UPPER COLUMBIA RIVER

Semi-Chronic Exposure of Early Life-Stages of White Sturgeon (*Acipenser transmontanus*) to Columbia River Water

Prepared for

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1 PURPOSE AND OBJECTIVES

The purpose of this study was to evaluate the toxicity of surface water from the Columbia River (CR) to early life-stages (ELS) of white sturgeon (*Acipenser transmontanus*). This study was designed to provide data needed to answer the following questions:

1. What are the hatch, survival, and development rates of ELS of white sturgeon raised in river water upstream and downstream of the Teck Metals Ltd. Trail smelter facility (the facility)?
2. What are the concentrations of slag-associated chemicals of interest (COIs) in surface water during the reproductive season of adult white sturgeon, and during the time when early white sturgeon life stages are present?
3. Are there significant differences in any of the biological endpoints measured among experimental sites and/or the control?

2 METHODS AND MATERIALS

2.1 TEST SPECIES AND SOURCE

Fertilized white sturgeon eggs were obtained from the Kootenay Trout Hatchery (KTH) located in Fort Steele, British Columbia on July 15, 2008. Hatchery staff collected eggs from four breeding pairs of adult white sturgeon captured in the CR near Waneta, Canada (Ron Ek 2008, pers. comm..). Fertilization of the eggs was harmonized in the hatchery by injecting the sturgeon with a gonadotropin analog on two subsequent days. Embryos were transported in oxygenated bags and received at the stream-side laboratories within 6-hours of fertilization. Embryos were acclimated to test waters for 1-hour before being incubated in McDonald-type hatching jars (Aquatic Ecosystems, Apopka, FL). Following neurulation (i.e., within 72 hours of fertilization), water velocity within hatching jars was increased to gently 'agitate' the embryos, ensure sufficient oxygen was present, and minimize the potential for fungus development. Hatching began and ended on July 21 and 25, respectively; approximately 6 to 10 days post-fertilization.

2.2 TREATMENT WATER SOURCES

During the course of the entire study, water directly from the CR was extracted and tested at two locations (i.e., one up- and one down-stream of the facility). The upstream field site (UFS) was located along the east bank of the CR at river mile (RM) 758. The downstream field site (DFS) was also located along the east bank of the CR but stationed at RM 746. Field sites are illustrated in Figure 1 and coordinates are provided in Table 1.

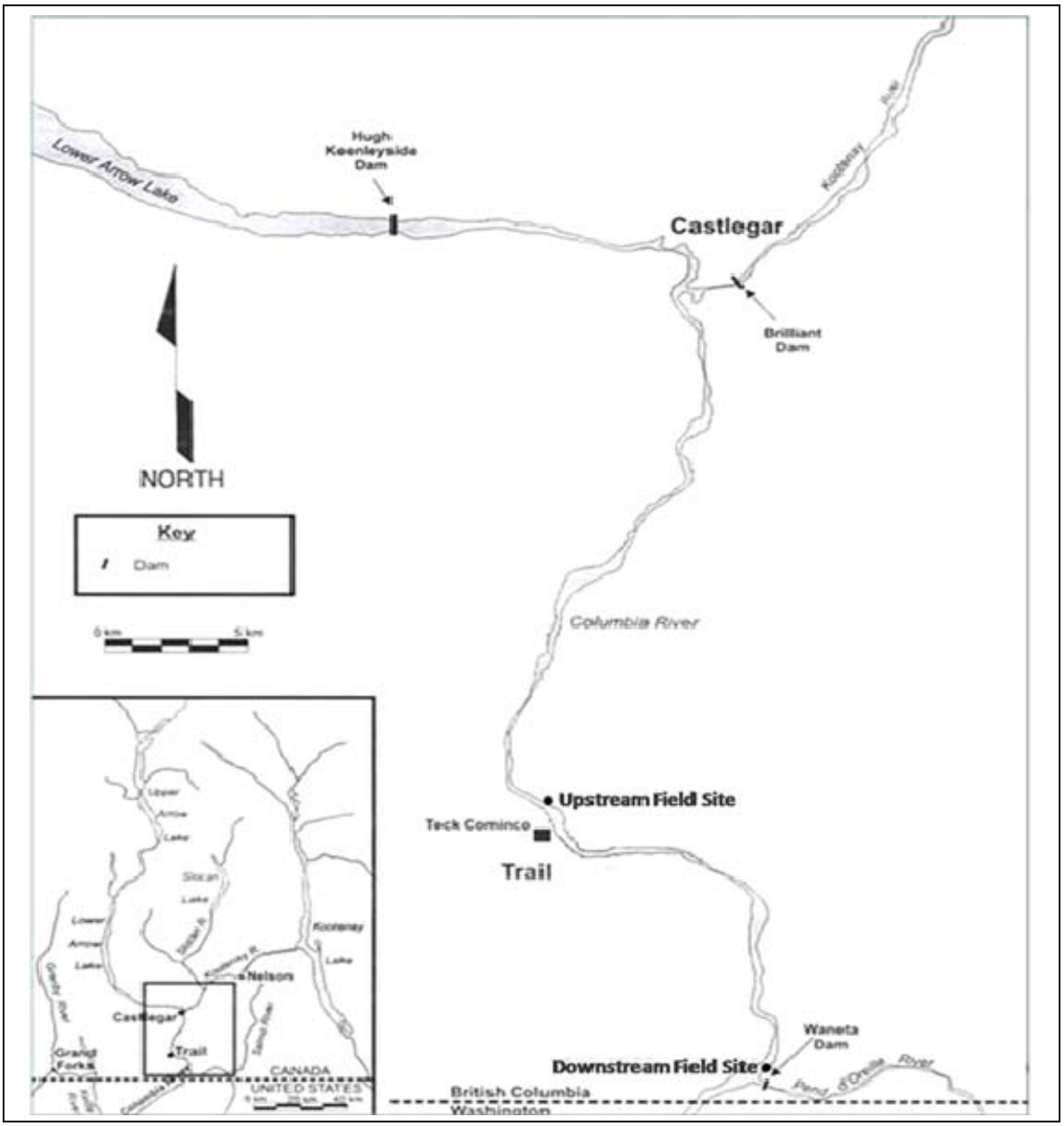

Figure 1. Sampling Area in the Columbia River

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

Treatment Group	Water Source	No. of Recirculating Systems	No. of Test Chambers per System	Location
CTR	Filtered Trail City Water	4	3	49°07'01.32" N 117°43'27.25" W
UFS	Columbia River Water Upstream from Teck Trail Facility	4	3	49°07'01.32" N 117°43'27.25" W
DFS	Columbia River Water Downstream from Teck Trail Facility	4	3	49°00'28.35"N 117°36'56.69"W

Notes :
CTR – control
UFS – upstream field site
DFS – downstream field site

Both sites were continuously supplied with river water in real-time by pumps located along the river channel. It should be noted that the UFS supplies the City of Trail with potable water; while the DFS serves as a water quality monitoring station for both the Provincial and Federal Canadian Governments. Filtered and dechlorinated City of Trail potable water was used as the control (CTR).

