UPPER COLUMBIA RIVER

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

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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 potential concern (COPCs) 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 14, 2009. Hatchery staff collected gametes from two female and six male adult white sturgeon captured in the CR near Waneta, Canada (Ek 2009, 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 (hrs) of fertilization. Embryos were acclimated to test waters for 1 hr before being incubated in McDonald-type hatching jars (Aquatic Ecosystems, Apopka, FL). Following neurulation (i.e., within 72 hrs 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 20 and 25, respectively; approximately 6 to 11 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., upstream and downstream 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 at RM 746. Field sites are illustrated in Figure 1 with coordinates provided in Table 1.

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. Carbon filtered City of Trail potable water was used as the control (CTR).

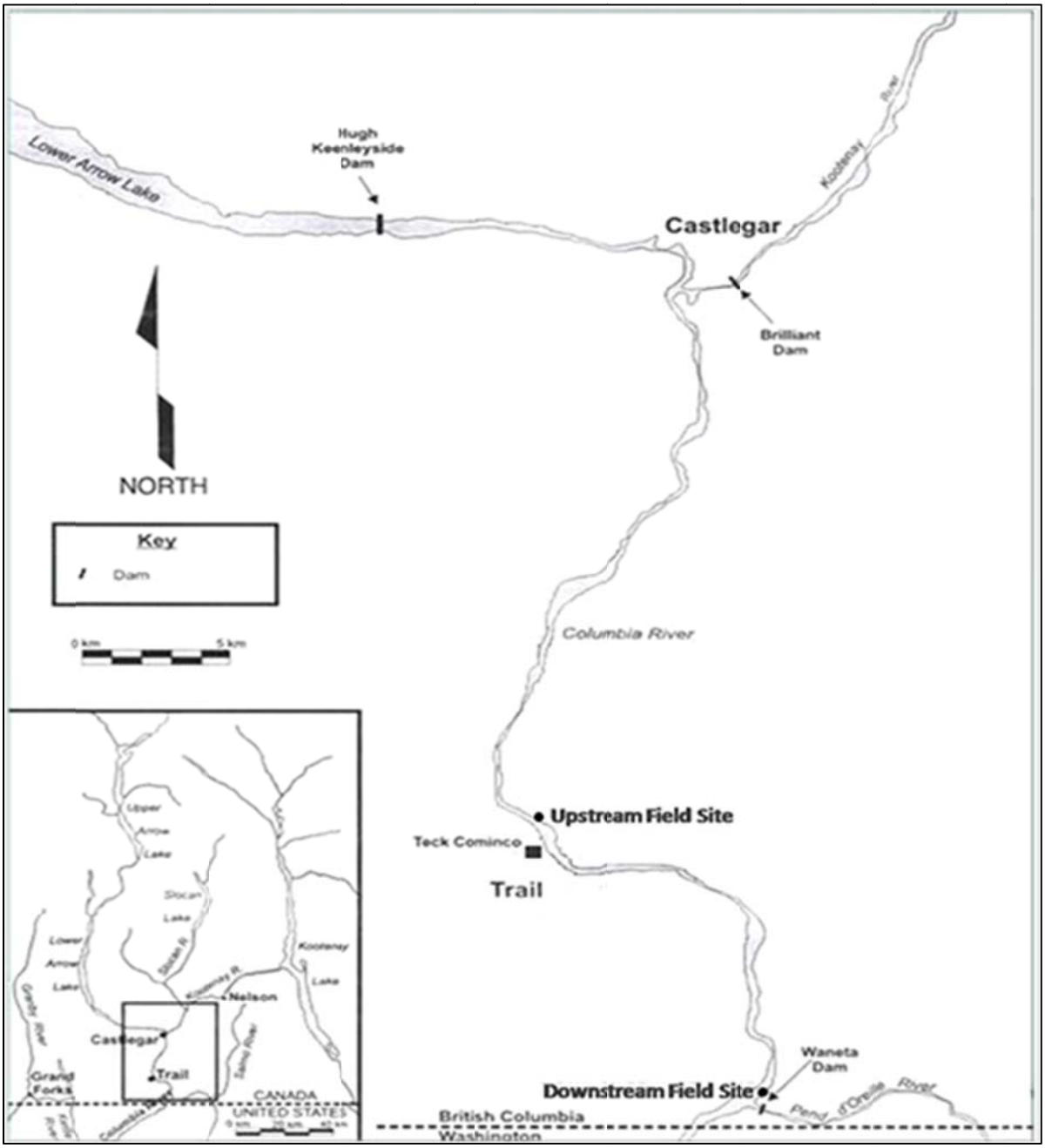

Figure 1. Sampling Area in the Columbia River (from Entrix 2011a)

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 City of Trail Potable 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

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 6 hrs. 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 hrs. All treatments were run in four replicate systems. A summary of the study design and associated treatments is presented within Tables 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 hrs
Exposure duration	69 days (> 40 days post swim-up)
Loading density/rate	< 0.1 g/L/24 hrs
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	≥ 4 times per day

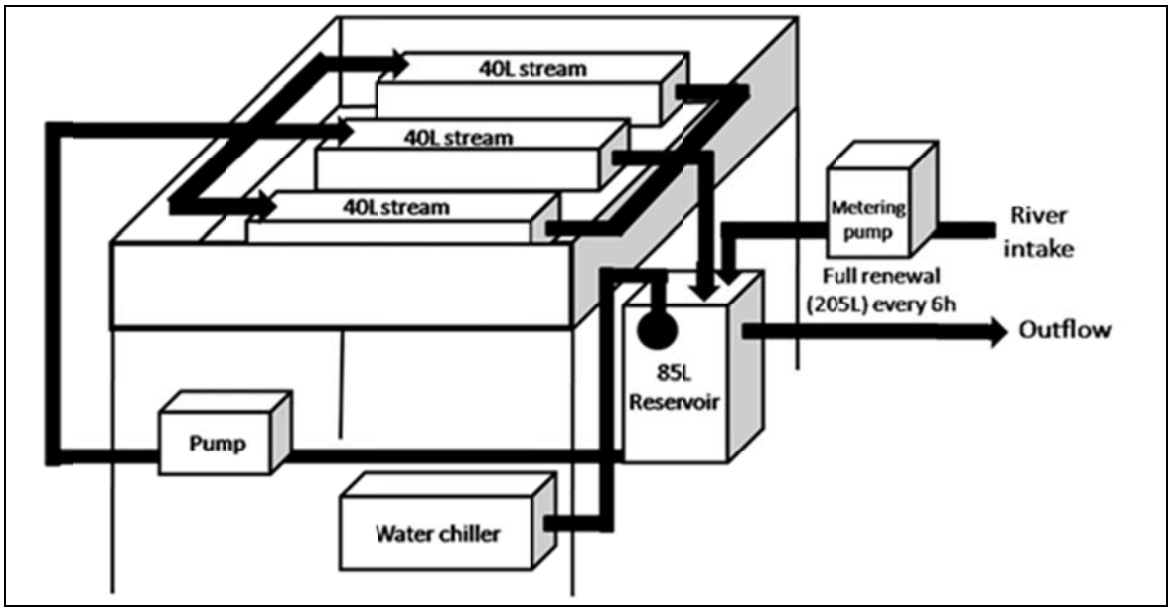

Figure 2. Flow-through Recirculating Exposure Systems

Day 0 of the study (July 14, 2009), coincided with the placement of 1500 embryos within dedicated McDonald hatching jars in each replicate per treatment group. Total test duration (exposure) extended for 69 days and concluded on September 20 through 21, 2009.

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 days post-hatch (dph) (July 31, 2009), food (live brine shrimp [*Artemia salina*] and frozen bloodworms), were 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 # 14002-860, 11388-374, and 11388-372). Following the first 2 weeks of the study where bi-weekly measurements were initially made, weekly measures of nitrate, nitrite, and total chlorine were made in each replicate system using LaMotte colorimetric and titrator test kits directly on site throughout the duration of the study (Table 3).

In addition to the above-mentioned routine water quality parameters measured on site, weekly water samples were collected from each exposure chamber and analyzed for total metals (i.e., target analyte list [TAL] metals), hardness, alkalinity, ammonia, sulfate, phosphorous, dissolved organic carbon (DOC), total organic carbon (TOC), total dissolved solids (TDS), total suspended solids (TSS), silica, and selected cations by Columbia Analytical Services (CAS) (Kelso, Washington, USA). All samples were collected using acid-cleaned polyethylene bottles, filtered through a 0.45 micrometer (µm) polycarbonate filter with Nalgene® filter holders and receivers as required (e.g., DOC). Samples for TAL, cations, ammonia, DOC, TOC, phosphorous, and hardness analysis were acidified with ultrapure sulfuric, nitric or hydrochloric acid to a pH <2 standard units (s.u.) as appropriate. All samples were maintained at approximately 4°C for shipment to the CAS analytical laboratory.

