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Cadmium biodynamics in the oligochaete *Lumbriculus variegatus* and its implications for trophic transfer

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Abstract

It has become increasingly apparent that diet can be a major source of trace metal bioaccumulation in aquatic organisms. In this study, we examined cadmium uptake, efflux, and subcellular compartmentalization dynamics in the freshwater oligochaete *Lumbriculus variegatus*. *L. variegatus* is an important component of freshwater food webs in Europe and North America and is potentially useful as a standard food source for laboratory-based trophic transfer studies. Cadmium accumulation and depuration were each followed for 10 days. Rate constants of uptake (k_u) and efflux (k_e) were estimated and subcellular Cd compartmentalization was followed over the course of uptake and efflux. The partitioning of Cd into operationally-defined subcellular compartments was relatively consistent throughout the 20-day experiment, with the majority of Cd accumulating in the cytosol. No major changes in Cd compartmentalization were observed over uptake or depuration, but there appeared to be some exchange between heat-stable and heat-labile cytosolic protein fractions. Cadmium accumulation from solution was strongly affected by ambient calcium concentrations, suggesting competition between Cd and Ca for uptake sites. Finally, we demonstrate the ability to manipulate the whole body calcium content of *L. variegatus* as a potential tool for examining calcium influences on dietary Cd dynamics. The potential for this species to be an important conduit of Cd to higher trophic levels is discussed, along with its potential as a standardized food source in metal trophic transfer studies.

Keywords: Cd; Uptake; Efflux; Subcellular distribution; Water hardness; *Lumbriculus variegatus*; Ca content

1. Introduction

A recent surge in publications related to dietary metal accumulation in a variety of organisms (e.g. Cheung and Wang, 2005; Rainbow et al., 2006; Sofyan et al., 2006; Martin et al., 2007) confirms earlier work (Munger and Hare, 1997; Reinfelder et al., 1998; Wang and Rainbow, 2000) that diet can be a major source of metal accumulation in aquatic organisms. The success of laboratory based, dietary metal accumulation studies often hinges on providing metal-loaded food that is representative of what the study organisms might ingest in nature—in terms of realistic metal concentrations, the composition of the food itself, and the bioavailability of the metal from the food. For example, the composition of some artificial foods may affect the uptake of the metal of interest due to the enrichment of food with essential metals (Wood et al., 2006). Similarly, recent studies suggest that the subcellular distribution of metals may influence metal bioavailability, with cytosolic metals tending to be highly bioavailable (Reinfelder and Fisher, 1991; Wallace et al., 2003; Wang and Rainbow, 2006; Martin et al., 2007). It is therefore important that food items used in metal bioaccumulation studies are well characterized and representative of what might be ingested in nature.

Lumbriculus variegatus is a freshwater oligochaete that is widely distributed in Europe and North America (Brinkhurst and Jamieson, 1971) and has been found at densities up to 10,000/m^2 in various aquatic systems (Cook, 1969). *L. variegatus* is an important component in freshwater food webs, both as a consumer of small food particles (such as live algae, decaying plant material, bacteria and fungi) accumulated in the benthic environment, and as a prey item for predatory species

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such as macroinvertebrates, fish and waterfowl (Brinkhurst and Jamieson, 1971). *L. variegatus* has long been used as a model organism in toxicity assessment of sediment-associated contaminants in the laboratory (Ankley et al., 1994; Phipps et al., 1995). This heartiness in the lab renders it amenable for laboratory-based work, but also highlights its potential importance as a potential source of contaminants to organisms who consume it in contaminated environments.

Not all species participate equally in the trophic transfer of contaminants. *L. variegatus* appears to have several characteristics that make it particularly important in this process, including (1) the tendency to accumulate contaminants (via pore water, overlaying water, and ingested food particles) (Ankley et al., 1994; Aisemberg et al., 2005; Piol et al., 2006)), (2) the ability to tolerate elevated exposure concentrations (Ankley et al., 1991; Phipps et al., 1995; Chapman et al., 1999; Chapman, 2001) and (3) is a food source to several different species in a community. However, Cd biodynamics are poorly understood in this species, and it remains unclear whether Cd accumulated in its tissues is highly bioavailable. These studies were designed, in part to assess these parameters, and also to evaluate the suitability of *L. variegatus* as a standardized food source in metal trophic transfer studies.