2.3 FISH EXPOSURES AND STUDY DESIGN

Individual and dedicated flow-through exposure systems were housed within retrofitted modular trailers as supplied by Britco, Structures Inc. Each exposure system consisted of two pumps, an 85 liter (L) high density polyethylene (HDPE) surge feed tank (reservoir), and three HDPE 40 L exposure chambers per replicate. Complete water exchange in each system occurred every six hours. Water temperatures within each reservoir were maintained at approximately 15 degrees Celsius (°C) using a titanium water chiller, and exposures were conducted under an illumination cycle of 16 light to 8 dark hours. All treatments were run in four replicate systems. A summary of the study design and associated treatments is presented within Table 1 and 2, and illustrated within Figure 2.

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

Parameter	Observation
Time of exposure initiation	≤ 12 h
Exposure duration	61 days (> 40 days post swim-up)
Loading density/rate	≤ 0.1 g/L/24h
Number of true replicates per treatment/dose	4 (in situ studies)
Number of fish per treatment at end of study	≥200
Observations	≥ 2 times per day
Feeding	≥ 3 times per day

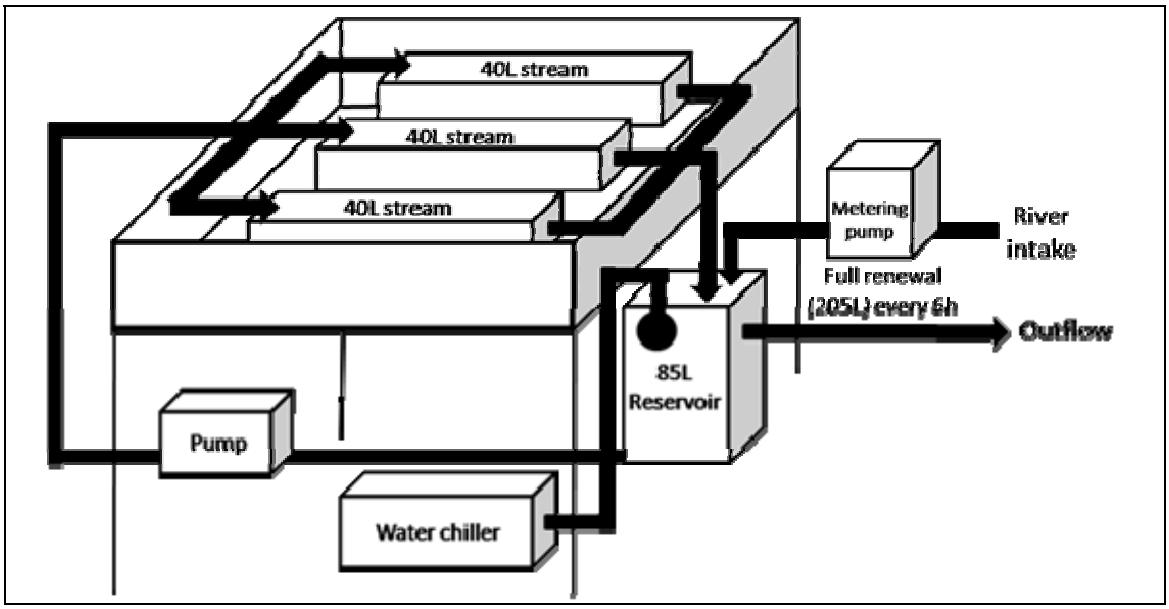

Figure 2. Flow-through Re-circulating Exposure Systems

Day 0 of the study (July 15, 2008), coincided with the placement of 800 embryos within dedicated McDonald-type hatching jars in each replicate per treatment group. Total test duration (exposure) extended for 61 days and concluded on September 13 through 14, 2008.

Post hatch larvae (i.e., 10 individuals at a time) were randomly divided into replicate chambers. Seeding density per replicate was maintained below 0.5 grams per liter (g/L), set by the American Society for Testing and Materials (ASTM) guidelines (ASTM 2009). At 10 dph (July 31, 2008), food (live brine shrimp [*Artemia salina*] and frozen bloodworms), was introduced into the test chambers 4 to 8 times throughout the day and into the evening; fish were allowed to feed *ad libitum*. Exposure chambers were cleaned twice daily; and any observed mortalities were removed, measured, weighed, and stored in formalin. At the conclusion of the study, all fish were euthanized, assigned a unique identifier, weighed, and measured.

2.4 WATER CHEMISTRY ANALYSES

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

In addition to the above-mentioned routine water quality parameters, weekly water samples were collected from each exposure chamber and analyzed for dissolved metals (i.e., target analyte list [TAL] metals). All samples were collected using acid-cleaned polyethylene bottles, filtered through a 0.45 µm polycarbonate filter with Nalgene® filter holders and receivers as required (e.g., dissolved metals); acidified with ultrapure nitric acid to a pH <2 standard units (s.u.), and maintained at approximately 4°C for shipment to the analytical laboratory (Liber Laboratory, Toxicology Centre, University of Saskatchewan).

Dissolved TAL metal analyses were performed using inductively coupled plasma mass spectrometry (ICP-MS) following Environmental Protection Agency (EPA) Method ILM05.2D (Creed et al. 1994). Dissolved organic carbon (DOC) analysis was performed weekly using a total organic carbon analyzer (TOC-5050A, Shimadzu, Mandel Scientific, Guelph, Ontario). Summaries of the blanks, analytical methods and associated method detection limits are presented in Tables 3 through 5.

