONS

Abstract

This chapter describes the basis for the design, construction, and utilization of artificial flow-through exposure systems to be used in field and laboratory studies under simulated fluvial conditions. The flow regime in the re-circulating exposure units can be adjusted such that it simulates fluvial conditions of interest (e.g. the flow-regime can be adjusted such that it accommodates the specific requirements for different life stages of riverine fish such as sturgeon).

Introduction

Similar to many species of fish, early life stage (ELS) white sturgeon undergo physiological and behavioural changes, often associated with environmental cues, and require distinctive and often differing habitats during early development (Coutant, 2004). During embryonic development, pre-neurulation for example, a temperate riverine environment is ideal for embryos in order to prevent developmental deformities. Post-neurulation, however, embryos are much hardier and a more turbid flow is preferred in order to prevent clustering and the development of fungus (Conte et al., 1988). Post hatch, white sturgeon larvae generally display negative phototaxis and tend to congregate, and hide when shelter is provided (Conte et al.,

1988). Prior to switching from yolk sac to exogenous food, larvae begin to swim up in the water column, presumably to be transported to a habitat more suitable for feeding (Coutant, 2004). In order to conduct effective chronic experiments with ELS fish a flexible experimental design that can be adapted to accommodate these various changes (while minimizing confounding variables e.g. fluctuating water chemistry) is desired.

Continuous flow-through exposure systems for controlled experiments of ELS white sturgeon under simulated fluvial conditions were designed and fabricated. The basic design was derived from a modular exposure system from Hruska and Dubé (2004) used in a previous experiment (Chapter 2) and adapted for our purposes. The modular exposure system from Hruska and Dubé (2004) had many qualities that were advantageous in a mobile experimental system, including flow-through and multi trophic exposure capabilities. For our purposes, however, a modified design was desired. Larger, independent exposure chambers, in which the flow regime could be manipulated for each individual unit, were more suitable for chronic exposures of ELS white sturgeon. In addition, a rectangular flow-through chamber design with a segregated drainage section proved superior as ELS white sturgeon experienced difficulties with currents and suction associated with other chamber and drainage designs. Furthermore, these portable, self-contained, exposure systems can be completely disassembled, easily transported, and set up directly in the field or in the laboratory to be used with a water source of choice (e.g. river-water, laboratory water, effluent mixtures, contaminant dilutions, etc.). Exposure systems consisted of holding tanks and reservoirs that allow for adjustable turnover rates of an aqueous exposure media. Each exposure chamber contained numerous screens, and inserts of varying size. This enabled the flow regime to be adjusted as the sturgeon larvae matured as well as being

able to increase the exposure area and adjust water height. In addition, modifications can be made to incorporate various exposure media, such as sediment.

Materials and Methods

The purpose of this section is to describe the basic design of the exposure system structures, and how the experimental setup was arranged. Each complete exposure system was comprised of a head tank, a reservoir, a metering pump, a march pump, a wet table and steel frame, a chiller (when not using the wet table to regulate water temperature), a delivery manifold, an egg hatching jar, and several exposure chambers. The method described herein covers the components required for a fully functioning exposure system as well as considerations that were taken when choosing an experimental layout. A more detailed explanation of building materials, fabrication, and suppliers is provided in the Environmental Toxicology Laboratory Standard Operating Procedure 6032 (SOP #6032) at the Toxicology Centre, University of Saskatchewan, Saskatoon, SK, Canada.

Equipment and Materials

Certain building materials and equipment were selected to reduce the chances of introducing possible sources of contamination. For example, polyvinyl chloride (PVC), high density polyethylene (HDPE) plastics, and stainless steel were usually selected when available or cost-effective. With the metal exposures, parts that contained galvanized steel and brass (as these contain zinc and copper, respectively) were avoided. Prior to initiation of the experiment, each exposure system was cleaned and flushed for a minimum of one week to remove any residues from the construction process.