Recently, Mount et al. (2006) showed that a *L. variegatus* diet provided sufficient nutrition to both rainbow trout and fathead minnows, and was well suited for trophic transfer studies for organic contaminants. The purpose of our study was to examine Cd bioaccumulation and subcellular distribution resulting from standard laboratory exposures. We also investigated the uptake of Cd in this species under different water chemistry conditions to determine the influence of ambient Ca concentrations on Cd uptake in the worms. Finally we explored the degree to which whole body calcium in this species could be manipulated by varying water hardness. The suitability of *L. variegatus* as a prey species for trophic transfer of Cd is discussed.

2. Materials and methods

Oligochaetes were purchased from Aquatic Foods (Fresno, California). Upon arrival, they were raised in reconstituted soft water (48 mg/L $NaHCO_3$, 30 mg/L $CaSO_4{\cdot}2H_2O$, 30 mg/L $MgSO_4$, and 2 mg/L KCl) (ASTM) (American Public Health Association et al., 1995) for a minimum of 1 week prior to exposure. All exposure containers were acid washed and thoroughly rinsed with deionized water. Food was withheld during exposures. Cd uptake, efflux, and subcellular fractionation experiments were performed independently in two laboratories – the US Geological Survey, Menlo Park, California (hereafter referred to as Exp. 1) and the Department of Environmental and Molecular Toxicology, North Carolina State University (hereafter referred to as Exp. 2).

All measurements of radioactivity in the worms and in the subcellular fractions (see below) were measured using a Perkin-Elmer Wallac Wizard 1480 automatic gamma counter (Shelton, Connecticut, USA). Samples were counted for 3 min. All counting errors were <5%. Appropriate corrections were made for radioactive decay and counting efficiency.

2.1. Cd accumulation

In both experiments, approximately 5 g of worms were placed into each of 1 L Nalgene® polypropylene beakers ($n = 6$ for Exp. 1 and $n = 3$ for Exp. 2) containing 1 L artificial soft water, Cd stock solution (both ^{109}Cd and stable), and NaOH (0.1N) to adjust pH to 6.85, yielding a final activity of approximately 0.585 μCi/L Cd. The final concentration of Cd was 0.52 μg/L. The exposures were conducted under 16 h:8 h (light:dark) photoperiod with moderate aeration at 14 °C for 10 days. The exposure medium was renewed daily. One milliliter water samples were taken daily to verify the radioactivity of both pre-and post-exposure water. The total Cd concentration in the exposure chamber was calculated based on the ratio between the stable Cd and ^{109}Cd. Cd concentrations were depleted on a daily basis, but the level of depletion decreased incrementally over time from 42% after day 1, to 18% after day 10 of uptake. At least 300 mg of worms were sub-sampled from each exposure chamber. The worms were blotted dry, weighed, and placed in 20 mL vials with 4 mL of water and measured for radioactivity *in vivo*. Measurements were performed daily in Exp. 1, and on days 1, 4, 8 and 10 in Exp. 2. After counting, the worms were immediately returned to their respective exposure chambers.

2.2. Cd efflux

After 10 days of Cd uptake, the remaining worms were transferred to clean 1 L beakers containing reconstituted soft water for 10 days of depuration. Water was changed daily. At least three replicates weighing approximately 0.1 g were sub-sampled (for Exp. 1, each day except day 9 of efflux; for Exp. 2, on days 2, 4, 8 and 10) during depuration in order to estimate efflux rate constants, k_e.

2.2.1. Stable Cd measurements

A parallel experiment with the same concentrations of unlabelled cadmium only was also carried out. In that experiment, worms were sub-sampled for measurement of Cd body burdens by inductively coupled optical emission spectrophotometry (ICP-OES) (Garbarino and Hoffman, 1999) on days 7, 8, 10 and 15. Duplicate exposures using stable Cd only were run simultaneously with Exp. 1. Worms were sampled on days 7, 8, 10 (uptake) and 15 (efflux) and prepared for elemental analysis. Tissues were weighed and then were digested by hot reflux in 16N HNO_3, after which the solutions were slowly dried. The digestate was reconstituted in 1% HNO_3 and filtered (0.45 μm) and subjected to ICP-OES for Cd determination. Acid-washed materials were used at all steps of the procedure to minimize contamination.