With the exception of mercury analysis, TAL metal analyses were performed using inductively coupled plasma mass spectrometry (ICP-MS) following Environmental Protection Agency (EPA) Method 6020. Mercury was analyzed following the methods described in EPA 1631E by means of cold vapor atomic fluorescence spectrometry. DOC and TOC analysis was performed weekly in accordance with method SM 5310 C. 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

Parameter	Laboratory	Method	Unit	LOD
Water Quality Parameters				
Temperature	U of S	VWR Symphony 14002-860	˚C	0
pH	U of S	VWR Symphony 14002-860	s.u.	0
DO	U of S	VWR Symphony 11388-374	mg/L	2
Conductivity	U of S	VWR Symphony 11388-372	µS/cm	1
Ammonia-Nitrogen	U of S	LaMotte Kit 3304	mg/L	0.05
Nitrate	U of S	LaMotte Kit 3319	mg/L	0.25
Nitrite	U of S	LaMotte Kit 7674	mg/L	0.02
Total Chlorine	U of S	LaMotte Kit 6905	mg/L	0.2
Alkalinity	CAS	SM2320B	mg/L	1.0
Ammonia N	CAS	SM5310C	mg/L	0.009
DOC	CAS	SM5310C	mg/L	0.07
Alkalinity	CAS	SM2320B	mg/L	1.0
Chloride	CAS	EPA300.0	mg/L	0.06
Fluoride	CAS	EPA300.0	mg/L	0.01
Hardness ($CaCO_3$)	CAS	SM2340C	mg/L	0.8
Nitrite-Nitrate	CAS	EPA300.0	mg/L	0.009
Phosphorus	CAS	EPA365.3	mg/L	0.004
Silica	CAS	EPA370.1	mg/L	0.08

Table 3. Analytical Methods and Associated Method Detection Limits (continued)

Parameter	Laboratory	Method	Unit	LOD
Water Quality Parameters (continued)				
Sulfate	CAS	EPA300.0	mg/L	0.02
TDS	CAS	SM2540	mg/L	5.0
TOC	CAS	SM5310C	mg/L	0.07
TSS	CAS	SM2540	mg/L	5.0
TAL Metals and Ions				
Aluminum	CAS	EPA6020	µg/L	0.3
Antimony	CAS	EPA6020	µg/L	0.05
Arsenic	CAS	EPA6020	µg/L	0.1
Barium	CAS	EPA6020	µg/L	0.02
Beryllium	CAS	EPA6020	µg/L	0.006
Cadmium	CAS	EPA6020	µg/L	0.005
Calcium	CAS	EPA6020	µg/L	6.0
Chromium	CAS	EPA6020	µg/L	0.02
Cobalt	CAS	EPA6020	µg/L	0.006
Copper	CAS	EPA6020	µg/L	0.02
Iron	CAS	EPA6010B	µg/L	3.0
Lead	CAS	EPA6020	µg/L	0.005
Magnesium	CAS	EPA6020	µg/L	2.0
Manganese	CAS	EPA6020	µg/L	0.06
Mercury	CAS	EPA1631E	µg/L	0.05
Nickel	CAS	EPA6020	µg/L	0.02
Potassium	CAS	EPA6020	µg/L	50
Selenium	CAS	EPA6020	µg/L	0.3
Silver	CAS	EPA6020	µg/L	0.004
Sodium	CAS	EPA6020	µg/L	20
Strontium	CAS	EPA6010B	µg/L	0.07
Thallium	CAS	EPA6020	µg/L	0.005
Titanium	CAS	EPA6010B	µg/L	0.4
Vanadium	CAS	EPA6020	µg/L	0.03
Zinc	CAS	EPA6020	µg/L	0.2

Notes:
All samples collected between 7/10/2009 and 9/21/2009
Measurements conducted in individual, randomly selected test chambers
$CaCO_3$ – calcium carbonate
CAS – Columbia Analytical Services
TAL – target analyte list
LOD – level of detection
DO – dissolved oxygen
DOC – dissolved organic carbon
TOC – total organic carbon
TDS – total dissolved solids
TSS – total suspended solids
U of S – University of Saskatchewan

Table 4. Minimum and Maximum Concentrations in Method Blanks

Parameter	Minimum	Maximum	Unit
Alkalinity	--	<1	mg/L
Ammonia-Nitrogen	--	<0.009	mg/L as N
TOC	<0.07	0.37	mg/L
Chloride	--	<0.06	mg/L
Fluoride	--	<0.01	mg/L
Hardness	--	<0.8	mg/L as $CaCO_3$
Nitrite-Nitrate	--	<0.009	mg/L as N
pH	5.6	7.22	s.u.
Phosphorus	--	<0.004	mg/L
Silica	--	<0.08	mg/L
TDS	--	≤5	mg/L
TSS	--	<5	mg/L
Sulfate	--	<0.02	mg/L
Mercury	0.032	4.5	ng/L
Aluminum	0.5	1.6	µg/L
Antimony	<0.05	0.006	µg/L
Arsenic	--	<0.1	µg/L
Barium	<0.02	0.086	µg/L
Beryllium	<0.006	0.011	µg/L
Cadmium	--	<0.005	µg/L
Calcium	<6	394	µg/L
Chromium	0.03	0.76	µg/L
Cobalt	<0.006	0.011	µg/L
Copper	<0.02	0.03	µg/L
Iron	<3	6.2	µg/L
Lead	<0.005	0.01	µg/L
Magnesium	<2	5.2	µg/L
Manganese	<0.06	0.10	µg/L
Nickel	<0.02	0.19	µg/L
Potassium	--	<50	µg/L
Selenium	--	<0.3	µg/L
Silver	--	<0.004	µg/L
Sodium	--	≤20	µg/L
Strontium	<0.07	0.1	µg/L
Thallium	--	<0.005	µg/L
Titanium	<0.4	0.6	µg/L
Vanadium	--	<0.03	µg/L
Zinc	<0.2	0.4	µg/L

Notes:
-- – Maximum concentration = level of detection (LOD)
$CaCO_3$ – calcium carbonate
N – nitrogen
TDS – total dissolved solids
TSS – total suspended solids

Table 5. Summary of Field Blanks in Deionized Water

Location	Parameter	Laboratory	Value (min-max)	Unit
Water Quality Parameters				
UFS	Ammonia-Nitrogen	U of S	<0.02 – 0.08[a]	mg/L
DFS	Ammonia-Nitrogen	U of S	<0.25[b]	mg/L
UFS	Nitrate	U of S	<0.25[b]	mg/L
DFS	Nitrate	U of S	<0.25[b]	mg/L
UFS	Nitrite	U of S	<0.02[b]	mg/L
DFS	Nitrite	U of S	<0.02[b]	mg/L
UFS	Hardness	CAS	<0.8[b]	mg/L
DFS	Hardness	CAS	<0.8[b]	mg/L
UFS	Alkalinity	CAS	<1.0[b]	mg/L
DFS	Alkalinity	CAS	<1.0[b]	mg/L
UFS	Total Chlorine	U of S	<0.02[b]	mg/L
DFS	Total Chlorine	U of S	<0.02[b]	mg/L
UFS	DOC	CAS	0.3-1.6	mg/L
DFS	DOC	CAS	0.28-2.5	mg/L
UFS	TOC	CAS	0.19-0.85	mg/L
DFS	TOC	CAS	0.07-0.32	mg/L
TAL Metals				
UFS	Aluminum	CAS	<0.5-1.5	µg/L
DFS	Aluminum	CAS	<0.5-18	µg/L
UFS	Antimony	CAS	<0.005-0.031	µg/L
DFS	Antimony	CAS	<0.005-0.009	µg/L
UFS	Arsenic	CAS	<0.1	µg/L
DFS	Arsenic	CAS	<0.1	µg/L
UFS	Barium	CAS	<0.02-0.07	µg/L
DFS	Barium	CAS	<0.02-0.11	µg/L
UFS	Beryllium	CAS	<0.006-0.013	µg/L
DFS	Beryllium	CAS	<0.006-0.009	µg/L
UFS	Boron	CAS	<0.020.067	µg/L
DFS	Boron	CAS	<0.02-0.11	µg/L
UFS	Cadmium	CAS	<0.005-0.025	µg/L
DFS	Cadmium	CAS	<0.005-0.178	µg/L
UFS	Chromium	CAS	0.04-0.08	µg/L
DFS	Chromium	CAS	0.04-0.08	µg/L
UFS	Cobalt	CAS	<0.006-0.016	µg/L
DFS	Cobalt	CAS	<0.006	µg/L
UFS	Copper	CAS	<0.02-0.05	µg/L
DFS	Copper	CAS	<0.02-0.12	µg/L
UFS	Iron	CAS	<3.0-3.9	µg/L

