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Mortality, bioaccumulation and physiological responses in juvenile freshwater mussels (*Lampsilis siliquoidea*) chronically exposed to copper

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ABSTRACT

Several studies have indicated that the early life stages of freshwater mussels are among the most sensitive aquatic organisms to inorganic chemicals, including copper. However, little is known about the toxic mode of action and sub-lethal effects of copper exposure in this group of imperiled animals. In this study, the physiological effects of long-term copper exposure (survival, growth, copper bioaccumulation, whole-body ion content, oxygen consumption, filtration rate, ATPase activities, and biomarkers of oxidative stress) were evaluated in juvenile (6 month old) mussels (*Lampsilis siliquoidea*). The mussels' recovery capacity and their ability to withstand further acute copper challenge were also evaluated in secondary experiments following the 28 day exposure by assessing survival, copper bioaccumulation and whole-body ion content. Mussels chronically exposed to 2 and 12 μg Cu/L showed significantly higher mortality than those held under control conditions (mortality 20.9, 69.9 and 12.5%, respectively), indicating that juvenile *L. siliquoidea* is underprotected by the U.S. Environmental Protection Agency (USEPA) biotic ligand model (BLM)-derived chronic water quality criteria (WQC) (2.18 μg Cu/L) and the hardness-derived USEPA WQC (12.16 μg Cu/L). Soft tissue copper burden increased equally for both copper exposures, suggesting that chronic toxicity is not associated with copper bioaccumulation. Several physiological disturbances were also observed during chronic copper exposure. Most relevant was a decrease in whole-body sodium content paralleled by an inhibition of Na^+ K^+-ATPase activity, indicating a metal-induced ionoregulatory disturbance. Filtration and oxygen consumption rates were also affected. Redox parameters (reactive oxygen production, antioxidant capacity against peroxyl radicals, glutathione-S-transferase (GST) activity, and glutathione (GSH) concentration) did not show clear responses, but membrane damage as lipid peroxidation (LPO) was observed in both copper exposures. Mussels previously held in control conditions or pre-exposed to 2 μg dissolved Cu/L were able to maintain their ionic homeostasis and did not experience mortality after the 4-d recovery period. In contrast, those previously exposed to 12 μg dissolved Cu/L exhibited 50% mortality indicating that they had already reached a 'point of no return'. Pre-exposure to copper did not influence mussel response to the copper challenge test. As observed for the chronic exposure, mortality of mussels held in the absence of copper and submitted to the challenge test was also associated with an ionoregulatory disturbance. These results indicate that ionoregulatory disruption in freshwater mussels chronically exposed to copper is the main mechanism of toxicity and that redox parameters do not appear to be useful as indicators of sub-lethal copper toxicity in these animals.

1. Introduction

During the past 30 years, species diversity and population density of native freshwater mussels (Unionidae) have declined substantively throughout the United States and Canada (Williams et al., 1993; Neves et al., 1997). This alarming decline has been attributed to a range of factors, including habitat destruction (Miller et al., 1989), changes in fish-host distribution (Isom and Yokley, 1968), invasive species (Gillis and Mackie, 1994), and environmental degradation associated with anthropogenic activities (Williams

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et al., 1993; Fuller, 1974; Havlik and Marking, 1987; Bogan, 1993). Surveys have revealed that toxic substances were one of the top five stressors cited as limiting freshwater mussels (Richter et al., 1997). It is known that chemical spills and other point sources of contaminants can cause localized mortality; however, the widespread decrease in freshwater mussel populations may result in part from the subtle, pervasive effects of chronic, low-level contamination (Strayer et al., 2004).

Several studies have indicated that the early life stages of freshwater mussels are among the most sensitive aquatic organisms to inorganic chemicals, including copper (Jacobson et al., 1993, 1997; Milam et al., 2005; Gillis et al., 2008). Notably, recent investigations (Wang et al., 2007a,b,c) have confirmed that juvenile freshwater mussels are more sensitive to acute and chronic copper exposure than most of the commonly tested freshwater organisms including cladocerans (*Daphnia magna* and *Ceriodaphnia dubia*), an amphipod (*Hyalella azteca*), fathead minnow (*Pimephales promelas*), and rainbow trout (*Oncorhynchus mykiss*). According to Wang et al. (2007a), the 50% effect concentration (EC_{50}) of copper for the early life stages of mussels often were at or below the final acute value (FAV) used to derive the United States Environmental Protection Agency (USEPA, 1996) 1996 acute Water Quality Criteria (WQC) for copper. March et al. (2007), using consensus-based methods, determined that multiple species of freshwater mussels are sensitive to particular waterborne contaminants (e.g. copper) at concentrations lower than the current federal U.S. Water Quality Criteria and the state of Oklahoma's water quality standards.

