Limnol. Oceanogr., 51(2), 2006, 1013–1020

Competition between protons and cadmium ions in the planktonic food chain leading to the phantom midge *Chaoborus*

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Abstract

Cadmium concentrations in the phantom midge *Chaoborus* can be related to those of the free Cd ion, Cd^{2+}, in lakewater provided that the competitive influence of H^+ on Cd uptake sites is considered. Because this predator takes up its Cd from plankton, competition between H^+ and Cd^{2+} ions could take place at several levels in the food chain to which this insect belongs. To identify at which trophic levels this occurs, we first measured H^+-Cd^{2+} competition in the gut of a given *Chaoborus* species (*Chaoborus americanus*) by exposing it to constant Cd concentrations in water and food but at various ambient pH levels (4.5, 5.5, 6.5). There was no difference in the efficiency with which this predator assimilated Cd at the various pHs, suggesting that H^+ swallowed by *Chaoborus* do not compete with Cd at uptake sites in its gut. pH-sensitive dyes showed that *C. americanus* is able to maintain its gut pH between 6.5 and 8.0, even when the pH of ambient water varies beyond this range (4.5–9.0). We then determined whether H^+-Cd^{2+} competition is likely to take place on the prey of *Chaoborus* by measuring the importance of water as a Cd source for the calanoid copepod *Diaptomus minutus*. Copepods fed with Cd-rich green algae and exposed to either a high $[Cd^{2+}]$ (5 nmol L^{-1}) or a low $[Cd^{2+}]$ (1.6 nmol L^{-1}) accumulated a majority of their Cd from water, suggesting that H^+ and Cd^{2+} ions are likely to compete at Cd uptake sites on these crustaceans. Last, we measured Cd accumulation by this copepod at various pHs. Copepods held at pH 4.8 accumulated less Cd than those at pH 5.5, suggesting that H^+-Cd^{2+} competition occurs in this animal. A bioaccumulation model designed to take into account H^+-Cd^{2+} competition was parameterized using our data for Cd accumulation by copepods at pH 4.8 and 5.5 and then used to predict measured responses at pH 6.4.

Mining, smelting, and other industrial activities have increased the flux of metals to aquatic ecosystems (Chapman et al. 2003). Because not all of the metals in these systems is available for uptake by organisms, total metal concentrations in water or sediment cannot usually be used to predict their bioaccumulation or effects (Campbell 1995; Hare et al. 2003). For this reason, a variety of freshwater and marine organisms have been used as biomonitors to estimate bioavailable metal concentrations in their surroundings (Phillips and Rainbow 1993). To use a biomonitor effectively, we need to be able to relate contaminant concentrations in the organism to those in its environment. Models that consider the characteristics of both the pollutant and the biomonitor organism provide the best means of achieving this goal (Pace 2001).

The phantom midge *Chaoborus* has been proposed as a Cd biomonitor in lakes by Hare and Tessier (1996, 1998) and Croteau et al. (1998, 2002). These researchers showed that by using the tenets of the free ion activity model (or its offshoot the biotic ligand model) they could predict Cd concentrations in *Chaoborus*, $[Cd]_{Chaoborus}$, from those of the free Cd ion, $[Cd^{2+}]$, in surrounding lakewater provided that they considered competition between hydrogen ions and free cadmium ions at biological uptake sites; that is,

$$[Cd]_{Chaoborus} = \frac{F \times [Cd^{2+}]}{[H^+] + K_a} \quad (1)$$

where K_a is a pseudoequilibrium constant for the binding of H ions to uptake sites on biological membranes and F is a proportionality constant specific to Cd and *Chaoborus*. However, because subsequent studies showed that larvae of this insect do not take up Cd from water but from their planktonic prey (Munger and Hare 1997; Munger et al. 1999), the trophic level(s) at which Cd^{2+}-H^+ competition occurs is open to doubt. We conducted laboratory experiments on *Chaoborus americanus* and its prey (a copepod) to determine at which of these trophic levels Cd and H ions compete, and thus to put Eq. 1 on a better mechanistic footing.

Methods

We conducted five experiments designed to determine (1) if H^+ and Cd^{2+} ions swallowed by *Chaoborus* compete for Cd-uptake sites on its gut wall; (2) if *Chaoborus* can control the pH of its gut; (3) if prey take up Cd from water; (4) the influence of pH on Cd accumulation by copepods; and (5) the rate at which copepods lose Cd. To answer these questions, we conducted experiments on the predator-prey pair of *C. americanus* (a large-sized insect that is abundant in fishless Canadian Shield lakes) and *Diaptomus minutus* (a common copepod in Shield lakes). We used the experimental data from experiments 3–5 to determine if a one-compartment model could predict Cd accumulation by the copepod and account for H^+-Cd^{2+} competition at this trophic level.