Table 5. Summary of Field Blanks in Deionized Water (continued)

Location	Parameter	Laboratory	Value (min-max)	Unit
TAL Metals (continued)				
DFS	Iron	CAS	<3.0-8.5	µg/L
UFS	Lead	CAS	<0.005-0.024	µg/L
DFS	Lead	CAS	<0.005-0.094	µg/L
UFS	Manganese	CAS	<0.006-0.034	µg/L
DFS	Manganese	CAS	<0.006-0.088	µg/L
UFS	Mercury	CAS	0.28-2.25	ng/L
DFS	Mercury	CAS	0.14-4.46	ng/L
UFS	Nickel	CAS	<0.02-0.03	µg/L
DFS	Nickel	CAS	<0.02-0.04	µg/L
UFS	Selenium	CAS	<0.3	µg/L
DFS	Selenium	CAS	<0.3	µg/L
UFS	Silver	CAS	<0.004-0.008	µg/L
DFS	Silver	CAS	<0.004-0.006	µg/L
UFS	Strontium	CAS	<0.07-<0.1	µg/L
DFS	Strontium	CAS	<0.07-<0.1	µg/L
UFS	Thallium	CAS	<0.005-0.026	µg/L
DFS	Thallium	CAS	<0.005	µg/L
UFS	Titanium	CAS	<0.4	µg/L
DFS	Titanium	CAS	<0.4-1.1	µg/L
UFS	Vanadium	CAS	<0.03	µg/L
DFS	Vanadium	CAS	<0.03	µg/L
UFS	Zinc	CAS	<0.2-0.5	µg/L
DFS	Zinc	CAS	<0.2-0.7	µg/L

Notes:
All metals represent unfiltered samples due to contamination issues with dissolved samples
[a] Majority of values below detection limit of assay
[b]All values below detection limit of assay
CAS – Columbia Analytical Services
DFS – downstream field site
DOC – dissolved organic carbon
TAL – target analyte list
TOC – total organic carbon
U of S – University of Saskatchewan
UFS – upstream field site

2.5 STATISTICAL ANALYSIS

Statistical analyses were conducted using either Systat 12 software (Systat, Chicago, IL, USA), R (R Development Core Team 2009), or Microsoft Excel. Statistical significance was accepted when $p < 0.05$. Mortalities were expressed as average percent of fish surviving in

a chamber. Furthermore, survival analysis was used to analyze mortality data and is detailed in Appendix A. Treatment means are expressed throughout as mean ± 1 standard deviation (SD). Comparisons between treatment and CTR (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. 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 temperature over the 69-day exposure period for all treatment groups was 15°C (± 0.7). Other measured water quality parameters 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 = 96 ± 16 percent
- pH = 7.6 s.u. (± 0.5)
- Conductivity = 127 µS/cm (± 8.8)
- Hardness = 56.8 mg/L as $CaCO_3$ (± 0.08)
- Total ammonia = 0.02 mg/L as N (± 0.03)
- DOC = 2.48 mg/L (± 0.36)
- TOC = 2.04 mg/L (±0.31)
- Alkalinity = 52.2 mg/L as $CaCO_3$ (± 1.6).

Details of all measurements are presented in Table 6.

3.2 CONCENTRATIONS OF SELECTED METALS

With few exceptions, there were no marked differences in selected metal concentrations among water types (Figure 3). The only three elements for which differences between the UFS and DFS exposure groups were observed were antimony, thallium and copper. Median concentrations and 95th centiles at the DFS were less that those reported for the UFS group for copper, and greater than those reported for antimony and thallium. Furthermore, median concentrations and 95th centiles for aluminum and barium were greater in CTR water than those measured in the DFS and UFS treatment groups. It should be noted that all comparisons of metal concentrations were performed on total

(not dissolved) metals, due to concerns about contamination of blanks during sampling and/or analyses of the dissolved samples.

Table 6. Average (AV) and Standard Deviation (SD) of Water Quality Parameter Measurements (n) over the 69-day Exposure Period

		CTR			UFS			DFS		
Parameter	Laboratory	AV	SD	n	AV	SD	n	AV	SD	n
Temp (°C)	U of S	15	0.98	233	15	0.38	209	15	0.52	229
DO (%)	U of S	95	16	233	96	16	209	99	14	254
pH	U of S	7.6	0.37	221	7.7	0.53	190	7.6	0.62	217
Cond (µS/cm)	U of S	130	8.8	228	126	8.6	205	127	8.5	225
Ammonia (mg/L)	U of S	0.028*	0.043	43	0.028*	0.037	36	<0.02*	0.006	39
Nitrate (mg/L)	U of S	0.46*	0.84	35	0.36*	0.072	30	<0.69*	1.5	30
Nitrite (mg/L)	U of S	<0.02*	0.019	46	<0.02*	0.002	39	<0.02*	0	38
Hard (mg/L)	CAS	57	2.0	50	57	1.6	48	57	2.0	45
Alk (mg/L)	CAS	50	1.6	49	53	1.9	47	53	1.2	45
SO_4 (mg/L)	CAS	12	0.86	48	9.9	0.38	48	10	0.45	44
Tot Cl (mg/L)	U of S	<0.2*	0	42	<0.2*	0	31	<0.02*	0	31
DOC (mg/L)	CAS	2.39	0.81	49	2.8	0.93	46	2.9	1.2	44
TOC (mg/L)	CAS	1.74	0.52	48	2.3	0.58	48	2.4	.86	43

Notes:
* Majority of measurements were below the method detection limit.
Alk – alkalinity
CAS – Columbia Analytical Services
Cond – conductivity
CTR – control
DFS – downstream field site
DO – dissolved oxygen
DOC – dissolved organic carbon
Hard – hardness as $CaCO_3$
PO_4 – phosphate
SO_4 – sulfate
Temp – temperature
Tot Cl – total chlorine
U of S – University of Saskatchewan
UFS – upstream field site