Table 3. Analytical Methods and Associated Method Detection Limits for Water Quality Parameters

Parameter	*Method*	*Unit*	*Level of Detection*
Temperature	VWR Symphony 14002-860	˚C	0
pH	VWR Symphony 14002-860	s.u.	0
DO	VWR Symphony 11388-374	mg/L	2
Conductivity	VWR Symphony 11388-372	µS/cm	1
Ammonia-Nitrogen	LaMotte Kit 3304	mg/L	0.02
Nitrate	LaMotte Kit 3319	mg/L	0.25
Nitrite	LaMotte Kit 7674	mg/L	0.02
Hardness	LaMotte Kit 4824-DR-LT	mg/L	20
Alkalinity	LaMotte Kit 4419-DR	mg/L	20
Sulfate	LaMotte Kit 7778	mg/L	20
Phosphate	LaMotte Kit 7416-01	mg/L	0.05
Total Chlorine	LaMotte Kit 6905	mg/L	0.2
DOC	EPA 415.3	mg/L	0.01-0.12
TOC	EPA 415.4	mg/L	0.01-0.12

Notes:
All samples collected between 7/09/2008 and 9/14/2008)
Measurements conducted in individual, randomly selected test chambers
DO – dissolved oxygen
DOC – dissolved organic carbon
TOC – total organic carbon

Table 4. Limits of Detection (LOD) and Method Blanks (MB) for TAL Metals (in µg/L)

Dissolved Metal	6/30/2008		7/25/2008		8/15/2008		11/24/2008	
	LOD	MB	LOD	MB	LOD	MB	LOD	MB
Be	1.539	<LOD	0.030	0.030	0.108	<LOD	0.057	0.069
B	0.000	<LOD	12.343	<LOD	14.589	<LOD	29.277	<LOD
Al	0.596	<LOD	0.715	<LOD	1.884	<LOD	2.691	<LOD
Ti	0.000	0.049	0.708	<LOD	1.636	<LOD	0.470	0.526
V	0.099	<LOD	0.026	<LOD	0.039	<LOD	0.057	<LOD
Cr	0.015	<LOD	0.011	0.020	0.016	<LOD	0.012	0.058
Mn	0.017	<LOD	0.114	<LOD	0.138	<LOD	0.058	0.089
Fe	0.189	<LOD	0.116	<LOD	0.196	<LOD	0.340	<LOD
Co	0.005	<LOD	0.023	0.029	0.034	<LOD	0.010	<LOD
Ni	0.048	<LOD	0.200	<LOD	0.515	<LOD	0.124	0.271
Cu	0.053	0.369	0.035	<LOD	0.068	<LOD	0.617	<LOD
Zn	0.276	<LOD	0.104	<LOD	0.158	<LOD	0.446	<LOD
As	0.124	<LOD	0.281	<LOD	0.302	<LOD	0.104	0.269
Se	6.577	<LOD	0.493	<LOD	0.272	<LOD	0.318	0.230
Sr	0.012	0.493	0.006	1.672	0.008	<LOD	0.007	<LOD
Mo	0.011	<LOD	0.029	0.064	0.028	<LOD	0.019	0.868
Ag	0.025	<LOD	0.005	<LOD	0.010	0.349	0.007	<LOD
Cd	0.009	<LOD	0.020	0.024	0.025	<LOD	0.011	<LOD
Sn	0.140	<LOD	0.091	<LOD	0.055	0.150	0.037	0.376
Sb	0.006	0.016	0.005	0.024	0.002	1.173	0.004	0.055
Ba	0.030	<LOD	0.013	<LOD	0.016	0.457	0.016	0.030
Hg	0.026	<LOD	0.016	0.018	0.016	0.052	0.017	<LOD
Tl	0.003	<LOD	0.002	0.604	0.002	<LOD	0.002	0.045
Pb	0.003	0.007	0.002	0.099	0.007	0.039	0.013	0.058
U	0.000	0.007	0.000	0.001	0.000	<LOD	0.000	0.001

Note:
Analyzed by EPA ILM05.2D

Table 5. Summary of DOC Blanks and Metal Field Blanks

Sample Type	Parameter	Date Analyzed	Method	Value (mg/L)
RO Water	DOC	7/30/2008	EPA 415.3	0.22
RO Water	TOC	7/30/2008	EPA 415.3	0.22
Tap Water	DOC	7/30/2008	EPA 415.3	2.98
Tap Water	TOC	7/30/2008	EPA 415.3	3.04
Blank 6	DOC/TOC	12/16/2008; 5/2/2009	EPA 415.3	5.95-6.02
Field Blank (DI Water)	Be	7/25/2008	EPA ILM05.2D	0.18
Field Blank (DI Water)	B	7/25/2008	EPA ILM05.2D	<12
Field Blank (DI Water)	Al	7/25/2008	EPA ILM05.2D	22
Field Blank (DI Water)	Ti	7/25/2008	EPA ILM05.2D	5.2
Field Blank (DI Water)	V	7/25/2008	EPA ILM05.2D	0.21
Field Blank (DI Water)	Cr	7/25/2008	EPA ILM05.2D	0.13
Field Blank (DI Water)	Mn	7/25/2008	EPA ILM05.2D	2.4
Field Blank (DI Water)	Fe	7/25/2008	EPA ILM05.2D	8.8
Field Blank (DI Water)	Co	7/25/2008	EPA ILM05.2D	0.18
Field Blank (DI Water)	Ni	7/25/2008	EPA ILM05.2D	0.94
Field Blank (DI Water)	Cu	7/25/2008	EPA ILM05.2D	0.52
Field Blank (DI Water)	Zn	7/25/2008	EPA ILM05.2D	20
Field Blank (DI Water)	As	7/25/2008	EPA ILM05.2D	0.33
Field Blank (DI Water)	Se	7/25/2008	EPA ILM05.2D	<0.49
Field Blank (DI Water)	Sr	7/25/2008	EPA ILM05.2D	107
Field Blank (DI Water)	Mo	7/25/2008	EPA ILM05.2D	0.57
Field Blank (DI Water)	Ag	7/25/2008	EPA ILM05.2D	<0.005
Field Blank (DI Water)	Cd	7/25/2008	EPA ILM05.2D	0.37
Field Blank (DI Water)	Sn	7/25/2008	EPA ILM05.2D	1.8
Field Blank (DI Water)	Sb	7/25/2008	EPA ILM05.2D	0.19
Field Blank (DI Water)	Ba	7/25/2008	EPA ILM05.2D	34
Field Blank (DI Water)	Hg	7/25/2008	EPA ILM05.2D	0.19
Field Blank (DI Water)	Tl	7/25/2008	EPA ILM05.2D	0.61
Field Blank (DI Water)	Pb	7/25/2008	EPA ILM05.2D	0.34
Field Blank (DI Water)	U	7/25/2008	EPA ILM05.2D	0.059

Notes:
DI – deionized
DOC – dissolved organic carbon
RO – reverse osmosis
TOC – total organic carbon

2.5 STATISTICAL ANALYSIS

Statistics were performed using Systat 12 software (Systat, Chicago, IL, USA). To avoid bias introduced by unequal seeding densities, survival analysis was used to analyze

mortality data. Treatment means were expressed throughout as mean ± 1 standard deviation (SD). Comparisons between treatment and control (filtered city water) group means were performed via analysis of variance (ANOVA) or Kruskal-Wallis tests, for parametric and non-parametric data, respectively. Where appropriate, parametric data were subjected to post-hoc Tukey's tests; data that did not meet the assumptions of parametric statistical techniques were analyzed with post-hoc Mann-Whitney U tests. Relationships between survival to exposure termination and initial seeding density were analyzed with a least squares linear regression. Calculations for recommended seeding density were performed based upon the average number of larvae surviving per chamber at termination and the average weight of larvae prior to swim-up. All calculations were performed using individual chambers as replicates, even though chambers were technically pseudo-replicates.