2.3. Cd subcellular compartmentalization

The subcellular compartmentalization of Cd was evaluated during both accumulation and efflux phases of the experiment. Subcellular fractions were obtained following the methods described by Wallace et al. (2003) and others (Cain et al., 2006;

Cheung and Wang, 2005; Giguere et al., 2006; Rainbow et al., 2006). Briefly, the homogenized tissue was centrifuged at 800 × *g* for 15 min at 4 °C. The resultant pellet contained cellular debris and nuclei (referred as cell debris fraction). The supernatant was subjected to centrifugation at 3000 × *g* for 20 min at 4 °C. This step resulted in a pellet containing organelles, such as mitochondria, some lysosomes and peroxisomes (referred to organelle fraction). The supernatant was then subjected to an ultracentrifugation at 100,000 × *g* for 60 min at 4 °C. The resultant pellet contained microsomes (referred to microsomal fraction). This supernatant was heat-treated (80 °C for 10 min), followed by placement on ice for 1 h. The supernatant was then centrifuged at 30,000 × *g* for 10 min at 4 °C. This step produced a pellet containing heat-denaturable proteins (HDP) and a supernatant containing heat-stable proteins fraction (HSP). It is generally assumed that metallothionein-like proteins (MTLPs) are the ligands most responsible for binding Cd in the HSP fraction; but the HSP fraction also consists of various cellular ligands including amino acids and low molecular weight peptides, such as glutathione (Kraemer et al., 2005). Recovery of radioactivity for subcellular distribution was 83 ± 1.9% in both experiments. Cadmium in each fraction is expressed as percentage of the total recovered Cd in the worm.

2.4. Cd uptake under different water hardnesses

To investigate the effect of ambient calcium concentration and free cadmium ion activity on Cd uptake, *Lumbriculus* were exposed to Cd (0.55 μg/L with a final activity of 1.14 μCi/L) under five water hardness conditions, ranging from very soft to very hard. Solutions were prepared according to the ASTM recipes (American Public Health Association et al., 1995). The corresponding hardness ranges (in terms of $CaCO_3$) for the various treatments were 10–13, 40–48, 80–100, 160–180, and 280–320 mg/L. 1 M phosphate buffer saline (pH 6.77) was used to adjust pHs of the exposure solutions to approximately pH 7.4. Approximately 0.4–0.5 g were placed in 50 mL Nalgene® polypropylene beakers at 4 °C without aeration. Four individual worms from each treatment were sampled for radioactivity measurement at 2, 5, 24, 49 and 67 h time points. Uptake rate constants of Cd in the worm under different water hardness conditions were calculated by incorporating the k_e estimates obtained from Exp. 1 and Exp. 2.

2.5. Effect of water hardness on the calcium content in the worm

To manipulate the calcium content of *Lumbriculus*, approximately 1 g of worms was placed into 50 mL Nalgene® polypropylene beakers containing the very soft, moderately hard, and very hard waters for 18, 23 and 29 days, respectively, at 4 °C without aeration or food. Individuals were then removed from their respective chambers, rinsed with deionized water, blotted dry and weighed. Samples were then digested in 1 mL ultrapure nitric acid at 80 °C overnight in a water bath. Sample volumes were brought to 7 mL with deionized water. Calcium analysis was performed using inductively coupled plasma atomic emission spectroscopy (ICP-AES) (Perkin-Elmer, Model 2100 DV, Waltham, MA, USA).

2.6. Statistical analysis

Cadmium efflux and uptake rate constants were calculated based on the method described by Schlekat et al. (2002). Cadmium efflux rate constants (k_e) were calculated based on Eq. (1).

$$C_t = C_i(1 - e^{-k_e t}) \quad (1)$$

where C_t = Cd concentration in the worms (ngCd/g wet weight) at time t, C_i = Cd concentration in the worms (ngCd/g wet weight) at the beginning of the efflux phase of the experiment, k_e = efflux rate constant (/day).

Uptake rate constants (k_u) were calculated from Eq. (2).

$$k_u = \frac{C_t \times k_e}{[1 - e^{-k_e t}] \times C_w} \quad (2)$$

where C_w = concentration of Cd in the exposure media, C_t = concentration of Cd observed in the worms after 1 day of exposure, k_u = uptake rate constant (L/g/day).

