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Linking Oxidative Stress and Magnitude of Compensatory Responses with Life-Stage Specific Differences in Sensitivity of White Sturgeon (*Acipenser transmontanus*) to Copper or Cadmium

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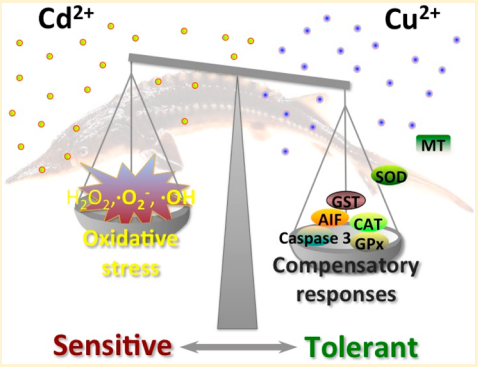
Supporting Information

ABSTRACT: Sensitivity of white sturgeon (*Acipenser transmontanus*) to copper (Cu) or cadmium (Cd) has been shown to significantly differ as a function of life-stage. This study investigated oxidative stress, metal homeostasis, and associated compensatory responses as potential mechanisms of this sensitivity pattern in three early life-stages. Sturgeon were most sensitive to Cu at 15 days post hatch (dph), which was accompanied by a significant increase in lipid peroxidation (LPO). Genes involved with amelioration of oxidative stress were significantly less inducible at this stage than in older, less sensitive fry. At 48 dph, acute lethality of sturgeon exposed to Cd was greatest and body LPO was significantly induced by 3.5-fold at 5 μg Cd/L. Moreover, there was a small but significant increase in antioxidative responses. At 139 dph, sturgeon were most tolerant to Cu and Cd and accumulation of these metals was least. Also, expression of metallothionein (MT) and apoptotic genes were greatest while expression of metal transporters was reduced and concentration of LPO was not different from controls. Our results suggest that life-stage specific sensitivity of white sturgeon to metals is complex, encompassing differences in the ability to mount compensatory responses important for metal homeostasis and combating oxidative stress and concomitant damages.



INTRODUCTION

Because of their toxic potencies and persistence in the environment, metals are contaminants of global concern to aquatic wildlife, including fishes. Occurrence of metals in freshwater ecosystems results from activities including discharge of municipal effluents, industrial discharges, and mining activities. Among metals, copper (Cu) and cadmium (Cd) are of particular concern as they are ubiquitous in the environment and can lead to a variety of adverse effects in aquatic wildlife, particularly fish.[1,2]

Among fishes, one species that has been shown to be particularly sensitivity to metals is the white sturgeon (*Acipenser transmontanus*). White sturgeon are one of the most archaic fish species currently residing on earth; however, many populations are considered endangered, and have been decreasing in North American river systems over the past few decades.[3] Among other factors, such as habitat destruction, genetic bottlenecks and overfishing, pollution with metals has been hypothesized as a potential factor contributing to these declines.[3−5] For some metals, including Cu, white sturgeon were shown to be even more sensitive than salmonids, which are considered to be among fishes most sensitive to metal pollution.[6,7] In general, fish are most sensitive to adverse effects of exposure to metals during early life-stages.[8,9] Whereas studies with white sturgeon have revealed that this is also true for this species, they also indicated that they exhibit a unique pattern in sensitivities to metals during physiologically distinct phases of early development, including yolksac, swim-up, and early or late juveniles.[6,7,10,11]

White sturgeon were shown to be most sensitive to acute waterborne exposure (96 h) to Cu during transition to

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exogenous feeding between 15 and 40 days post hatch (dph); however, yolksac (8 dph) and later juvenile (89−167 dph) sturgeon were found to be approximately 3- and 6-fold more tolerant to Cu, respectively.[6,7,12−14] Compared to Cu, white sturgeon demonstrated greater sensitivities to Cd at later stages of development. They were most sensitive to Cd at 61 dph and were relatively tolerant beyond 89 dph.[7] These observations raised questions as to the specific mechanism(s) that result in differences in sensitivity to Cu or Cd among life-stages of white sturgeon. Understanding these differences and their potential causes are of interest to risk assessors because it provides important information for identification of the most sensitive life-stages across species of concern such as critically endangered sturgeons or other receptors of interest.

Metals are known to cause toxicity to fishes through several mechanisms, including disruption of ion homeostasis and induction of oxidative stress. Metals can be taken up and transported through dedicated metal transporters and isomorphic substitution, which can cause a net loss of Na^+, K^+, Ca^{2+}, and Cl^- and result in an increase in blood viscosity and pressure, compensatory tachycardia, and ultimately death by cardiac failure.[15−19] Another major mechanism of toxicity common to metals is generation of reactive oxygen species (ROS), which can damage DNA, proteins, and membrane lipids, leading to pathological injury and mortality.[20,21] To ameliorate adverse effects caused by metals, organisms have developed a number of compensatory response pathways that are conserved among taxa.[22] Compensatory responses to metals include regulating the expression of (1) metal-binding proteins known as metallothioneins (MTs) that are involved in both transport and detoxification of metals through binding and removal of their redox potential, as well as scavenging of superoxide radicals;[23,24] (2) metal transporters such as copper transporter (CTR1), divalent metal transporter (DMT1), and copper-transporting ATPases (ATP7a) that limit metal uptake, which in turn slows onset of toxicity;[25] (3) antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPx), superoxide dismutases (SOD), and glutathione S-transferases (GST) that can remove free radicals and oxidative damage;[20,26] and (4) caspase-dependent or -independent apoptotic signaling pathways that eliminate damaged cells.[27]

Complexity of differential life-stage sensitivity notwithstanding, a number of studies have demonstrated that capacity to mount compensatory responses, especially regulation of metal and ion homeostasis pathways and activities of the antioxidant response systems, are important factors contributing to intraspecies and interspecies differences in metal sensitivity to fishes.[25,28−31] To date, however, metal-induced oxidative stress and associated compensatory responses among early life-stages of fishes are largely unknown. A recent study using in vitro liver explants with white sturgeon revealed that while expression of the MT mRNA was induced in response to some metals its responsiveness was less than that in most other species of fish.[32] This led us to hypothesize that oxidative stress and capacities of compensatory responses, including the limited capacity of white sturgeon to induce MTs, might result in less capacity to detoxify metals and therefore could be drivers of the differences in life-stage specific sensitivity of fishes to metals.

The goal of the current comparative study was to investigate differences in metal-induced oxidative stress and associated compensatory responses as possible mechanisms responsible for life-stage-specific differences in sensitivity of white sturgeon to Cu or Cd. Larvae at transition to exogenous feeding (15 dph), early juveniles (48 dph), and later juveniles (139 dph) that spanned sensitive and tolerant life-stages were exposed to serial concentrations of either Cu or Cd for 96 h. Accumulation of metals was compared among life-stages, and differences in magnitudes of compensatory responses in pathways of metal homeostasis and the antioxidant system, specifically focusing on changes in expression of MT, ATP7a, CTR1, DMT1, GPx, GST, CAT, SOD, CASP3, and AIF, as well as oxidative damage (lipid peroxidation, LPO) were quantified and correlated with differences in acute lethality for each life-stage.

MATERIALS AND METHODS

Experimental Organisms. Embryos of white sturgeon were obtained from the Kootenay Trout Hatchery (Fort Steele, BC, Canada) and were reared in the Aquatic Toxicology Research Facility at the University of Saskatchewan in a flow-through system maintained at approximately 15 °C. Fish were fed a diet of commercial frozen bloodworms (San Francisco Bay Brand, Newark, CA, USA). Proper permits were obtained before research commenced (Fisheries and Oceans Canada SARA Permit XRSF 20 2013/SARA 305), and all procedures involving animals were approved by the University of Saskatchewan's University Council on Animal Care and Supply (Protocol 20070049).

Exposure Protocol. Stock solutions of Cu and Cd were prepared in reverse osmosis water and dechlorinated city of Saskatoon municipal tap water to approximate conditions found in the transboundary reach of the Columbia River from which the breeding stock of the fish used in this study originated. Exposures were conducted for 96 h in quadruplicate tanks with fish at ages of 15 and 48 dph, and in triplicate tanks with fish at 139 dph under static renewal conditions in 0.5-, 5, and 25-L polypropylene containers, respectively, with 10 or 12 individuals per replicate tank. Seeding densities were in accordance with requirements set forth by the American Society for Testing and Materials (ASTM) guidelines for testing early life-stage of fishes.[33,34] At both 15 and 48 dph, sturgeon were exposed to 0, 1.25, 2.5, 5, 10, or 20 μg/L of Cu or Cd. At 139 dph, sturgeon were exposed to 0, 4, 10, 25, 62.5, and 156.3 μg/L of Cu or 1.6, 4, 10, 25, and 62.5 μg/L of Cd. Exposures were conducted under a 16:8 h light/dark cycle and at a temperature of 15 ± 1 °C. Sturgeon were acclimated in exposure systems for 24 h prior to initiation of the exposure and were not fed during the study. Mortalities were recorded every 12 h. Upon termination of the exposure, fish were euthanized by cervical dislocation. At 15 dph, whole individuals were used for analysis due to small size. At 48 and 139 dph, head, body, and tail were separated. Head and body were used as proxies for gills and livers, respectively, in subsequent analysis, due to individuals being too small to separate these organs. Samples were stored at −80 °C.

MT is one of the major drivers in determining the freely available metal and the toxic effect of metals in fishes.[25,28−30] Therefore, for quantification of other compensatory and biochemical responses, concentrations were selected on the basis of the maximum or near to maximum responses observed for MT gene expression and where sufficient fish were available in each life-stage.

Water Chemistry and Metal Uptake. Water quality parameters including temperature, pH, dissolved oxygen (DO), hardness, ammonia, inorganic cations (Na^+, K^+, Mg^{2+}, and Ca^{2+}) and anions (Cl^-, SO_4^{2-}, and PO_4^{3-}), and dissolved organic carbon (DOC) were determined every 24 h during the

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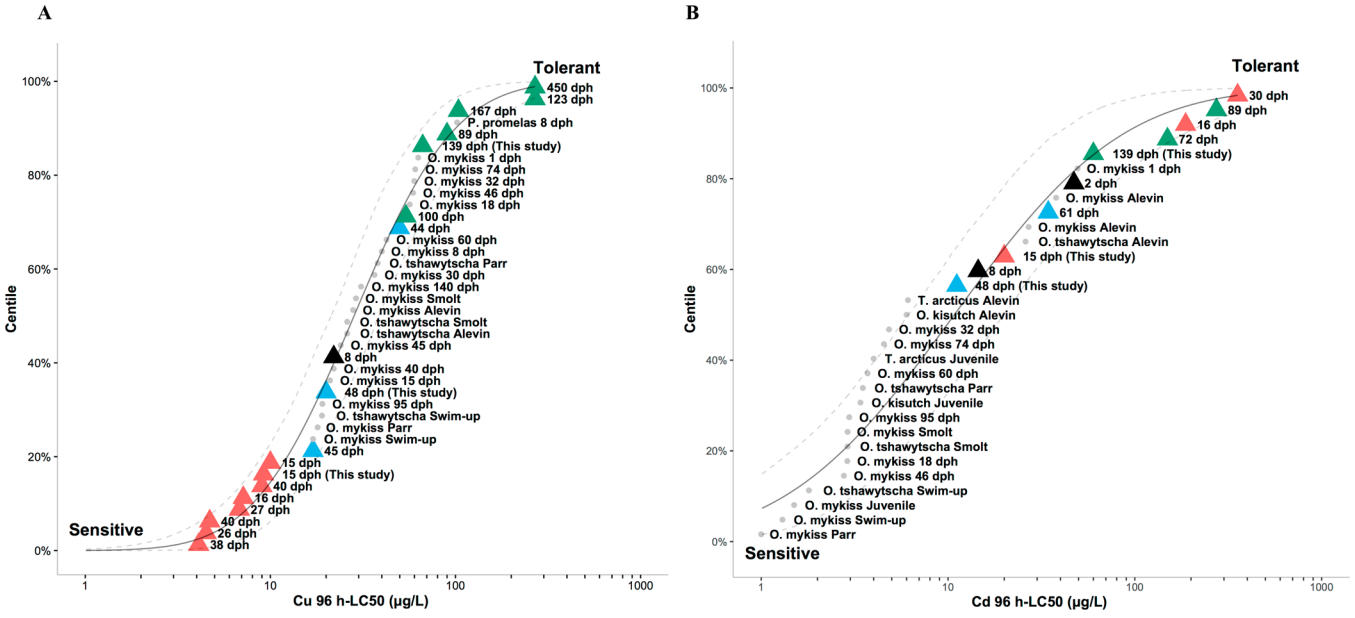

Figure 1. Species sensitivity distributions (SSDs) for life-stage acute toxicity (96 h) of Cu (A) and Cd (B). Values for 96 h-LC_{50} (μg/L) and 95% confidence interval for life-stages (black: 2−8 dph; red: 15−40 dph; blue: 44−61 dph; green: 72−450 dph) of white sturgeon (triangles) are from this study and the literature (detailed references listed in Table S5). The raw data (triangles and dots), predictions (solid line), and 95% confidence intervals (dashed lines) are shown. The other 96 h-LC_{50} values of early life-stages of different fish species including rainbow trout, chinook salmon, coho salmon, arctic grayling, and fathead minnows (gray dots) are from the literature (Table S5). When an LC_{50} value could not be calculated because mortality was less than 50% at the greatest concentration tested, the LC_{50} was reported as the greatest test concentration. Control survival for the 15 and 16 dph tests of white sturgeon was less than 90%, thus the LC_{50} calculation is not definitive.

exposure. Concentrations of Cd or Cu were measured in exposure solutions at initiation and termination of each exposure, as well as in tissues at termination of each exposure by use of inductively coupled plasma mass spectrometry (ICP-MS, Thermo X Series, Thermo-Fisher, Bremen, Germany). These data were then used to determine the speciation of Cu or Cd using biotic ligand model (BLM) visual MINTEQ (version 3.0). Detailed methods are available in the Supporting Information.

RNA Isolation and Quantitative Real-Time PCR. Total RNA was extracted from the whole body (15 dph), or head and body (48 and 139 dph) by use of the RNeasy Lipid Tissue Kit (Qiagen, Mississauga, ON, Canada) according to the manufacturer's protocol. Primers for β-actin and MT were described previously,[32] and primers for all other genes were designed by use of Primer3 software (Table S7) based on sequences from *de novo* assembly and annotation of a transcriptome of white sturgeon that was generated in house by use of a MiSeq instrument (Illumina, San Diego, CA), as described previously.[35,36] Abundances of transcripts of genes of interest were quantified by normalizing to β-actin according to the $2^{-\Delta\Delta Ct}$ method.[37] Detailed descriptions of methods used for RT-PCR are available in the Supporting Information.

Lipid Hydroperoxide (LPO) assay. Concentrations of LPO in whole body (15 dph) or head and body (48 and 139 dph) of white sturgeon were determined by use of a Lipid Hydroperoxide Assay kit (Cat 705002, Cayman Chemical Company, Ann Arbor, MI, USA) according to the manufacturer's protocol. Hydroperoxides were detected based on absorption at 500 nm by use of a 96-well plate spectrometer (SpectraMax 190, Molecular Devices, Sunnyvale, CA, USA) operated at room temperature.

Statistical Analysis. Statistical analyses were conducted by use of either "drc", "MASS", "clustsig", "corrplot", "vegan", and "ggplot2" packages in R version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria) or GraphPad Prism version 5.0 (GraphPad, La Jolla, CA, USA). Detailed descriptions of statistical methods are available in the Supporting Information.

RESULTS

Exposure Verification and Water Quality. Measured concentrations of Cu and Cd in water were comparable to nominal concentrations in all exposure experiments, and on average, were within 90% of each other (Tables S1 and S2). The relative percentage of free Cu or Cd ion and other metal species as determined by the BLM model were comparable among exposure media used for different life-stages (Tables S1 and S2).

Life-Stage Specific Sensitivities. Survival curves and 96 h-LC_{50} values indicated dose- and life-stage-specific mortality of sturgeon after exposure to each metal (Figure S1 and Tables S4 and S5). For Cu, white sturgeon were most sensitive at 15 dph with a 96 h-LC_{50} of 9.2 μg/L (95% CI 5.5−12.8), compared to >20 μg/L and 66.5 μg/L (95% CI 56.7−76.3) at 48 dph and 139 dph, respectively. For Cd, white sturgeon were more sensitive at 48 dph with a 96 h-LC_{50} of 11.14 μg/L (95% CI 6.3−16), compared to 15 dph with a 96 h-LC_{50} > 20 μg/L and 139 dph with a 96 h-LC_{50} > 60 μg/L. Species sensitivity distributions (SSDs) for Cu or Cd that were developed based on available 96 h-LC_{50} values for early life-stage of freshwater fishes (Table S5) demonstrated that sensitivities of white sturgeon to Cu at 15 to 40 dph were approximately at the fifth centile among all life-stages of species tested (Figure 1). For Cd, white sturgeon were most sensitive at 48 dph, which was approximately at the 56th centile.

Life-Stage Specific Accumulation of Cu and Cd. Concentrations of metals in tissues of sturgeon exposed to 10

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μg/L Cu or Cd were significantly less at 139 dph (4.4 ± 0.2 μg/g in head and 6 ± 0.6 μg/g in body for Cu; 1.1 ± 0.5 μg/g in head and 1.1 ± 0.3 μg/g in body for Cd) relative to 15 dph (21.4 ± 0.1 for Cu; 4 ± 0.2 for Cd) and 48 dph (9.7 ± 2.1 μg/g in head and 13.2 ± 2.2 μg/g in body for Cu; 2.6 ± 0.5 μg/g in head and 2.6 ± 0.3 μg/g in body for Cd) (Figure S2 and Table S6).