3 RESULTS AND DISCUSSION

3.1 ROUTINE WATER QUALITY PARAMETERS

Average water temperatures over the 61-day exposure period for all treatment groups were 15°C (± 0.13). Other measured water quality parameter were within appropriate and applicable ranges for fish culture (e.g., ASTM 2009); and were generally comparable among all treatments. Averages across all treatment groups for key water quality parameters were as follows:

- DO = 95 ± 1.7 percent
- pH = 7.7 s.u. (± 0.11)
- Conductivity = 132 μS/cm (± 21)
- Hardness = 72.2 mg/L as $CaCO_3$ (± 10.4)
- Total ammonia = 0.07 mg as N/L (± 0.1)
- DOC = 2.21 mg/L (± 0.30).

Details of all measurements are presented in Table 6.

3.2 CONCENTRATIONS OF SELECTED METALS

With few exceptions, there were no statistically significant differences in selected metal concentrations among water types (Figure 3). The only two elements for which significant differences among exposure groups were observed were zinc and lead, with median concentrations and 95th centiles at the DFS being less that those reported for the CTR and UFS groups.

Table 6. Average (AV) and Standard Deviation (SD) of Water Quality Parameter Measurements (n) over the 61-day Exposure Period

Parameter	City Water			UFS			DFS		
	AV	SD	n	AV	SD	n	AV	SD	n
Temp (°C)	15	0.94	507	15	0.55	503	15	0.4	488
DO (%)	96	11	500	96	11	497	93	7.4	486
pH	7.6	0.37	503	7.7	0.34	501	7.8	0.21	482
Cond (µS/cm)	147	6.2	508	142	5.0	503	108	3.3	479
Ammonia (mg/L)	<0.056*	0.1	87	<0.067*	0.15	81	<0.073*	0.12	88
Nitrate (mg/L)	<0.13*	0.021	67	<0.17*	0.08	66	<0.16*	0.063	68
Nitrite (mg/L)	<0.01*	0.002	70	<0.01*	0.002	70	<0.01*	0	65
Hard (mg/L)	72	10	72	72	10	74	72	10	70
Alk (mg/L)	53	5.7	51	57	6	53	56	5.0	62
SO_4 (mg/L)	<0*	0	56	<0.35*	2.6	58	<0*	0	70
PO_4 (mg/L)	<0.025*	0	48	<0.025*	0	45	<0.025*	0	48
Tot Cl (mg/L)	<0.1*	0	73	<0.1*	0	70	<0.099*	0.009	72
DOC (mg/L)	1.9	0.63	17	2.4	0.88	21	2.4	0.88	17

Notes:
Temp – temperature
DO – dissolved oxygen
DOC – dissolved organic carbon
Hard – hardness as $CaCO_3$
SO_4 – sulfate
PO_4 – phosphate
Tot Cl – total chlorine
* The majority of measurements were below the method detection limit.

3.3 HATCHABILITY

For statistical analysis, survival to hatch was expressed as percent hatch

$$\%\,Hatch = \left(\frac{Eggs_T - Eggs_D - Eggs_{FI}}{Eggs_T} \right) \times 100\,\% \qquad \text{Eq. (1)}$$

Where:

$Eggs_T$ = total number of eggs
$Eggs_D$ = Number of dead eggs
$Eggs_{FI}$ = Number of fungus infected eggs

Percent hatch values were, in increasing order, 75.8 percent in downstream river water, 76.2 percent in upstream river water, and 82.2 percent in filtered city water (Figure 4). Although river water treatments tended to have slightly lower hatch rates, this difference was not significant (ANOVA, p = 0.203).

