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Sensitivity of early life stages of white sturgeon, rainbow trout, and fathead minnow to copper

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Abstract Populations of white sturgeon (WS; *Acipenser transmontanus*) are in decline in several parts of the United States and Canada, attributed primarily to poor recruitment caused by degradation of habitats, including pollution with contaminants such as metals. Little is known about sensitivity of WS to contaminants or metals such as copper (Cu). Here, acute (96 h) mortalities of WS early life stages due to exposure to Cu under laboratory conditions are reported. Two standard test species, rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*), were exposed in parallel to determine relative sensitivity among species. Swim-up larvae [15 days post-hatch (dph)] and early juveniles (40–45 dph) of WS were more sensitive to Cu ($LC_{50} = 10$ and 9–17 µg/L, respectively) than were yolksac larvae (8 dph; $LC_{50} = 22$ µg/L) and the later juvenile life stage (100 dph; $LC_{50} = 54$ µg/L). WS were more sensitive to Cu than rainbow trout and fathead minnow at all comparable life stages tested. Yolksac larvae of rainbow trout and fathead minnow were 1.8 and 4.6 times, respectively, more tolerant than WS, while swim-up and juvenile life stages of rainbow trout were between 1.4- and 2.4-times more tolerant than WS. When plotted in a species sensitivity distribution with other fishes, the mean acute toxicity value for early life stage WS was ranked between the 1st and 2nd centile. The WS life stage of greatest Cu sensitivity coincides with the beginning of active feeding and close association with sediment, possibly increasing risk. WS early life stages are sensitive to aqueous copper exposure and site-specific water quality guidelines and criteria should be evaluated closely to ensure adequate protection.

Keywords Fish · Metal · Ecotoxicology · Early life stage sensitivity

Introduction

Sturgeon (Acipenseridae) are among the largest freshwater fish in the world. Some species can live more than

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100 years, weigh more than 800 kg and reach lengths of more than 6 m. Sturgeon are also among the most archaic fish species with prehistoric ancestors dating back an estimated 175 million years (UCWSRI 2002). Presently, however, populations of sturgeon are threatened globally and have been decreasing over the past century in Northern Europe, Asia, and North America (Birstein 1993; Coutant 2004; Gisbert and Williot 2002). In North America, populations of white sturgeon (WS; *Acipenser transmontanus*) have been reported to be declining in the northwestern United States and British Columbia, Canada. Populations of WS have been listed as endangered in parts of Canada (COSEWIC 2012) and the USA (U.S. Fish and Wildlife Service 2012). Decreases in populations of sturgeon in the Columbia, Fraser, and Sacramento–San Joaquin rivers and their tributaries have been attributed primarily to poor annual recruitment (Coutant 2004; DFO 2007; Scott and Crossman 1998; UCWSRI 2002). Results of some simulation models of population trends and demographics have predicted that without implementation of successful remedial efforts WS will become virtually extinct in these rivers within 50 years (DFO 2007; Irvine et al. 2007; Paragamian et al. 2005; Paragamian and Hansen 2008; UCWSRI 2002).

Possible hypotheses for failures of recruitment of WS include, among others, overharvesting, habitat alteration, changes in flow regime, decreased water quality, such as temperature, turbidity, total dissolved gases, pollution, poor nutrition, genetic bottlenecks or inbreeding depression, predation by introduced species such as walleye (*Sander vitreus*), inter-specific competition, pathogens, and disease (Birstein 1993; Coutant 2004; Gisbert and Williot 2002; Irvine et al. 2007; Kruse and Scarnecchia 2002; Luk'yanenko et al. 1999; Paragamian and Hansen 2008; UCWSRI 2002). In some of the larger North American rivers, such as the Columbia, metals are of particular concern due to past and present activities of mines, metallurgical facilities, pulp and paper mills, as well as other industrial and municipal sources (UCWSRI 2002). Copper (Cu), for example, is often found in contaminated systems at concentrations that are greater than naturally occurring levels (Grosell 2012; Kamunde and Wood 2003; Niyogi and Wood 2003). Concentrations of Cu in clean natural freshwaters are typically in the lower µg/L range (e.g. 0.2–2 µg/L), but thresholds for lethality on fishes can occur at concentrations that are only 10-fold greater (Grosell 2012; Wood 2001). In addition, effects on more sensitive endpoints, including behaviour, chemosensory, and olfaction, have been recorded within the lower µg/L concentration range (Grosell 2012). In general, little is known about the potential toxicity of metals, such as Cu, to WS, or the tolerance of WS relative to other fishes.