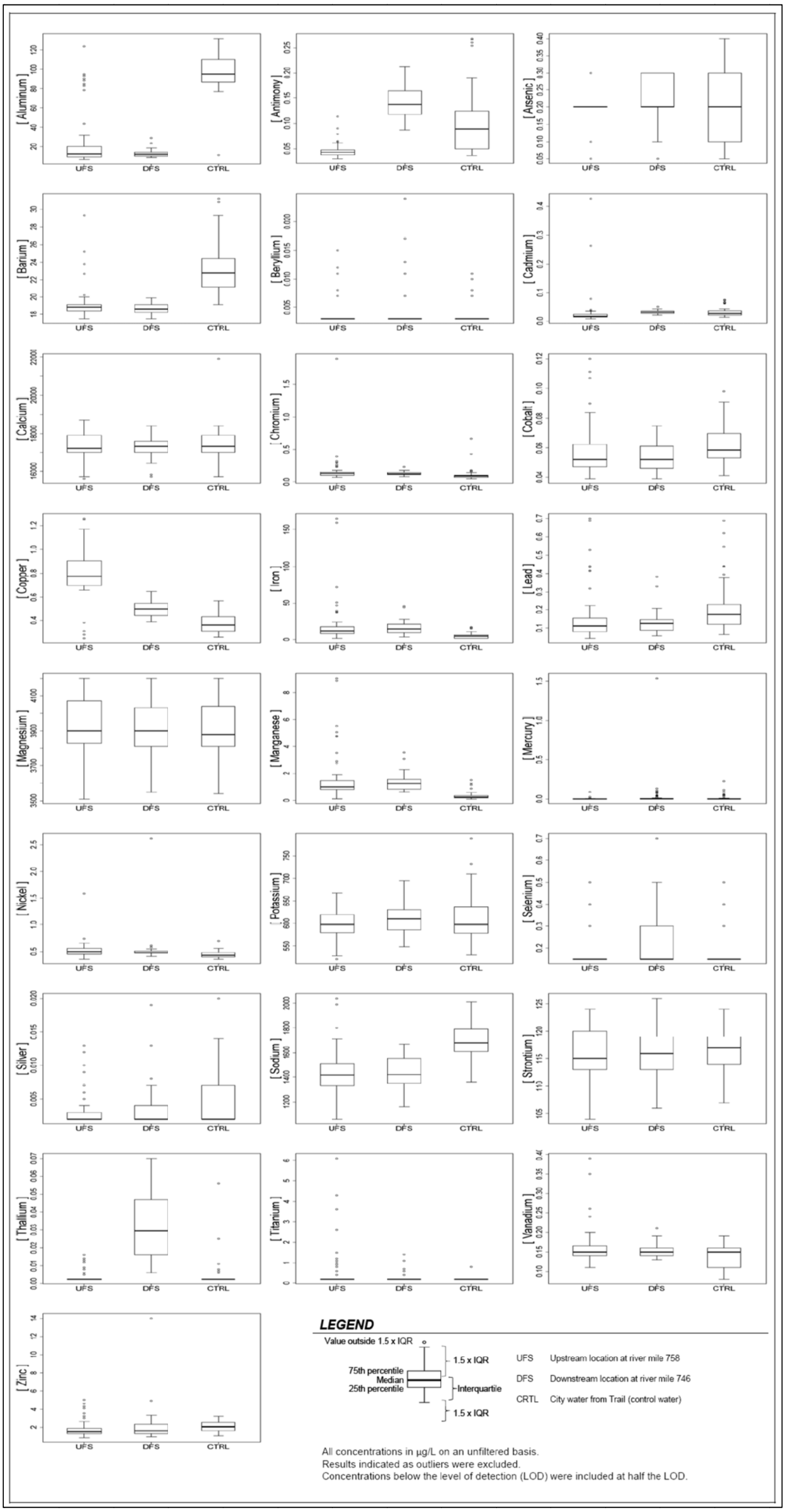

Figure 3. Concentrations of Total Metals in River (UFS and DFS) and Filtered City (CTR) Water During the 69-day Exposure Period

3.3 MORTALITY

Average mortality over the entire study duration of ELS white sturgeon in all treatment groups was between 58 and 60 percent (Figure 4). No statistically significant differences were observed among any of the treatment groups. Variation among replicate test systems of the same treatment group was 8.7, 4.0 and 3.8 percent in the CTR, UFS and DFS treatments, respectively.

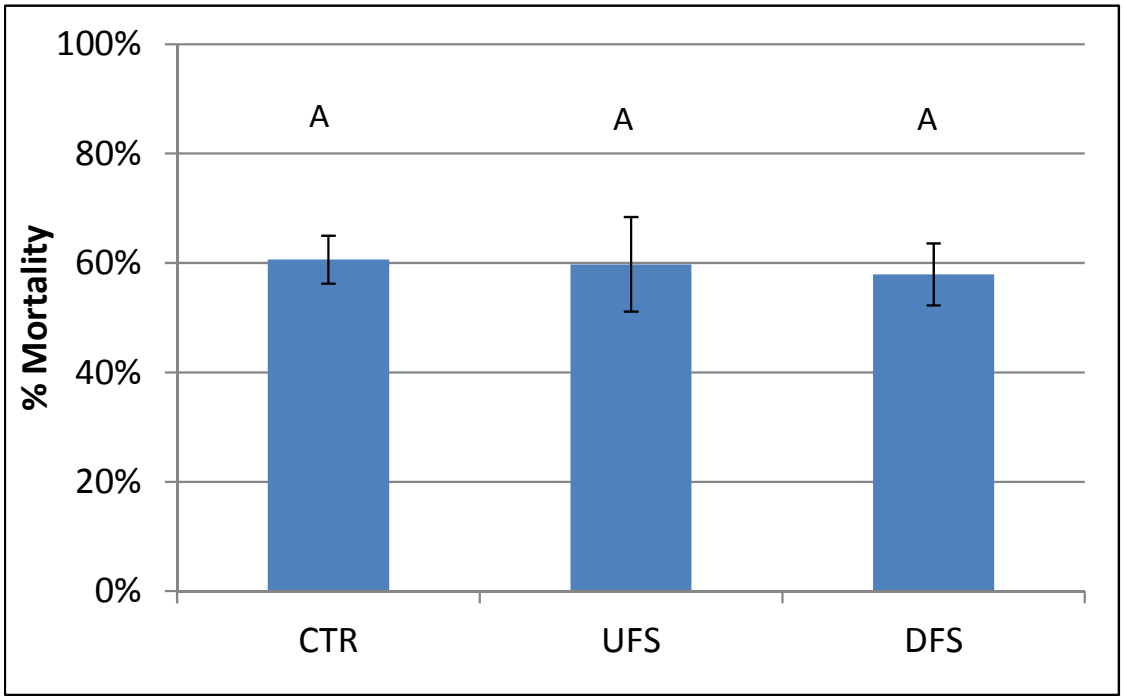

Figure 4. Percent Mortality (mean ± 1 SD) of ELS White Sturgeon Fry in River (UFS and DFS) and Filtered City (CTR) Water

Note: Different letters indicate statistically significant differences between treatment groups.

In addition to overall mortality, data were also analyzed to estimate the average time to death in each test chamber over the course of the study. Data were not censored for this analysis. The mean number of days to death did not statistically differ among treatments. Average days to death were 25.1 and 25.5 days for fish exposed to UFS and DFS water, respectively. Average days to death in filtered city water (CTR) was slightly greater at 27.9 days (Figure 5). This calculation is based on a simple average of the day in the exposure that mortality occurred considering only the fish that died. Since only dead fish are considered in this calculation, the fact that fewer fish died in the DFS exposure is not taken into consideration. A more comprehensive survival analysis that considers both the time and the number of fish that died is presented in Appendix A. Neither the mortality

percentages shown (Figure 4), nor the average days of survival for fish that died (Figure 5) show any significant differences, suggesting that overall trends in observed mortality did not depend on the source water.

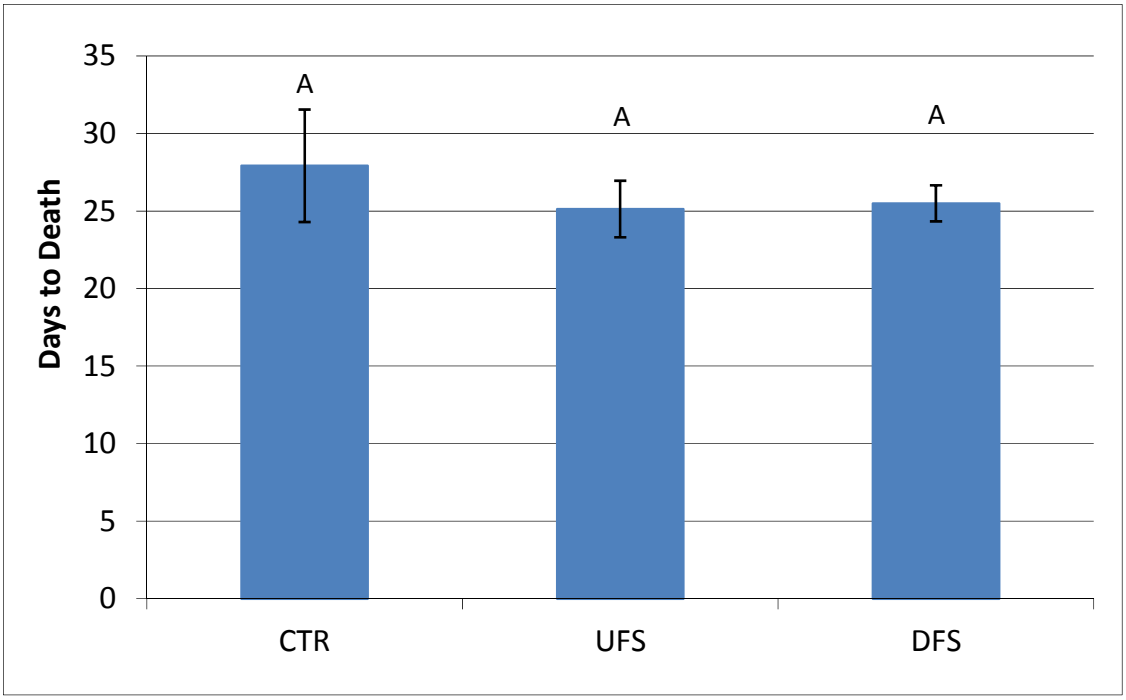

Figure 5. Average Number of Days to Death (mean ± 1 SD) of Fish in River (UFS and DFS) and Filtered City (CTR) Water

Note: Different letters indicate statistically significant differences between treatment groups.

As in the 2008 in situ study (ENTRIX 2011a), fish survival depended on life-stage, and most fry died during the transition to exogenous feeding between Days 22 and 30 after initiation of the experiment (approximately 14 through 21 days post hatch). 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. 1988; Bennett and Farrell 1998; Gisbert and Williot 1997; Mohler et al. 2000). The variability in survival during this transition period is generally attributed to the type of diet provided and whether or not it is readily accepted by the fry (Bardi et al. 1998; Bennett and Farrell 1998; Lutes et al. 1990). Some studies suggest that sturgeon undergo morpho-physiological changes to the digestive system during and/or just prior to this transition phase and proper timing and development are necessary for survival (Bardi et al. 1998; Buddington 1991; Buddington and Christofferson 1985). Groups that routinely spawn and breed sturgeon such as the Kootenay Trout Hatchery, the Columbia Basin Hatchery, and the University of California, also experience die offs during this transition phase (Figure 6).