Flow-Through Exposure Chambers

Artificial flow-through exposure chambers, removable screen dividers, and removable inserts were constructed from $\frac{1}{4}$ " acrylic plexiglass. The acrylic should be a light transparent colour (e.g. aqua-green) to allow some light to pass through but dark enough so that the fish are not affected by their surroundings. The basic dimensions are give in Fig. A.1. Each exposure chamber contained six removable screen dividers, two inserts, an opaque HDPE lid to provide shade for the fish, and a HDPE treatment divider to prevent cross contamination.

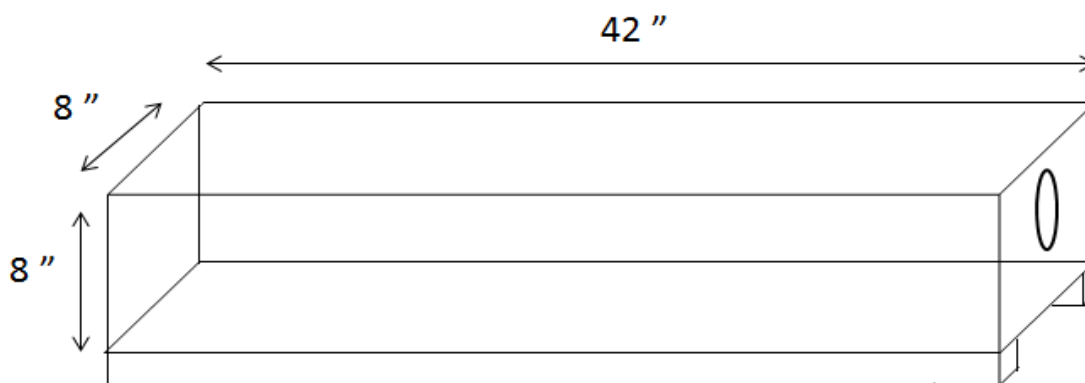


Figure A.1. Dimensions of exposure chamber.

Experimental Design Setup

A head tank contained the water/solution mixture of interest to test in the experimental exposure system. The mixture was delivered from the head tank to the 85-L exposure system reservoir via a metering pump. The mixture delivery rate from the reservoir to the exposure chambers was regulated by a delivery manifold attached to a re-circulating march pump. The mixture flowed from the delivery manifold to an exposure chamber inflow nozzle, through the

exposure chamber, and exited through an outflow drain hole, which in turn was connected back to the 85-L reservoir. There was an overflow drain at the back of each reservoir that discarded wastewater and a baffle within each reservoir that prevented short-circuiting of the inflow to the overflow drain (Fig. A.2). The test solution was cooled to the desired temperature by re-circulating chilled water through the wet table. To do so, one large, separate chilled water bath was fabricated, cooled to temperature, and then re-circulated through all the wet tables (alternatively, the test solution mixture may be cooled to the desired temperature by placing a chiller unit inside the 85-L reservoirs; Fig. A.2).