Water quality guidelines and criteria are typically based upon effects concentrations (e.g. LC_{50}s) for aquatic organisms and are estimates of the concentration of a contaminant in the environment that is expected to protect 95 % of a group of diverse genera, assuming an appropriate number and variety of taxa are used for calculations (CCME 2007, EPA 1985). In cases where a species is deemed commercially or recreationally important and its threshold value is more sensitive than the calculated guideline or criteria, that particular species mean acute value (SMAV) will supersede (EPA 1985). These estimates, however, are only based upon species for which there are existing toxicity data that meet acceptable standards and often do not consider life stage specific sensitivities unless existing data indicates significant differences. Consequently, there is uncertainty whether an endangered species such as the WS that is recreationally, commercially, and culturally important (UCWSRI 2002) but has little to no existing toxicity data is protected by current guidelines and criteria.

Fish are generally most sensitive to effects of contaminants such as metals during early life stages (Hutchinson et al. 1998; McKim 1977). Previous work has indicated differences in sensitivity among early life stages of WS (Vardy et al. 2011), and studies of the effects of contaminants and life stage-specific sensitivities are important for making informed regulatory decisions. Life stage-specific sensitivity of WS is of particular interest given their early life history strategies. Early life stages of sturgeon inhabit benthic habitats, on surface sediments or in interstitial space between stones. There is some debate among researchers over the exact timing and sequence of certain behavioral events during WS early life stage development, these events possibly being influenced by differences in availability of appropriate substrata (McAdam 2011), but there is a general acceptance that early life stages of WS are in close contact with the substratum and exhibit distinct hiding and drifting phases (Brannon et al. 1983, 1985; Deng et al. 2002; Kynard and Parker 2005). Yolksac stage WS tend to hide/burrow in refugia (Brannon et al. 1983, 1985; Gessner et al. 2009; McAdam 2011; Richmond and Kynard 1995; personal observation in the laboratory). Prior to transitioning to exogenous feeding, sturgeon swim up in the water column (presumably to be transported by currents to more suitable foraging grounds; Auer and Baker 2002; Gessner et al. 2009; McAdam 2011) before returning to the bottom during the juvenile life stage, where they begin to scavenge and prey on benthic species and spend much of their life closely associated with sediments. Therefore, in addition to exposure to pollutants in the water column, sturgeon can be exposed to contaminants associated with sediments (Feist et al. 2005; Kruse and Scarnecchia 2002) or contaminants released into the sediment–water interface. Sediments are sinks for pollutants and often contain high concentrations of metals, which can be released back into porewater and the water column following remobilization

(Salomons et al. 1987; Sullivan and Taylor 2003). Thus, WS could be exposed chronically to lesser concentrations of metals, or, during certain life stages and for shorter periods of time, to greater concentrations of metals at the sediment–water interface. For this reason, and to generate data to develop species sensitivity distributions (SSDs) in support of deriving protective acute water quality guidelines and criteria, it is necessary to determine both acute and chronic toxicity of metals to WS. The results of chronic studies on survival and growth have been presented previously (Vardy et al. 2011).

The primary objective of this study was to establish acute toxicity data for the effect of Cu on early life stages of WS that can be used in risk assessments. Early life stage WS were exposed to increasing concentrations of dissolved Cu, bracketing environmentally relevant concentrations and those expected to be lethal. In addition, rainbow trout (*Oncorhynchus mykiss*) and fathead minnow (*Pimephales promelas*) were exposed to Cu in the laboratory, in parallel to WS, to provide paired information for use in species sensitivity comparisons.

Methods

Test materials

Copper(II) sulfate pentahydrate (Chemical Abstracts Service (CAS) number 7758-99-8; purity 99.995 %) was obtained from Sigma-Aldrich (Oakville, ON, Canada) and was dissolved in laboratory reverse osmosis water.