Life-Stage Specific Lipid Peroxidation. Compared to controls, LPO was not significantly different in head and body tissues of sturgeon exposed to 10 μg Cu/L at 48 dph and 25 μg Cu/L at 139 dph, or 10 μg Cd/L at 15 dph and 62.5 μg Cd/L at 139 dph (Figure 2). LPO was significantly greater by 1.5-fold

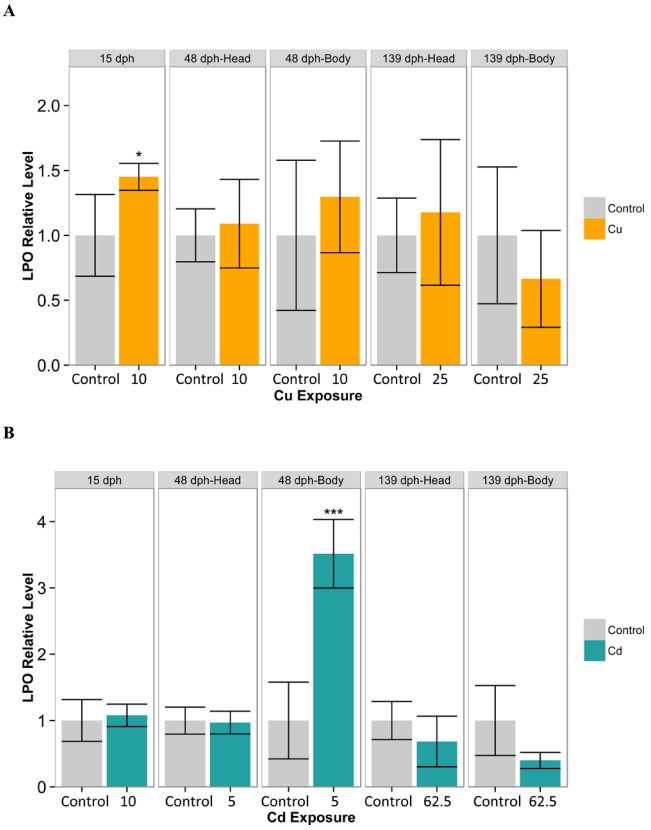

Figure 2. Levels of lipid peroxidation (LPO) across different life-stages of white sturgeon following 96 h exposure to either Cu (A) or Cd (B) (μg/L; nominal concentration). All data were normalized to the control group and are presented as mean ± SD (3 or 4 replicate measures with 1 or 2 fish per replicate). Significant differences compared to controls are indicated by an asterisk (*p < 0.05 and ***p < 0.001).

in sturgeon exposed to 10 μg Cu/L at 15 dph. LPO in body tissue of sturgeon exposed to 5 μg Cd/L at 48 dph was significantly greater by 3.5-fold.

Life-Stage Specific Transcriptional Responses to Cu. Expression of MT was up-regulated in a dose- and life-stage-dependent manner by exposure to Cu (Figure 3). With the exception of expression in body at 48 dph, abundance of transcripts of MT was least at 15 dph (1.4-fold increase at 10 μg/L), followed by 48 dph (2.1-fold increase in head at 10 μg/L) and 139 dph (2- and 2.9-fold greater in head and 4- and 7.8-fold greater in body, respectively, at 10 or 25 μg/L). Abundances of transcripts of metal transporters were generally not affected at 15 or 48 dph; however, expressions of ATP7a, CTR1, and DMT1 were significantly decreased to 0.54-, 0.35-

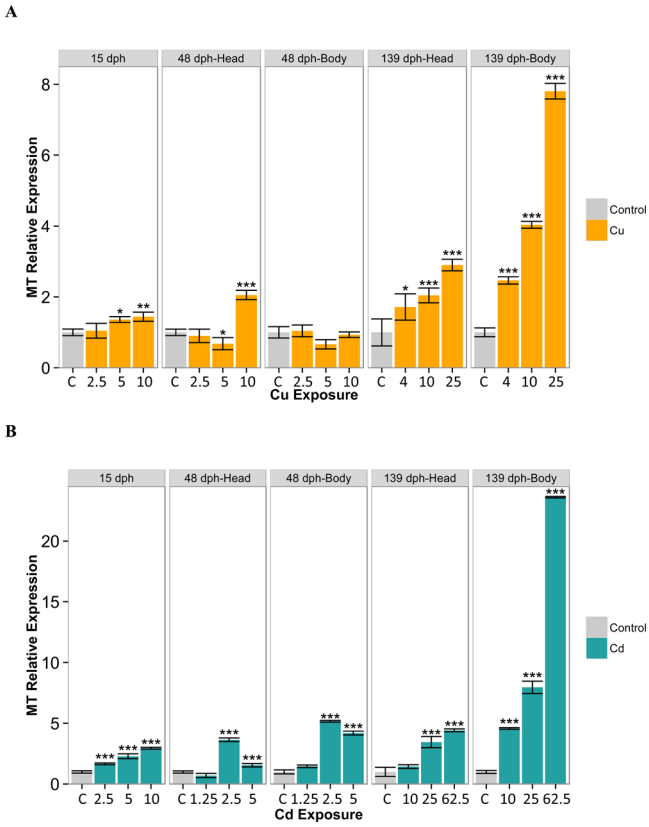

Figure 3. Abundance of transcripts of MT across different life-stages of white sturgeon following 96 h exposure to either Cu (A) or Cd (B) (μg/L; nominal concentration). All data are expressed as relative changes normalized to the control group (C) and are presented as mean ± SD (3 or 4 replicate measures with 1 or 2 fish per replicate). Significant differences compared to controls are indicated by asterisks (*p < 0.05, **p < 0.01, and ***p < 0.001).

and 0.27-fold in the head of 139 dph fish, respectively (Figures 4 and S5). Rank order of magnitudes of changes in abundances of transcripts of genes related to response to oxidative stress (GPx, GST, CAT, and SOD) and apoptosis (CASP3 and AIF) were 15 dph (0.7- to 1.6-fold) < 48 dph (1- to 1.5-fold in head; 1- to 1.8-fold in body) < 139 dph (0.7- to 2.7-fold in head; 1- to 2.9-fold in body) (Figures 4 and S5).

Life-Stage Specific Transcriptional Responses to Cd. Effects of exposure to Cd on expression of MT at different life-stages were similar to effects of Cu, but the magnitude of response was greater in sturgeon exposed to Cd. Among the three life-stages, magnitudes of changes in abundance of transcripts of MT were 15 dph (2.3- and 2.9-fold increase at 5 or 10 μg/L, respectively) < 48 dph (3.6- and 1.6-fold increase in head; 5.2- and 4.2-fold increase in body at 2.5 or 5 μg/L, respectively) ≪ 139 dph (4.4- and 23.6-fold induction at 62.5 μg/L in head and body, respectively) (Figure 3). Abundances of transcripts of metal transporters were generally not affected in sturgeon at 15 or 48 dph; however, abundances of transcripts of ATP7a, CTR1 and DMT1 were significantly decreased by 0.55-, 0.2-, and 0.45-fold in the head of 139 dph fish, respectively (Figures 4 and S5). Expression of genes related to the response to oxidative stress and apoptosis were significantly affected in sturgeon exposed to Cd. Among the three life-stages, magnitudes of changes in abundances of these transcripts were 15 dph (0.9- to 1.3-fold) < 48 dph (0.9- to 1.7-

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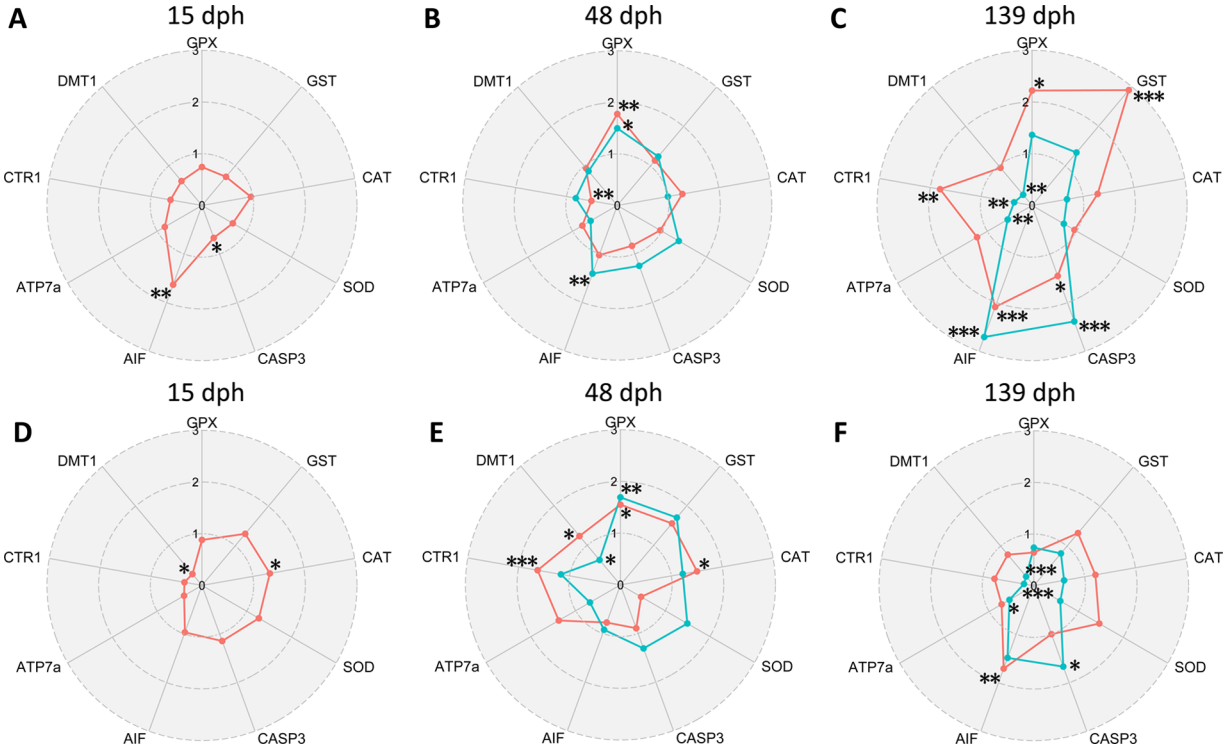

Figure 4. Radar plots illustrating changes in abundances of transcripts of metal transporters (ATP7a, CTR1, and DMT1), antioxidative (GPx, GST, CAT and SOD) and apoptosis (CASP3 and AIF) genes in different life-stages of white sturgeon following 96 h exposure to either Cu or Cd. (A) 10 μg/L of Cu; (B) 10 μg/L of Cu (green: head and red: body); (C) 25 μg/L of Cu (green: head and red: body); (D) 10 μg/L of Cd; (E) 5 μg/L of Cd (green: head and red: body); (F) 62.5 μg/L of Cd (green: head and red: body). Data are presented as the mean of gene transcription levels (3 or 4 replicate measures with 1 or 2 fish per replicate) after normalization to the control. Significant differences compared to controls are indicated by asterisks (*p < 0.05, **p < 0.01, and ***p < 0.001).

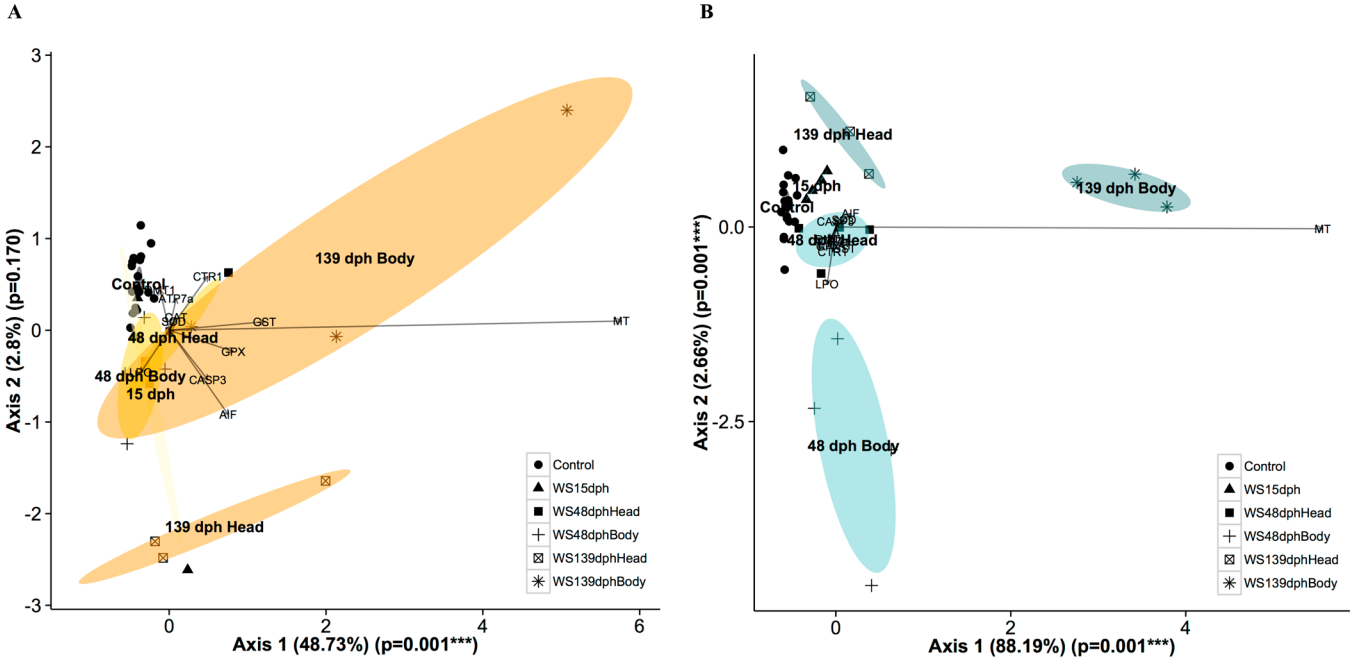

Figure 5. Biplot illustrating distance-based redundancy analysis (dbRDA) of the measured responses in white sturgeon along different life-stages following 96 h exposure to either Cu (A) or Cd (B). Each data point represents a sample, and controls were from all three life-stages. The proximity of points in this biplot is an approximation of the similarity for samples with respect to their responses. Significant differences among the groupings were observed (p = 0.001***). The percentage of variability by each axis is indicated. Ovals represent the 95% confidence ellipse around the group (life-stage and tissue) centroids, and vector length and direction are indicative of the relative contribution of the measured responses to the ordination.

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fold in head; 0.5- to 1.6-fold in body) ≈ 139 dph (0.6- to 1.7-fold in head; 0.6- to 1.7-fold in body) (Figures 4 and S5).

Hierarchical Cluster, Correlation, and Distance-Based Redundancy Analysis (dbRDA). Hierarchical clustering analysis of both Cu and Cd showed that changes in expression of MT and concentrations of LPO were most significant ($p < 0.05$) and significantly different from changes in expression of metal transporters, and genes important for the response to oxidative stress or apoptosis (Figure S6). Furthermore, genes within a certain pathway were grouped in the same cluster. In addition, dbRDA analysis demonstrated a strong and statistically significant ($p = 0.001$) shift in compensatory responses among life-stages following exposure to Cu or Cd (Figure 5). For Cu, responses of the least sensitive life-stage of 139 dph were different from those of the other two life-stages and the controls ($p = 0.001$), and changes in expression of MT, GST, and AIF contributed most to this difference (Figure 5A). In contrast, responses in body tissue at the most sensitive life-stage of 48 dph and the least sensitive 139 dph to Cd were different from that in head tissue at all life-stages and controls ($p = 0.001$), and changes in LPO and MT were the main drivers of differences in responses in bodies of 48 and 139 dph fish, respectively (Figure 5B).

DISCUSSION

This study provides some of the first evidence suggesting that differences in life-stage specific sensitivity of white sturgeon to Cu or Cd is correlated with developmental variation of molecular and biochemical responses. Relative percentage of free Cu or Cd ion and other metal species showed no differences among test solutions used in different life-stage exposure experiments, indicating that water chemistry did not significantly contribute to the observed differences in toxicities.

Oxidative Stress and Compensatory Responses to Cu among Early Life-Stages. The significant increase in LPO in white sturgeon exposed to Cu at the most sensitive life-stage based on acute lethality (15 dph) indicated that exposure to Cu might have caused an increase in ROS that overwhelmed intrinsic compensatory responses in these fish. In contrast, LPO levels in 48 or 139 dph sturgeon exposed to Cu were not different from controls. Therefore, it is hypothesized that white sturgeon at later life-stages were capable of mounting an effective compensatory response to oxidative stress, thus limiting the accumulation of hydroperoxides. This is consistent with induction of genes associated with compensatory responses to metals at these later life-stages that were not observed in 15 dph sturgeon. Changes in expression of genes important for responses to oxidative stress were almost negligible at 15 dph, which indicates that at this age white sturgeon might not be able to mount a compensatory response to a toxic insult by Cu.