Based on these observations, it appears that the transition to exogenous feeding represents a period during the ELS of white sturgeon that often is characterized by naturally great mortality.

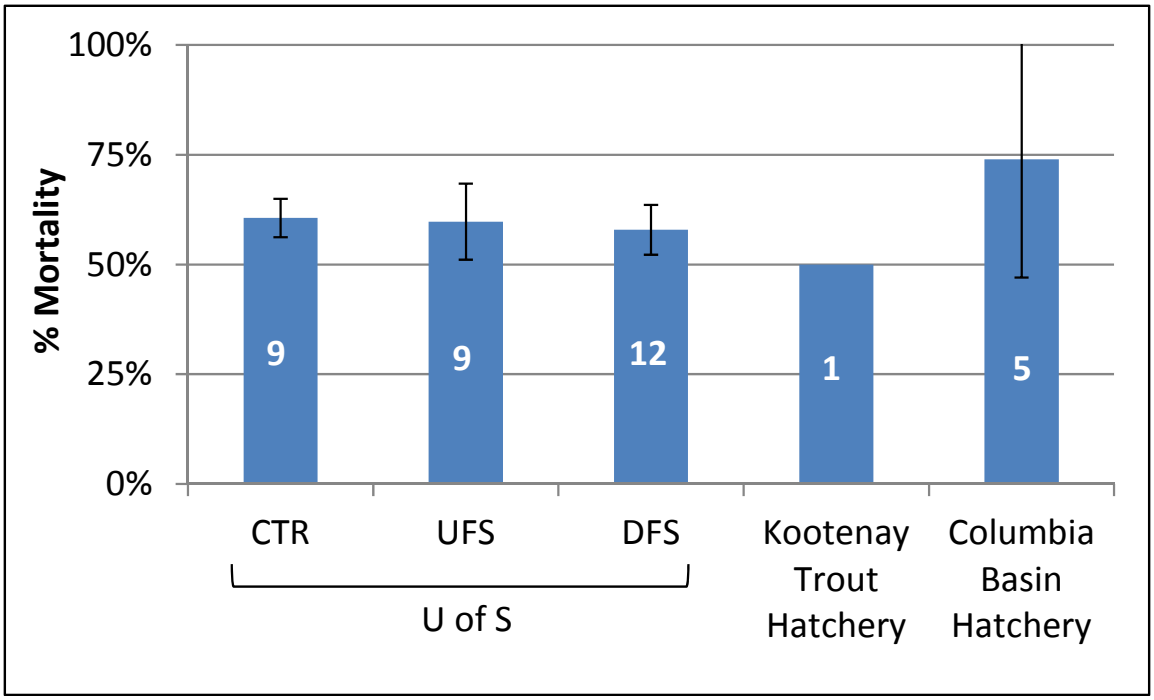

Figure 6. Mortality Rates (mean ± 1 SD) of ELS of White Sturgeon Observed During the 2009 Surface Water Toxicity Studies and at Two Other Institutions
Note: Numbers in bars represent number of replicates used to calculate average values.

3.4 LENGTH AND WEIGHT

There were statistically significant differences for both weight and length values among treatment groups. Fish raised in UFS water were significantly lighter and shorter than fry from the DFS group. Mean weight per fish was 0.95 g in CTR, 0.71 g in UFS, and 1.15 g in DFS water (Figure 7). Mean length values were 51.3 millimeters (mm) in CTR, 46.1 mm in UFS, and 53.7 mm in DFS water (Figure 8). To investigate whether the difference in size may be related to changes in the condition of the fish we calculated a condition index (CI) for each fish (Equation 1):

$$CI = \frac{weight(g)}{\left(length(mm)/10\right)^3} \qquad (1)$$

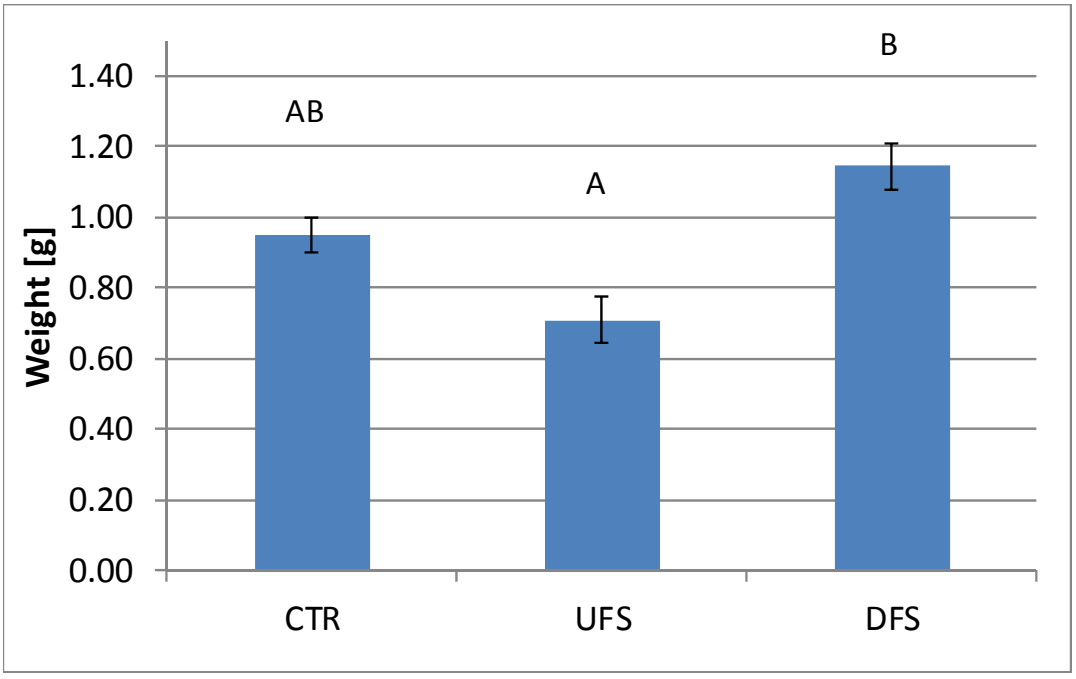

Figure 7. Average Weight (mean ± 1 SD) of White Sturgeon Fry in River (UFS and DFS) and Filtered City (CTR) Water at Termination of Experiment

Note: Different letters indicate statistically significant differences between treatment groups.

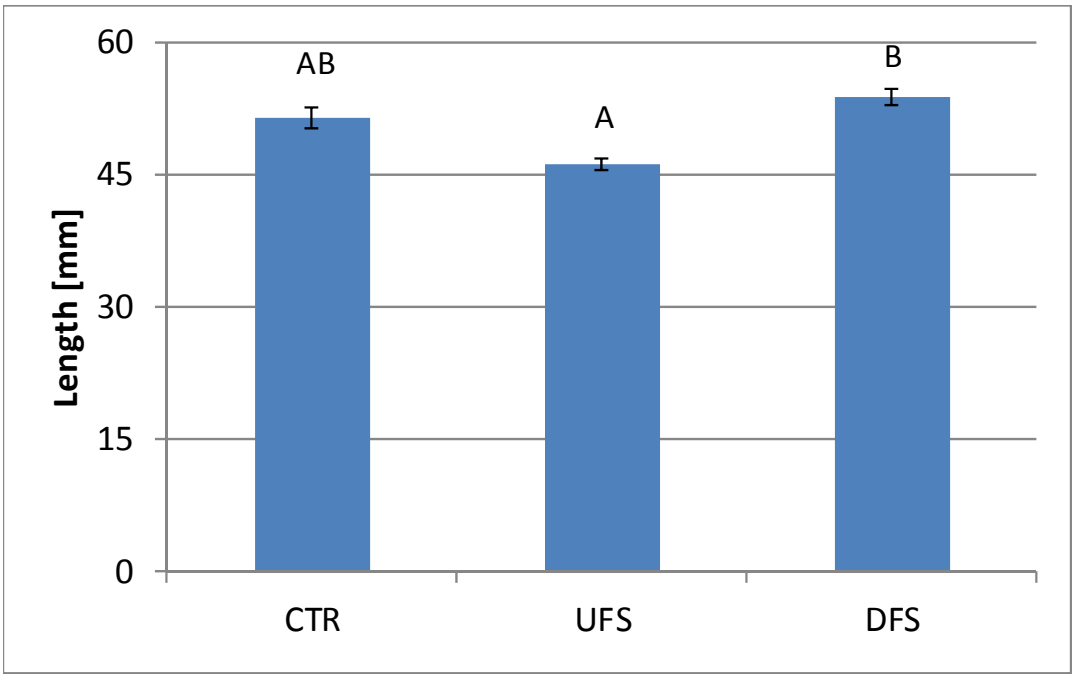

Figure 8. Average Length (mean ± 1 SD) of White Sturgeon Fry in River (UFS and DFS) and Filtered City (CTR) Water at Termination of Experiment

Note: Different letters indicate statistically significant differences between treatment groups.