Data are expressed as mean ± standard error unless otherwise stated. All analyses were performed using SAS (version 8.02). All data met the assumptions of equal variance and normal distribution when analysis of variance (ANOVA) was applied. Regression analysis was used to determine the relationship between Cd concentrations in the worms and exposure duration in the 10-day uptake experiment and under different hardness conditions. Pairwise comparison was used to compare Cd levels in the worm in the efflux experiment and also Cd in different subcellular compartments in the worm. Calcium levels in the worms were compared using Tukey's honestly significant difference (HSD) test.

Analysis of covariance (ANCOVA) was used to test the difference in Cd uptake rates in the worms under different water hardness conditions with time and treatment as dependent variables (in SAS, using PROC GLM and ESTIMATE statement). Free ion concentrations of each ion species were calculated using the visual MINTEQ software (version 2.52, a Windows version of MINTEQA2 version 4.0, which was released by the US Environmental Protection Agency in 1999. Visual MINTEQ is available for free downloaded at http://www.lwr.kth.se/English/OurSoftware/vminteq/). Stepwise regression analysis was used to determine the relationship between the concentrations of free ion species and Cd uptake rates.

3. Results and discussion

3.1. Cd uptake and efflux in reconstituted soft water

L. variegatus exhibited strongly linear increase in Cd accumulation over 10 days of exposure in both Experiments 1 and 2 (Fig. 1). Cadmium accumulation rates in the worms were comparable with the two experiments. The average Cd uptake rate constant (k_u) of the two experiments was 0.058 ± 0.012 L/g/day.

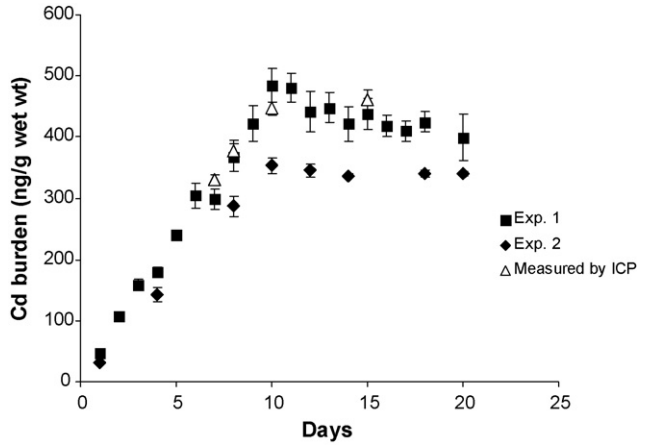

Fig. 1. Cadmium uptake and efflux in *L. variegatus* exposed to 0.52 μg/L Cd for 10 days and depurated for 10 days in clean soft water. Exp. 1 was conducted at USGS, Menlo Park, CA and Exp. 2 was conducted at NCSU ($n = 6$ for Exp. 1 and $n = 3$ for Exp. 2). Cadmium burdens in these experiments were calculated based on ^{109}Cd activities. Independent measures of Cd body burdens are represented by open triangles (days 7, 8, 10 and 15) and were measured by inductively coupled optical emission spectrophotometry (ICP-OES). Data were expressed as means ± standard error.

Slight differences in Cd uptake rates between these two experiments were potentially due to the use of different *Lumbriculus* cultures. Nevertheless, these results indicate that Cd accumulation in *L. variegatus* is predictable and reproducible. These results are consistent with other studies showing that *L. variegatus* and other oligochaetes such as *Tubifex tubifex* accumulate Cd in a linear fashion when exposed to Cd-spiked sediments or Cd in solution (Redeker et al., 2004; Gillis et al., 2004; Piol et al., 2006).

Cadmium efflux was slow in both experiments with an average rate constant of loss (k_e) calculated at 0.002 ± 0.0004/day. After 10 days efflux, worms retained approximately 82% (Exp. 1) and 96.6% (Exp. 2) of their pre-efflux phase body burdens (Fig. 1). Approximately 55% was still retained in the worm after 61 days of efflux in Exp. 1 (results not shown). Slow depuration was also reported in *L. variegatus* exposed to Cd via sediment (Dawson et al., 2003). Compared to other species, Cd elimination in *L. variegatus* is relatively slow (Luoma and Rainbow, 2005).

Based on these k_u and k_e estimates, a steady state Cd body burden derived from the aqueous route of exposure (assuming ambient dissolved Cd concentration of 0.52 μg/L) in *L. variegatus* was calculated to be approximately 15 μg/g wet weight. Considering that in nature this species would also accumulate Cd from its diet, Cd bioaccumulation in this animal would be predicted to be quite high. This tendency to bioaccumulate Cd coupled with its apparent ability to tolerate high levels of Cd exposure (Chapman et al., 1999; Phipps et al., 1995) would seem to indicate that this species could play an important role in the trophic transfer of Cd in aquatic ecosystems.