Significant up-regulation of expression of GPx in 48 or 139 dph life-stages, which were relatively more tolerant to Cu, implies that this enzyme might play an important role in defense against H_2O_2 in white sturgeon resulting from exposure to Cu. Interestingly, no significant changes in expression of SOD or CAT were found at any life-stages exposed to Cu, indicating these enzymes might have a limited role in removing Cu-induced ROS in white sturgeon. Among the three different life-stages, the amount of LPO was negatively correlated with expression of genes encoding for antioxidant enzymes; however, this was only statistically significant for GST ($r = -0.97$, $p < 0.05$). Greater expression of GST at 139 dph might have prevented formation of LPO, offering another explanation for the greater tolerance at this stage of development.

In this study, induction of MT expression occurred in a concentration-dependent manner, predominantly at later life-stages. This effect was similar to that reported by other studies with other species of fish, including Persian sturgeon (*Acipenser persicus*).[38] There was a significant inverse relationship ($r = -0.93$, $p < 0.05$) between MT expression and concentration of LPO across all three life-stages exposed to Cu, which emphasizes the role of MT in sequestering Cu to reduce oxidative stress and in scavenging ROS to protect sturgeon against oxidative damage. Although basal expression of MT did not change among life-stages, MT responded in a life-stage-specific manner to Cu. At 15 dph, induction of MT was least (less than 1.5-fold) but concentrations of LPO were significantly greater than those in controls. In contrast, at 48 dph when sturgeon were relatively less sensitive to Cu, greater expression of MT was observed in head but not in body, and concentrations of LPO in either head or body were not significantly greater than in controls. At the most tolerant life-stage of 139 dph, expression of MT was greater in both head and body tissues, and there was no increase in LPO. The greater induction of MT at more developed life-stages could be a contributing factor to the decreases in sensitivity to Cu of these life-stages. Although information on post-transcriptional regulation and translation are important in order to derive definite conclusions regarding the relationship between amounts of mRNA and protein,[39] previous studies have shown a significantly positive correlation between abundances of MT mRNA transcript and protein in aquatic organisms exposed to metals, and thus, it is hypothesized that the here-observed increase in MT mRNA is also indicative of an increase in MT protein.[40]

ATP7a and CTR1 are two of the main Cu transporters in fish.[18] Expressions of both of these genes were significantly down-regulated in head from white sturgeons exposed to Cu at 139 dph, suggesting that they might be involved in a regulatory mechanism that reduces uptake of Cu. However, no changes in expression of these genes were observed at earlier life-stages, indicating that this compensatory response does not occur at 15 and 48 dph. Taken together, these results suggest an increasing tolerance of sturgeon to Cu with increasing age, and that the greater sensitivity to Cu at 15 dph might be partially due to the inability to up-regulate compensatory responses at this life-stage, which could result in greater oxidative stress and associated damages.

In this study, heads were used as a proxy for gills, which might be the primary target site for Cu toxicity in white sturgeon at 15 dph. Gills have extensive surface areas and are the first site of direct and continuous contact with the aqueous environment, and therefore, represent an important target for waterborne Cu.[41,42] They are the primary organ of ion-regulation in freshwater fish and are prone to oxidative injury, which can impact respiratory, excretory, or osmoregulatory functions, particularly during stress caused by exposure to Cu.[43] This might be particularly true during early life-stages when gills become functional and are still very delicate. For example, results of previous studies indicated that 8-d-old larvae of fathead minnow (*Pimephales promelas*) were most sensitive to Cu during the period when they are transitioning from cutaneous to branchial respiration.[44,45] Gills of white sturgeon start to develop at 4 dph and development is completed by approximately 14 dph, at which time they transition to gill

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respiration.[46] Earlier studies with white sturgeon demonstrated that this species was relatively insensitive to exposure to Cu at earlier life-stages (8 dph), a time during which gill development is incomplete and where skin is the primary organ for respiration, excretion, and osmoregulation.[13] However, at 15 dph gills become the predominant organ for these processes. Because completion of gill development and the period of transition to gill respiration seem to parallel greater sensitivity to Cu observed at 15 dph, it is hypothesized that newly developed gills are likely to be structurally delicate and have a lesser capacity to mount compensatory responses against Cu toxicity. Furthermore, differences in kinetics of metal uptake and ionoregulatory homeostasis between the skin (primary organ of respiration prior to 15 dph) and gill (primary organ of respiration at and after 15 dph) might play an important role in life-stage specific differences in metal sensitivity. Relatively high whole body Cu accumulation at 15 dph in our study might suggest high Cu uptake and it might be one of the factors responsible for higher sensitivity of white sturgeon to Cu at 15 dph.

Oxidative Stress and Compensatory Responses to Cd among Early Life-Stages. Similar to effects of exposure to Cu, exposure to Cd resulted in significant impacts on molecular and biochemical processes associated with metal sequestration and antioxidant responses in early life-stages of white sturgeon. This result is consistent with results of previous studies in which exposure of fish to waterborne Cd was associated with oxidative stress, including increases in concentrations of LPO.[47,48] In the present work, unlike Cu, exposure to 5 μg Cd/L led to 3.5-fold greater concentrations of LPO in the body of sturgeon at 48 dph, which was the most sensitive life-stage, but not at 15 or 139 dph. The weak or absence of LPO response in 15 dph fish exposed to Cu and Cd, respectively, may indicate that the pathways responsible for this response are still developing at that life stage. Another possible explanation for this difference in effect between Cu and Cd is that these metals differ in their mechanisms of toxic action, and that Cd is a much stronger inducer of oxidative stress and associated physiological damages in white sturgeon. Cu is an essential element and is a cofactor for a number of enzymes that play a crucial role in important cellular and enzymatic mechanisms,[49] while Cd has no known role in organisms. The essential nature of Cu might be one reason for greater tolerance toward oxidative damage as compared to that of Cd. Differences in their affinities toward biological ligands might also be responsible for differences in oxidative damage in different life-stages of white sturgeon. For Cd, greater amounts of LPO occurred in body, rather than head, and redundancy analysis further confirmed that LPO contributed most to the significant difference between head and body. Differences in development of tissues (head vs body) and susceptibility to oxidative stress can be explained by their ability to mount a response to oxidative stress as well as the different routes of exposure to Cd.

The pattern of induction of MT in response to exposure to Cd was very similar compared to exposure to Cu, and the magnitude of responses of MT increased as a function of developmental stage. Specifically, response of MT to Cd was least at 15 dph; however, LPO levels were not altered at this life-stage, which was different compared to fish exposed to Cu. Expression of MT was greater in head and body tissues of white sturgeon exposed to Cd at 48 dph, but the lesser MT response at 5 compared to 2.5 μg Cd/L indicated that the capacity for induction of MT was already being exceeded. Induction of MT was greatest at 139 dph, which represents the most tolerant life-stage. Cd is known to be assimilated and transported by DMT1 and epithelial Ca^{2+} channels.[25] Similar to effects of Cu on Cu transporters, a comparison of expression of DMT-1 among life-stages showed DMT-1 was significantly down-regulated in head at 139 dph, which might be a mechanism of lesser uptake of Cd at this later life-stage compared to 15 and 48 dph, and which may explain the lesser toxicity at that life-stage.

During exposures to either Cu or Cd, the greatest apoptotic response occurred in the most tolerant life-stage of 139 dph. This indicates a different defense strategy might be used in later less sensitive life-stages compared to the more sensitive earlier life-stages. The greater capacity for removing damaged cells through apoptosis is often compensated for by a subsequent increase in cell proliferation to maintain tissue structure and function,[50,51] which leads us to hypothesize that white sturgeon at later stages of development have a strong recovery capability. The greatest induction of MT and apoptotic response by fish at 139 dph appeared efficient in preventing LPO in tissues, thereby preserving tissue integrity and rendering the resistance to adverse effects of metals. Taken together, while the mechanism(s) of greater resistance is not known, it is hypothesized that the decreased expression of metal transporters, more efficient sequestering of Cd, and greater capacity to detoxify ROS play important roles at the most tolerant life-stage.

Livers of fishes are known to be one of the main tissues for storage of Cd and the primary site where toxicity manifests.[25,52,53] Hence, a fully developed liver is expected to be required for the complete manifestation of Cd-induced oxidative injury. Therefore, liver is the preferred organ for assessing oxidative stress. Because liver is the predominant metabolic organ in higher organisms such as fish, it is expected to gain full functionality after the transition to exogenous feeding. These assumptions are supported by studies demonstrating that in zebrafish the liver is established by 5 dpf, which coincides with transition to exogenous feeding.[54] Assuming a similar pattern, the liver of white sturgeon is likely to develop at onset of exogenous feeding, which occurs approximately at 21−34 dph.[13] This appears to overlap with the manifestation of increasing sensitivity and levels of LPO in response to exposure with Cd at 48 dph. Collectively, these findings lead to the hypothesis that white sturgeon at 15 dph are less sensitive to Cd because the target organ, the liver, is underdeveloped and metabolically less important. It was hypothesized that at 21−48 dph, the liver is further developed but still cannot mount a strong compensatory response, while by 139 dph white sturgeon have fully developed gills and livers that have a greater capacity to detoxify Cd and prevent adverse effects through the greatest expression of MT and apoptotic genes.

Another important organ for metal accumulation and storage is the kidney. Thus, in addition to liver, it might also play an important role with regard to contributing to metal toxicity in white sturgeon.[55] However, ontogeny of the kidney is not well characterized in white sturgeon, and the very small size of larvae during early life stage makes it almost impossible to study metal accumulation in this organ. Future studies describing kidney development in white sturgeon larvae should provide a better understanding of the role of kidney in life stage specific metal accumulation and associated toxicities in this species.

Accumulation of Cu or Cd among Early Life-Stages. Accumulation of metals is subject to competitive dynamic

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processes of uptake and excretion, and represents a crucial metric related to adverse outcomes in exposed organisms, and thus, their sensitivity. In this study, accumulation of Cu or Cd varied markedly and there was an inverse relation with age (15 dph > 48 dph > 139 dph), although accumulation was not always coincident with sensitivity. Whole-body metal concentrations measured in this study were comparable to the reported 96-h-critical body residue (96-h-CBR).[56] For Cd, the values of white sturgeon (1.07 μg/g at 139 dph to 3.96 μg/g at 15 dph) were approximately in the same range as those reported for larvae of rainbow trout and Java barb (1.1 and 3.2 μg/g, respectively).[56] For Cu, values for white sturgeon (5.43 μg/g at 139 dph to 21.41 μg/g at 15 dph) were comparable to a 96-h value of 6.8 μg/g previously reported for pink salmon larvae.[56] The same trends in whole-body metal concentrations among life-stages were also observed when they were normalized to basal metal concentrations in controls (increase over control for 15 dph fish was 2.6- and 68-fold, for 48 dph fish was 2.1- and 13.4-fold, and for 139 dph fish was 1.9- and 17-fold for exposure to Cu and Cd, respectively).The observation of greatest accumulation of Cd and Cu at 15 dph supported the hypothesis that white sturgeon at this life-stage lack effective biochemical processes necessary for elimination of these metals because of poorly developed organs such as the liver, and possibly the kidney. Other factors such as presence of permeable skin,[57] and the greater surface area to volume ratio also might have contributed to the increased metal uptake. The most tolerant life-stage of 139 dph accumulated 4 times less metals than did individuals at 15 dph when exposed to 10 μg/L of Cu or Cd. This might be attributed to greater induction of MT that sequestered more metals and down-regulation of metal transporters that reduced metal uptake in this life-stage,[58−60] as discussed above, as well as the presence of well-developed organs involved in processing, detoxification, storage, and elimination of metals.

Implications and Uncertainties. Current guidance for conducting risk assessments and developing water quality criteria (WQC) for metals in the aquatic environment does not consider evaluation of most sensitive life-stages.[61] However, determination of the most sensitive life-stage is critical for the establishment of more appropriate WQC that are protective of fishes. Results of the present work revealed that exposure to Cu or Cd provoked different oxidative stress and compensatory responses among three major life-stages of white sturgeon, and which likely represent important factors contributing to the significant differences in the life-stage specific sensitivities of this species. The apparent divergence in life-stage specific sensitivity to Cu or Cd was paralleled by the pattern of oxidative stress and associated tissue damage, which was likely a result of developmental differences in tissues/organs and a balance in metal detoxification through induction of MT and oxidant homeostasis among fish of different stages. During exposure to Cu or Cd, expressions of all metal transporters were down-regulated in the head of the later life-stage (139 dph) fish. This result indicates that regulation of metal transporters might be an important compensatory mechanism by which fully developed fish slow the uptake of metals, and thus confers tolerance to metals, as suggested previously.[25] Disruption of osmotic balance is one of the most important mechanisms of Cu and Cd toxicity, and further studies focused on kinetic interactions of these metals with Na^+ and Ca^{2+} homeostatic processes are recommended to confirm this as one of the key mechanisms in life-stage specific sensitivity of white sturgeon. It is also important to note that the biochemical and some compensatory responses were measured at only one concentration, and hence establishing a dose−response relationship can provide a better understanding of proposed mechanisms in future studies. Investigations into mechanisms of differences among life-stages of fishes to metals are ongoing and could streamline ecological risk assessment of metals by allowing the prediction of which species have life-stage specific differences in sensitivity and prediction of which life-stages have greatest sensitivity.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.6b03301.

Detailed experimental materials and methods, data tables, and additional figures as mentioned in the text (PDF)

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

(1) Dang, F.; Wang, W. X.; Rainbow, P. S. Unifying prolonged copper exposure, accumulation, and toxicity from food and water in a marine fish. *Environ. Sci. Technol.* **2012**, *46* (6), 3465−3471.

(2) Yi, Y.; Yang, Z.; Zhang, S. Ecological risk assessment of heavy metals in sediment and human health risk assessment of heavy metals in fishes in the middle and lower reaches of the Yangtze River basin. *Environ. Pollut.* **2011**, *159* (10), 2575−2585.

(3) Birstein, V. J. Sturgeons and Paddlefishes - Threatened Fishes in Need of Conservation. *Conserv. Biol.* **1993**, *7* (4), 773−787.

(4) Kruse, G. O.; Scarnecchia, D. L. Assessment of bioaccumulated metal and organochlorine compounds in relation to physiological biomarkers in Kootenai River white sturgeon. *J. Appl. Ichthyol.* **2002**, *18* (4−6), 430−438.

(5) Pikitch, E. K.; Doukakis, P.; Lauck, L.; Chakrabarty, P.; Erickson, D. L. Status, trends and management of sturgeon and paddlefish fisheries. *Fish Fish.* **2005**, *6* (3), 233−265.

(6) Vardy, D. W.; Oellers, J.; Doering, J. A.; Hollert, H.; Giesy, J. P.; Hecker, M. Sensitivity of early life stages of white sturgeon, rainbow