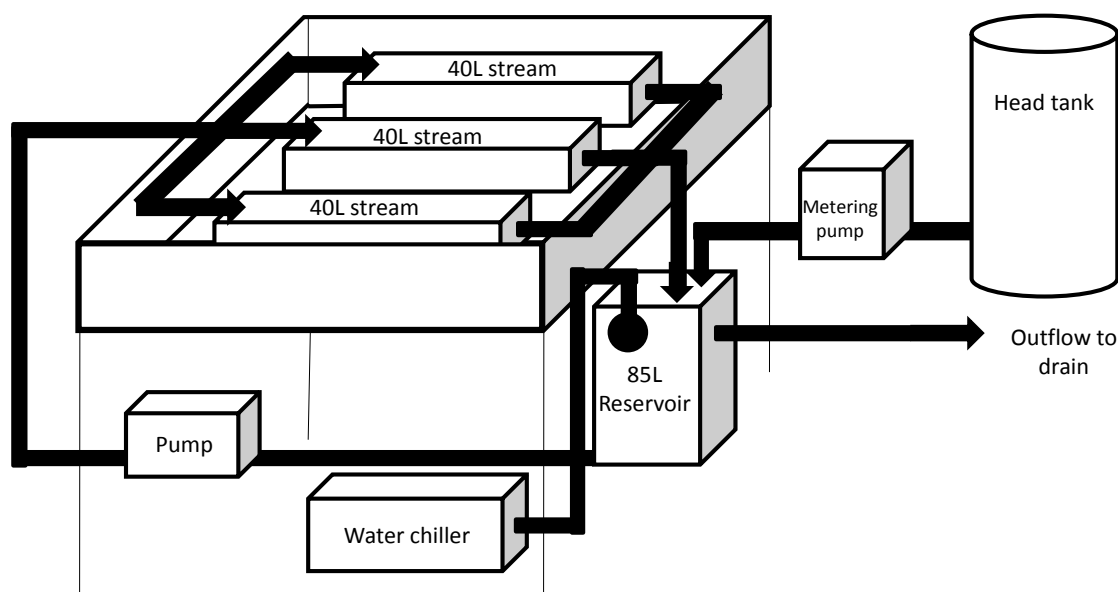


Figure A.2. Schematic of flow-through system layout showing 3 exposure chambers (figure adapted from A. Tompsett, Toxicology Centre, University of Saskatchewan)

Exposure Chambers, Incubation/Hatching Jars, and Exposure System Inflow Manifold

Each wet table could hold up to six plexiglass exposure chambers side by side. Each treatment chamber had six removable screens and two removable inserts to control the flow

regime. Fiberglass mosquito netting was attached by use of silicone sealer over the rectangular holes of the inserts in order to reduce water flow and prevented the fish from passing through. Egg hatching jars were placed in the inflow end of the exposure chambers. The first screen at the inflow end of the chamber was removed and the test solution was pumped through the top of the standpipe of the hatching jar (from the reservoir) when the experiment was initiated (Fig. A.3). Once the embryos began to hatch the circular screen on the top of the hatching jar was raised 1” to allow the larvae to escape and flow into the exposure chamber. The egg hatching jar screen was cleaned regularly as it collected hatched egg shells. Post hatch, the egg hatching jar was removed and the exposure system inflow nozzle was attached. Transparent HDPE board dividers were placed between each true replicate in the wet table to prevent cross contamination.

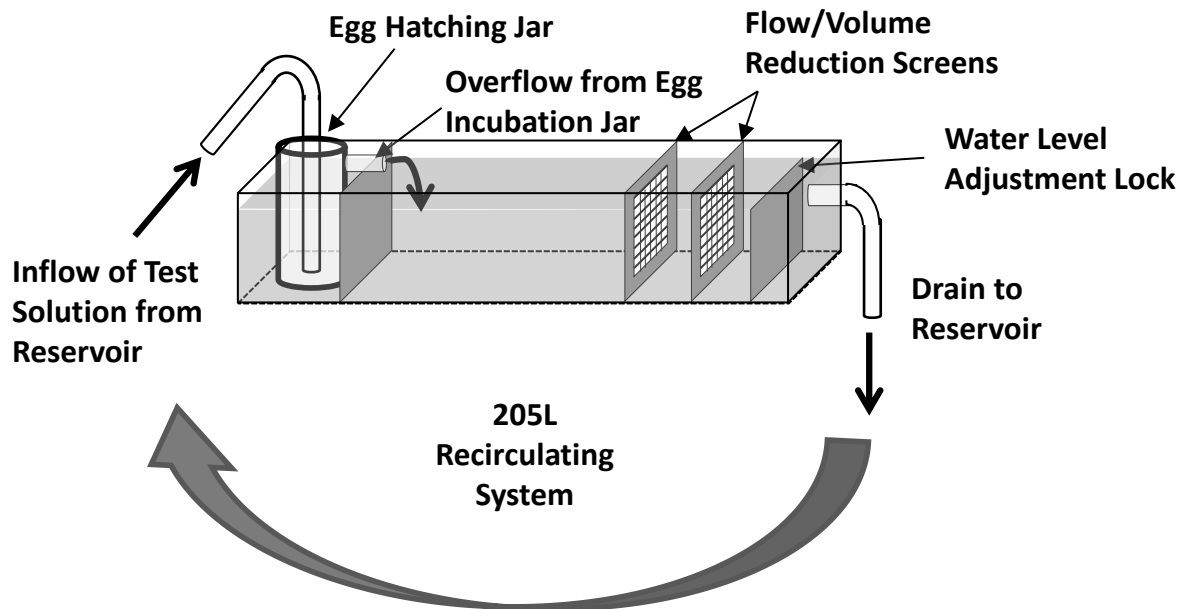


Figure A.3. Exposure chamber design with egg hatching jar.