As benthic filter-feeding organisms, freshwater mussels are exposed to metals that are dissolved in water, associated with suspended particles and deposited in sediments. Because of these multiple routes of exposure, freshwater mussels can bioaccumulate certain metals to concentrations that substantially exceed those dissolved in water (Ravera, 1984; Ray, 1984). The acute toxicity of metals to freshwater mussels has been examined in a number of studies, but the sub-lethal effects of long-term exposure to low level, environmentally relevant concentrations are little understood. Sub-lethal effects of metal exposure include impairment in growth, filtration efficiency, enzyme activity and behaviour (Naimo, 1995; Vijayavel et al., 2007; March et al., 2007), with subsequent ecological impacts. Therefore, there is a need to investigate the physiological effects of long-term copper exposure, particularly biomarker responses capable of predicting ecological impacts from metal exposure in freshwater mussels. In contrast to the simple measurement of toxicant bioaccumulation, biomarkers can offer biologically relevant information on the potential impact of toxic pollutants on the health of organisms (Van der Oost et al., 1996). Copper toxicity, for example, can be attributed to a range of biological dysfunction like the generation of reactive oxygen species (Harris and Gitlin, 1996), ionoregulatory disturbance (Laurén and McDonald, 1985, 1987a,b; Vitale et al., 1999; Grosell and Wood, 2002; Bianchini et al., 2004; Grosell et al., 2004a), acid–base balance disturbances (Vitale et al., 1999; Skaggs and Henry, 2002; Grosell et al., 2003, 2004b) and impairment of ammonia excretion (Blanchard and Grosell, 2006; Grosell et al., 2003, 2004b; Wilson and Taylor, 1993).

In light of the above, the objectives of the present study were to evaluate the physiological effects of chronic copper exposure (28 days) to juvenile freshwater mussels (*Lampsilis siliquoidea*) by assessing survival, biometric parameters, copper bioaccumulation, whole-body ion content (Na^+, K^+, Mg^{2+}, Ca^{2+}), oxygen consumption, filtration rate, ATPase activities, and biomarkers of oxidative stress. To evaluate the recovery capacity of freshwater mussels and their ability to withstand further acute copper challenge (20 μg/L), 4 day recovery tests and acute copper exposures were performed following the 28 day chronic copper exposure by assessing survival, copper bioaccumulation and whole-body ion content (Na^+, K^+, Mg^{2+}, Ca^{2+}). Chronic exposures were conducted with three groups of mussels, control (0 μg Cu/L), low concentration of copper (2 μg Cu/L) and high concentration (12 μg Cu/L). The recovery test and acute copper challenge were performed with mussels that had survived the 28 d chronic exposure.

Lampsilis siliquoidea was chosen for this study because it is widespread throughout North America (Watters et al., 2009) and because it is closely related, and thus a potential surrogate for *Lampsilis fasciola*, Canadian Species at Risk. Because of its broad distribution and success in laboratory culture, *L. siliquoidea* has been frequently used in toxicity studies (Wang et al., 2007a,b,c, 2010; Gillis et al., 2008, 2010; Gillis, 2011; Bringolf et al., 2007). Although *L. siliquoidea* has been shown to be more sensitive to some contaminants than standard aquatic test organisms (Wang et al., 2007b) very little is known about the actual mechanisms of toxicity and thus the physiological reasons for their heightened sensitivity.

2. Materials and methods

2.1. Experimental animals

Juvenile freshwater mussels (*Lampsilis siliquoidea*, Barnes, 1823) (Fatmucket), were obtained from laboratory culture at Missouri State University (Springfield, MO, USA), where glochidia isolated from at least three females were pooled for production of juvenile mussels with host fish (Wang et al., 2007a). Juvenile mussels were reared in the laboratory for six months with live algae (*Neochloris oleoabundans*) in a compact system (Barnhart, 2006) before shipping to Environment Canada (Burlington, ON) for testing. Once in the laboratory, mussels were kept in an aerated 3-L polyethylene aquarium with 2 L of reconstituted moderately hard water (80–100 mg/L $CaCO_3$) (USEPA, 1994). About 70% of the culture water was replaced every 2 days over a 20-day acclimation period. Water temperature and photoperiod were maintained at 20 ± 1 °C and 16:8 h light:dark, respectively. Mussels were fed twice daily during the acclimation period with commercially nonviable algae (Shellfish Diet, Reed Mariculture, Campbell, CA, USA), according to Ingersoll et al. (2006) and Wang et al. (2007c). They were fed at a rate of 1 μL concentrated food per 1 L of moderately hard reconstituted water.

2.2. Chronic toxicity tests

An early-life stage chronic copper exposure (28 d) was performed using juvenile mussels (~7 month-old). Copper exposures were performed in reconstituted moderately hard water (80–100 mg/L $CaCO_3$) (USEPA, 1994) under semi-static conditions with full renewal of test solutions every day. The test was conducted with three groups of mussels: control (n = 75), exposed to a low copper concentration (nominal 5 μg Cu/L) (n = 105), and exposed to a high copper concentration (nominal 15 μg Cu/L) (n = 135). Fifteen juvenile mussels were randomly distributed in 1-L glass beakers for each treatment. Exposure vessels were kept at 20 ± 1 °C and under a 16:8 h (light:dark) photoperiod regime. Exposure media were gently aerated using 10 μL pipette tips attached to airlines to maintain an acceptable concentration of dissolved oxygen (>6.8 mg/L).

At the beginning of the experiment, juveniles with foot movement were randomly weighed, measured (length and width) and identified with numbered tags attached to their shells. Exposure solutions were prepared by adding a copper stock solution (see below) to reconstituted moderately hard water which had been previously aerated for 24 h to achieve CO_2 equilibrium and stabilization (USEPA, 1994). Exposure solutions were kept at 20 °C to equilibrate for 24 h prior to adding mussels. Copper chloride (Baker