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The effect of extreme waterborne cadmium exposure on the internal concentrations of cadmium, calcium, and sodium in *Chironomus riparius* larvae ☆

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Abstract

Chironomus riparius larvae (3rd–4th instar) were extremely resistant to waterborne Cd with 48 h LC50s of 331 mg Cd/L in soft water (10 mg/L $CaCO_3$) and 1106 mg Cd/L in moderately hard (140 mg $CaCO_3$/L) water. Unexposed larvae had whole body Ca and Na concentrations of 11.2(0.3) and 84.5(3.0) μmol/g, respectively. The larvae exposed through acute toxicity tests accumulated massive amounts of Cd, reaching >50 μmol/g in larvae exposed to 437 mg Cd/L, though burdens were lower at higher exposure concentrations. These Cd burdens were approximately fivefold greater than whole-body Ca concentrations. Cd exposure also had a significant negative effect on internal Ca: whole-body Ca declined by over 70% in larvae exposed to Cd above the LC50 concentration. The effect of Cd exposure on whole-body Na was much less dramatic as levels dropped by 10–28% in the acutely exposed larvae. Time series exposures (up to 72 h) across a range of Cd concentrations (0.1–865 mg/L) revealed that internal Ca dropped within the first hour of exposure regardless of the concentration of Cd. In all but the highest (865 mg Cd/L) exposure, internal Ca eventually recovered to the control level. Cd resistance in *C. riparius* may lie in its ability to maintain internal Ca balance even when exposed to extreme (>100 mg/L) levels of Cd, coupled with remarkable capacities for storage–detoxification and excretion of Cd.

Keywords: Cadmium; Calcium regulation; Sodium regulation; Chironomid; *Chironomus riparius*; Metal resistance; Tolerant invertebrate

1. Introduction

Chironomid larvae have been successful at inhabiting a wide range of aquatic habitats including those that have been heavily impacted by environmental contamination. Benthic surveys often find that chironomids, along with the comparably tolerant aquatic oligochaetes, are the dominant organisms in significantly polluted areas (Wentsel et al., 1977; Winner et al., 1980). In fact, the number of Chironomidae genera has been shown to increase in response to an increase in metal contamination (Canfield et al., 1994). Some studies suggest that chironomids develop tolerance to metal exposure which enables them to survive in metal polluted environments (Wentsel et al., 1978; Krantzberg and Stokes, 1989; Postma et al., 1996). Chironomid larvae are notably resistant to a number of waterborne metals including Pb (Qureshi et al., 1980; Rao and Saxena, 1981), Cu (Nebeker et al., 1984), and Cd (Williams et al., 1986; Postma et al., 1996). Indeed a species sensitivity distribution for Cd produced by USEPA (2000) illustrated that 4th instar *Chironomus riparius* larvae were the least sensitive of all the aquatic organisms for which data were compiled.

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Despite their well documented tolerance to metal exposure, there has been relatively little research into the mechanism responsible for that tolerance. Although both Krantzberg and Stokes (1989) and Timmermans and Walker (1989) reported that chironomid larvae do not regulate Cd uptake, there is evidence that chironomid larvae employ a range of other strategies to deal with Cd exposure and accumulation. A number of studies have found that chironomid larvae are able to detoxify accumulated metal through the induction of metal binding proteins such as metallothionein-like proteins (MTLP) (Yamarura et al., 1983; Seidman et al., 1986; Gillis et al., 2002, 2006) while others suggest that their tolerance is based on their ability to excrete significant amounts of accumulated metal (Timmermans and Walker, 1989; Postma et al., 1996). Therefore, there is a body of evidence which demonstrates that chironomid larvae can successfully handle, either through sequestration and/or excretion, the accumulated metal, but there is only a limited understanding of the effect of metal uptake on ion regulation and balance in the exposed larvae. Disturbances of ionoregulatory homeostasis appear to be the proximate mechanisms of acute toxicity for most metals in both aquatic vertebrates and invertebrates, with different metals targeting the regulation of different essential ions (Niyogi and Wood, 2004). Calcium regulation in particular is often the target of acute Cd toxicity because of ionic mimicry (Bury et al., 2003) and there is some evidence that Cd may be taken up through calcium channels in at least one chironomid species (*Chironomus staegeri*) (Craig et al., 1999). Furthermore, Bervoets et al. (1995) observed a significant decrease in Cd uptake with an increase in the calcium concentration and salinity of the exposure water in *C. riparius*. However, there has been little or no research conducted to elucidate why these organisms are able to withstand such high levels of Cd exposure without succumbing to hypocalcemia as do most other aquatic organisms when they are exposed to much lower levels of Cd.