Experimental fish

Fertilized WS eggs were collected at the Kootenay Trout Hatchery, Fort Steele, BC, Canada, from a minimum of four breeding pairs of adult WS caught in the Columbia River near Waneta, Canada. Fertilized eggs were transported to the Aquatic Toxicology Research Facility (ATRF), University of Saskatchewan, Saskatoon, SK, Canada where the embryos were raised under standard culturing conditions (Conte et al. 1988) until the desired life stages were achieved. Eyed embryos of rainbow trout were obtained from the Trout Lodge (Summer, WA, USA) and incubated in McDonald-type hatching jars (Aquatic Ecosystems, Apopka, FL, USA) until hatch. Fathead minnows were obtained from Osage Catfisheries (Osage Beach, MO, USA) and several generations were produced to insure healthy progeny.

Exposure methods

Acute (96 h) toxicity of Cu was determined in accordance with the methods described by the American Society for Testing and Materials (ASTM 2007), with minor modifications. The exposure design consisted of sets of laboratory-based 96 h static renewal tests with mortality as the measurement endpoint. Laboratory water (carbon and biofiltered city water) was adjusted to simulate natural conditions of the Columbia River near Trail, BC, Canada. Target hardness of $\sim$65 mg/L and dissolved organic carbon (DOC) concentrations of $\sim$2.5 mg/L were achieved by mixing laboratory water with reverse osmosis water in a 1:1 ratio. Target temperatures of 12 ± 1, 16 ± 1 and 20 ± 1 °C for rainbow trout, WS, and fathead minnows, respectively, were achieved by immersing the exposure chambers in chilled or heated water baths or by use of environmental control chambers. All fish were tested under a 16:8 h light:dark cycle of illumination by use of standard daylight fluorescent lighting. Culture conditions for the fish (DOC, hardness, temperature, photoperiod) were the same as exposure conditions with the difference that fish were fed between one and four times a day, depending on life stage. For testing the yolksac life stage (8 dph) fish were exposed to increasing concentrations of Cu in 0.5 L high-density polyethylene (HDPE) test containers. Identical but larger 5 and 20 L test containers were used during toxicity tests with later life stages. Loading densities remained less than the recommended 0.5 g/L and fish were not fed during the acclimation and exposure period (ASTM 2007). The various life stages, expressed as dph, and species tested are described in Table 1.

Concentrated stock solutions of Cu were prepared separately in individual HDPE carboys and allowed to equilibrate for 48 h prior to making dilutions to obtain test solutions. Exposures were conducted in triplicate or quadruplicate for each treatment group; each test chamber contained 10–15 individuals with 50 % solution renewal every 12 h. Fish were acclimatized to the exposure chambers for 24 h prior to the addition of test solutions. Exposure chambers were cleaned once a day and dead fish were removed, length and weight measured, and preserved for potential use in future experiments.

Water chemistry analyses

Basic water quality parameters, including temperature, pH, dissolved oxygen and conductivity, were measured daily by use of Symphony Electrodes (VWR, Mississauga, ON, Canada, Cat No. 11388-328) or YSR electrodes (YSR Inc., Yellow Springs, OH, USA). Typically, subsamples were collected during water changes from each replicate of each concentration and used for individual analysis. Hardness, alkalinity, ammonia, nitrates, nitrites, and chlorine were collected following a similar sampling scheme but only at the initiation and termination of experiments, and analyzed by use of LaMotte colorimetric and titrator test kits

Table 1 Acute median lethal concentrations (LC_{50}s) for Cu exposure for white sturgeon (*Acipenser transmontanus*), rainbow trout (*Oncorhynchus mykiss*), and fathead minnow (*Pimephales promelas*) early life stages expressed in days post-hatch (dph)

96 h LC_{50} for copper exposure (μg/L)

Fish species	Life stage						Water quality criteria	
	Yolksac (8 dph)	Swim-up (15 dph)	Juvenile (40 dph)	Juvenile (45 dph)	Later Juvenile (100 dph)	SMAV[a]	CMC[b]	CWQG[c]
White sturgeon (*Acipenser transmontanus*)	22 (20–25)	10 (8–12)	9 (7–12)	17 (14–21)	54 (47–62)	18	7.9 (±1.5)	2
Rainbow trout (*Oncorhynchus mykiss*)	40 (34–46)	21 (18–23)	22 (20–25)	24 (20–28)		26	8.5 (±3.0)	2
Fathead minnows (*Pimephales promelas*)	102 (78–135)					102	11.8	2

Values in parentheses for LCs represent 95 % CI, values in parentheses for water quality criteria represent SD

[a] SMAV refers to the species mean acute value

[b] CMC refers to the Criteria Maximum Concentration for fresh water species. The mean freshwater criteria is presented and calculated from the various life stage experiments for each specie using the Biotic Ligand Model (EPA 2007b)