This analysis revealed that there were no statistical differences in the condition of fish among treatment groups (Figure 9). It was found, however, that both weight and length were significantly and positively correlated with temperature (Figure 10 panels A and B). No such correlations were observed between water temperature and mortalities ($R^2 = 0.23$).

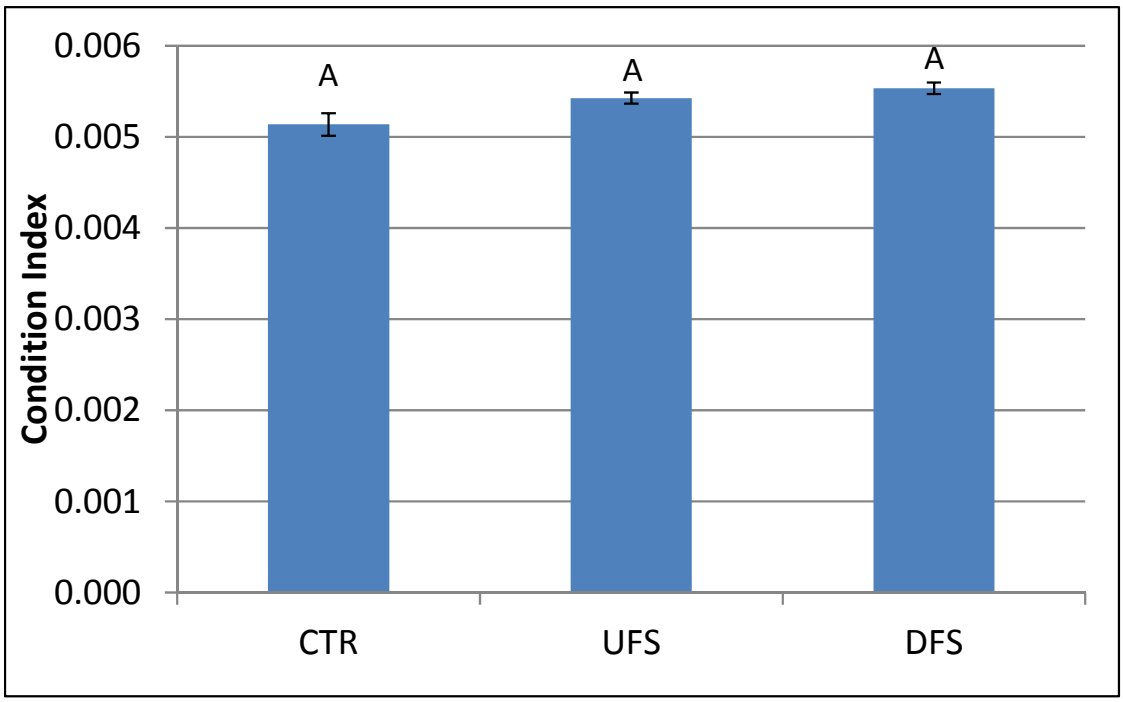

Figure 9. Average Condition Indices (CI; mean ± 1 SD) of White Sturgeon Fry in River (UFS and DFS) and Filtered City (CTR) Water at Termination of Experiment

Note: Different letters indicate statistically significant differences between treatment groups.

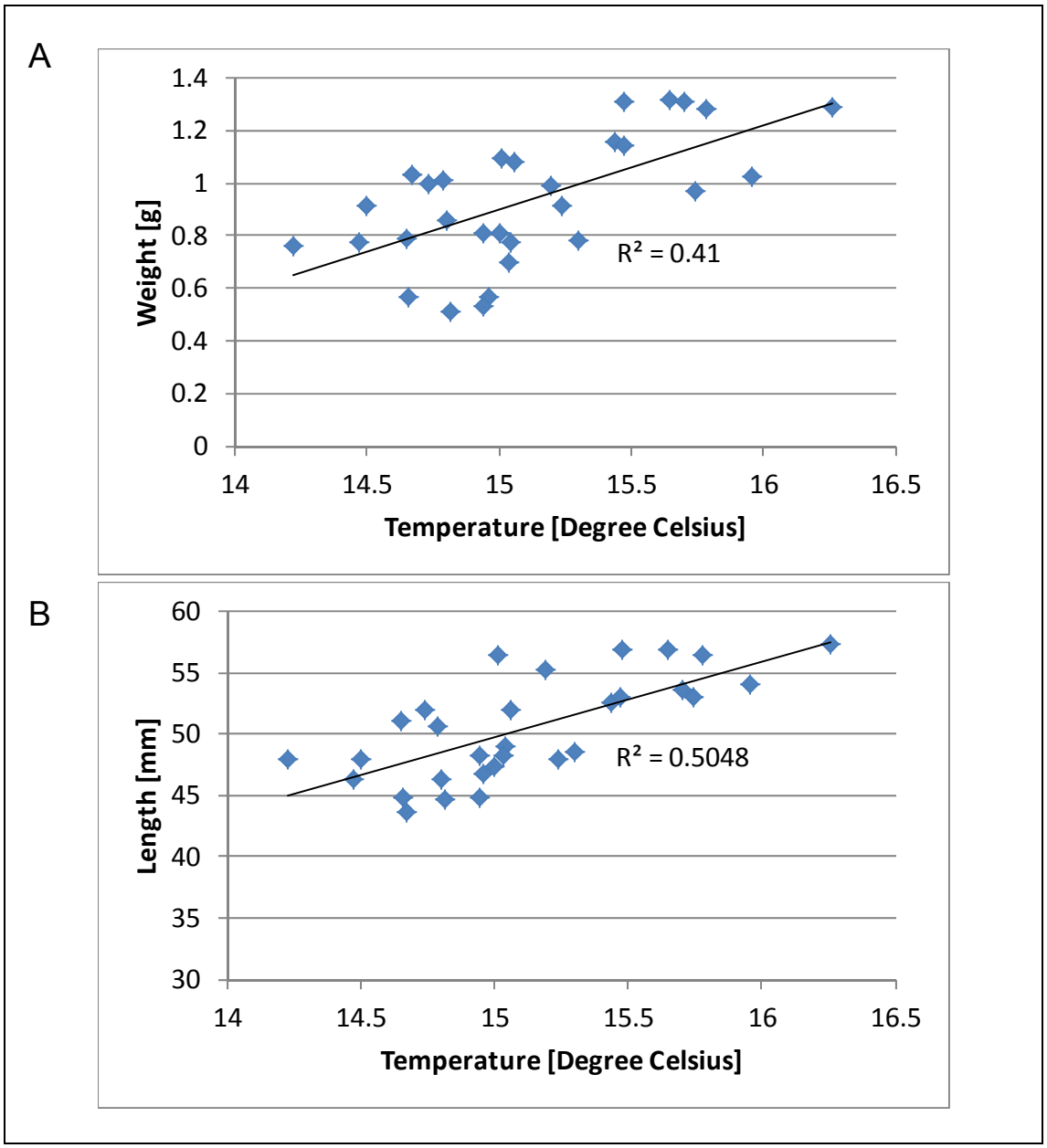

Figure 10. Relationship Between Average Water Temperatures Measured Over the 69 day Exposure Period and Average Weight (A) and Length (B) of White Sturgeon Fry in Each Exposure Chamber

3.5 BIOTIC LIGAND MODEL 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 ELS white sturgeon (Entrix 2011b). Additional BLM calibrations were

performed for copper and lead with the acute data from the 2009 acute ELS sturgeon study (Entrix 2011c). 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) and acute mortality (LC50). Predicted average LC20 and LC50 values and average water concentrations for these metals are shown in Table 7 for CTR, and UFS and DFS locations. Average concentrations of cadmium, copper, zinc, and lead in both the CTR and 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. It should be noted that comparisons were made to total metal concentrations in river water, which represent a more conservative estimate than the exposure to dissolved elements that are considered bioavailable.