Discussion

Chronic ELS toxicity tests are useful bioassays that help to characterize species sensitivity. Embryos, larvae, fry, and juvenile fish are often more sensitive to contaminants than their adult counterparts, yet certain chemicals induce varying degrees of life stage specific adverse effects (Hutchinson et al., 1998). This often complicates study design when working with a test species or chemical where little toxicity data exists. Embryos through juvenile exposure studies are useful in this regard as they investigate the sensitive periods (especially during early ontogenic development). Difficulties still arise, however, when trying to incorporate several life stages into a single experimental set up (especially when varying environmental conditions are required). The ability to manipulate an exposure system design as the experiment progresses and thereby maintain animal health and minimize unwanted variables is a great asset.

White sturgeon ELS's are sensitive to environmental fluctuations (Buddington, 1991) and the exposure system presented here helps to accommodate their changing requirements. Flow-through systems are the preferred choice in chronic aquatic exposure experiments as they tend to maintain high levels of oxygen and reduce build-up of toxic wastes such as ammonia-nitrogen (Rand, 1995). Turn-over rates can be adjusted in the present system to balance between desired exposure durations of the contaminant of interest and flushing of unwanted wastes. In addition, a funnel and filter can be easily installed in the lid of the reservoirs so that the outflow of the exposure chamber is filtered before being re-circulated. Depending on the material, the filter can simply remove excess food or can be selective (e.g. carbon filters, cations/anions exchangers etc.). In addition, inflow nozzles can be adjusted so that the output of water is above the water

line, thereby creating a bubbling effect and increasing dissolved oxygen levels if required. Air stones may be placed in the reservoirs or exposure chambers (segregated from the fish to minimize disturbance) if needed. This can be useful during exposures where ammonia levels are of concern but are not the contaminant of interest and need to be reduced. The exposure system can also be arranged so that the head tank is continuously receiving a supply of the water/mixture source. This enables 24 h composites of the water source to be tested in real time in the exposure chambers. This design is particularly useful when conducting whole water toxicity tests, especially under fluvial field conditions where spikes in contaminants downstream of a contaminant source may not be captured otherwise. Alternatively, static renewal of the head tanks is another option that may be more useful when conducting dose-response experiments where exposures to increasing dilutions are desired.

Sturgeon hatcheries have found that the McDonald-type hatching jar is an effective method to incubate and hatch white sturgeon embryos (Conte et al., 1988). The design developed here allows for the hatching jar to be fitted securely into the exposure system by removing the first screen. The delivery manifold also allows for finite adjustments of flow velocities, a crucial step in preventing the development of fungal diseases. In addition, this allows the embryos to be exposed to the contaminants of interest immediately after fertilization, thereby capturing the most sensitive period in some cases. Once the embryos hatch they swim directly into the exposure portion of the chamber, eliminating any need for fish handling and its associated stress.

Post-hatch, sturgeon larvae can be sensitive to the physical conditions of their environment, and under experimental conditions survival can be greatly dependent on their

ability to transition to later life stages within the constraints of their tanks. For example, yolk sac larvae are easily pushed around by heavy currents and will often become entangled or stuck in crevices or holes if given the opportunity. In addition, larvae display negative phototaxis and tend to prefer to congregate in areas of shade if provided. Once they absorb their yolk sac they swim up in the water column and tend to graze for food along the walls of their tanks. If food is not easily found during early onset of exogenous feeding, larvae can experience difficulties in transitioning from yolk sac and often die of starvation (Conte et al., 1988). From past experience, it has been found that the classic flow-through tank design with a standpipe drain was inappropriate for rearing of white sturgeon ELS's under experimental conditions as larvae would become stuck on the drainage screens of the standpipe as the suction would be too great.