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Acknowledgments

Funding was provided by the Fonds de Recherche du Québec sur la Nature et les Technologies, the Metals in the Environment Research Network (contribution 52), the Natural Sciences and Engineering Research Council of Canada, Ontario Power Generation, and the Mining Association of Canada. Assistance from A. Boullemant, P. Boucher, C. Dion, C. Durand, S. Duval, L. Rancourt, and R. Rodrigue is gratefully acknowledged, as are comments by L. Croisetière, C. Fortin, and J. McGeer.

Table 1. Composition of the media and treatment levels used in our experiments.

Experiment	Alga (*Pseudokirchneriella subcapitata*)	Copepod (herbivore) (*Diaptomus minutus*)	Insect (predator) (*Chaoborus americanus*)
Experiment 1: Cd^{2+}-H^+ competition in the gut of *Chaoborus*	Simplified BBM* MES† 10^{-2} mol L^{-1} NTA‡ 10 μmol L^{-1} NaOH 10^{-2} mol L^{-1} pH 6.3 $[Cd^{2+}]$§ 10 nmol L^{-1} [Cd] 640 nmol L^{-1}	Artificial lakewater‖ [Cd] = $[Cd^{2+}]$ = 10 nmol L^{-1} pH 6.3 Cd-contaminated algae	3 treatment levels: pH 4.5, 5.5, and 6.5¶ artificial lakewater‖ [Cd]=$[Cd^{2+}]$§=10 nmol L^{-1} Cd-contaminated prey
Experiment 3: water and food as Cd sources for copepods	Simplified BBM* MES† 10^{-2} mol L^{-1} NTA‡ 10 μmol L^{-1} NaOH 10^{-2} mol L^{-1} pH 6.3	2 treatment levels: Cd-contaminated algae in artificial lakewater‖ at pH 5.5 with either low $[Cd^{2+}]$§ (1.6 nmol L^{-1}) or high $[Cd^{2+}]$§ (5 nmol L^{-1})	
Experiment 4: Cd^{2+}-H^+ competition on copepods	$[Cd^{2+}]$§ 5 nmol L^{-1} [Cd] 320 nmol L^{-1}	3 treatment levels: pH 4.8, 5.5, or 6.4#; artificial lakewater‖; Cd-contaminated algae; nominal $[Cd^{2+}]$=4 nmol L^{-1}	

* Bold basal medium (Starr and Zeikus 1993) without metals and EDTA.
† 2-(N-morpholino)ethanesulphonic acid, pK_a = 6.1, an inert biological pH buffer.
‡ Nitrilotriacetic acid.
§ $[Cd^{2+}]$ calculated using the chemical speciation program MINEQL+ (Schecher and McAvoy 1998).
‖ Artificial lakewater for all uses was prepared using the concentrations of major ions (μmol L^{-1}) measured in a lake representative of those sampled; that is, Ca^{2+} (21), Mg^{2+} (9.2), Na^+ (23.6), K^+ (2.3), SO_4^{2-} (21), Cl^- (7.5), NO_3^- (6.3).
¶ pH adjusted every 12 h using NaOH or HNO_3.
# pH adjusted every 6 h using NaOH or HNO_3.

Collection of invertebrates—We collected invertebrates for our experiments from two low-Cd lakes located near Quebec City, Quebec. The calanoid copepod *D. minutus* was collected from Lake Bleu (pH 5.6, 46°55′N, 71°58′W), and larvae of the phantom midge *C. americanus* were collected from Lake Pelouse (pH 5.6, 47°01′N, 72°05′W). These species are common residents of many Canadian Shield lakes (Borkent 1981; Keller and Pitblado 1984), and copepods are an important prey for *Chaoborus* larvae (Fedorenko 1975; Hare and Carter 1987). Both invertebrates were taken by hauling a 104-μm mesh-aperture plankton net horizontally in the water column during the day. Plankton samples were transported to the laboratory in a cooler and then held at 15°C in a temperature-controlled environmental chamber where all experiments took place. When *D. minutus* were required for an experiment, they were removed from the bulk plankton using a Pasteur pipette under a microscope. Conditions for the various experimental treatments described below are summarized in Table 1. Artificial lakewater for all experiments was prepared according to the information given in the footnote to Table 1. Speciation calculations (MINEQL+; Schecher and McAvoy 1998) showed that all of the Cd in the artificial lakewater was present as the free Cd ion.