[c] CWQG refers to the Canadian Water Quality Guidelines for the protection of aquatic life adjusted to the present study's hardness (CCME 2003)

(Chestertown, MD, USA) or samples were sent to Columbia Analytical Services (CAS; Kelso, WA, USA) for external analyses. Water samples for analysis of concentrations of Cu in exposure chambers were also collected following the same sampling scheme at initiation and termination of the experiment. Water for Cu analysis was collected from each treatment group into acid-cleaned polyethylene bottles and filtered through a 0.45 μm polycarbonate filter. Filtered water was acidified with ultrapure nitric acid to pH < 2. Quantification of Cu was performed by use of inductively coupled plasma mass spectrometry (ICP-MS) following EPA method 6020 and ILM05.2D (Creed et al. 1994). All calculations and reported values pertaining to Cu concentrations are based on the average measured concentrations in the treatment groups. DOC analysis was performed using a TOC analyzer (TOC-5050A, Shimadzu, Mandel Scientific, Guelph, ON, Canada).

Data analysis and statistics

Mortality was calculated and the proportion of fish dead in each of the exposure chambers of a given Cu concentration was compared to that of the controls. LC_{50}s for each of the species were calculated by use of TOXSTAT® software (Western EcoSystems Technology 1996). To assess the relative sensitivity of early life stages of WS to Cu, relative to those of other fishes, a species sensitivity distribution (SSD) was calculated for Cu (Posthuma et al. 2002). The SSD for freshwater fishes was derived based on toxicity data obtained from: EPA's ECOTOX database (EPA 2007a), information on Cu sensitivity of three different sturgeon species published by Dwyer et al. (2005), and the data obtained during this study. Data considered for the derivation of the SSD were exclusively from 96 h toxicity studies that reported LC_{50} values. Species mean (geomean) acute values (SMAVs) were calculated where data from multiple studies were available. If only one data point was available for a species, this was used as the SMAV in the SSD. To facilitate comparisons among tests without confounding the comparison by differences in hardness, all data included in the SSD were adjusted to a hardness of 50 mg $CaCO_3$/L by use of the Criteria Maximum Concentration (CMC) regression equation for Cu, as outlined by the US EPA for calculating freshwater dissolved metals criteria that are hardness dependent (EPA 2009).

Results

Exposure verification

Measured concentrations of Cu (Table 2) were comparable to nominal concentrations, and on average, were within 95 % of each other (see Supplementary Data). However, there were small detectible concentrations of Cu in the controls, but these concentrations were less than the least dose of each metal concentration. Generally, measured concentrations were less than nominal concentrations.

Water quality

Average water temperatures for all treatment groups during the WS, rainbow trout, and fathead minnow exposures were 16 °C (±0.9), 13 °C (±0.5), and 22 °C (±0.1), respectively. The average dissolved oxygen saturation, pH,

Table 2 Mean ± standard deviation (SD; numbers in parentheses) measured exposure concentrations for copper during acute (96 h) static renewal exposure experiments with white sturgeon (WS; *Acipenser transmontanus*), rainbow trout (RT; *Oncorhynchus mykiss*), and fathead minnow (FM; *Pimephales promelas*) early life stages expressed as days post-hatch (dph)