The exposure chamber design used in these studies incorporates several divider screens that enable the exposure enclosure area to be increased as the fish mature. The extra screens decrease the suction that the larvae experience while maintaining adequate flow rates. In addition, removable screens are fitted to the chamber walls in router grooves, providing smooth, tight fits that prevent larvae from becoming entangled while maintaining uninterrupted flow regimes. The removable screen dividers and inserts also provide versatility in adjusting flow regimes as fish mature. Screen dividers provide a smaller enclosure during the transition to feed stage and increase chances of fish contact with food. Particles of food also collect on the screens and facilitate grazing. Some hatcheries employ an initial feeding technique whereby the water level in the tanks is dropped, a semi-moist powder food is applied to the edges, and then the water level is raised again slowly allowing the fry to graze (R. Ek, Kootenay Trout Hatchery, Fort Steele, BC, Canada). A similar technique can be applied in the exposure system used here

by adjusting the inserts. In addition, each exposure chamber had an adjustable cover that can be used to provide varying degrees of shade refuge for the fish, can be used to attach automatic feeders, as well as being useful during cleaning, water sampling, and water chemistry analyses.

Overall, the exposure systems for field and laboratory based studies of ELS white sturgeon under fluvial conditions are extremely versatile and easy to use. They are easy to ship, assemble, clean, and store. Fluctuations in water chemistry that are often encountered with static renewal systems are virtually non-existent. The general flow-through design of the system and exposure chambers can be manipulated and applied to a variety of exposure scenarios (e.g. incorporation of substrates).

APPENDIX B

PROTOCOLS FOR WHITE STURGEON REARING UNDER EXPERIMENTAL CONDITIONS

Abstract

The purpose of this chapter is to outline protocols that are beneficial in ensuring health and growth of embryos, larvae and juveniles under experimental conditions. The methods described herein focus on embryo incubation techniques and feeding regimes that have proven successful in white sturgeon early life stage development.

Introduction

White sturgeon are threatened throughout North America and there is a growing initiative in recent years to help preserve this species (UCRWSRI, 2002). Recruitment failure is a common factor in population declines and studies investigating early life stage (ELS) white sturgeon sensitivity to anthropogenic stressors are needed. In particular, studies involving white sturgeon embryos, larvae, and juveniles, periods often considered sensitive during fish development (Hutchinson et al., 1998), would greatly improve our knowledge on biology of the species and increase chances of restoring natural populations by improving recruitment rates. There are few guidelines, however, that outline proper techniques and protocols for white sturgeon rearing, especially under laboratory conditions. White sturgeon are not commonly used in toxicity testing and applicable ASTM or EPA guidelines do not exist. White sturgeon are particularly sensitive, however, to environmental stress during ELS's (Buddington, 1991) and

transition from yolk sac to exogenous food can be particularly difficult. The goal of this chapter is to provide guidance for white sturgeon rearing under experimental conditions, gained from several years experience involving long term studies raising embryos to 60 days post-hatch (dph). In particular, effective embryo incubation techniques and feeding regimes to transition larvae to exogenous food, under experimental exposure conditions, will be discussed.

Methods and Materials

Embryo Exposure/Incubation

The embryo is most sensitive within the first few hours post fertilization before the chorion hardens and experiments were initiated as soon as possible when conducting embryo exposures. Ideally, eggs were collected from four-six breeding pairs of adult white sturgeon. Fertilization of the eggs was harmonized in the hatchery by injecting adult sturgeon with a gonadotropin analog (LHRH analog, Syndel International Inc, Qualicum Beach, BC, Canada) on two subsequent days. Embryos were then transported in oxygenated bags and received at the exposure facility within hours of fertilization. Embryos were randomly assigned to their treatment groups and gradually acclimated to their solution waters for 1 h before being transferred to 6 L McDonald-type hatching jars (Aquatic Ecosystems, Apopka, FL, USA). Embryos were incubated under a low flow velocity (~ 3 L/min so that the embryos were just barely rolling over top of each other) for ~ 72 h until neurulation occurred. Development of a white band down the centre of the embryo indicated successful neurulation. After neurulation, the flow velocity was increased to gently agitate the embryos at first (~18 h to ensure complete neurulation), followed by increased flow velocity causing embryos to be evenly circulated ~