In this study we investigate the effects of waterborne Cd exposure on the accumulation of Cd and on the levels of internal Ca and Na in late (3rd–4th) instar *C. riparius* larvae. After determining acute Cd LC50 s in both a moderately hard and an ion-poor soft water, the whole-body concentrations of Cd, Ca, and Na were measured in the exposed larvae. Also, the effects of Cd exposure on internal concentrations of Cd, Ca and Na were followed over time (up to 72 h) in larvae exposed to a range of Cd concentrations from very low, non-toxic levels to acutely toxic concentrations of waterborne Cd. The overall goal of this study was to determine the effect of Cd exposure on the internal ion balance in *C. riparius* in an effort to understand the mechanism responsible for its Cd resistance. Although this study investigates cadmium acclimation in laboratory exposed chironomid larvae, it does not address the issue of natural populations of chironomids which have become adapted to metal exposure.

2. Materials and methods

2.1. Chironomid cultures

A continuous culture of the non-biting midge *C. riparius* was initiated with egg masses from the National Water Research Institute (Environment Canada), Burlington, ON. *C. riparius* were cultured in 10 L glass aquaria fitted with an equal size lid for adult flight and mating. The upper aquarium was fitted with a mesh sleeve to allow for the removal of egg masses. Silica sand was used as a substrate and Hamilton city tap water (Lake Ontario) as the overlying culture water. This water was dechlorinated on site and routinely monitored for chlorine, cadmium, and major ions. The ionic composition of the Hamilton city tap water in mM was $[Na^+] = 0.6$, $[Cl^-] = 0.8$, $[Ca^{2+}] = 1.8$, $[K^+] = 0.4$, $[Mg^{2+}] = 0.5$, $[Cd] < 5.0 \times 10^{-7}$. Water hardness was approximately 140 mg/L (as $CaCO_3$ equivalents), pH 7.8 to 8.0, and dissolved organic carbon (DOC) was approximately 3.0 mg/L. The cultures were aerated, and held at $21 \pm 2\,^{\circ}C$ under a 16:8 h light:dark photoperiod regime. New culture tanks were initiated with first instar larvae and fed crushed Nutrafin^{TM} fish flakes *ad libitum*. Under these conditions the larvae reached the 3rd instar approximately two weeks after a culture tank was initiated.

2.2. Acute cadmium exposures for 48 h LC50 studies

Exposures were conducted in 250 mL glass beakers and held under the same conditions (temperature, light, etc.) as the cultures except that no substrate or food was added to any of the exposures. Acute (48 h) Cd LC50 s were determined in both an ion-poor soft water created by reverse osmosis (Ca 50 μM, Na 50 μM, Mg 20 μM, pH 7.2, DOC 0.7 mg/L, approximate hardness 10 mg/L as $CaCO_3$ equivalents) and the moderately hard, Hamilton city tap water (composition above). Cd exposure solutions were made from a stock of reagent grade $Cd(NO_3)_2$ (Fisher Scientific). Seven Cd concentrations (treatments) were included in each acute toxicity test.

Twenty-four hours prior to use in an exposure, the larvae were transferred to clean culture water in order to purge their gut contents. Ten 3rd to 4th instar larvae were added to each of five replicate beakers for each of the exposure treatments. The measured (dissolved) Cd concentrations were 0, 38, 437, 989, 1279, 1495, 1879, and 2152 mg Cd/L in the hard water exposures and 0, 10, 98, 185, 277, 456, and 905 mg Cd/L in the soft water exposures. Water samples (5 mL) were taken at initiation of the exposure to determine the concentration of total Cd (unfiltered) and dissolved Cd (filtered through an Acrodisk^{TM} 0.45 μm in-line-syringe-tip filter) in the exposures. Mortality was assessed at 24 and 48 h. After 48 h all surviving larvae were removed from the exposure solutions and transferred to dechlorinated Hamilton city tap water for 5 min of rinsing. Following rinsing, all larvae from a replicate beaker were blotted dry on filter paper and weighed to the nearest 0.01 mg as a composite sample for the replicate.

2.3. Time series cadmium exposures

In order to determine the pattern of Cd accumulation over time and any subsequent effect on internal Ca and Na, *C. riparius* larvae were exposed to a range of Cd concentrations (0.1–865 mg/L) in a time series manner using the moderately hard water. For the 0.1, 1.0, and 18 mg/L experiments the Cd exposures were created using a solution of $Cd(NO_3)_2$ spiked with ^{109}Cd (as $CdCl_2$, Perkin-Elmer) as a radio-tracer. For the 865 mg/L, only 'cold' Cd (i.e. no radio-tracer) was used. Based on the results of the acute toxicity tests which yielded a 48 h LC50 of 1106 mg/L in moderately hard water, the highest concentration, 865 mg Cd/L was chosen to represent a toxic exposure and the second concentration at 18 mg/L was approximately 2% of the LC50. In these two 'high' level exposures, Cd accumulation and internal Ca and Na were followed for up to 48 h. Two other 'lower' time series Cd exposures were conducted at 1.0