Treatment	Fish species									
	WS life stages (dph)					RT life stages (dph)				FM life stages (dph)
	8 Measured (µg/L)	15 Measured (µg/L)	40 Measured (µg/L)	45 Measured (µg/L)	100 Measured (µg/L)	8 Measured (µg/L)	15 Measured (µg/L)	40 Measured (µg/L)	45 Measured (µg/L)	8 Measured (µg/L)
Control	1.3 (±0.6)	0.4 (±0.0)	0.4 (±0.0)	0.4 (±0.0)	0.8 (±0.2)	1.3 (±0.7)	0.3 (±0.1)	2.4 (±2.5)	1.2 (±0.6)	1.4 (±0.8)
1	1.8 (±0.2)	2.8 (±0.1)	1.6 (±0.1)	2.0 (±0.1)	8.3 (±0.1)	1.8 (±0.6)	2.1 (±0.1)	9.7 (±0.7)	2.6 (±0.1)	2.1 (±0.5)
2	2.7 (±N/A)[a]	4.7 (±0.2)	3.8 (±0.2)	3.7 (±0.1)	17 (±N/A)[a]	3.5 (±0.6)	3.8 (±0.0)	20 (±1.4)	4.0 (±0.0)	3.6 (±0.7)
3	5.9 (±0.1)	8.3 (±0.1)	11 (±0.4)	7.3 (±0.1)	36 (±N/A)*	8.0 (±0.7)	7.6 (±0.0)	40 (±2.2)	8.4 (±0.3)	8.6 (±0.7)
4	8.2 (±N/A)[a]	20 (±0.9)	21 (±1.3)	15 (±0.2)	74 (±21.6)	19 (±3.6)	15 (±0.2)	74 (±8.5)	15 (±0.4)	23 (±1.9)
5	21 (± 1.8)	30 (± 0.1)	42 (± 1.7)	29 (± 0.1)	150 (± N/A)*	38 (± 2.4)	29 (± 0.0)	146 (± 14.6)	30 (± 0.5)	43 (± 1.0)
6	38 (±0.2)	52 (±3.2)	85 (±2.1)	56 (±0.4)	282 (±24.4)	78 (±11.5)	59 (±0.5)	296 (±18.7)	63 (±2.4)	85 (±12.4)
7	86 (±10.7)		171 (±2.1)			155 (±32.3)				195 (±9.3)
8	151 (±0.4)									
9	382 (±5.2)									

[a] SD was not calculated due to lack of concentration measurements

and conductivity for all treatment groups were 86 % (±8.7), 7.5 (±0.2), and 187 µS/cm (±25.5), respectively. Mean hardness was 57 mg/L $CaCO_3$ (±12.4) and the concentration of dissolved organic carbon was 2.2 mg/L (±0.5). The average total concentration of ammonia, expressed as nitrogen (N) for all treatment groups was less than the limit of detection (<0.025 mg/L). There were no significant differences in all other measured water quality parameters among treatment groups of any given experiment (summary of analytical methods, method detection limits, method blanks, and mean water quality parameters for individual exposures are provided as Supplementary Data).

Lethal concentrations

LC_{50}s were successfully calculated for all life stages of each species that was tested (Table 1). Average survival of unexposed control fish was 90 % or greater in all experiments (see supplementary data for summary of mean mortality for individual exposures). WS were most sensitive to Cu at 15 and 40 dph, followed by 45 and then 8 dph. WS exposed to Cu at a later life stage (100 dph) were more tolerant than were earlier life stages (Fig. 1). LC_{50}s for toxicity of Cu to WS were less than those for rainbow trout and fathead minnows for all comparable life stages tested (Fig. 1; Table 1). LC_{50}s for WS swim-up larvae and juvenile life stages were between 1.4- and 2.4-times more sensitive than rainbow trout. Rainbow trout were least sensitive at 8 dph, followed by the later life stages, which exhibited comparable sensitivities to each other. Fathead minnow (8 dph) were more tolerant than WS and rainbow trout at all life stages tested.

Species sensitivity distribution

The SSD that was developed based on 59 freshwater fishes, including the data of the various life stages of the three species studied here (Fig. 2), demonstrated that WS that were 8, 15, 40, 45, or 100 dph were ranked in the 14th, 1st, 2nd, 3rd, and 28th centile, respectively. The SMAV for WS ranked in the 2nd centile. Rainbow trout from the present study at 8, 15, 40, and 45 dph ranked in the 22nd, 13th, 9th, and 10th centile, respectively. The SMAV for rainbow trout calculated solely from the present study, when averaged among all life stages, ranked in the 8th centile, the SMAV for rainbow trout calculated solely from the ECOTOX database ranked in the 23rd centile, and the overall SMAV for rainbow trout, calculated from the ECOTOX database and values generated during the present study, ranked in the 16th centile. Based on the results of the present study, fathead minnow (8 dph) ranked in the 46th centile, while the SMAV for fathead minnow,