The physiological traits that result in high levels of Cd bioaccumulation in *L. variegatus* also make it amenable to use as a food item in laboratory based dietary metal accumulation studies. While dissolved uptake rates are moderate, the slow efflux makes this animal ideal for dietary studies. First, it allows for more accurate estimates of metal concentrations in the diet and provides the flexibility of being able to offer prepared food for longer periods of time. Second, it reduces a potentially confounding factor of aqueous Cd exposure to the consumer resulting from release of Cd from the food. Finally, if researchers are using food labeled with gamma emitting isotopes, slow efflux allows for the concentrations of metal in the food to be precisely standardized. For example, an exposure can be terminated when the desired concentration is reached to ensure uniform Cd levels in the prey. This is essential in trophic transfer studies in which experimental results depend on consistency of exposure conditions.

3.2. Cd subcellular distribution

Another important factor in the trophic transfer of trace metals is the bioavailability of metals in the food. In our experiments Cd was predominately distributed with the cytosol through the accumulation and depuration phases. Cadmium associated with the heat denatured protein (HDP) fraction represented a consistently high proportion of the body burden, averaging 48% (39–51%) and 53% (44–57%) in Exp. 1 and Exp. 2, respectively (Fig. 2). The HSP fraction was the only fraction that showed any significant variation between the two independent experiments. For both experiments, the percentage of Cd in the HSP averaged 16.2%. However, the proportions of Cd observed on day 10 of accumulation in Exp. 1 (23%) and on day 10 of depuration in Exp. 2 (32%) were significantly greater ($P < 0.05$, pairwise comparison) from proportions on other days, except for day 18 in Exp. 1. Overall, the proportion of Cd associated with the HSP

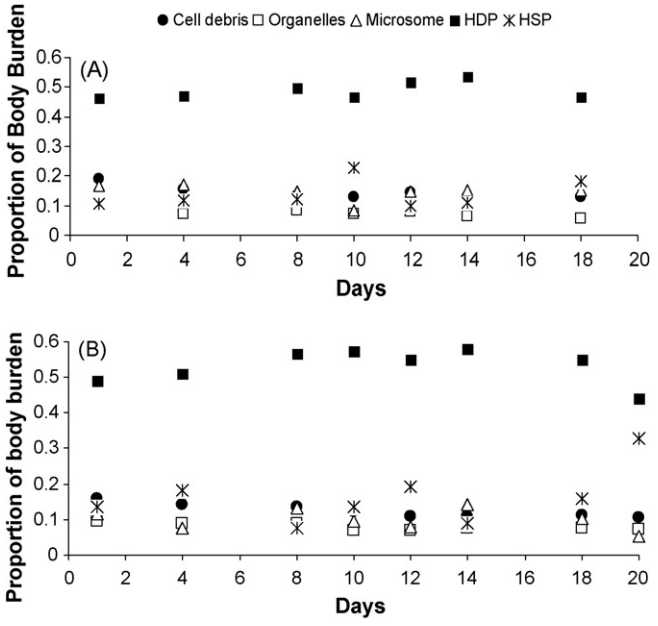

Fig. 2. Proportional distribution of Cd in subcellular compartments in *L. variegatus* over 10 days of accumulation and 10 days of depuration. HDP: heat-denatured proteins, HSP: heat-stable proteins. Error bars were omitted for clarity of presentation but estimates of variances are included in text. Top and bottom panels represent Exp. 1 and Exp. 2, respectively.

fraction varied modestly and showed no clear trend during either the accumulation or depuration phases of the experiments. The proportion of Cd in the cell debris fraction ranged from 12.7 to 19% (Exp. 1) and 10.6 to 16% (with 16% on day 1 of accumulation, implying adsorption on the worm body surface) (Exp. 2). The proportion of Cd in the organelles (approximately $7.8 \pm 1\%$ (Exp. 1) and $7.9 \pm 1\%$ (Exp. 2) was almost identical. Cadmium in the microsomal fraction was relatively constant during accumulation and depuration, with $15 \pm 2\%$ in Exp. 1 and $10 \pm 2\%$ in Exp. 2, respectively.