Table 7. Average BLM-predicted LC20 (chronic) and LC50 (acute) Values for Cadmium (Cd), Copper (Cu), Zinc (Zn), and Lead (Pb) Compared with Average Measured Metal Concentrations from the Same Water Sources

	Cd (µg/L)			Cu (µg/L)			Zn (µg/L)			Pb (µg/L)		
	LC20	LC50	Water	LC20	LC50	Water	LC20	LC50	Water	LC20*	LC50	Water
CTR	2.7	12	0.03	2.2	11	0.37	119	116	2.1	200	215	0.21
UFS	3.0	14	0.04	3.4	17	0.84	144	140	1.8	271	290	0.15
DFS	3.0	14	0.03	3.4	17	0.50	146	143	2.2	278	298	0.12

Notes:
*LC20 for Pb is BLM-predicted acute LC20.
CTR – control
DFS – downstream field site
UFS – upstream field site

4 CONCLUSIONS

This study did not find differences in mortality of ELS of white sturgeon raised in Columbia River water upstream or downstream of Teck's Trail Facility. Concentrations of slag-associated COPCs (i.e., cadmium, copper, lead, and zinc) in river water upstream and downstream of the Trail facility were below BLM predictions for chronic effects (i.e., predicted LC20 concentrations are much higher than average concentrations for these metals in test waters).

Elevated mortality was observed across all treatment groups during the transition to exogenous feeding. This elevated life-stage specific mortality appears to be a natural phenomenon, and is in accordance with observations by other groups working with this species.

A survival analysis effectively accounts for censoring (e.g., culling), allows for estimates of time-to-death, and provides a basis for making statistical comparisons of survival among treatment groups. As detailed within Appendix A, the survival analysis illustrates that source water has virtually no affect on observed survival.

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APPENDIX A

APPLICATION OF SURVIVAL ANALYSIS

1 BACKGROUND AND OBJECTIVES

The purpose of this work was to apply a survival analysis to compare the survival in each of the source waters to see if there were significant differences as a more comprehensive alternative to comparison of percent morality estimates (as shown in Figure 6 of the report). Traditional quantification of mortality percentages can be misleading when applied to these chronic exposures, since previous work (Entrix 2011a) has shown that in the course of these tests, mortality due to the transition to exogenous feeding is expected to affect a significant percentage of the fish in the study. In addition, a large number of fish were removed (or "culled") mid-way through the test to maintain a target fish density. Changes in total fish number as the tests proceed makes it difficult to directly compare mortality estimates among treatments using percent mortality estimates defined by number of dead fish divided by number of fish present at test initiation. High mortality during specific life stages introduces an uncertainty regarding cause of mortality, i.e. is it due to a treatment effect or is it due to a naturally occurring life-stage-specific mortality rate? When evaluating the effect of various water sources on white sturgeon survival it would be advantageous to apply an analysis (e.g., survival analysis) that is capable of dealing with these issues.

The objective of this analysis was to apply survival analysis to evaluate the effect of three different water sources on white sturgeon survival while utilizing the data to the fullest extent possible. Survival analysis was chosen because it effectively deals with issues such as culling and high natural mortality by evaluating the number of fish that die within a daily time interval relative to the number of fish at risk of dying. The number of fish at risk of dying at any given time point is based on the number of fish at test initiation and is adjusted to account for those that died and for those that have been culled (which in survival analysis terms is described as right-censored). Once survival curves have been developed for white sturgeon exposed to each water source (treatment), there are multiple options for evaluating the effect of water source including a log-rank test, or an analysis of variance (ANOVA). These approaches will be discussed below.

2 METHODS AND MATERIALS

The method used here to derive survival curves was the Kaplan-Meier (KM) method. This approach calculates the probabilities of survival at various time-points of exposure by incorporating information about time-to-death and censoring to determine the cumulative fraction of surviving organisms (Kleinbaum and Klein 2005). The KM formula is a product limit formula, and can be described by (Equation 1):

$$\hat{S}(t_{(j)}) = \prod_{i=1}^{j} \hat{\Pr}[T > t_{(i)} \mid T \geq t_{(i)}], \quad (1)$$

where:

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

i = the index of multiplication

T = a random variable that represents survival time

t = a time-point of interest

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

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

where:

d_i = the number of deaths at time *i*

n_i = the number or organisms at risk of dying at time i

The effect of censoring can be incorporated by defining $n_i = n_{i-1} - c_{i-1} - d_{i-1}$, such that the number of organisms that were censored (*c*) or died (*d*) at the previous time (i.e., time *i-1*) are removed from the risk set of interest (i.e. at time *i*). The ability to consider censored observations in the survival probability function provides a direct means of accounting for the effects of culling, while still allowing the culled fish to contribute to survival estimates up to the point at which those fish were removed from the test systems.

Survival curves were constructed for each of the waters tested utilizing the KM method described above. The log-rank test and an ANOVA were used to assess the effect of the different water sources on white sturgeon survival. For the log-rank test, it was assumed that the various test chambers for a given water source were not independent, and all of the replicate data were therefore pooled. For the ANOVA approach, the individual test chambers were assumed to be independent, and the effect of water source was investigated by comparing KM survival estimates at test termination with test chambers representing experimental units. Two replicates (one from the upstream field site [UFS] exposure and one from the downstream field site [DFS] exposure) were excluded from the described analyses because of a high incidence of death due to infection. All data analysis and statistical procedures were conducted with R (R Development Core Team 2009).

3 RESULTS AND DISCUSSION

Overall survival curves for the three test waters are shown in Figure A1 panel A using pooled information from all individual replicates. Survival curves for these three test waters are remarkably similar. All test waters show significant mortality between 10 and 20 days of exposure, and this has been previously associated with natural mortality coincident with the transition to exogenous feeding (Entrix 2011a). After 28 days, there is very little additional mortality observed in exposures regardless of the source water. At the end of the test, the fraction surviving in the DFS exposure water was higher than the other two waters. Using the log-rank test to test for differences in the survival curves, it is concluded that the survival curves are statistically different among treatment groups when data from replicates are pooled (p-value = 0.000039). From this survival analysis, point estimates of survival times (e.g., median survival times) can be extracted from the KM curves. Median survival times for the three test waters were: 22 days for control (CTR), 38 days for DFS, and 22 days for UFS.

The replicate and pseudo-replicate tests for each source water are shown separately for CTR (Figure A-1B), DFS (Figure A-1C), and UFS (Figure A-1D). Considerable variability is seen at the replicate and pseudo-replicate level. This variability somewhat tempers the conclusion in Figure A1 panel A that differences exist between exposure waters. An ANOVA was applied to test for the significance of water source on white sturgeon survival at test termination. Table A1 shows the mean survival at test termination for each water source, and the ANOVA results demonstrate that when the survival variability from each test chamber is considered, there is no statistical difference in survival of white sturgeon in the waters tested (p-value = 0.32). As above, median survival times can be estimated for each of the exposure chambers, but it would be inappropriate to compare those results with an ANOVA approach because some of the median survival time estimates are beyond the length of the study (e.g., see panels B through D on Figure A1).

Table A 1. Mean white sturgeon survival at test termination for each of the water sources tested (standard deviations are shown in parentheses). Mean survival was not significantly different on the basis of water source (ANOVA; p-value = 0.32).

Water Source	Mean Survival (s.d.)
CTR 0.42	(0.15)
DFS 0.49	(0.08)
UFS 0.43	(0.10)