DOI: 10.1021/acs.est.6b03301
Environ. Sci. Technol. 2016, 50, 9717−9726

trout, and fathead minnow to copper. *Ecotoxicology* **2013**, *22* (1), 139−147.
(7) Calfee, R. D.; Little, E. E.; Puglis, H. J.; Scott, E.; Brumbaugh, W. G.; Mebane, C. A. Acute sensitivity of white sturgeon (Acipenser transmontanus) and rainbow trout (Oncorhynchus mykiss) to copper, cadmium, or zinc in water-only laboratory exposures. *Environ. Toxicol. Chem.* **2014**, *33* (10), 2259−2272.
(8) Hutchinson, T. H.; Solbe, J.; Kloepper-Sams, P. J. Analysis of the ECETOC aquatic toxicity (EAT) database - III - Comparative toxicity of chemical substances to different life stages of aquatic organisms. *Chemosphere* **1998**, *36* (1), 129−142.
(9) Mckim, J. M. Evaluation of Tests with Early Life Stages of Fish for Predicting Long-Term Toxicity. *J. Fish. Res. Board Can.* **1977**, *34* (8), 1148−1154.
(10) *Upper Columbia White Sturgeon Recovery Plan*; Upper Columbia White Sturgeon Recovery Initiative, Technical Report: BC, Canada, 2002; http://www.uppercolumbiasturgeon.org/planning/.
(11) Coutant, C. C. A riparian habitat hypothesis for successful reproduction of white sturgeon. *Rev. Fish. Sci.* **2004**, *12* (1), 23−73.
(12) Vardy, D. W.; Santore, R.; Ryan, A.; Giesy, J. P.; Hecker, M. Acute toxicity of copper, lead, cadmium, and zinc to early life stages of white sturgeon (Acipenser transmontanus) in laboratory and Columbia River water. *Environ. Sci. Pollut. Res.* **2014**, *21* (13), 8176−8187.
(13) Vardy, D. W.; Tompsett, A. R.; Sigurdson, J. L.; Doering, J. A.; Zhang, X.; Giesy, J. P.; Hecker, M. Effects of subchronic exposure of early life stages of white sturgeon (Acipenser transmontanus) to copper, cadmium, and zinc. *Environ. Toxicol. Chem.* **2011**, *30* (11), 2497−2505.
(14) Little, E. E.; Calfee, R. D.; Linder, G. Toxicity of copper to early-life stage Kootenai River white sturgeon, Columbia River white sturgeon, and rainbow trout. *Arch. Environ. Contam. Toxicol.* **2012**, *63* (3), 400−408.
(15) Bridges, C. C.; Zalups, R. K. Molecular and ionic mimicry and the transport of toxic metals. *Toxicol. Appl. Pharmacol.* **2005**, *204* (3), 274−308.
(16) Baldisserotto, B.; Chowdhury, M. J.; Wood, C. A. Effects of dietary calcium and cadmium on cadmium accumulation, calcium and cadmium uptake from the water, and their interactions in juvenile rainbow trout. *Aquat. Toxicol.* **2005**, *72* (1−2), 99−117.
(17) Wood, C. M. An introduction to metals in fish physiology and toxicology: Basic principles. In *Homeostasis and Toxicity of Non-Essential Metals*; Wood, C. M., Farrel, A. P., Brauner, C. J., Eds.; Elsevier Academic Press: San Diego, CA, 2011; pp 1−51.
(18) Komjarova, I.; Bury, N. R. Evidence of common cadmium and copper uptake routes in zebrafish Danio rerio. *Environ. Sci. Technol.* **2014**, *48* (21), 12946−12951.
(19) Eyckmans, M.; Tudorache, C.; Darras, V. M.; Blust, R.; De Boeck, G. Hormonal and ion regulatory response in three freshwater fish species following waterborne copper exposure. *Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol.* **2010**, *152* (3), 270−278.
(20) Sevcikova, M.; Modra, H.; Slaninova, A.; Svobodova, Z. Metals as a cause of oxidative stress in fish: A review. *Vet. Med. (Prague, Czech. Repub.)* **2011**, *56* (11), 537−546.
(21) Tang, S.; Cai, Q.; Chibli, H.; Allagadda, V.; Nadeau, J. L.; Mayer, G. D. Cadmium sulfate and CdTe-quantum dots alter DNA repair in zebrafish (Danio rerio) liver cells. *Toxicol. Appl. Pharmacol.* **2013**, *272* (2), 443−452.
(22) Lushchak, V. I. Adaptive response to oxidative stress: Bacteria, fungi, plants and animals. *Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol.* **2011**, *153* (2), 175−190.
(23) Sutherland, D. E. K.; Stillman, M. J. The "magic numbers" of metallothionein. *Metallomics* **2011**, *3* (5), 444−463.
(24) Tang, S.; Allagadda, V.; Chibli, H.; Nadeau, J. L.; Mayer, G. D. Comparison of cytotoxicity and expression of metal regulatory genes in zebrafish (Danio rerio) liver cells exposed to cadmium sulfate, zinc sulfate and quantum dots. *Metallomics* **2013**, *5* (10), 1411−1422.
(25) Uren Webster, T. M.; Bury, N.; van Aerle, R.; Santos, E. M. Global transcriptome profiling reveals molecular mechanisms of metal tolerance in a chronically exposed wild population of brown trout. *Environ. Sci. Technol.* **2013**, *47* (15), 8869−8877.
(26) Tang, S.; Wu, Y.; Ryan, C. N.; Yu, S.; Qin, G.; Edwards, D. S.; Mayer, G. D. Distinct expression profiles of stress defense and DNA repair genes in Daphnia pulex exposed to cadmium, zinc, and quantum dots. *Chemosphere* **2015**, *120*, 92−99.
(27) Krumschnabel, G.; Podrabsky, J. E. Fish as model systems for the study of vertebrate apoptosis. *Apoptosis* **2009**, *14* (1), 1−21.
(28) Benson, W. H.; Birge, W. J. Heavy-Metal Tolerance and Metallothionein Induction in Fathead Minnows - Results from Field and Laboratory Investigations. *Environ. Toxicol. Chem.* **1985**, *4* (2), 209−217.
(29) Olsson, P. E.; Kille, P. Functional comparison of the metal-regulated transcriptional control regions of metallothionein genes from cadmium-sensitive and tolerant fish species. *Biochim. Biophys. Acta, Gene Struct. Expression* **1997**, *1350* (3), 325−334.
(30) De Boeck, G.; Ngo, T. T. H.; Van Campenhout, K.; Blust, R. Differential metallothionein induction patterns in three freshwater fish during sublethal copper exposure. *Aquat. Toxicol.* **2003**, *65* (4), 413−424.
(31) Eyckmans, M.; Celis, N.; Horemans, N.; Blust, R.; De Boeck, G. Exposure to waterborne copper reveals differences in oxidative stress response in three freshwater fish species. *Aquat. Toxicol.* **2011**, *103* (1−2), 112−120.
(32) Doering, J. A.; Beitel, S. C.; Eisner, B. K.; Heide, T.; Hollert, H.; Giesy, J. P.; Hecker, M.; Wiseman, S. B. Identification and response to metals of metallothionein in two ancient fishes: White sturgeon (Acipenser transmontanus) and lake sturgeon (Acipenser fulvescens). *Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol.* **2015**, *171*, 41.
(33) *Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates, and Amphibians*; American Society for Testing and Materials: West Conshohocken, PA, 2007; Vol. *E*, pp 729−796.
(34) *Standard Guide for Conducting Early Life-Stage Toxicity Tests with Fishes*; American Society for Testing and Materials: West Conshohocken, PA, 2009; Vol. *E*, pp 1241−1205.
(35) Doering, J. A.; Tang, S.; Peng, H.; Eisner, B. K.; Sun, J.; Giesy, J. P.; Wiseman, S.; Hecker, M. High Conservation in Transcriptomic and Proteomic Response of White Sturgeon to Equipotent Concentrations of 2,3,7,8-TCDD, PCB 77, and Benzo[a]pyrene. *Environ. Sci. Technol.* **2016**, *50* (9), 4826−4835.
(36) Zee, J.; Patterson, S.; Wiseman, S.; Hecker, M. Is hepatic oxidative stress a main driver of dietary selenium toxicity in white sturgeon (Acipenser transmontanus)? *Ecotoxicol. Environ. Saf.* **2016**, *133*, 334−440.
(37) Livak, K. J.; Schmittgen, T. D. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **2001**, *25* (4), 402−408.
(38) Shariati, F.; Esaili Sari, A.; Mashinchian, A.; Pourkazemi, M. Metallothionein as Potential Biomarker of Cadmium Exposure in Persian Sturgeon (Acipenser persicus). *Biol. Trace Elem. Res.* **2011**, *143* (1), 281−291.
(39) Vogel, C.; Marcotte, E. M. Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. *Nat. Rev. Genet.* **2012**, *13* (4), 227−232.
(40) Knapen, D.; Reynders, H.; Bervoets, L.; Verheyen, E.; Blust, R. Metallothionein gene and protein expression as a biomarker for metal pollution in natural gudgeon populations. *Aquat. Toxicol.* **2007**, *82* (3), 163−172.
(41) Dang, Z.; Lock, R. A.; Flik, G.; Wendelaar Bonga, S. E. Na(+)/K(+)-ATPase immunoreactivity in branchial chloride cells of Oreochromis mossambicus exposed to copper. *J. Exp. Biol.* **2000**, *203*, 379−387.
(42) Pandey, S.; Parvez, S.; Ansari, R. A.; Ali, M.; Kaur, M.; Hayat, F.; Ahmad, F.; Raisuddin, S. Effects of exposure to multiple trace metals on biochemical, histological and ultrastructural features of gills of a freshwater fish, Channa punctata Bloch. *Chem.-Biol. Interact.* **2008**, *174* (3), 183−192.

DOI: 10.1021/acs.est.6b03301
Environ. Sci. Technol. 2016, 50, 9717−9726

(43) Sinha, A. K.; AbdElgawad, H.; Giblen, T.; Zinta, G.; De Rop, M.; Asard, H.; Blust, R.; De Boeck, G. Anti-oxidative defences are modulated differentially in three freshwater teleosts in response to ammonia-induced oxidative stress. *PLoS One* **2014**, *9* (4), e95319.
(44) Sellin, M. K.; Tate-Boldt, E.; Kolok, A. S. Acclimation to Cu in fathead minnows: Does age influence the response? *Aquat. Toxicol.* **2005**, *74* (2), 97−109.
(45) McNulty, H. R.; Anderson, B. S.; Hunt, J. W.; Turpen, S. L.; Singer, M. M. Age-specific toxicity of copper to larval topsmelt Ather1Nops Affinis. *Environ. Toxicol. Chem.* **1994**, *13* (3), 487−492.
(46) Hochleithner, M.; Gessner, J. *The Sturgeons and Paddlefishes (Acipenseriformes) of the World: Biology and Aquaculture*. AquaTech Publciaitons: Kitzbuehl, Austria, 2001.
(47) Adiele, R. C.; Stevens, D.; Kamunde, C. Differential inhibition of electron transport chain enzyme complexes by cadmium and calcium in isolated rainbow trout (Oncorhynchus mykiss) hepatic mitochondria. *Toxicol. Sci.* **2012**, *127* (1), 110−119.
(48) Dugmonits, K.; Ferencz, A.; Jancso, Z.; Juhasz, R.; Hermesz, E. Major distinctions in the antioxidant responses in liver and kidney of Cd(2+)-treated common carp (Cyprinus carpio). *Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol.* **2013**, *158* (4), 225−230.
(49) Rahman, K. Studies on free radicals, antioxidants, and co-factors. *Clin. Interv. Aging* **2007**, *2* (2), 219−236.
(50) Habeebu, S. S. M.; Liu, J.; Klaassen, C. D. Cadmium-induced apoptosis in mouse liver. *Toxicol. Appl. Pharmacol.* **1998**, *149* (2), 203−209.
(51) Berntssen, M. H. G.; Aspholm, O. O.; Hylland, K.; Wendelaar Bonga, S. E.; Lundebye, A. K. Tissue metallothionein, apoptosis and cell proliferation responses in Atlantic salmon (Salmo salar L.) parr fed elevated dietary cadmium. *Comp. Biochem. Physiol., Part C: Toxicol. Pharmacol.* **2001**, *128* (3), 299−310.
(52) Harrison, S. E.; Klaverkamp, J. F. Uptake, Elimination and Tissue Distribution of Dietary and Aqueous Cadmium by Rainbow-Trout (Salmo-Gairdneri Richardson) and Lake Whitefish (Coregonus-Clupeaformis Mitchill). *Environ. Toxicol. Chem.* **1989**, *8* (1), 87−97.
(53) Hogstrand, C.; Haux, C. Binding and Detoxification of Heavy-Metals in Lower-Vertebrates with Reference to Metallothionein. *Comp. Biochem. Physiol., C: Comp. Pharmacol.* **1991**, *100* (1−2), 137−141.
(54) Chu, J.; Sadler, K. C. New school in liver development: lessons from zebrafish. *Hepatology* **2009**, *50* (5), 1656−1663.
(55) Kondera, E.; Lugowska, K.; Sarnowski, P. High affinity of cadmium and copper to head kidney of common carp (Cyprinus carpio L.). *Fish Physiol. Biochem.* **2014**, *40* (1), 9−22.
(56) Meador, J. P. Tissue concentrations as the dose metric to assess potential toxic effects of metals in field-collected fish: Copper and cadmium. *Environ. Toxicol. Chem.* **2015**, *34* (6), 1309−1319.
(57) Mohammed, A. Why are Early Life Stages of Aquatic Organisms more Sensitive to Toxicants than Adults? In *New Insights into Toxicity and Drug Testing*; Gowder, S., Ed.; InTech: Rijeka, Croatia, 2013.
(58) Mitane, Y.; Tohyama, C.; Saito, H. The role of metallothionein in the elevated excretion of copper in urine from people living in a cadmium-polluted area. *Toxicol. Sci.* **1986**, *6* (2), 285−291.
(59) Hauser-Davis, R. A.; Bastos, F. F.; Tuton, B.; Chavez Rocha, R.; Saint' Pierre, T.; Ziolli, R. L.; Arruda, M. A. Bile and liver metallothionein behavior in copper-exposed fish. *J. Trace Elem. Med. Biol.* **2014**, *28* (1), 70−74.
(60) Ahearn, G. A.; Mandal, P. K.; Mandal, A. Mechanisms of heavy-metal sequestration and detoxification in crustaceans: a review. *J. Comp. Physiol., B* **2004**, *174* (6), 439−452.
(61) *Canadian Water Quality Guidelines for the Protection of Aquatic Life*; Canadian Environmental Quality Guidelines; Canadian Council of Ministers of the Environment: Winnipeg, MB, 1999.

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Supporting Information Cover Page

Linking oxidative stress and magnitude of compensatory responses with life-stage specific differences in sensitivity of white sturgeon (*Acipenser transmontanus*) to copper or cadmium

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Supporting Information Contents

26 Pages, 7 Figures, 7 Tables

MATERIALS AND METHODS

Test chemicals

Copper (II) sulfate pentahydrate (Chemical Abstracts Service CAS 7758-99-8; purity 99.995%) and cadmium chloride hemi-pentahydrate (CAS 7790-78-5; purity 99.999%) were obtained from Sigma-Aldrich (Oakville, ON, Canada). Stock solutions of Cu and Cd were prepared in reverse osmosis water and dechlorinated city of Saskatoon municipal tap water to approximate conditions (hardness, alkalinity and dissolved organic carbon [DOC]) found in the transboundary reach of the Columbia River where the breeding stock of the fish used in this study originated from. Stock and working solutions were allowed to equilibrate for a minimum of 24 h prior to use.

Water chemistry

Water quality parameters including temperature, pH and dissolved oxygen (DO) were measured every 24 h after water change by use of an YSI Quatro Multi-Parameter probe (Yellow Springs, OH, USA). Hardness and ammonia were determined every 24 h by use of Nutrafin Test kits (Hagen, Montreal, QC). Concentrations of Cd or Cu in exposure solutions were measured at initiation and termination of each exposure by use of inductively coupled plasma mass spectrometry (ICP-MS, Thermo X Series, Thermo-Fisher, Bremen, Germany) following USEPA Method ILM05.2D. Concentrations of inorganic cations (Na^+, K^+, Mg^{2+} and Ca^{2+}) and anions (Cl^-, SO_4^{2-} and PO_4^{3-}) were determined according to USEPA Method 300.1 by use of a Dionex ICS-3000 dual-ion chromatography system (Dionex, Sunnyvale, CA, USA). Dissolved organic carbon (DOC) was analyzed by use of a TOC-V CPN (model 5000, Shimanzu Co., Kyoto, Japan). Based on the measured water quality parameters, speciation analysis of Cd or Cu was performed using biotic ligand model (BLM) Visual MINTEQ version 3.0. All calculations and reported values pertaining to the dosing of metals are based on measured concentrations.

Metal uptake

Samples of whole body (15 dph; four samples), and head or body (48 or 139 dph; two samples each) from sturgeon exposed to 10 µg Cu/L or 10 µg Cd/L (5 µg Cd/L at 48 dph) for 96 h were dried at 60 ^oC for 72 h in an oven (Thermo Scientific). Samples from each treatment group were pooled and approximately 0.01 to 0.1 g of tissue were cold digested for 5 h by use of

5 mL of ultra-pure concentrated 70% nitric acid and 1.5 mL of 30% hydrogen peroxide (trace metal grade, Fisher Scientific). Digested samples including method blanks and a standard (TORT-2 lobster hepatopancreas, National Research Council of Canada, Ottawa, ON, Canada) were evaporated at 65 °C and reconstituted in 5 mL of 2% nitric acid. Samples were analyzed by use of ICP-MS. Results were normalized to dry mass and expressed as µg Cu or Cd /g tissue.

RNA isolation and quantitative real-time PCR

Total RNA was extracted from the whole body (15 dph), or head or body (48 and 139 dph) by use of the RNeasy Lipid Tissue Kit (Qiagen, Mississauga, ON, Canada) according to the manufacturer's protocol. Concentrations of RNA were determined by use of a NanoDrop ND-1000 Spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA) and samples of RNA were stored at -80 ºC. First-strand cDNA was synthesised from 1 µg of RNA by use of the QuantiTect Reverse Transcription Kit (Qiagen) according to the manufacturer's protocol. Quantitative real-time polymerase chain reaction (qPCR) was performed in 96-well plates by use of an ABI 7300 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). A 50 µL reaction mixture of 2x concentrated QuantiFast SYBR green master mix, 2.5 µL of cDNA, 10 pmol gene-specific primers, and nuclease free water was prepared for each cDNA sample and primer combination. Primers for *β-actin* and *MT* were described previously,[1] and primers for genes involved with responses to oxidative stress (*GPx*, *GST*, *SOD* and *CAT*) and genes associated with apoptosis (*CASP3* and *AIF*) were designed by use of Primer3 software (Table S7) based on sequences from *de novo* assembly and annotation of the transcriptome of white sturgeon that was generated in house by use of a MiSeq instrument (*Illumina*, San Diego, CA) as described previously.[2, 3] Each sample of cDNA was analyzed in duplicate with 20 µL reaction volumes per well. The reaction mixture for qPCR was denatured at 95 ºC for 10 min followed by a thermal cycle profile consisting of denaturing at 95 ºC for 10 s and extension for 1 min at 60 ºC for a total of 40 PCR cycles. Abundance of transcripts of genes of interest was quantified by normalizing to *β-actin* according to the $2^{-\Delta\Delta Ct}$ method.[4]

Statistical analysis

The LC_{50}s were calculated by use of the two-parameter log-logistic function (LL.2) in 'drc' package (drc: Analysis of dose-response curve data) of R software version 3.1.2 (R Foundation

for Statistical Computing, Vienna, Austria). In cases where a LC_{50} could not be estimated because mortality at the greatest concentration tested was less than 50%, the LC_{50} was reported as being greater than the greatest concentration tested. A 'fitdistr' function from the 'MASS' package in R was used to fit a distribution to the species sensitivity data. Internal concentrations of metals were log-transformed and tested for normality and homogeneity of variance using the Shapiro-Wilk test and Bartlett's test, respectively. Three-way analysis of variance (ANOVA) was then used to indicate significant differences in transformed internal concentrations among life-stages, tissues and treatments by R. To make qPCR data more intuitive, radar plots of fold-changes in abundances of transcripts were constructed by use of the function CreateRadialPlot (http://pcwww.liv.ac.uk/%7Ewilliam/Geodemographic%20Classifiability/func%20CreateRadialPlot.r) via R. A clustering analysis was used to compare the assemblages of measured effects at different life-stages by use of the Bray-Curtis similarity matrix through the 'clustsig' package (clustsig: Significant Cluster Analysis) in R. Pearson's correlation coefficients (PCC) were calculated on the measured effects across the three life-stages exposed to Cu or Cd for the correlation analysis via the R package 'corrplot'. Distance-based redundancy analysis (dbRDA) was conducted in R to examine the measured effects in different life-stages of white sturgeon exposed to Cu or Cd. An ANOVA-like permutation test available in the 'vegan' package (vegan: Community Ecology, version 2.2-1) was performed to determine which axes explained the greatest percentage of sample variation and if sample clustering was statistically different. All other statistical analyses were conducted by use of either R or GraphPad Prism version 5.0 (GraphPad, La Jolla, CA, USA). When statistically significant differences were found, a Dunnett's post-hoc test was used to determine which treatments were significantly different from control. Differences were considered statistically significant at a $p \leqslant 0.05$.