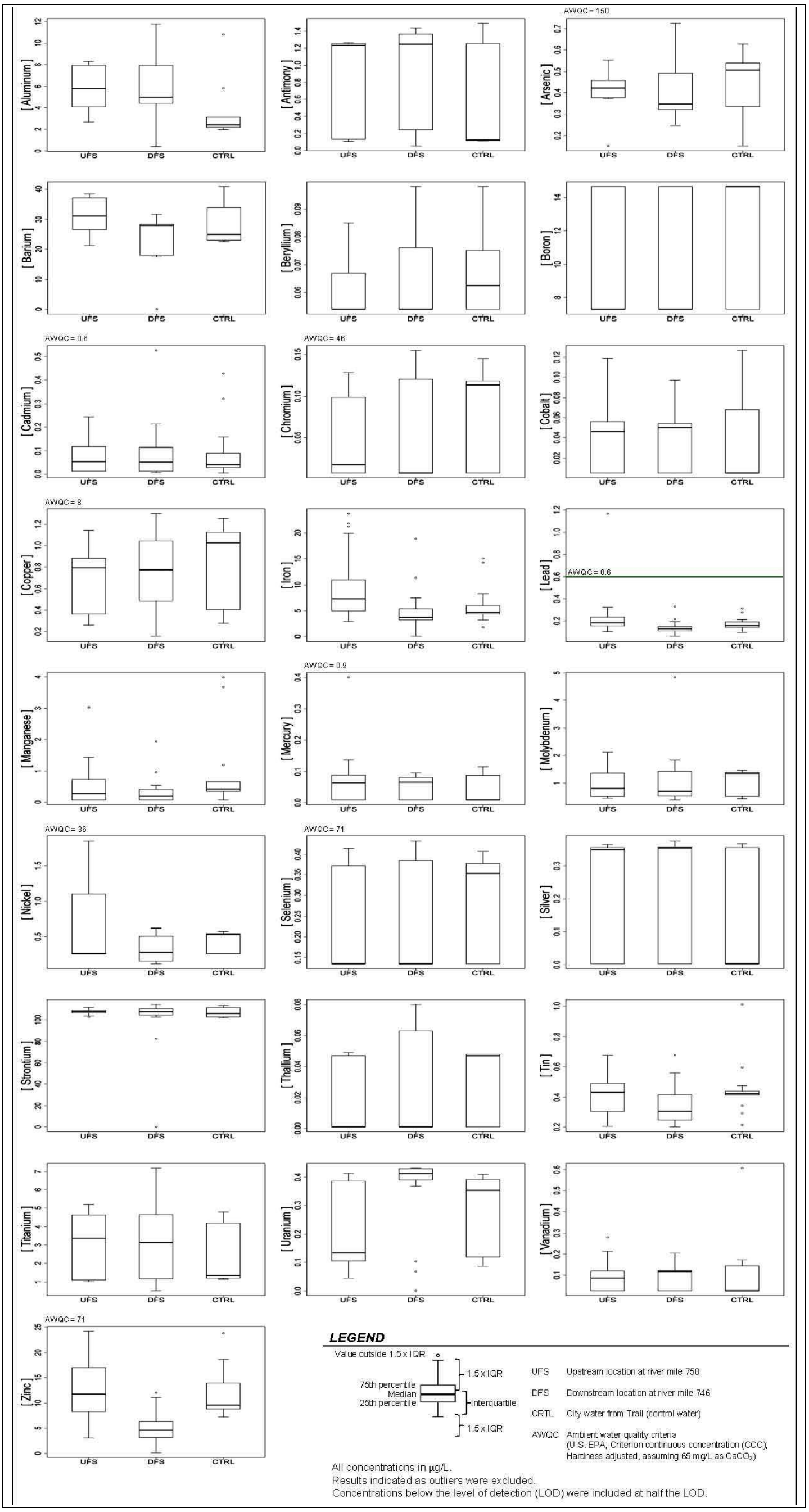

Figure 3. Concentrations of Selected Metals in UFS, DFS and Filtered City (CRTL) Water During the 61-day Exposure Period.

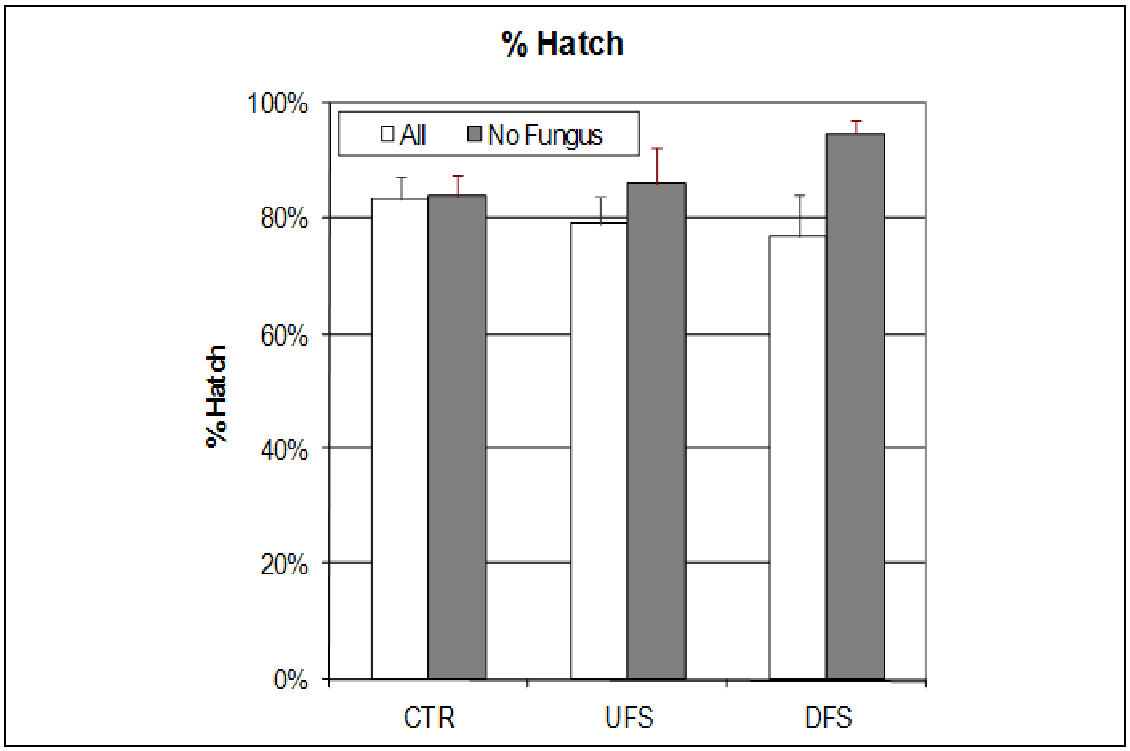

Figure 4. Hatching Success of Fertilized Eggs Incubated in UFS, DFS and Filtered City (CTR) Water
Notes:
Bars represent means ± 1 SD
White bars – all eggs considered
Grey bars – fungus infected eggs excluded

3.4 MORTALITY

Mortality over the course of the experiment was analyzed using a survival analysis, to determine the mean number of days that white sturgeon larvae in each chamber survived. Data were not censored for the survival analysis because unequal seeding led to density-dependent tank effects. The mean number of days of survival did not statistically differ among treatments (Kruskal-Wallis, $p = 0.57$). In fact, the average survival time showed little variation with a range of 28.4 to 29 days post-fertilization inclusive of all treatments (Figure 5). This time period coincides with the complete resorption of the yolk-sac in white sturgeon larvae.

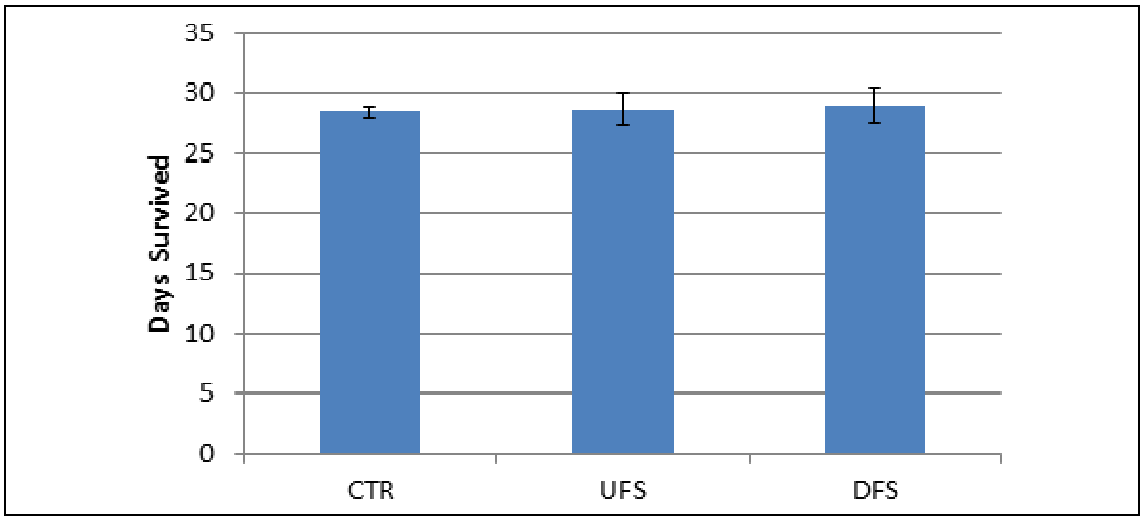

Figure 5. Average Number of Days (mean ± 1 SD) Fish Survived in River (UFS and DFS) and Filtered City (CTR) Water

Although an unequal number of fish were seeded into the tanks at exposure initiation, the number of fish surviving at experiment termination did not differ between treatments (ANOVA, $p = 0.065$). Individual chambers, although technically pseudo-replicates, were treated as true replicates for this analysis since survival seemed to be more dependent upon density than treatment water. The average number of fish surviving per chamber across all treatments was 100.9 fish. Within treatments, the average number of fish surviving ranged from 91.3 at the UFS to 106.2 at the DFS, and 105.2 in the CTR.