halfway up the side of the hatching jar. At this stage of development, embryos were relatively hardy and suspending them in the water column greatly reduced the chances of fungal infection. Increasing flow velocity before complete neurulation has occurred, however, can result in increased deformities. Hatching jars were inspected and cleaned a minimum of 4 times a day for fungus. It should be noted that once fungus developed within a system it spread very quickly, especially in waters with greater organic content, so diligent cleaning is necessary. Common turkey basters were an effective tool used to remove individual eggs from the hatching jar with minimal disturbance. The optimal temperature for embryo incubation was 15 °C, but it was found that temperatures could be lowered to as little as 11 °C if delayed hatching is preferred. At 15 °C hatching began approximately 5-7 d post-fertilization. Once the embryos began to hatch larvae would swim up the water column in the hatching jar and flow out into the exposure chamber.

Feeding Regime

At 7 dph food was introduced to the chambers to familiarize the larvae with a food scent. When familiarizing the larvae with food it was found that newly hatched brine shrimp (*Artemia salina*) and “blood juice” collected from thawed bloodworms (Hagen, San Francisco Bay Brand, Edmonton, AB, Canada) worked best. In addition, a semi-moist powder diet was developed containing one part # 0 trout chow, three parts cyclopeze, two parts krill and one part tubifex. This semi-moist food was introduced into the exposure chambers by pinching small amounts between the fingers and distributing it along the sides of the exposure chambers as well as along the screens where larvae transitioning to feed were found to actively forage. At ~10 dph larvae

rejected their black, corkscrew-like, yolksac plug and began to swim up and actively feed. Feeding rates were increased when larvae were transitioning to feed since this has been shown to be a critical period for survival (Conte et al., 1988). The most effective feeding regime for transitioning larvae to exogenous food included 24 h feeding at approximately 2 h intervals. In addition, it was noted that larvae sometimes clump together in groups, and feeding (especially with bloodworms) were targeted to these areas. Feed was ground to a fine size at the onset of feeding since larvae mouths were not yet fully developed. A meat or food processor was an effective tool to help grind frozen bloodworms to appropriate sizes.

As stated above, fry were fed a combination of live brine shrimp and frozen bloodworms, *ad libitum*, every 2 h throughout the day and into the evening during onset of exogenous feeding. Constant feeding throughout the day was by far the best method to reduce mortalities during the transition period. If possible, it is recommended that stock solutions of live, < 48 h old, brine shrimp be established and constantly delivered into the exposure chambers. Metering pumps can be used to establish slow drips of brine shrimp, and are an easy and effective system to design. Once fish were established on food, feeding frequency was reduced to four times a day and eventually down to once per day, at around 60 dph. An effective but more time consuming option to deliver the semi-moist diet was to lower the water level in the chambers, smear the food on the walls at the previous water line, then slowly raise the water level again. Fish actively foraged for food along the sides. This method worked best if the sides of the exposure chamber were not smooth so the semi-moist food stuck. Roughing the sides of the exposure chamber around the water line with low grit sandpaper worked well, or using a Dremel® tool to grind a shallow groove, “feeding tray”, along the length of the chamber was also effective. The semi-

moist diet was fed throughout the night with automatic feeders since it was noted that ELS white sturgeon tend to actively feed during this time. Depending on feeding rates, exposure tanks were monitored for ammonia and nitrite levels and cleaned frequently (\geq twice per day at minimum). Table B.1 outlines the most effective culturing regime for ELS white sturgeon under experimental conditions.