Table S1. Concentrations of Cd measured by ICP-MS and estimated speciation (%) of Cd in acute toxicity experiments to different life-stages of white sturgeon. Measured concentrations (Mean ± S.D.) were the average Cd concentration in exposure solutions at initiation (0 h) and termination (96 h) of each exposure. Speciation of Cd with common inorganic ligands (as a percentage of total Cd species present) was estimated based on water chemistry variables using biotic ligand model (BLM) visual MINTEQ (version 3.0).

Life Stage	Nominal Concentrations (µg/L)	Measured Concentrations (µg/L) Mean ± S.D.	Cd Speciation (%)					
			Cd^{+2}	$CdOH^{+}$	$CdCl^{+}$	$CdSO_4$	$CdHCO_3^{+}$	$CdCO_3$
15 dph	0	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
15 dph	1.25	1.30 ± 0.06	87.78	0.10	0.76	3.10	1.44	1.54
15 dph	2.5	2.48 ± 0.10	89.00	0.09	0.77	3.14	1.46	1.33
15 dph	5	4.75 ± 0.18	90.30	0.08	0.78	3.17	1.48	1.25
15 dph	10	10.12 ± 0.49	91.14	0.08	0.78	3.15	1.48	1.25
15 dph	20	20.92 ± 0.20	91.80	0.08	0.78	3.09	1.47	1.21
48 dph	0	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
48 dph	1.25	1.11 ± 0.19	88.92	0.10	0.76	3.05	1.46	1.63
48 dph	2.5	2.57 ± 0.06	89.74	0.13	0.77	3.06	1.46	1.97
48 dph	5	5.04 ± 0.17	90.78	0.09	0.77	3.08	1.48	1.47
48 dph	10	10.26 ± 0.12	91.34	0.10	0.77	3.06	1.48	1.57
48 dph	20	20.31 ± 0.29	91.75	0.11	0.77	3.00	1.46	1.63
139 dph	0	0.16 ± 0.02	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
139 dph	1.6	1.65 ± 0.01	88.51	0.12	0.62	2.70	1.40	2.07
139 dph	4	4.14 ± 0.18	88.71	0.13	0.62	2.69	1.41	2.07
139 dph	10	9.92 ± 0.13	90.50	0.10	0.63	2.71	1.42	1.63
139 dph	25	24.48 ± 0.70	91.95	0.08	0.63	2.66	1.43	1.23
139 dph	62.5	59.68 ± 1.45	92.92	0.08	0.61	2.49	1.39	1.28

n.d. and n.a. indicate below detection limit and not available, respectively.

Table S2. Concentrations of Cu measured by ICP-MS and estimate speciation (%) of Cu in acute toxicity experiments to different life-stages of white sturgeon. Measured concentrations (Mean ± S.D.) were the average Cu concentration in exposure solutions at initiation (0 h) and termination (96 h) of each exposure. Speciation of Cu with common inorganic ligands (as a percentage of total Cu species present) was estimated based on water chemistry variables using biotic ligand model (BLM) visual MINTEQ (version 3.0).

Life Stage	Nominal Concentrations (µg/L)	Measured Concentrations (µg/L) Mean ± S.D.	Cu Speciation (%)						
			Cu^{+2}	$CuOH^{+}$	$Cu(OH)_2$	$CuSO_4$	$CuCO_3$	$CuHCO_3^{+}$	$Cu(CO3)_2^{-2}$
15 dph	0	1.37 ± 0.38	11.81	8.52	0.30	0.40	63.71	0.37	0.23
15 dph	1.25	2.47 ± 0.22	15.32	8.88	0.25	0.53	64.37	0.47	0.18
15 dph	2.5	4.00 ± 0.89	15.74	9.13	0.26	0.54	63.23	0.46	0.17
15 dph	5	7.22 ± 0.49	16.23	9.25	0.26	0.56	57.98	0.43	0.14
15 dph	10	10.75 ± 0.39	17.67	8.97	0.22	0.61	50.73	0.42	0.10
15 dph	20	21.08 ± 1.01	19.53	8.08	0.17	0.67	33.01	0.34	0.04
48 dph	0	1.35 ± 0.22	12.10	8.97	0.33	0.41	67.14	0.38	0.25
48 dph	1.25	2.18 ± 0.29	14.88	9.00	0.27	0.50	65.89	0.46	0.20
48 dph	2.5	3.62 ± 0.24	15.00	9.28	0.28	0.50	65.09	0.44	0.19
48 dph	5	6.11 ± 0.48	13.68	9.50	0.32	0.46	61.49	0.37	0.19
48 dph	10	10.92 ± 0.33	13.43	9.12	0.30	0.45	50.58	0.31	0.13
48 dph	20	20.59 ± 0.98	12.93	8.03	0.24	0.43	31.46	0.21	0.05
139 dph	0	n.d.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
139 dph	4	4.83 ± 0.24	14.20	9.11	0.28	0.43	63.34	0.39	0.19
139 dph	10	10.76 ± 0.68	14.92	8.93	0.25	0.45	51.52	0.34	0.12
139 dph	25	24.44 ± 0.11	15.76	7.65	0.18	0.47	27.28	0.22	0.03
139 dph	62.5	57.60 ± 0.52	12.06	5.30	0.11	0.34	0.08	n.a.	n.a.
139 dph	156.25	148.50 ± 1.35	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

n.d. and n.a. indicate below detection limit and not available, respectively.

Table S3. Water quality including temperature, DO, pH, DOC, ammonia, and inorganic cations and anions (Mean ± S.D.) in all exposure water samples at each life-stage.

Life Stage	Temperature	DO%	pH	DOC (mg/L)	Ammonia	Anions (mg/L)			Cations (mg/L)			
						Cl^-	SO_4^{2-}	PO_4^{3-}	Na^+	K^+	Mg^{2+}	Ca^{2+}
15 dph	16.05 ± 0.22	87.74 ± 8.10	7.48 ± 0.11	1.45 ± 0.16	n.d.	4.05 ± 0.07	25.46 ± 0.06	n.d.	10.39 ± 0.11	1.01 ± 0.02	5.43 ± 0.06	9.38 ± 0.30
48 dph	15.91 ± 0.08	81.49 ± 5.04	7.55 ± 0.08	1.12 ± 0.05	n.d.	3.98 ± 0.14	24.63 ± 0.50	n.d.	10.04 ± 0.15	1.00 ± 0.02	5.46 ± 0.10	9.11 ± 0.29
139 dph	14.84 ± 0.15	105.86 ± 3.09	7.54 ± 0.14	1.85 ± 0.37	n.d.	3.29 ± 0.12	22.35 ± 0.28	n.d.	8.89 ± 0.15	0.89 ± 0.02	5.21 ± 0.10	11.15 ± 0.21

n.d. indicates below detection limit.

Table S4. Acute toxicity (survival) data of different life-stages of white sturgeon exposed to Cu and Cd for 96 h.

Exposure	Life Stage	Tank	Measured Concentrations	Alive	Dead
Cu	15 dph	1	1.37	11	1
	15 dph	2	1.37	9	3
	15 dph	3	1.37	7	5
	15 dph	4	1.37	5	7
	15 dph	1	2.47	10	2
	15 dph	2	2.47	10	2
	15 dph	3	2.47	9	3
	15 dph	4	2.47	11	1
	15 dph	1	4.00	9	3
	15 dph	2	4.00	8	4
	15 dph	3	4.00	9	3
	15 dph	4	4.00	8	4
	15 dph	1	7.23	5	7
	15 dph	2	7.23	7	5
	15 dph	3	7.23	10	2
	15 dph	4	7.23	5	7
	15 dph	1	10.75	6	6
	15 dph	2	10.75	7	5
	15 dph	3	10.75	6	6
	15 dph	4	10.75	7	5
	15 dph	1	21.08	1	11
	15 dph	2	21.08	4	8
	15 dph	3	21.08	5	7
	15 dph	4	21.08	1	11
	48 dph	1	1.35	10	0

48 dph	2	1.35	10	0
48 dph	3	1.35	10	0
48 dph	4	1.35	10	0
48 dph	1	2.18	10	0
48 dph	2	2.18	10	0
48 dph	3	2.18	10	0
48 dph	4	2.18	10	0
48 dph	1	3.62	10	0
48 dph	2	3.62	10	0
48 dph	3	3.62	10	0
48 dph	4	3.62	10	0
48 dph	1	6.11	10	0
48 dph	2	6.11	10	0
48 dph	3	6.11	10	0
48 dph	4	6.11	10	0
48 dph	1	10.92	10	0
48 dph	2	10.92	10	0
48 dph	3	10.92	8	2
48 dph	4	10.92	9	1
48 dph	1	20.59	10	0
48 dph	2	20.59	10	0
48 dph	3	20.59	9	1
48 dph	4	20.59	8	2
139 dph	1	0.00	10	0
139 dph	2	0.00	10	0
139 dph	3	0.00	10	0
139 dph	1	4.83	10	0
139 dph	2	4.83	10	0

	139 dph	3	4.83	10	0
	139 dph	1	10.76	10	0
	139 dph	2	10.76	10	0
	139 dph	3	10.76	10	0
	139 dph	1	24.44	10	0
	139 dph	2	24.44	10	0
	139 dph	3	24.44	9	1
	139 dph	1	57.60	8	2
	139 dph	2	57.60	7	3
	139 dph	3	57.60	6	4
	139 dph	1	148.50	0	10
	139 dph	2	148.50	0	10
	139 dph	3	148.50	0	10
Cd	15 dph	1	0.00	11	1
	15 dph	2	0.00	9	3
	15 dph	3	0.00	7	5
	15 dph	4	0.00	5	7
	15 dph	1	1.30	10	2
	15 dph	2	1.30	10	2
	15 dph	3	1.30	10	2
	15 dph	4	1.30	9	3
	15 dph	1	2.48	11	1
	15 dph	2	2.48	9	3
	15 dph	3	2.48	8	4
	15 dph	4	2.48	12	0
	15 dph	1	4.75	11	1
	15 dph	2	4.75	11	1
	15 dph	3	4.75	10	2

	15 dph	4	4.75	10	2
	15 dph	1	10.12	10	2
	15 dph	2	10.12	11	1
	15 dph	3	10.12	11	1
	15 dph	4	10.12	9	3
	15 dph	1	20.92	11	1
	15 dph	2	20.92	12	0
	15 dph	3	20.92	12	0
	15 dph	4	20.92	12	0
	48 dph	1	0.00	10	0
	48 dph	2	0.00	10	0
	48 dph	3	0.00	10	0
	48 dph	4	0.00	10	0
	48 dph	1	1.11	10	0
	48 dph	2	1.11	10	0
	48 dph	3	1.11	10	0
	48 dph	4	1.11	10	0
	48 dph	1	2.58	9	1
	48 dph	2	2.58	10	0
	48 dph	3	2.58	10	0
	48 dph	4	2.58	10	0
	48 dph	1	5.04	3	7
	48 dph	2	5.04	6	4
	48 dph	3	5.04	7	3
	48 dph	4	5.04	8	2
	48 dph	1	10.26	3	7
	48 dph	2	10.26	6	4
	48 dph	3	10.26	5	5

	48 dph	4	10.26	3	7
	48 dph	1	20.31	6	4
	48 dph	2	20.31	2	8
	48 dph	3	20.31	8	2
	48 dph	4	20.31	2	8
	139 dph	1	0.16	10	0
	139 dph	2	0.16	10	0
	139 dph	3	0.16	10	0
	139 dph	1	1.65	10	0
	139 dph	2	1.65	10	0
	139 dph	3	1.65	10	0
	139 dph	1	4.14	10	0
	139 dph	2	4.14	10	0
	139 dph	3	4.14	10	0
	139 dph	1	9.92	10	0
	139 dph	2	9.92	10	0
	139 dph	3	9.92	10	0
	139 dph	1	24.48	10	0
	139 dph	2	24.48	10	0
	139 dph	3	24.48	10	0
	139 dph	1	59.68	10	0
	139 dph	2	59.68	10	0
	139 dph	3	59.68	10	0

Table S5. Summary of acute toxicity (96 h) of Cu and Cd to different life-stages of white sturgeon and other fish species from this study and the literature. LC_{50} (µg/L) values, 95% confidence interval (95% CI), rank and centile for SSD curves are given. When an LC_{50} value could not be calculated because mortality was less than 50% at the greatest concentration tested, the LC_{50} was reported as the greatest test concentration. Control survival for the 15 and 16 dph tests was less than 90 percent, thus the LC_{50} calculation is not definitive.

Exposure	Species	Life-Stage	96 h-LC_{50}	95%CI	Rank	Centile	Reference
Cu	White sturgeon	38 dph	4.1	3.4-4.8	1	0.02	[5]
	White sturgeon	26 dph	4.5	3.4-6.1	2	0.05	[5]
	White sturgeon	40 dph	4.7	3.9-5.4	3	0.07	[5]
	White sturgeon	27 dph	6.8	5.2-8.9	4	0.10	[5]
	White sturgeon	16 dph	7.1	5.0-10.1	5	0.12	[6]
	White sturgeon	40 dph	9	7-12	6	0.15	[7]
	White sturgeon	15 dph	9.2	5.5-12.8	7	0.17	This study
	White sturgeon	15 dph	10	8-12	8	0.20	[7]
	Rainbow trout	Swim-up	17	15-19	9	0.22	[8]
	White sturgeon	45 dph	17	14-21	10	0.24	[7]
	Rainbow trout	Parr	18	15-22	11	0.27	[8]
	Chinook salmon	Swim-up	19	18-21	12	0.29	[8]
	Rainbow trout	95 dph	19.1	15.8-23	13	0.32	[6]
	White sturgeon	48 dph	20	>20	14	0.34	This study
	Rainbow trout	15 dph	21	18-23	15	0.37	[7]
	Rainbow trout	40 dph	22	20-25	16	0.39	[7]
	White sturgeon	8 dph	22	20-25	17	0.41	[7]
	Rainbow trout	45 dph	24	20-28	18	0.44	[7]
	Chinook salmon	Alevin	26	24-33	19	0.46	[8]
	Chinook salmon	Smolt	26	23-35	20	0.49	[8]
	Rainbow trout	Alevin	28	27-30	21	0.51	[8]
	Rainbow trout	Smolt	29	>20	22	0.54	[8]
	Rainbow trout	160 dph	30.9	23.6-40.6	23	0.56	[5]

	Rainbow trout	30 dph	36.5	28-45.1	24	0.59	[5]
	Chinook salmon	Parr	38	35-44	25	0.61	[8]
	Rainbow trout	8 dph	40	34-46	26	0.63	[7]
	Rainbow trout	60 dph	42.4	34.7-51.8	27	0.66	[6]
	White sturgeon	44 dph	49.8	>49.8	28	0.68	[6]
	White sturgeon	100 dph	54	47-62	29	0.71	[7]
	Rainbow trout	18 dph	56.6	50.6-63.4	30	0.73	[6]
	Rainbow trout	46 dph	59	49.2-70.9	31	0.76	[6]
	Rainbow trout	32 dph	59.9	53.1-67.7	32	0.78	[6]
	Rainbow trout	74 dph	60.6	54.9-66.2	33	0.80	[6]
	Rainbow trout	1 dph	62.9	56.6-69.9	34	0.83	[6]
	White sturgeon	139 dph	66.5	56.7-76.3	35	0.85	This study
	White sturgeon	89 dph	90	77-108	36	0.88	[6]
	Fathead minnows	8 dph	102	78-135	37	0.90	[7]
	White sturgeon	167 dph	103.7	79.4-135.5	38	0.93	[5]
	White sturgeon	123 dph	268.9	204.8-352.9	39	0.95	[5]
	White sturgeon	450 dph	269	205-353	40	0.98	[5]
Cd	Rainbow trout	Parr	1	0.8-1.1	1	0.03	[8]
	Rainbow trout	Swim-up	1.3	1.2-1.4	2	0.06	[8]
	Rainbow trout	Juvenile	1.5	1.2-1.8	3	0.09	[9]
	Chinook salmon	Swim-up	1.8	1.7-2	4	0.13	[8]
	Rainbow trout	46 dph	2.8	2.1-3.7	5	0.16	[6]
	Rainbow trout	18 dph	2.9	2.2-3.8	6	0.19	[6]
	Chinook salmon	Smolt	2.9	>2.9	7	0.22	[2]
	Rainbow trout	Smolt	2.9	>2.3	8	0.25	[5]
	Rainbow trout	95 dph	2.96	2.2-4	9	0.28	[6]
	Coho salmon	Juvenile	3.4	2.2-5.1	10	0.31	[9]
	Chinook salmon	Parr	3.5	2.8-5.6	11	0.34	[8]
	Rainbow trout	60 dph	3.7	3.3-4.2	12	0.38	[6]
	Arctic grayling	Juvenile	4	1.5-9.7	13	0.41	[9]
	Rainbow trout	74 dph	4.5	4.1-5	14	0.44	[6]

Rainbow trout	32 dph	4.8	4.3-5.4	15	0.47	[6]
Coho salmon	Alevin	6	5.2-7.1	16	0.50	[9]
Arctic grayling	Alevin	6.1	4.7-7.8	17	0.53	[9]
White sturgeon	48 dph	11.1	6.3-16	18	0.56	This study
White sturgeon	8 dph	14.5	13.7-15.3	19	0.59	[10]
White sturgeon	15 dph	20	>20	20	0.63	This study
Chinook salmon	Alevin	26	>26	21	0.66	[8]
Rainbow trout	Alevin	27	>27	22	0.69	[8]
White sturgeon	61 dph	34.4	<34.4	23	0.72	[6]
Rainbow trout	Alevin	37.9	27.1-53.1	24	0.75	[9]
White sturgeon	2 dph	47.2	>47.2	25	0.78	[6]
Rainbow trout	1 dph	49.4	>49.4	26	0.81	[6]
White sturgeon	139 dph	60	>60	27	0.84	This study
White sturgeon	72 dph	149.5	>149.5	28	0.88	[6]
White sturgeon	16 dph	187	>187	29	0.91	[6]
White sturgeon	89 dph	273.5	>273.5	30	0.94	[6]
White sturgeon	30 dph	355	>355	31	0.97	[6]

Table S6. Measured internal doses (µg/g; dry mass) among life-stages of white sturgeon following 96 h exposure to either 10 µg/L of Cu or Cd. Data are presented from 2 replicated measures (2 or 4 fish per replicate) of either Cu or Cd plus control.