Since mortality was suspected to be dependent upon initial seeding density, the relationship between the number of fish that died during the experiment and seeding density was explored. 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 ($R^2 = 0.983$; $p < 0.001$). In general, if more fish were seeded initially, more fish died during the experiment. To determine whether the relationship between seeding density and mortality was more widely applicable than just the current experiment, control data from the U of S laboratory experiment run concurrently in 2008 was added to the curve (ENTRIX 2011). Data from these chambers fell on the regression line of the original curve, and their inclusion slightly strengthened the relationship (Figure 6; $R^2 = 0.9857$).

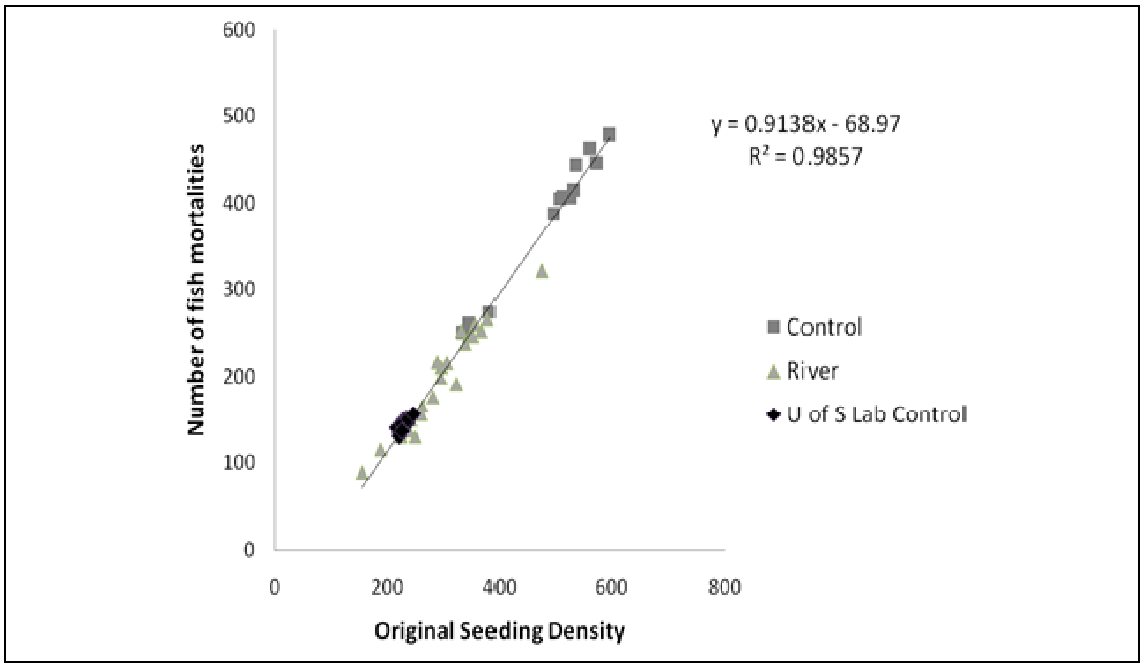

Figure 6. Fish Mortality at Study Termination as a Function of Original Seeding Density in Experimental Chambers

Notes:
River – exposure in river water
Control – exposure in control water
U of S Lab Control – exposure in control water of a parallel chronic laboratory study at the University of Saskatchewan using the same batch of fish.

When stratified by life stage, it is evident that the relationship of seeding density to fish survival is unique to the transition of larva to exogenous feeding between Day 23 and 34 after initiation of the experiment. This may be an indication that early life-stages of white sturgeon are particularly sensitive to competition during the transition to feeding. The mortality observed is consistent with the expected mortality of sturgeon during this period when the larvae are transitioning from yolk sac to exogenous feed (Conte et al. 1998; Bennett and Farrell 1998; 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. The variability in survival during this transition period is generally 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 morphophysiological changes 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). Groups that routinely spawn and breed sturgeon such as the Kootenay Trout Hatchery, Canada, the Columbia Basin Hatchery, USA, and the University of California, USA also experience die offs during this transition phase

(Figure 7), and ongoing work is focused on developing an optimal sturgeon feed to maximize survival (Ek 2008, pers. comm.; Lyon 2008, pers. comm.; Van Eenennaam 2008, pers. comm.). Based on these observations, it appears that the transition to exogenous feeding represents a period during the early development of white sturgeon that often is characterized by naturally great mortality.