Table B.1. General activity schedule for conducting a chronic early life stage study with white sturgeon (*A. transmontanus*)

Day	Conditions
0	<ul style="list-style-type: none"> -Collect freshly fertilized embryos from hatchery and transport to experimental facility in sealed oxygenated bags -Acclimate embryos to exposure water temperature by floating sealed bag in exposure chamber -Acclimate embryos to exposure water by opening bag and gradually adding small volumes of exposure water over a 1 h period. DO should be monitored once the bag is opened and airstones may need to be added -Transfer acclimated embryos to McDonald-type hatching jars. If possible, ≥ 1000 embryos per jar is optimal for incubation. Adjust flow velocity through hatching jars so that the top layer of embryos rolls very gently but does not get pushed up in the water column
1-3	<ul style="list-style-type: none"> -Monitor hatching jars a minimum of 4 times per day here after; look for dead spaces in embryo circulation and remove any clumps of fungus -Measure DO, Temperature, pH and conductivity daily and all other water quality parameters at least every 7 days here after
4	<ul style="list-style-type: none"> -Ensure that neurulation has been completed; a white band will develop across the grey/black embryo -Increase water flow to hatching jars such that embryos are circulated ~ 4inches up the height of the jar
5	<ul style="list-style-type: none"> -Increase water flow to hatching jars such that embryos are vigorously circulated; ~ 7-8inches up the height of the jar
6-7	<ul style="list-style-type: none"> -Once hatch begins, raise the top screen of the hatching jar and allow larvae to flow out into exposure chamber -Remove hatched embryos shells that accumulate in the hatching jar
8-12	<ul style="list-style-type: none"> -Observe larvae 2-3 times per day here after and record any behavioral abnormalities - Remove dead larvae; count, weigh, measure length, and preserve dead fish in formalin here after -Clean exposure chambers at least 2 times a day here after
13	<ul style="list-style-type: none"> -Begin adding diet to the chambers to condition fry to food -Monitor ammonia/nitrite levels here after
14-15	<ul style="list-style-type: none"> -Monitor for swim up and presence/rejection of black yolk sac plugs
16-17	<ul style="list-style-type: none"> -Begin full feeding regime: 24 h metering of brine shrimp, bloodworms in morning, afternoon, evening (and throughout the night if possible), semi-moist food in morning, afternoon, evening and throughout the night -Diligent cleaning and monitoring of ammonia/nitrites
40-onwards	<ul style="list-style-type: none"> -Reduce feeding frequency; begin to cut out semi-moist diet then brine shrimp, eventually feed only bloodworms

Discussion

ELS white sturgeon experience greater than normal natural mortality under hatchery and laboratory conditions than that which would be expected from a standard test species such as a rainbow trout (R. Ek, Kootenay Trout Hatchery, Fort Steele, BC, Canada). Little is known about white sturgeon biology in the wild, but given their life history profile, ELS mortality may be an inherent characteristic (Coutant, 2004). White sturgeon embryos are susceptible to fungal infections during incubation (Conte et al., 1988), and larvae often experience difficulties transitioning to exogenous feeding. Acceptable chronic test criteria under EPA guidelines stipulate that control mortality must not exceed a certain percentage (usually < 40%) in order for the test to be valid. Although these guidelines do not specifically include white sturgeon, and are likely not suitable for white sturgeon toxicity testing, they are currently the standard guidelines to follow. The protocols presented here for white sturgeon ELS rearing under experimental conditions will help to reduce mortality unrelated to treatment effects. Diligent cleaning and intensive feeding schedules, including a variety of food options throughout the day and night, will greatly improve chances of conducting successful chronic ELS toxicity tests with white sturgeon. A 24 h feeding regime of live food such as brine shrimp, in combination with intermittent feedings of frozen bloodworms, proved most effective. With the introduction of food, decreases in water quality can be an issue and should be monitored frequently for changes. Optimal conditions for sturgeon health include water temperatures of 15°C (\pm 1), a pH of \sim 7, dissolved oxygen levels of \geq 80%, and negligible nitrite/ammonia levels. Spikes in nitrite levels can lead to mortalities very quickly and prompt corrective action such as increasing turnover rates and/or extra aeration are a few examples of non-invasive solutions.