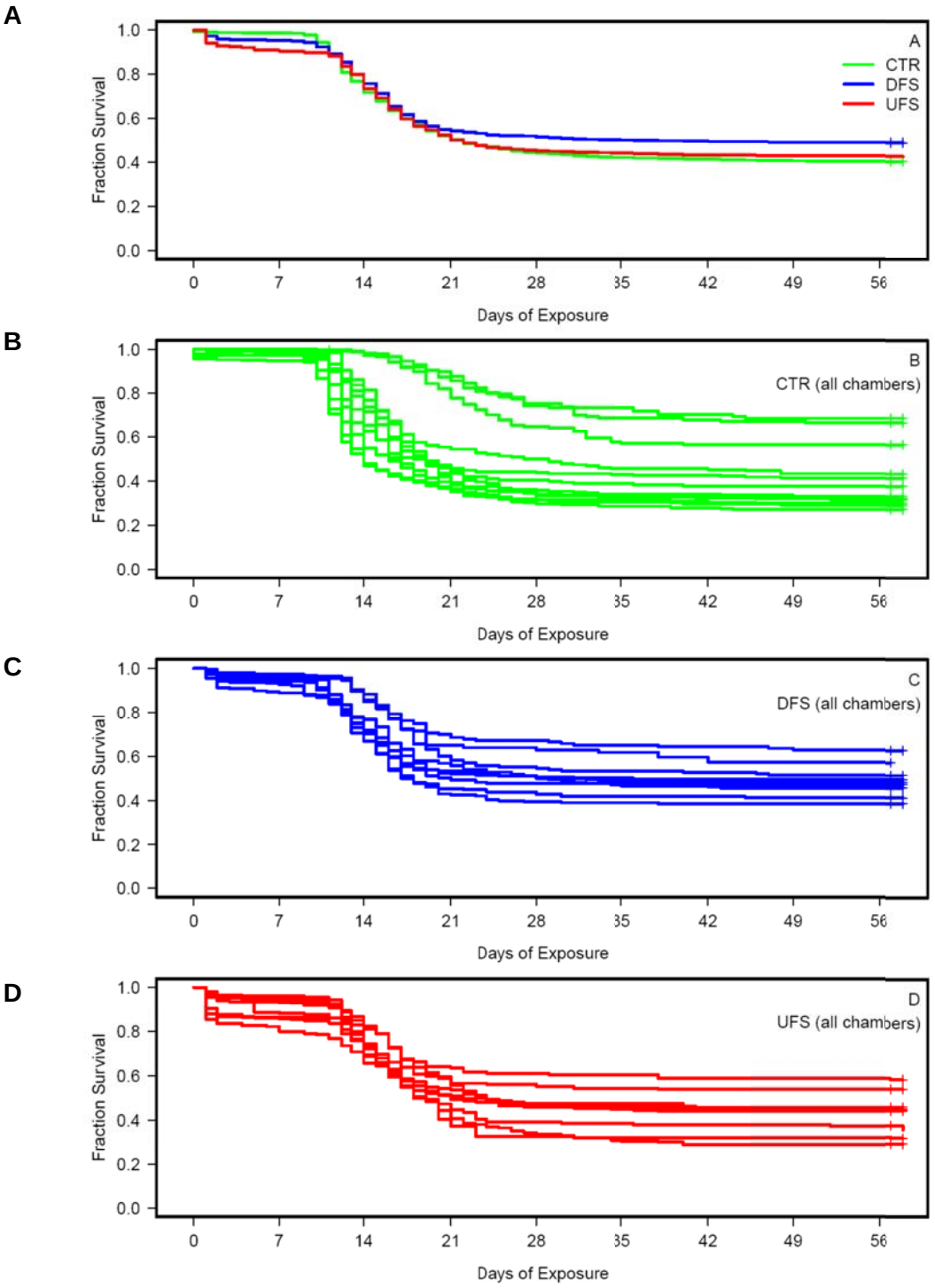

Figure A1. Kaplan-Meier survival curves for the CTR, DFS, and UFS. Panel A shows the overall survival curves for each water source. Panels B, C, and D show survival curves for the individual test chambers for the CTR, DFS, and UFS water sources, respectively.

The conclusion that the observed mortality is primarily associated with the transition of fish to exogenous feeding regardless of treatment group, and therefore is not related to source water composition, is further supported by measurements of metal concentrations in these exposures. Figure A2 shows measured total metal concentrations as well as biotic ligand model (BLM) adjusted acute (LC50) and chronic (LC20) estimates of dissolved metal effects for each metal in each test water. These BLM predictions for acute and chronic responses were developed from concentration-response data measured as part of the 2008 and 2009 acute and chronic white sturgeon exposures (Entrix 2011a,b,c,d). Since total concentrations are expected to be higher than dissolved concentrations, the use of totals to characterize exposure provides additional conservatism in this comparison. Total concentrations for zinc (Figure A2 panel A), copper (Figure A2 panel B), cadmium (Figure A2 panel C), and lead (Figure A2 panel D) are all well below the BLM-estimated acute and chronic dissolved effects estimates (note that for lead, only a BLM-predicted acute estimate is available) and this is true for river samples taken both upstream and downstream of the Trail facility. Metal concentrations in the exposure waters, therefore, are not expected to contribute to the observed mortality in these tests.

4 CONCLUSIONS

A survival analysis applied to observed sturgeon mortality for chronic exposures to Columbia River water and a control show that source water had virtually no affect on observed survival.

Observed mortality was consistent with previous reports of high natural mortality associated with the transition to exogenous feeding in these developing fish.

Total concentrations of cadmium, copper, lead, and zinc in river water samples upstream and downstream of the Trail facility were well below BLM estimates of metal concentrations that would be associated with conservative chronic effects, and therefore it is unlikely that exposure to these metals contributed to observed mortality.

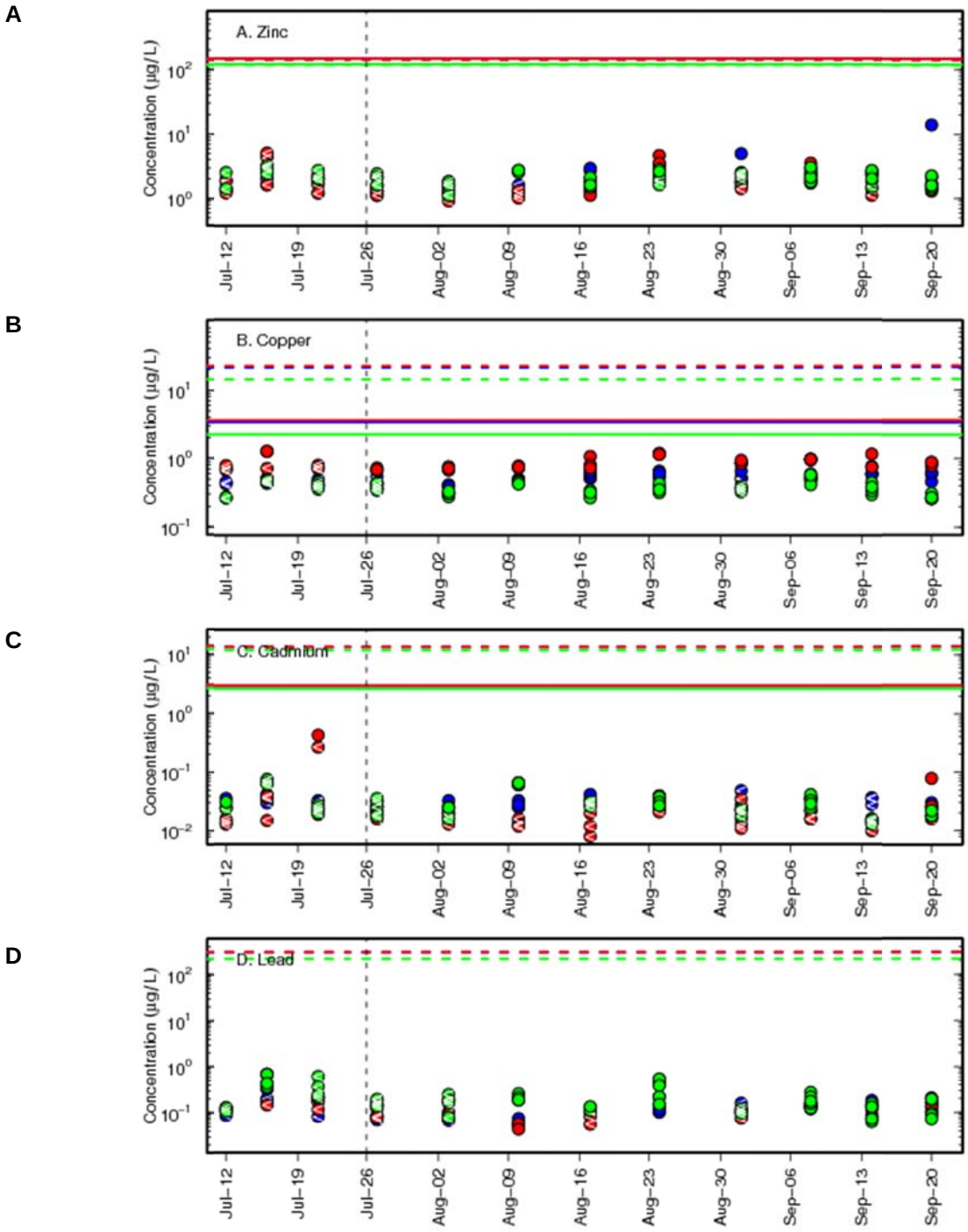

Figure A2. Total metal concentrations measured within exposure chambers for each of the waters used for the chronic sturgeon exposures. Measurements that produce values below detection limits are shown as a "<". The dotted vertical line at Jul-26 indicates test initiation. Horizontal lines show the BLM predicted acute EC50 (dashed line) or chronic EC20 (solid line) for each metal. Symbol and line color indicates source water (as in Figure A1); CTR – green, UFS – red, DFS – blue.

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