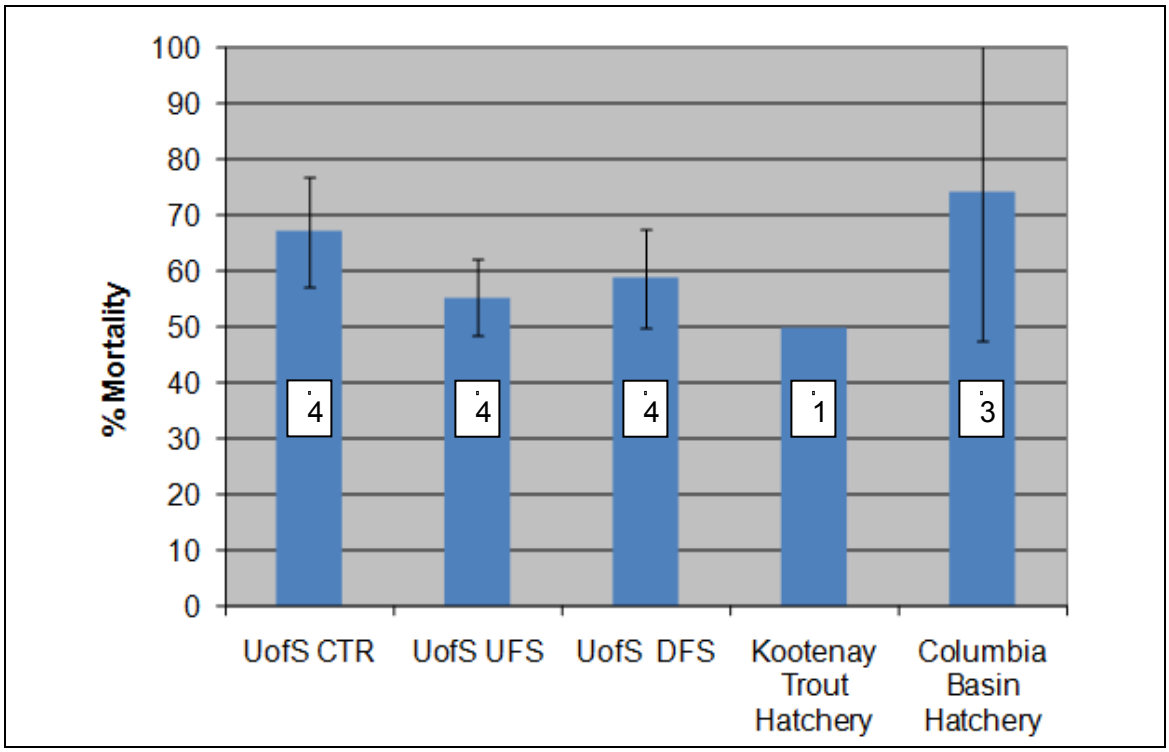

Figure 7. Mortality Rates (mean ± 1 SD) of Early Life Stages of White Sturgeon Observed During the 2008 Surface Water Toxicity Studies and at Two Other Institutions: Kootenay Trout Hatchery, Columbia Basin Hatchery
Note:
Numbers in bars represent number of replicates used to calculate average values.

However, as soon as sturgeon successfully adapt to exogenous food, they seem to be more robust with regard to this factor, demonstrated by the lack of a relationship between seeding density and number of dead fish at the juvenile life-stage. 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. However, one hypothesis is that the surface area is an important factor in density related mortality. It is assumed that the surface area is proportional to the amount of available biofilm and algae that likely are important "primer" food items during the very early stage of transition to feeding. This hypothesis has not yet been tested, but may be important in understanding the causes of mortality in early life stages of white sturgeon. Furthermore, there were no statistical differences in water quality (DO, ammonia, nitrate, nitrite, pH, temperature, phosphate, DOC, total organic carbon [TOC], etc.) among the

treatments eliminating the possibility that elevated mortalities in the exposure systems with greater fish densities were due to differences in water quality.

3.5 LENGTH AND WEIGHT

Weight and length values were relatively consistent among chambers, and therefore, chamber size and number of fish per chamber did not appear to have affected growth. As a consequence, chambers were treated as pseudo-replicates for this part of the analysis. Accordingly, Kruskal-Wallis tests were performed on the three pseudo-replicated chambers in each replicate first. All chambers that were not significantly different from the others were pooled. Chambers with significant differences were treated as true replicates in subsequent analyses.

Replicates, as determined above, were compared to determine differences among treatments in weight and length at exposure termination. There were no treatment differences in fish weight at exposure termination (ANOVA, $p = 0.683$). Mean mass per replicate values were 0.379g in CTR, 0.396g in UFS, and 0.375g in DFS water (Figure 8). In addition, there were no significant differences in fish length at exposure termination (ANOVA, $p = 0.908$). Mean length per replicate values were 40.61 mm in CTR water, 40.9 mm in UFS water, and 40.51 mm in DFS water (Figure 9).

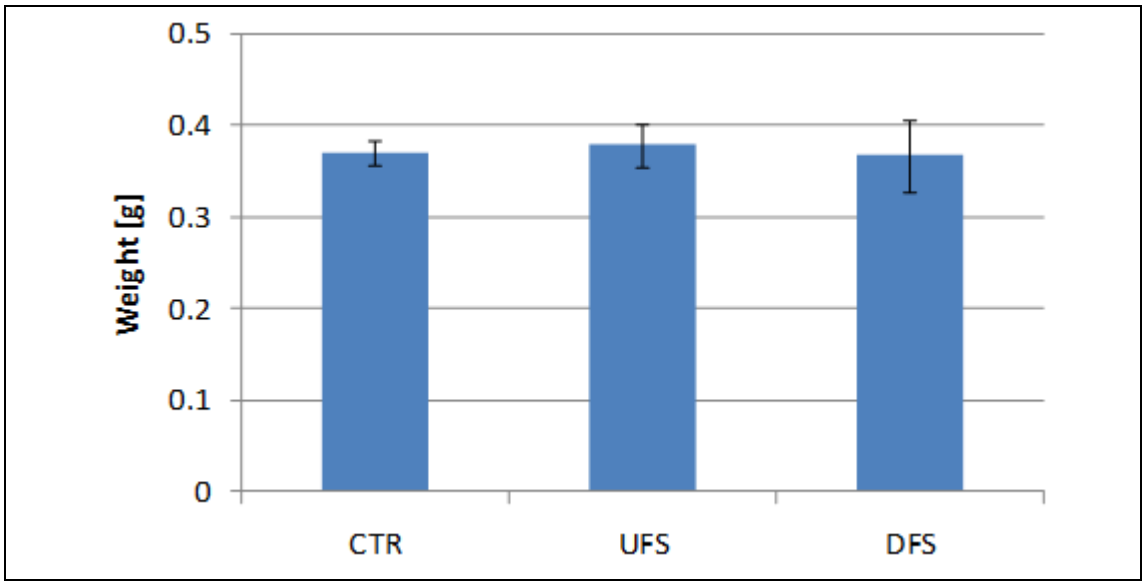

Figure 8. Average Weight (mean ± 1 SD) of White Sturgeon Fry at Termination of Experiment