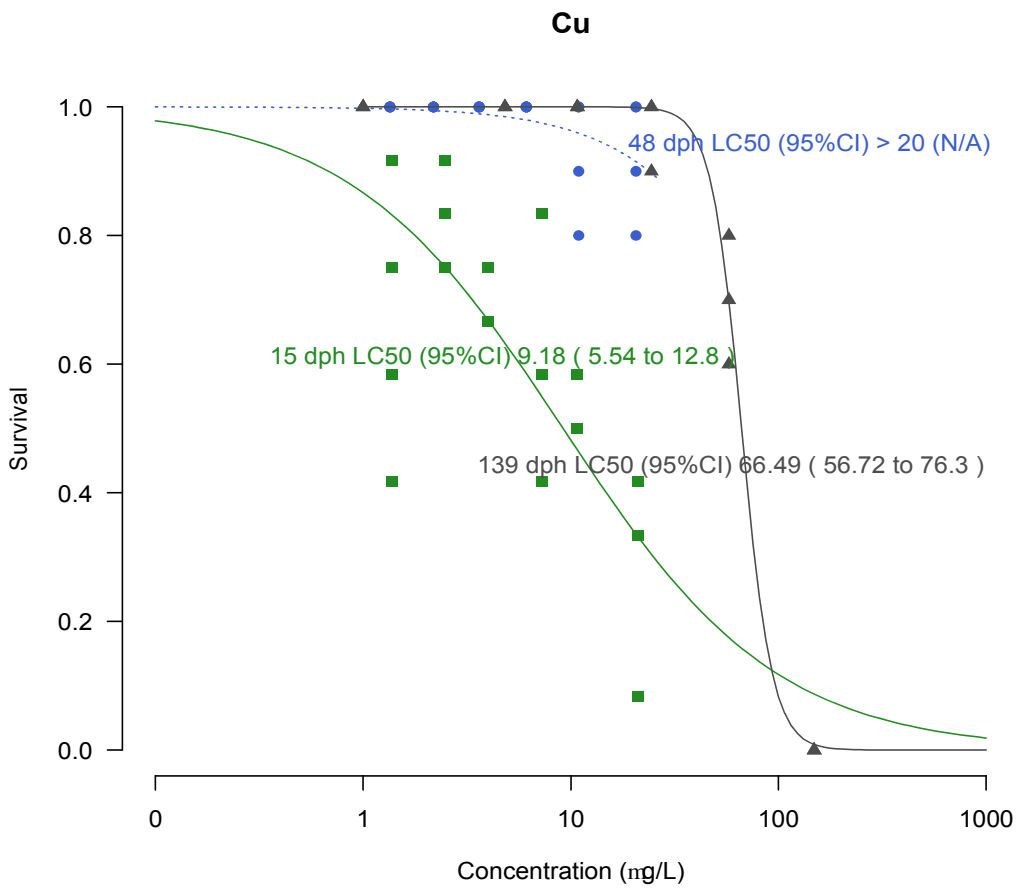
Metal	Life Stage	Treatment	Internal Dose (µg/g, dm) Mean ± S.D.
Cu		Blank	0.002 ± 0.003
	15 dph	Control	8.26 ± 0.75
	15 dph	Cu 10	21.41 ± 0.13
	48 dph	Control-Head	4.38 ± 1.60
	48 dph	Cu 10-Head	9.66 ± 2.18
	48 dph	Control-Body	6.45 ± 1.16
	48 dph	Cu 10-Body	13.23 ± 2.23
	139 dph	Control-Head	2.28 ± 0.51
	139 dph	Cu 10-Head	4.35 ± 0.17
	139 dph	Control-Body	3.35 ± 0.35
	139 dph	Cu 10-Body	5.99 ± 0.58
Cd		Blank	0.0001 ± 0.0002
	15 dph	Control	0.058 ± 0.01
	15 dph	Cd 10	3.96 ± 0.22
	48 dph	Control-Head	0.06 ± 0.01
	48 dph	Cd 10-Head	2.57 ± 0.53
	48 dph	Control-Body	0.28 ± 0.05
	48 dph	Cd 10-Body	2.58 ± 0.33
	139 dph	Control-Head	0.05 ± 0.002
	139 dph	Cd 10-Head	1.14 ± 0.46
	139 dph	Control-Body	0.07 ± 0.02
	139 dph	Cd 10-Body	1.08 ± 0.31

Table S7. Sequences, efficiencies, annealing temperatures, and corresponding target gene Genbank accession number of white sturgeon oligonucleotide primers used in RT-PCR. Sequences with no available accession # are indicated with '-'.

Target Gene	Function	Accession #	Primer Sequence (5' - 3')	Efficiency	Annealing Temp (ºC)
β-actin	Reference	FJ205611	Forward: ACTGCAAGTGCACAGACTG Reverse: AGGAGCAGCAGCTTTTCTTG	1.96	60
MT	Ion homeostasis	KP164836	Forward: CCGAGCACAATGAAAATGA Reverse: ACATCTGCTGGAAGGTGGA	1.89	60
GPx	Oxidative Stress	-	Forward: AGTTGATGTGAACGGGAAGG Reverse: ACTTGGGGTCAGTCATCAGG	2.0	60
GST	Oxidative Stress	-	Forward: CTCCAGGATGAAAACCTTGG Reverse: ACTCAATCCCATGCAAAAGG	2.0	60
CAT	Oxidative Stress	-	Forward: GAACGAAGAAGAGCGCCAG Reverse: GATGCGGCTCCCATAGTCT	1.92	60
SOD	Oxidative Stress	-	Forward: GCAGGTCCGTGGTGATTCAT Reverse: TTCCGATGACACAGCAAGCT	1.87	60
CASP3	Apoptosis	-	Forward: TCACACAGGGACTGGATGAA Reverse: AGTGACAGCTCTCCCCAGAA	1.98	60
AIF	Apoptosis	-	Forward: ATCGTGGGTGGAGGATTTGG Reverse: GCCCCTACGTTGTGATGGAA	1.91	60
ATP7a	Metal Transporter	-	Forward: GCAACAGAACAGGCTCACAA Reverse: CTTGCAACCAACAAAGCTCA	1.94	60
CTR1	Metal Transporter	-	Forward: TAGAGCACAGCCAGCAAGAA Reverse: CTTTGCCCTGGAAACGACTA	2.0	60
DMT1	Metal Transporter	-	Forward: TGATCCCAATCCTCACCTTC Reverse: ATACCCTACGAAGCCCAGGT	2.0	60

Figure S1. Acute toxicity (96 h) of Cu (**A**) and Cd (**B**) to different life-stages of white sturgeon. LC_{50} (µg/L) values and 95% confidence interval (95% CI) of the observed data were estimated via the two-parameter log-logistic function (LL.2) in 'drc' package of R. Cu or Cd concentrations (mean concentration of the initiation-0 h and termination-96 h of experiments) that measured by ICP-MS were used for the calculation. Data are presented from 3 or 4 replicated measures [each green square (15 dph), blue circle (48 dph) and black triangle (139 dph) represent 10 individuals] per exposure concentration of either Cu or Cd. Solid or dashed lines indicate the fits of the experimental data to logit model. Control survival for the 15 dph test was less than 90 percent, thus the LC_{50} calculation is not definitive.

A



B

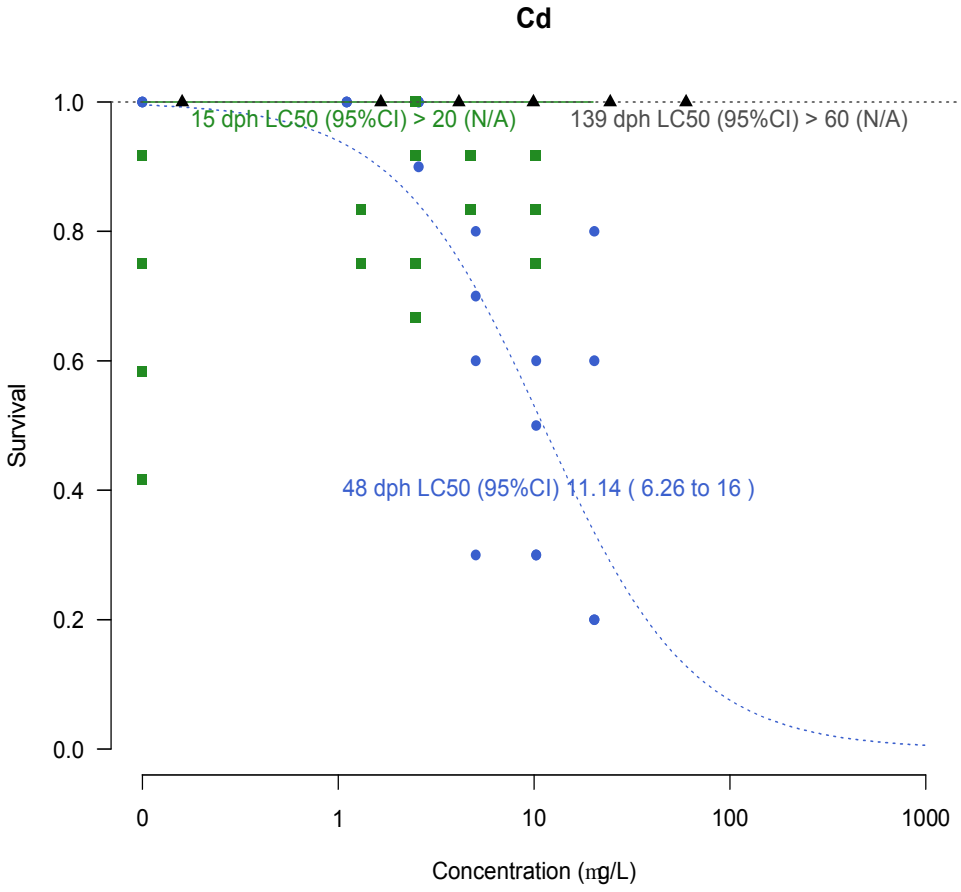
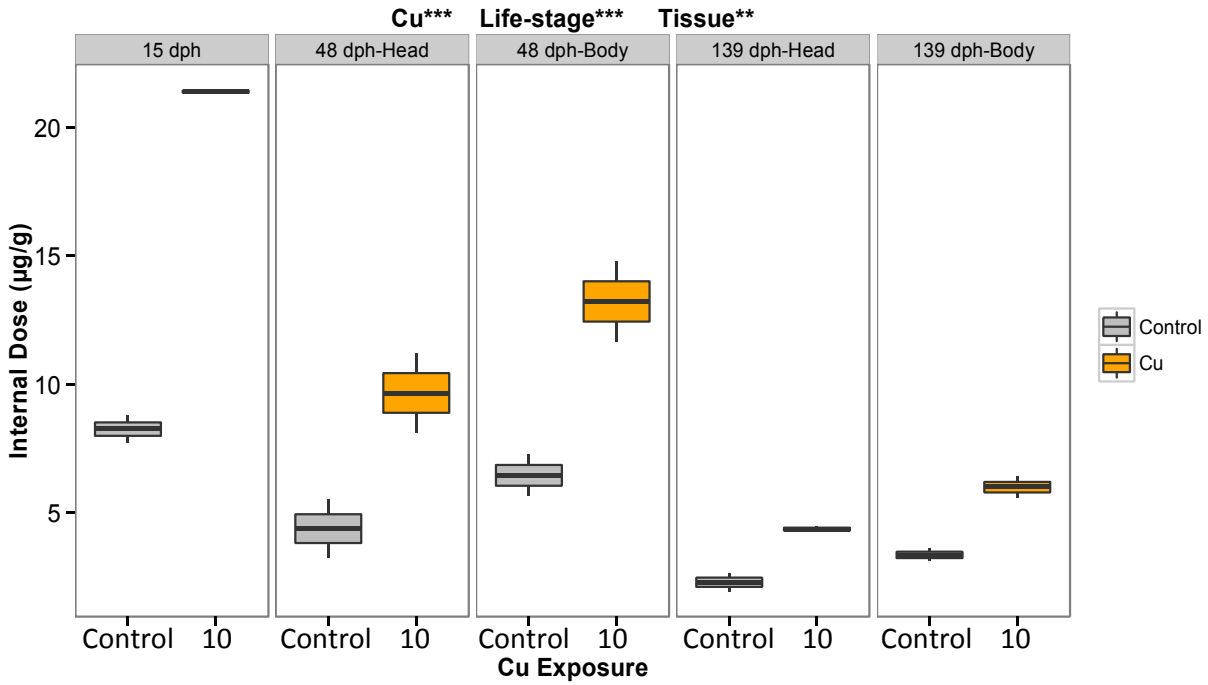


Figure S2. Comparison of internal doses (µg/g; dry mass) among life-stages of white sturgeon following 96 h exposure of 10 µg/L of either Cu (**A**) or Cd (**B**). Box-and-whisker plots (median and interquartile range) are presented from 2 replicated measures (2 or 4 fish per replicate) of either Cu or Cd plus control. Three-way ANOVA was used to compare between Cu or Cd, life-stage, and tissue as well as their interaction effects (**p<0.01 and ***p<0.001).

A



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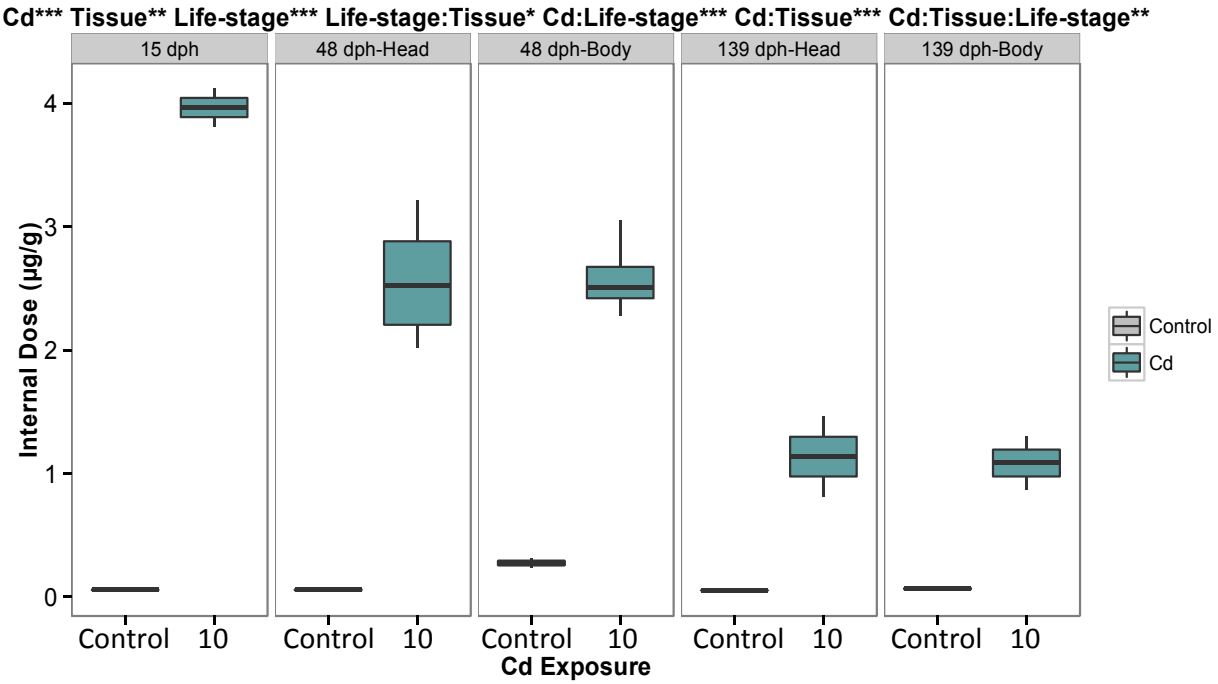


Figure S3. Comparison of dry body mass (g) of white sturgeon controls at different life-stages. Data are presented as mean ± S.D. (2 replicate measures with 2 fish per replicate). Significant difference (**p<0.01 and $^{***}p$<0.001) relative to control at 15 dph.