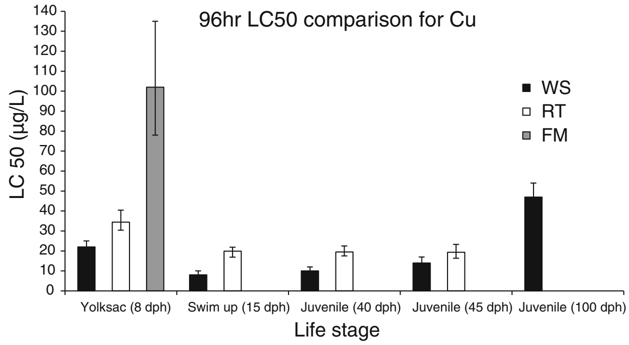

Fig. 1 Comparison of median lethal concentrations (LC_{50}s) of white sturgeon (WS; *Acipenser transmontanus*), rainbow trout (RT; *Oncorhynchus mykiss*), and fathead minnow (FM; *Pimephales promelas*) life stages [days post-hatch(dph)] exposed to copper. *Error bars* represent confidence intervals for measured LC_{50}s

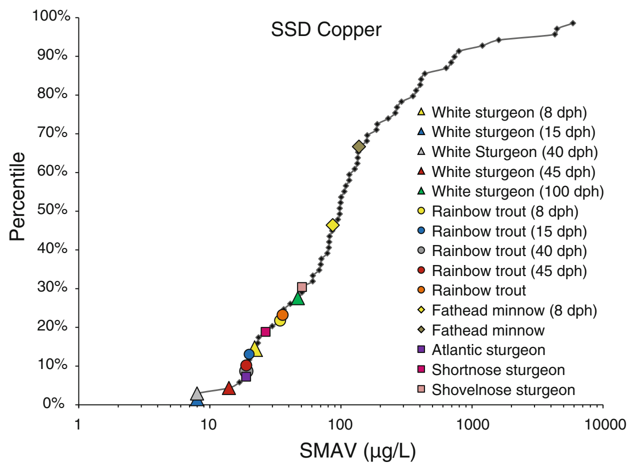

Fig. 2 Species sensitivity distribution (SSDs) for copper. Values for species with life stages, expressed as days post-hatch (dph), are from experiments conducted at the University of Saskatchewan. Atlantic, shortnose, and shovelnose sturgeon values are from Dwyer et al. (2005), and all other species values are from the ECOTOX database (EPA 2007a). The Species Mean Acute Value (SMAV) is the geometrical mean LC_{50} for a given species

calculated from the ECOTOX database, ranked in the 67th centile. The overall SMAV for fathead minnow, calculated from the ECOTOX database and the present study's findings, ranked in the 59th centile. Early life stage Atlantic (*Acipenser oxyrinchus*), shortnose (*Acipenser brevirostrum*), and shovelnose (*Scaphirhynchus platorynchus*) sturgeon based on data from Dwyer et al. (2005) ranked in the 7th, 19th, and 30th centile, respectively.

Discussion

Based on findings of the present study, early life stage WS appear to be among the most sensitive fishes to acute Cu exposure, relative to other freshwater fishes. Three of the five life stages tested for WS were the most sensitive fishes in the SSD. The SMAV for WS was calculated and plotted in the SSD and WS were ranked the most sensitive species overall. Similarly, all other early life stage sturgeon incorporated in the same SSD, including Atlantic, shortnose, and shovelnose sturgeon, were relatively sensitive and ranked in the 23rd centile or less. In studies conducted by Dwyer et al. (2005), it was concluded that sturgeon in general should be considered a sensitive species in contaminant assessments, and results from the present study are consistent with these findings. Results of previous studies have shown that some standard test species, such as rainbow trout, are relatively sensitive to certain metals, whereas others, such as fathead minnow, are more tolerant (Besser et al. 2007; Dwyer et al. 2005; Taylor et al. 2000). LC values for the effects of Cu on rainbow trout and fathead minnow determined during the present study were slightly less, but generally consistent with previously reported SMAVs.

Post-hatch, early life stages of fish are generally considered more sensitive to contaminants than adults (Hutchinson et al. 1998; McKim 1977, McKim et al. 1978). In the present study, five early life stages of WS and four early life stages of rainbow trout were exposed to Cu to compare life stage specific sensitivity. For both species, the later larval/early juvenile life stages (15–45 dph) were more sensitive to the effects of Cu than was the yolksac (8 dph) life stage, and in the case of WS, the later juvenile life stage (100 dph). Greater sensitivity to Cu following the initial yolksac life stage and greater tolerance during the later juvenile life stage was observed for WS (Fig. 1). The observed differences in tolerance might be due to the fact that 8 dph larvae are still absorbing their yolksacs whereas at 15 dph larvae have begun to switch to exogenous feeding and are more physically active, leading to greater exposure since rates of respiration are increased and more water is forced over the gills. Rainbow trout, however, did not display a similar trend in sensitivity to Cu following 8 dph that was observed in WS. This might be due to differences in duration and timing of development of rainbow trout and WS, such that the observed sensitivities to Cu among the time periods (dph) tested might not be entirely comparable between species. Under culture conditions, rainbow trout embryos are typically incubated much longer than WS (4–14 weeks, depending on water temperatures, compared to 1 week for WS), and absorption of the yolksac can occur over a period twice as long. Therefore, longer development might result in less of a difference in sensitivity to Cu of post-yolksac larvae because rainbow trout are not transitioning through similar developmental stages as WS at comparable ages and at the same speed.