The results of our Cd compartmentalization studies suggest that each subcellular fraction or pool accumulates Cd at relatively constant rates. The "spillover" hypothesis (Langston and Zhou, 1986; Mason and Jenkins, 1995) invokes the idea that the MLTP pool may saturate at high metal doses leading to the "spill over" of excess metal to other pools (e.g. HDP and organelle fractions) and consequently, a shift in metal partitioning within the animal. This phenomenon was not observed in our study. Similar results have been reported in a marine mussel experimentally exposed to cadmium for 24 h (Ng and Wang, 2005), and other studies have demonstrated similar relationships in animals collected along metal gradients in the field (Cain et al., 2004; Giguere et al., 2003). However, Kraemer et al. (2005) showed that the percent contribution of Cd in the HSP fraction in the liver of yellow perch increased with exposure duration, while percent contribution of Cd from the HDP fraction decreased over time. Thus, shifts in metal partitioning that might affect Cd bioavailability to consumers (e.g. Wallace et al., 2003) were not problematic for *L. variegatus*, at least under our experimental conditions.

There remains some uncertainty regarding the bioavailability of metals from subcellular fractions. For example, Wallace et al. (2003) defined total bioavailable metals (TAM) as metals partitioning in organelles, HDP fraction, and HSP fractions (Wallace et al., 2003). This TAM excludes metals in insoluble fractions, for instance, cell debris and metal-rich granules (MRG), and metals adsorbed to the cell walls and integument. The general implication of this definition is that metals in the insoluble fractions are less bioavailable for trophic transfer (Rainbow et al., 2006; Wallace et al., 2003). However, Cheung and Wang (2005) revealed that the marine snail *Thais clavigera* assimilated metals (including Ag, Cd, and Zn) bound to the insoluble fractions or MRG with high efficiency (40–85%), which was similar to that of metals in the soluble fractions. In another study, Robinson et al. (2003) suggested that metals bound to the carapace of *Daphnia magna* and *Ceriodaphnia dubia* might be trophically available to predatory organisms. Our observations with aquatic insects lead us to speculate that metals adsorbed to the integument are likely bioavailable. There does however seem to be a consensus that metals associated with the cytosolic fractions are highly bioavailable for trophic transfer. Therefore, the Cd compartmentalization patterns observed in *L. variegatus* suggest that a large proportion of its body burden will be bioavailable for trophic transfer. Results from recent work in our lab indicate that Cd accumulated in *L. variegatus* is highly bioavailable to predatory stoneflies (Martin et al., 2007).

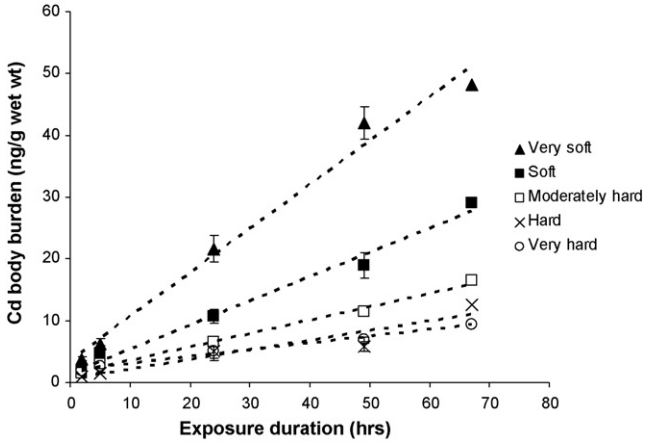

Fig. 3. Cadmium uptake in worms exposed to 0.52 μg/L of Cd under five different water hardness conditions for 3 days.

3.3. Cd uptake in the worm under different water hardness conditions

Under the five different water hardness conditions, uptake of Cd in the worm exhibited a strong linear relationship with the exposure duration ($R^2 > 0.90$, $P < 0.0001$ in all cases; Fig. 3). The Cd uptake rates (slopes of the lines: ng/g/h) for the worms in very soft, soft, moderately hard, hard, and very hard media were 0.74, 0.38, 0.21, 0.14 and 0.11 ng/g/h, respectively. These rates were significantly different except between the hard and very hard conditions (ANCOVA; $P < 0.05$ for all pairwise comparisons). Stepwise regression analysis showed that $\log([Cd^{2+}]/[Ca^{2+}])$ (free ion concentrations estimated using the MINTEQ software) was the most significant factor affecting k_u ($P = 0.0084$). Uptake rate constants showed an exponential decrease with increased water hardness and $-\log([Cd^{2+}]/[Ca^{2+}])$ (Fig. 4). The accumulation of Cd via Ca channels is well established in bivalves (Bondgaard and Bjerregaard, 2005; Wang and Fisher, 1999), insects (Craig et al., 1999) and fish (Wood et al., 2006). Competition between Ca^{2+} and Cd^{2+} for binding sites on the Ca channels is the most logical explanation for Ca mediated differences in Cd accumulation in our studies.