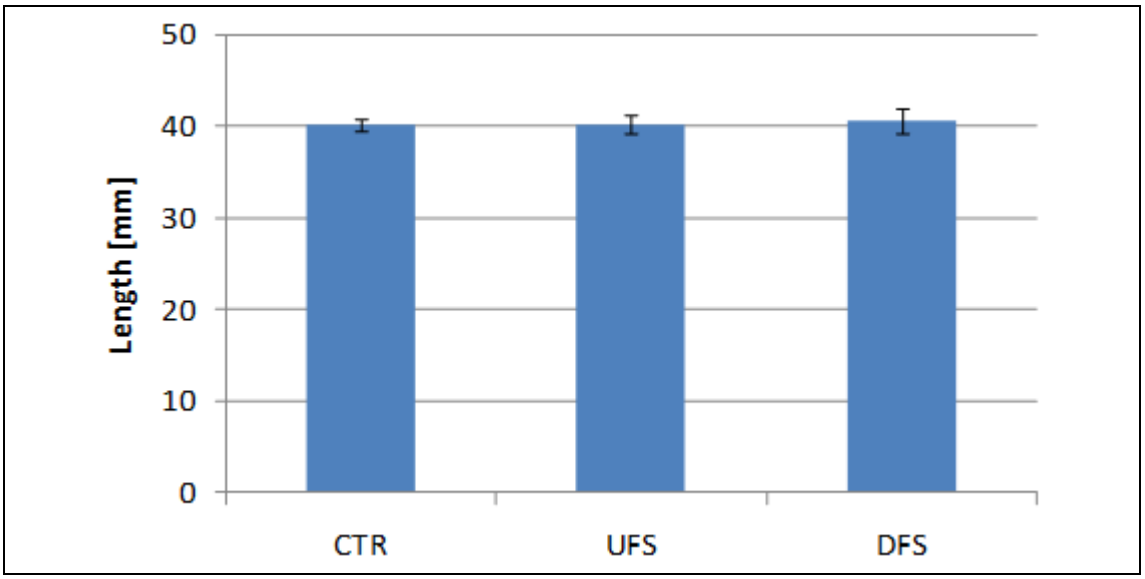

Figure 9. Average Length (mean ± 1 SD) of White Sturgeon Fry at Termination of Experiment

3.6 BLM PREDICTIONS OF METAL CONCENTRATIONS ASSOCIATED WITH CHRONIC EFFECTS

The biotic ligand model (BLM) was previously calibrated using the metal concentrations and associated toxicological responses for cadmium, copper, and zinc effects in chronic exposures to white sturgeon (Entrix 2011). The calibrated model was used in this study to predict the concentrations of these metals that would be associated with chronic mortality (as indicated by the LC20). Predicted average LC20 values and average river concentrations for these metals are shown in Table 7 for the UFS and DFS. Average concentrations of cadmium, copper, and zinc in river water are well below the LC20 values, suggesting that no chronic mortality would be expected as a result of metal exposures in these tests.

Table 7. Average Biotic Ligand Model (BLM)-predicted LC20 Values for Cadmium (Cd), Copper (Cu), and Zinc (Zn) Compared with Average Measured Metal Concentrations at the Same Field Stations

	Cd (µg/L)		Cu (µg/L)		Zn (µg/L)	
	LC20	River	LC20	River	LC20	River
UFS	3.3	0.08	3.3	0.69	148	12.8
DFS	3.7	0.09	4.6	0.79	168	5.1

4 CONCLUSIONS

In conclusion, this study did not find differences in biological responses of white sturgeon life-stages raised in Columbia River water upstream or downstream of Teck's Trail

smelter. In fact, survival, weight and length of fry were comparable not only between fish exposed to the two riverine waters (UFS and DFS) but also between the filtered clean control water. This finding is consistent with BLM predictions for chronic effects from cadmium, copper, and zinc, since predicted LC20s are much higher than average concentrations for these metals in the test waters.

There was a relatively great mortality across all treatment groups that occurred during the transition of fish to exogenous feeding regardless of treatment group, and an apparent density-dependent effect on mortality due to unequal seeding of larvae in the test tanks.

5 REFERENCES

ASTM (American Society for Testing and Materials). 2009. Standard guide for conducting early life-stage toxicity tests with fishes. ASTM E 1241 – 05. American Society for Testing and Materials, West Conshohocken, Pennsylvania, USA.

Bardi, R.W., F.A. Chapman, F.T. Barrows. 1998. Feeding trials with hatchery-produced Gulf of Mexico sturgeon larvae. *Prog. Fish-Cult*. 60, 25-31.

Bennett, W.R. and A.P. Farrell. 1998. Acute toxicity testing with juvenile white sturgeon (*Acipenser transmontanus*). *Water Qual. Res. J. Can*. 33(1), 95-110.

Buddington, R.K. 1991. Ontogenic development of sturgeons: selected physiological examples, in: Williot, P., (Ed.), *Acipenser*, actes de premier colloque international sur l'esturgeon. CEMAGREF-DICOVA, Bordeaux, France, 53-63.

Buddington, R.K. and J.P. Christofferson. 1985. Digestive and feeding characteristics of the chondrosteans. In: Binkowski, F.P., and S.I. Doroshov, (Eds.), North American sturgeons: biology and aquaculture potential. Dr. W. Junk, Dordrecht, Netherlands, 31-41.

Conte, F.S., S.I. Doroshov, P.B. Lutes, and E.M. Strange. 1988. Hatchery manual for the white sturgeon *Acipenser transmontanus* Richardson with application to other north American Acipenseridae. University of California, Division of Agriculture and Natural Resources, Oakland, California.

Creed, J.T., C.A. Brockhoff, and T.D. Martin. 1994. Determination of trace elements in water and wastes by inductively coupled plasma-mass spectrometry. Environmental Monitoring System Laboratory Office of Research and Development U.S. Environmental Protection Agency. Method 200.8.

Ek, R. 2008. Personal communication. Senior Fish Culturist, Kootenay Sturgeon Conservation Hatchery, Fort Steele, British Columbia.

ENTRIX. 2011. Semi-chronic water exposures of cadmium, copper, and zinc to early life-stages of white sturgeon (*Acipenser transmontanus*). Prepared for Teck American Inc. by Entrix and University of Saskatchewan. January 2011.

Gisbert, E. and P. Williot. 1997. Larval behaviour and effect of the timing of initial feeding on growth and survival of Siberian sturgeon (*Acipenser baeri)* larvae under small scale hatchery production. *Aquaculture*. 156, 63-76.

Howell, M.D. and J.G. McLellan. 2006. Lake Roosevelt white sturgeon recovery project, annual report January 2004 – March 2005. Bonneville Power Administration, Project No. 1995-027-00. Portland, Oregon.

Lutes, P.B., S.S.O. Hung, and F.S. Conte. 1990. Survival, growth, and body composition of white sturgeon larvae fed purified and commercial diets at 14.7 and 18.4°C. *Prog. Fish-Cult*. 52(3), 192-196.

Lyon, B. 2008. Personal communication. Fish Culturist, Columbian Basin Hatchery, Moses Lake, Washington.

Mohler, J.W., M.K. King, and P.R. Farrell. 2000. Growth and survival of first-feeding and fingerling atlantic sturgeon under culture conditions. *N. Am. J. Aquacult*. 62(3), 174-183.

Van Eenennaam, J. 2008. Personal communication. Research Associate, University of California, Davis, California.