Significant differences in sensitivities among early life stages could have major implications in risk assessment and development of water quality guidelines and criteria. Risk assessments based on the assumption that younger fish tend to be more sensitive to contaminants than older fish could result in considerable underestimations of sensitivity if post-yolksac early life stages are not considered, as demonstrated in the present study, and lead to under-protection of certain species. Currently, in Canada and the United States, there is no requirement to evaluate differences in sensitivities among life stages when developing water quality guidelines and criteria. In the United States, differences in life stage sensitivity are taken into account but only if existing data demonstrates that there are differences of more than a factor of two (EPA 1985). If no toxicity data exist for various life stages of a species, then differences in sensitivities among life stages are not considered when calculating criteria or assessing potential for effects. To overlook these potential differences with a vulnerable population of fish could result in significant under-protection. Yolksac larvae of WS have greater tolerance to Cu toxicity compared to succeeding life stages, but since these earlier life stages might be in more intimate contact with biologically available metals in contaminated sediments, they might be more at risk than would be indicated solely by their tolerance to Cu concentrations. Greater exposure to Cu during the transition to exogenous feeding, however, is detrimental since previous studies have shown that WS are inherently sensitive during this period of development (Vardy et al. 2011). This poses an increased threat to early life stage juveniles that return to the bottom to feed and are at greater risk of exposure to sediment bound contaminants.

Early life stages of WS are sensitive to aqueous copper exposure and site-specific water quality guidelines and criteria should be evaluated closely to ensure adequate protection when sturgeons are of concern. The Canadian water quality guideline for the protection of aquatic life for Cu, adjusted to the present study's hardness of 57 mg/L $CaCO_3$, is 2 μg/L (CCME 2003). In the United States, criteria for protection of aquatic life for Cu are site-specific and freshwater criteria are calculated by use of the biotic ligand model (BLM; EPA 2007b). Based on the present study's water quality parameters for WS, the water quality acute criteria for Cu, recommended by the US EPA for protection of aquatic life (CMC; EPA 2007b, 2009), would be between 6.4 and 9.5 μg/L. In order to assess the degree of protection of WS in relation to water quality guidelines and criteria, one half the species mean acute value (½ SMAV) was calculated. This is similar, but on a species level, to EPA water quality criteria methods where one half final acute values (FAVs) are calculated. In the present study, ½ SMAV for WS is above the Canadian water quality guideline but falls within the calculated range for US criteria (Table 1). When half the LC_{50} values for the individual life stages of WS are examined, some thresholds are less than US criteria. This merits further investigation, especially at the more sensitive life stages, to assess the level of protection in relation to water quality criteria for Cu.

This study provides a portion of much needed toxicity data for early life stage WS and identified significant differences in sensitivities among early life stages of fish. LC_{50} values from the present study predicted similar trends in early life stage WS sensitivity when compared to chronic early life stage WS threshold values for Cu (chronic values: 19 dph = 9.9 μg/L and 58 dph = 12.4 μg/L; Vardy et al. 2011). When feasible, contaminant exposure studies should include different life stages to help elucidate possible differences in life stage sensitivities in order to develop more comprehensive water quality guidelines and criteria. WS are sensitive to Cu exposure and water quality guidelines and criteria may need to be evaluated on a site-by-site basis when WS early life stages are present in order to ensure protection. Other endpoints, such as effects of Cu exposure on olfaction, chemosensory, and/or behavior, for example, could also be investigated with WS because these endpoints have been shown to be the most sensitive endpoints in other fish species (Grosell 2012). In addition, alternate routes of exposure, such as from contaminated sediment or dietary uptake, warrant further investigation as water-only exposures may represent variable proportions of total exposure depending upon life stage.

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