3.4. Effects of water hardness on the calcium content in the worm

Our biodynamics and compartmentalization studies suggest that *Lumbriculus* is an ideal prey item for trophic transfer studies. Another goal of our studies was to determine whether whole body calcium concentrations in *Lumbriculus* can be manipulated. Our motivation for exploring this question is that relative to our understanding of Cd uptake processes from solution, our understanding of uptake processes in the gastrointestinal tract is poorly understood. Competition experiments between Ca and Cd, for example, are easily performed in aqueous exposures, but are very difficult to conduct in dietary studies. The manipulation of Ca content in *Lumbriculus* allows researchers to explore whether Cd accumulation in the GI tract of their study animal is mediated by calcium transporters. We successfully manip-

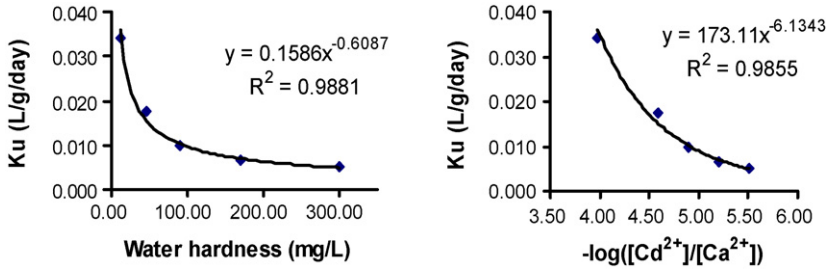

Fig. 4. Cadmium uptake rates as a function of log([Cd^{2+}]/[Ca^{2+}]) and water hardness. Both [Cd^{2+}] and [Ca^{2+}] were calculated using the visual MINTEQ software.

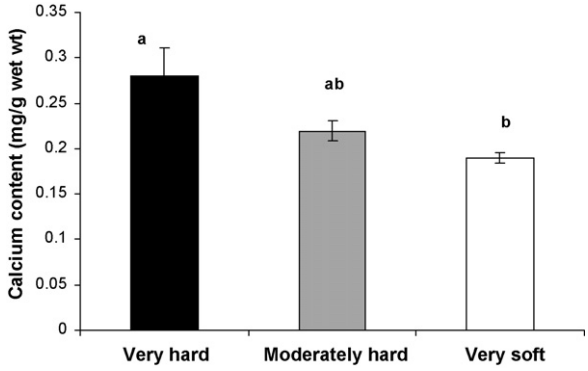

Fig. 5. Calcium content in the worms acclimated for 10 days in the very soft, moderately hard, and very hard water treatments. Data with different letters indicate significant differences.

ulated whole body calcium concentrations in *Lumbriculus* by holding the worms under different water chemistry conditions. Worms held in very hard water had Ca concentrations that were 43% higher than worms held in very soft water ($P = 0.0192$, Fig. 5). Preliminary work suggested that calcium content may affect Cd availability in predatory stoneflies (Martin and Buchwalter, unpublished). The ability to manipulate whole body Ca enhances the utility of this species as a food source for trophic transfer studies.

In summary, two independent experiments demonstrate that *L. variegatus* exhibits several characteristics that indicate its potential importance as a conduit of Cd to higher trophic levels. The apparent tendency for *L. variegatus* to accumulate and tolerate high concentrations of Cd in readily bioavailable forms suggest that this species is potentially very important in the trophic transfer of this toxic metal in aquatic ecosystems.

In addition, *L. variegatus* appears particularly well suited for use in laboratory-based trophic transfer studies. Efficient metal loading coupled with slow efflux make it easy to manipulate Cd concentrations in this species. Furthermore, the distributions of accumulated Cd in different subcellular fractions remained relatively stable over time. Finally, the ability to manipulate Ca content in this species makes it potentially useful for studying mechanisms of dietary Cd accumulation.

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