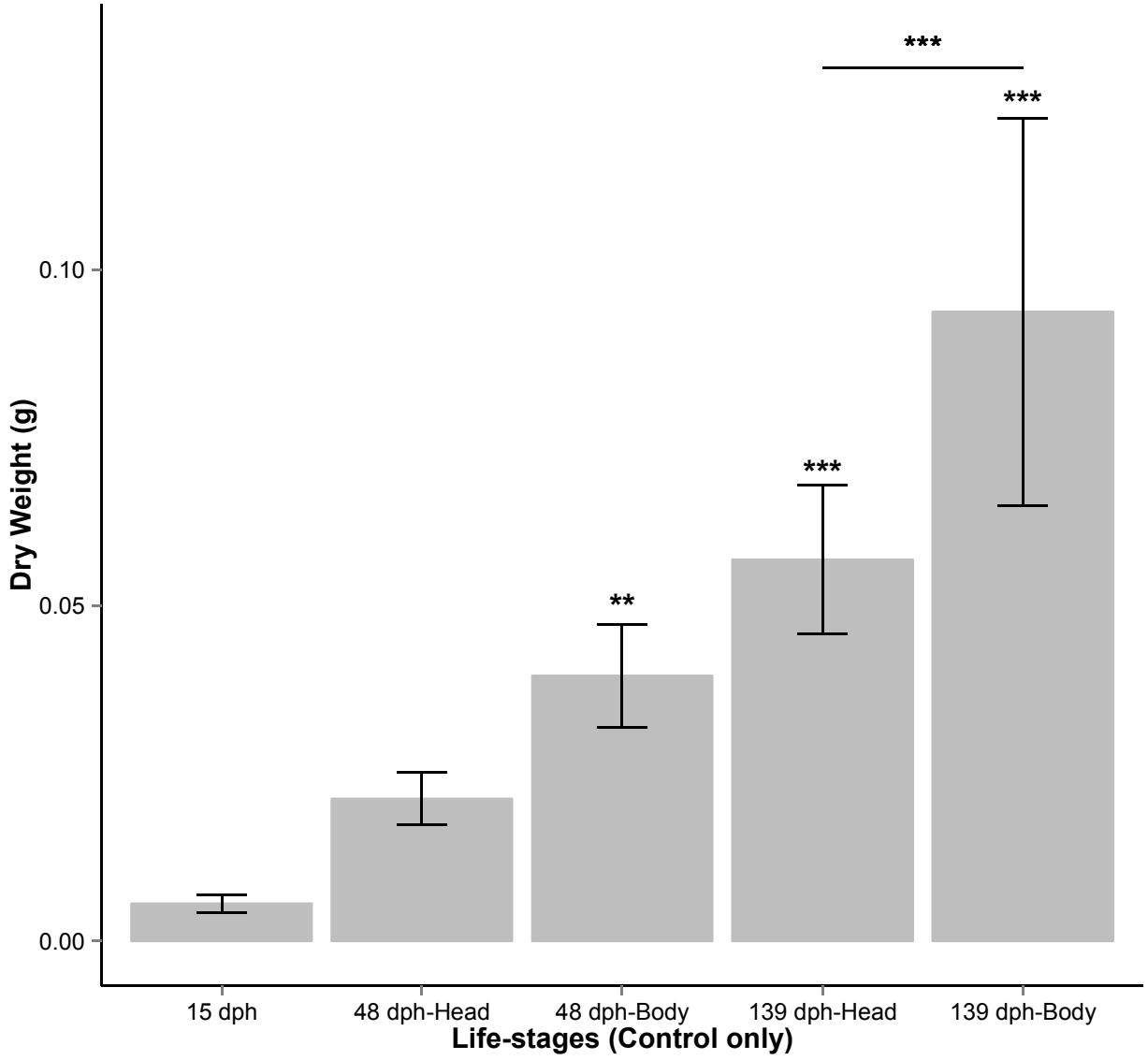


Figure S4. Comparison of basal abundance of transcripts of *MT* in white sturgeon controls at 15, 48 and 139 days post hatch (dph). Data are presented as mean ± S.D. (3 or 4 replicated measures with 1 or 2 fish per replicate). Significant difference (**p<0.01 and $^{***}p$<0.001) relative to 15 dph.

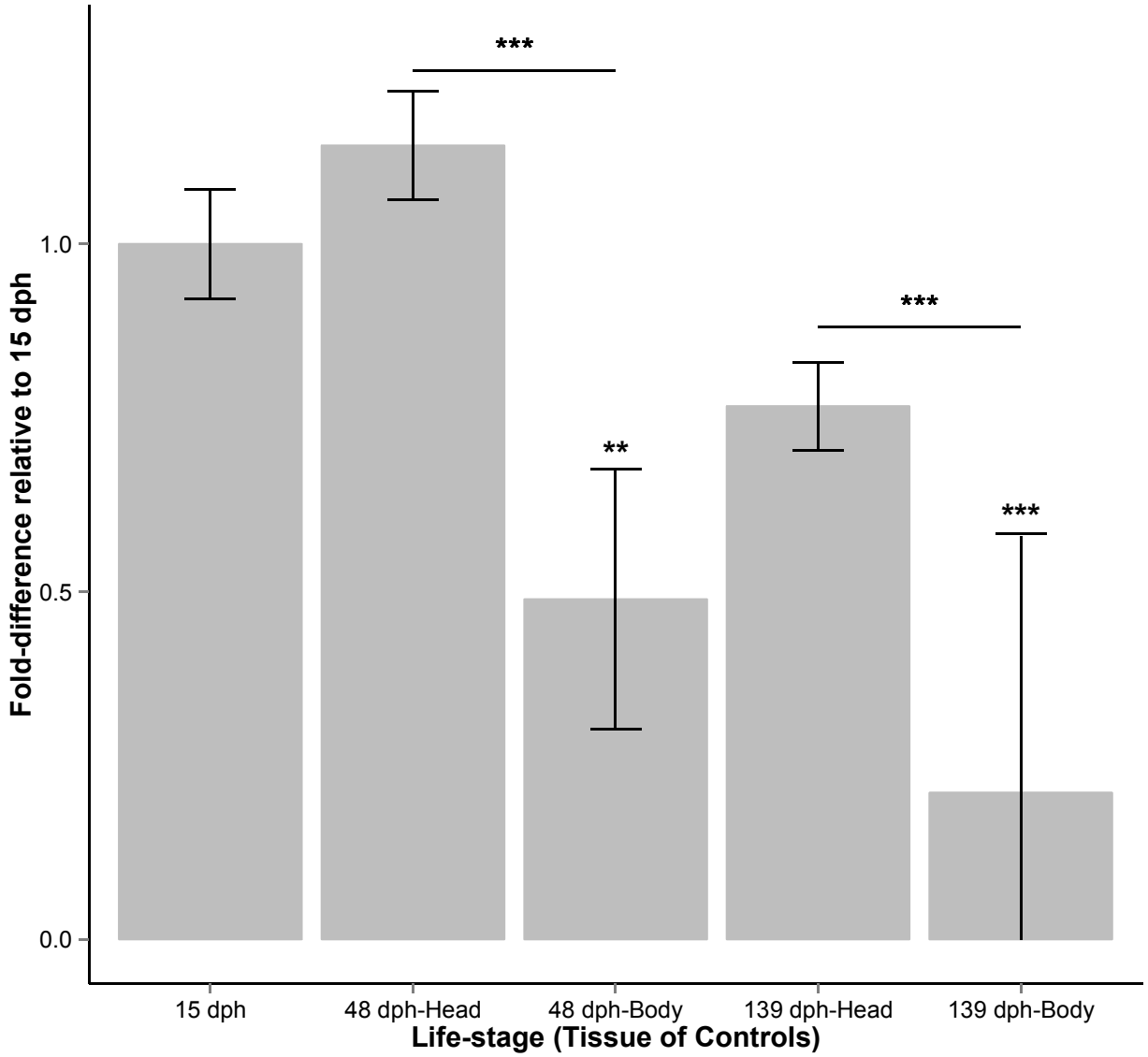
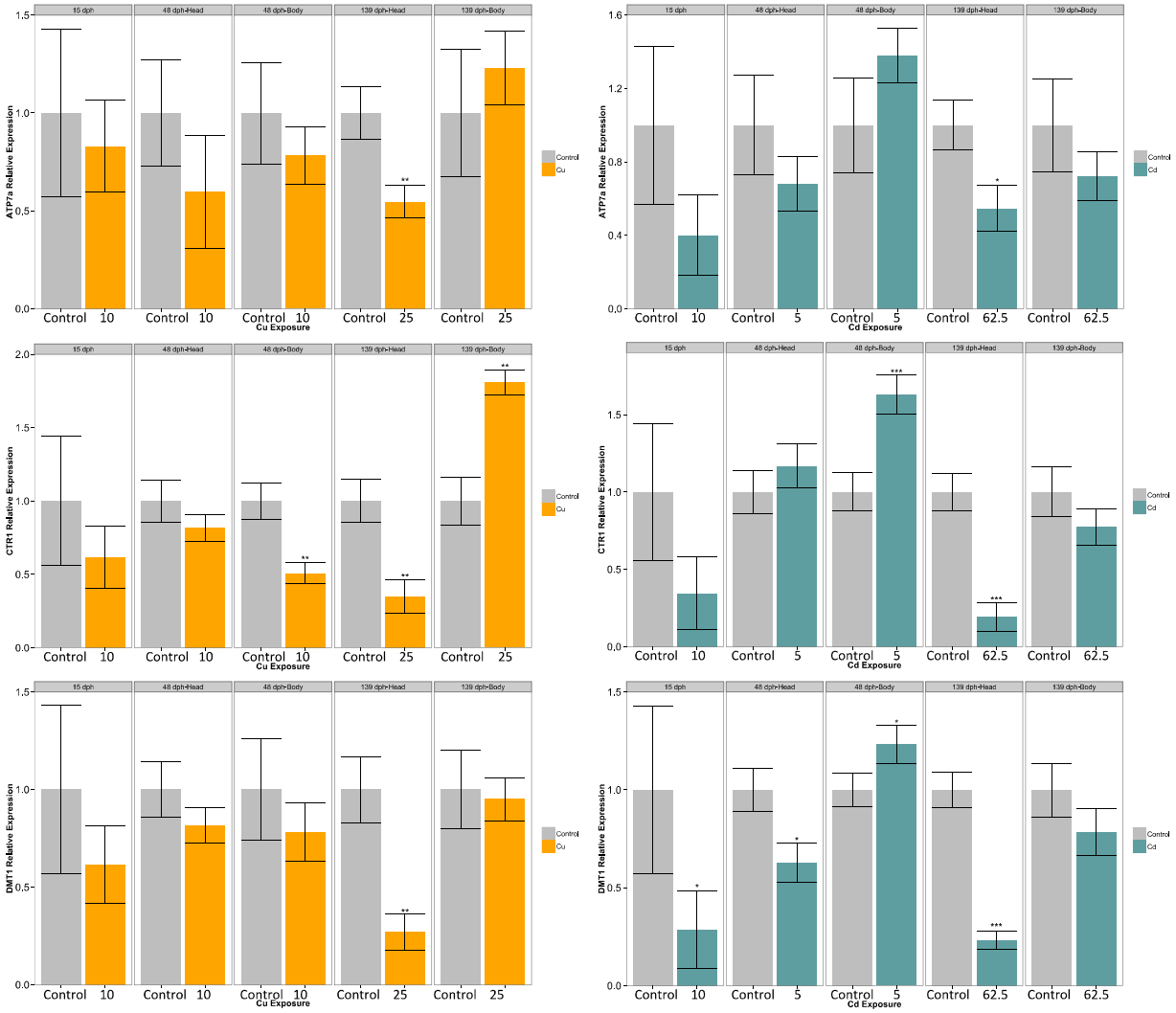
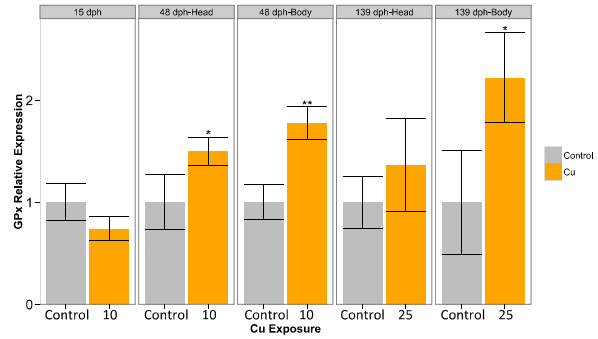


Figure S5. Abundances of transcripts of metal transporters (*ATP7a*, *CTR1*, and *DMT1*), antioxidative (*GPx*, *GST*, *CAT*, and *SOD*) and apoptosis (*CASP3* and *AIF*) genes in life-stages of white sturgeon following 96 h exposure to either Cu or Cd. Data are presented as mean ± S.D. (3 or 4 replicated measures with 1 or 2 fish per replicate). Significant difference (*p<0.05, **p<0.01 and ***p<0.001) relative to controls.

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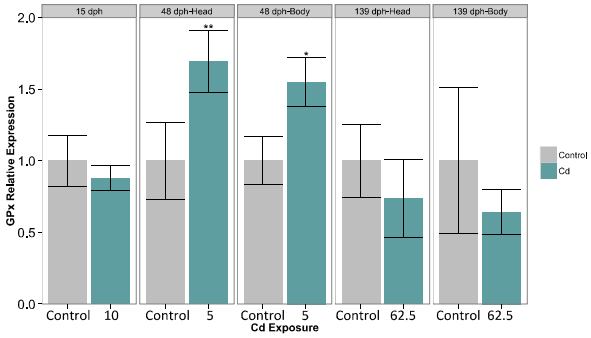
48 dph-Head
48 dph-Body
139 dph-Head
139 dph-Body
GPx Relative Expression
Control
Cu
Cu Exposure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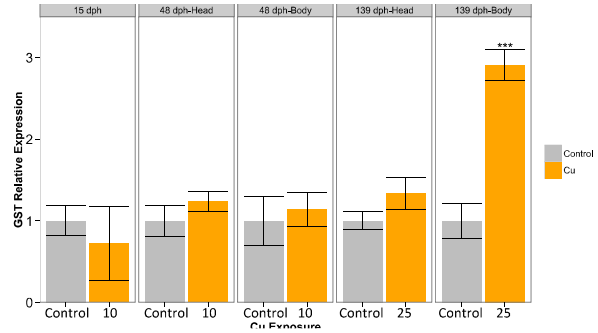
48 dph-Head
48 dph-Body
139 dph-Head
139 dph-Body
GPx Relative Expression
Control
Cd
Cd Exposure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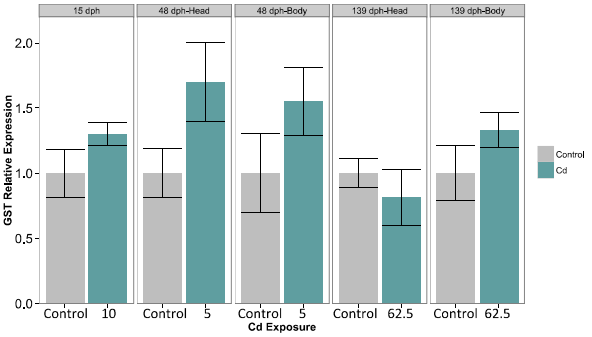
48 dph-Head
48 dph-Body
139 dph-Head
139 dph-Body
GST Relative Expression
Control
Cu
Cu Exposure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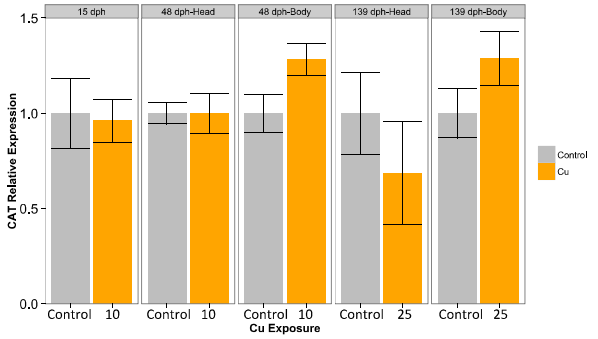
48 dph-Head
48 dph-Body
139 dph-Head
139 dph-Body
GST Relative Expression
Control
Cd
Cd Exposure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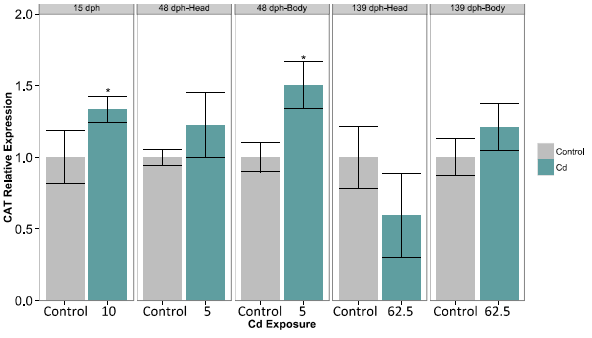
48 dph-Head
48 dph-Body
139 dph-Head
139 dph-Body
CAT Relative Expression
Control
Cu
Cu Exposure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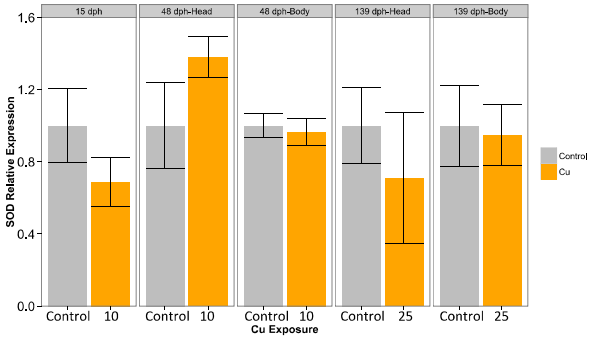
48 dph-Body
139 dph-Head
139 dph-Body
CAT Relative Expression
Control
Cd
Cd Exposure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48 dph-Body
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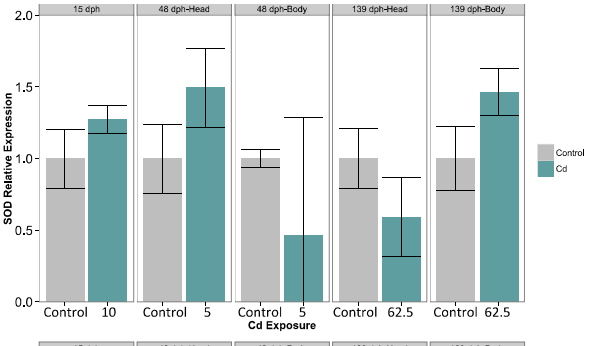
139 dph-Body
SOD Relative Expression
Control
Cu
Cu Exposure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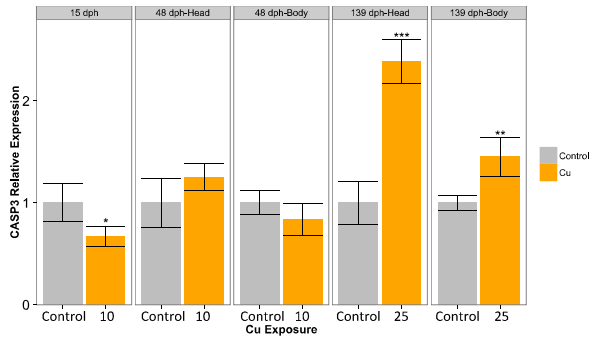
SOD Relative Expression
Control
Cd
Cd Exposure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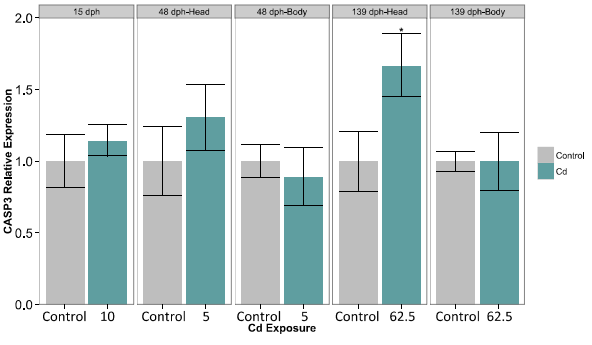
139 dph-Body
CASP3 Relative Expression
Control
Cu
Cu Exposure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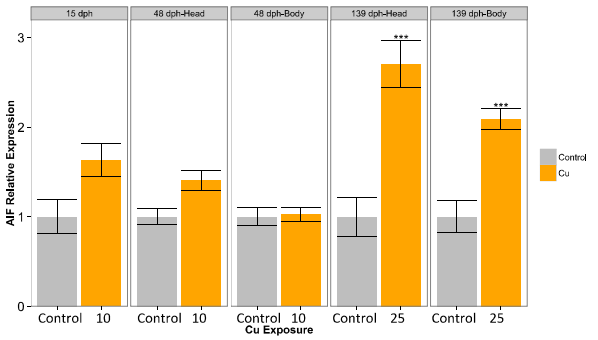
139 dph-Body
CASP3 Relative Expression
Control
Cd
Cd Exposure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48 dph-Body
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139 dph-Body
AIF Relative Expression
Control
Cu
Cu Exposure

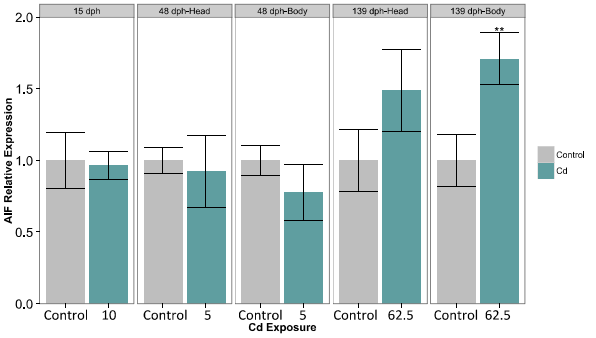

15 dph
48 dph-Head
48 dph-Body
139 dph-Head
139 dph-Body
AIF Relative Expression
Control
Cd
Cd Exposure

Figure S6. Dendrogram displaying similarities of measured effects in white sturgeon across three different life-stages following 96 h exposure to either Cu (**A**) or Cd (**B**). The analysis was performed using the Bray-Curtis similarity matrix through the 'clustsig' package in R. Different colors indicate the statistically different clusters using a similarity profile (simprof) test (p<0.05).

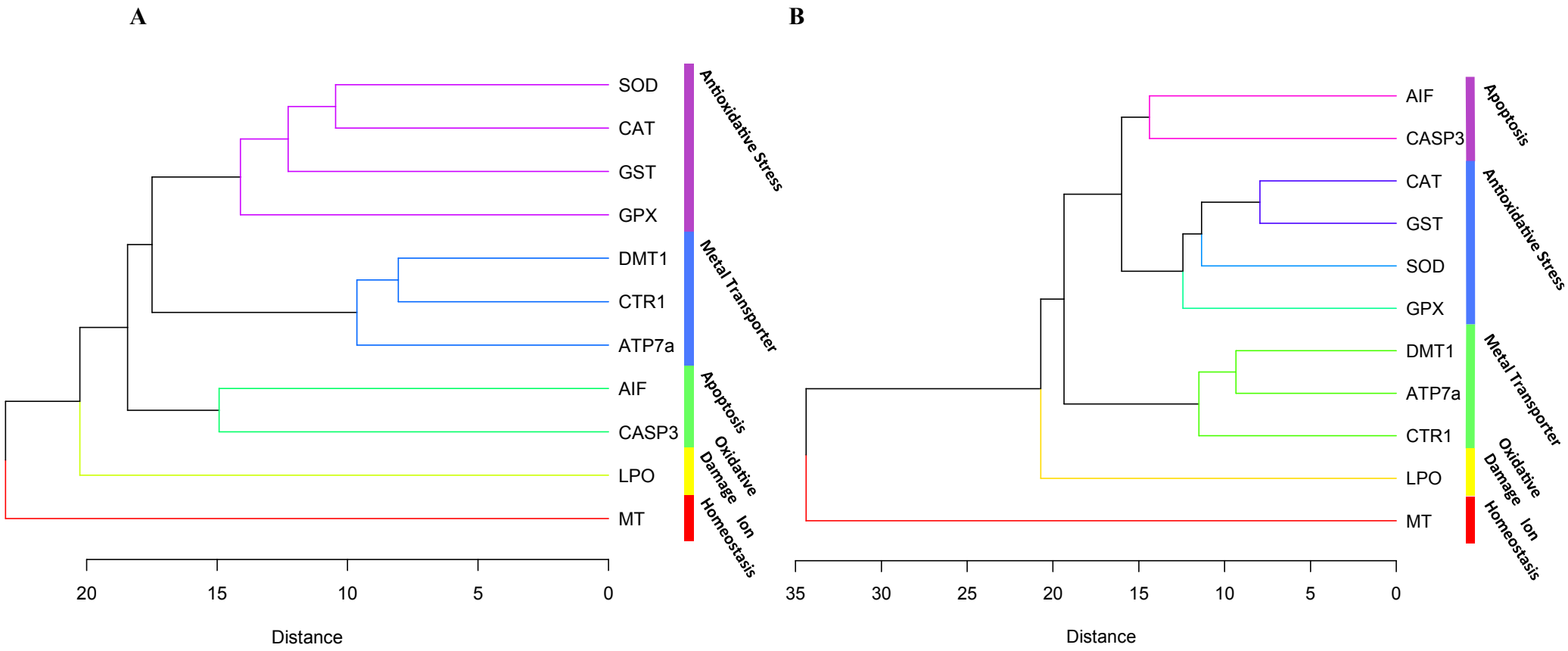
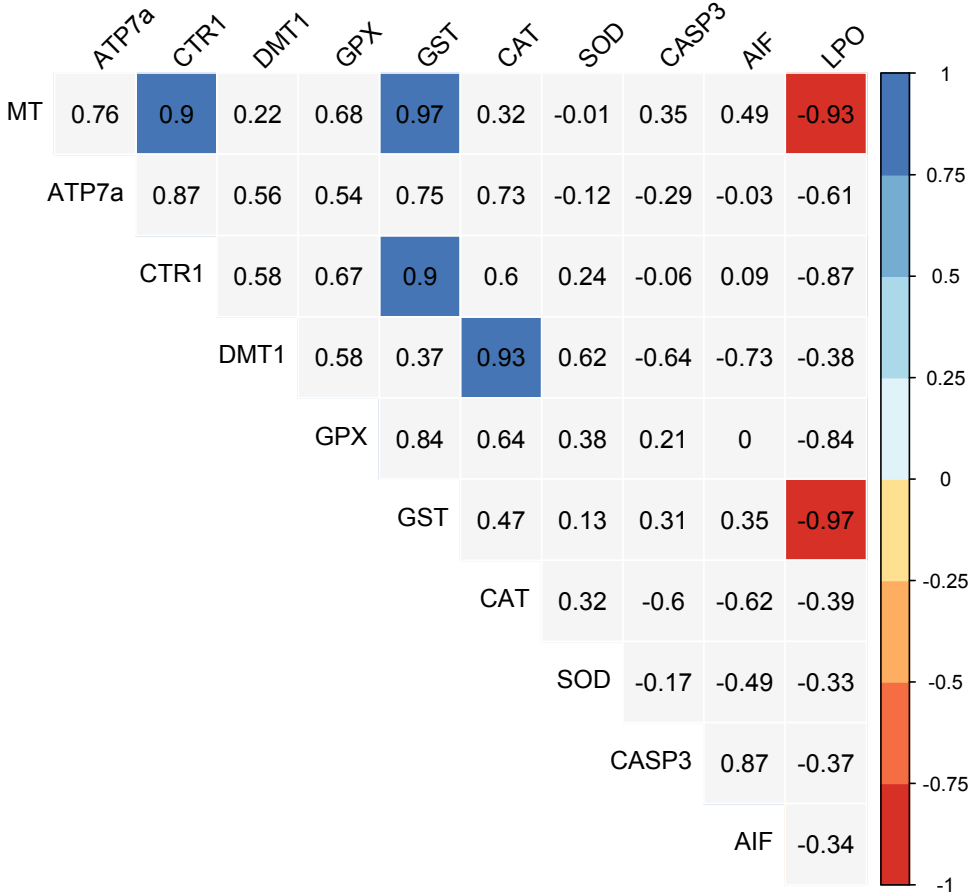
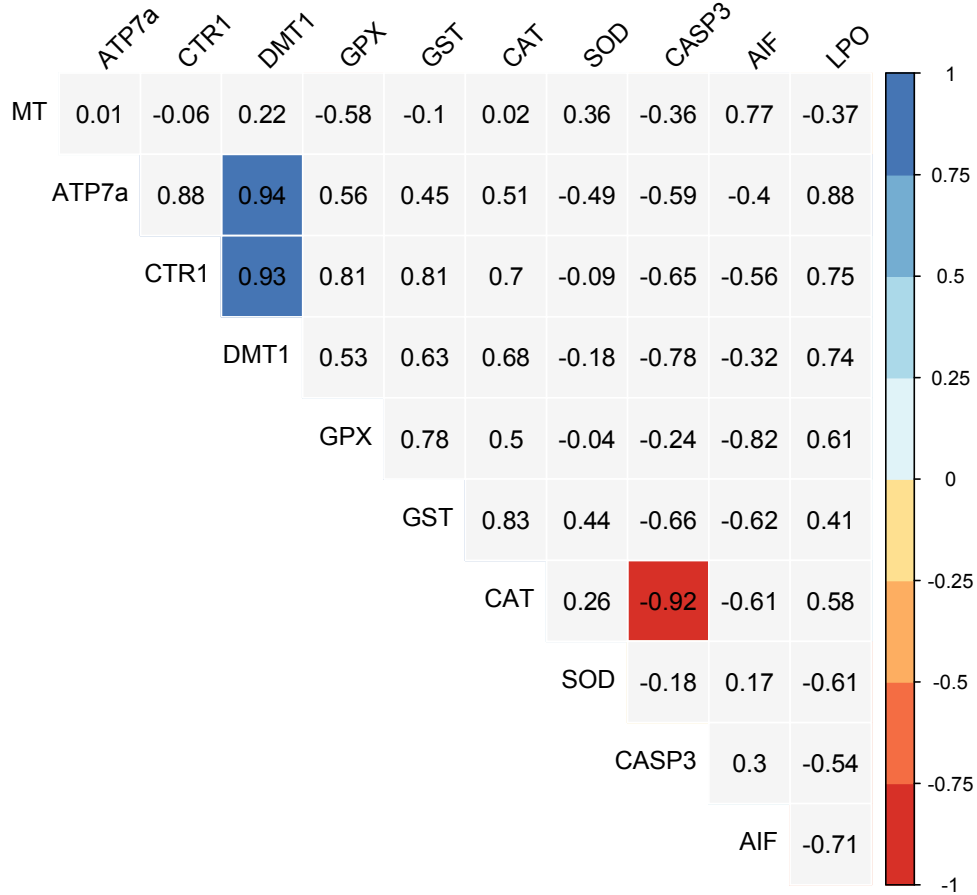


Figure S7. Correlogram displays of correlation matrices for measured effects in white sturgeon across three different life-stages following 96 h exposure to either Cu (**A**) or Cd (**B**). The analysis was performed through the 'corrplot' package in R. Pearson's correlation coefficient (PCC) values are given. Color (either blue-positive correlation or red-negative correlation) indicates the significance (p<0.05).

A



B



References

1. Doering, J. A.; Beitel, S. C.; Eisner, B. K.; Heide, T.; Hollert, H.; Giesy, J. P.; Hecker, M.; Wiseman, S. B., Identification and response to metals of metallothionein in two ancient fishes: White sturgeon (Acipenser transmontanus) and lake sturgeon (Acipenser fulvescens). *Comp Biochem Physiol C Toxicol Pharmacol* **2015**.
2. Doering, J. A.; Wiseman, S.; Beitel, S. C.; Giesy, J. P.; Hecker, M., Identification and expression of aryl hydrocarbon receptors (AhR1 and AhR2) provide insight in an evolutionary context regarding sensitivity of white sturgeon (Acipenser transmontanus) to dioxin-like compounds. *Aquat Toxicol* **2014,** *150*, 27-35.
3. Zee, J.; Patterson, S.; Wiseman, S.; Hecker, M., Is hepatic oxidative stress a main driver of dietary selenium toxicity in white sturgeon (Acipenser transmontanus)? *Ecotoxicology and Environmental Safety* **2016,** *133*, 334-440.
4. Livak, K. J.; Schmittgen, T. D., Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **2001,** *25*, (4), 402-408.
5. Little, E. E.; Calfee, R. D.; Linder, G., Toxicity of copper to early-life stage Kootenai River white sturgeon, Columbia River white sturgeon, and rainbow trout. *Arch Environ Contam Toxicol* **2012,** *63*, (3), 400-408.
6. Calfee, R. D.; Little, E. E.; Puglis, H. J.; Scott, E.; Brumbaugh, W. G.; Mebane, C. A., Acute sensitivity of white sturgeon (Acipenser transmontanus) and rainbow trout (Oncorhynchus mykiss) to copper, cadmium, or zinc in water-only laboratory exposures. *Environ Toxicol Chem* **2014,** *33*, (10), 2259-2272.
7. Vardy, D. W.; Oellers, J.; Doering, J. A.; Hollert, H.; Giesy, J. P.; Hecker, M., Sensitivity of early life stages of white sturgeon, rainbow trout, and fathead minnow to copper. *Ecotoxicology* **2013,** *22*, (1), 139-147.
8. Chapman, G. A., Toxicities of Cadmium, Copper, and Zinc to Four Juvenile Stages of Chinook Salmon and Steelhead. *Transactions of the American Fisheries Society* **1978,** *107*, (6), 841-847.
9. Buhl, K. J.; Hamilton, S. J., Relative sensitivity of early life stages of Arctic grayling, Coho salmon, and rainbow trout to nine inorganics. *Ecotoxicol Environ Saf* **1991,** *22*, (2), 184-197.
10. Vardy, D. W.; Santore, R.; Ryan, A.; Giesy, J. P.; Hecker, M., Acute toxicity of copper, lead, cadmium, and zinc to early life stages of white sturgeon (Acipenser transmontanus) in laboratory and Columbia River water. *Environ Sci Pollut Res Int* **2014,** *21*, (13), 8176-8187.