Experiment 1: Cd^{2+}-H^+ competition in the gut of Chaoborus americanus—Fourth instar *C. americanus* larvae were selected on the basis of their head-capsule length (~1 mm; Fedorenko and Swift 1972) and held for a 1-d acclimation period in 30-mL, high-density polyethylene (HDPE) containers filled with Cd-free artificial lakewater and a mixture of copepods collected from Lake Bleu as food. At the same time, nine basins lined with Teflon sheeting were each filled with 1.5 liters of artificial lakewater (Table 1) and their pH was adjusted to 4.5, 5.5, or 6.5 (±0.1 pH unit, three basins per pH). We then added sufficient ^{109}Cd as $CdCl_2$ (2.6 MBq $mmol^{-1}$ specific activity) to attain a Cd^{2+} concentration of 10 nmol L^{-1}, which is reported for some metal-contaminated lakes (Croteau et al. 1998). Cadmium speciation in the basin was estimated using the MINEQL+ speciation code (Schecher and McAvoy 1998). Three 10-mL HDPE containers were suspended in each basin so that their openings were above the water level; the bottoms of these containers had been previously removed and replaced with 100-μm mesh-aperture netting to allow water to circulate through each container. After allowing the exposure system to stabilize for 1 d, we placed one *C. americanus* in each container.

Every 24 h we prepared fresh prey for *C. americanus* by exposing ~500 *D. minutus* to ^{109}Cd-rich green algae (*Pseudokirchneriella subcapitata,* cultured as described below) at a density of ~8×10^4 cells mL^{-1} in artificial lakewater (Table 1) containing ~10 nmol L^{-1} dissolved Cd. We did not rigorously control the Cd concentrations in algae and water because our goal was simply to produce prey of known Cd concentration. At the end of the 24-h prey exposure period, we held copepods for 4 h in Cd-contaminated water without food so that they would eliminate their gut contents (verified by microscope); we did this to ensure that *C. americanus* would not consume prey containing variable amounts of algae in their guts.

We fed these depurated prey to *C. americanus* at the daily rate of ~10 copepods per predator. Uneaten prey were removed after 5 h and replaced with the same number of fresh prey to ensure that prey Cd concentrations remained con-

stant. The exact number of prey consumed by each predator was noted so that ingestion rates could be calculated. On days 4 and 9, *C. americanus* larvae were removed and held for 24 h in Cd-contaminated artificial lakewater without food to eliminate any Cd-contaminated crop contents (verified under a microscope). Larvae were then rinsed five times with uncontaminated artificial lakewater (Table 1) and placed in a counting vial for 30 min to measure their ^{109}Cd content (*see following*). On day 4, *C. americanus* were replaced in the Cd-exposure medium and feeding was continued as described above. We measured ^{109}Cd in depurated prey by pooling 60 copepods in each of three samples on days 4 and 9.

Experiment 2: Gut pH of C. americanus—To measure the gut pH of *C. americanus* larvae, we used two pH-sensitive dyes, the colors of which would be visible through the transparent body of this insect. The first dye, lacmoides, is wine-red at pH $<$ 4.5; as the pH rises, it gradually changes to dark blue at pH 6.5. The second pH-sensitive dye, alizarin yellow, is orange-red at pH 9 and higher but gradually changes to yellow at pHs below 8. We held 10 *C. americanus* larvae in artificial lakewater at pH 4.5 containing lacmoides or at pH 9 containing alizarin yellow. After 24 h, we compared, under a microscope, the color of the larval gut fluid to samples of water containing the dyes at various pHs between 4.5 and 9. Prey were not present in these experiments.

Experiment 3: Relative importance of water and food as Cd sources for copepods—To acclimate copepods to the experimental conditions (Table 1), we held 50 *D. minutus* in each of twelve 30-mL HDPE containers filled with Cd-free artificial lakewater and the alga *P. subcapitata* for 24 h in the dark on a shaker (to maintain algae in suspension). In the first treatment level, copepods from six containers were transferred to six similar containers filled with ^{109}Cd-labeled artificial lakewater (5 $\pm$ 0.8 [SD, standard deviation] nmol L^{-1} Cd^{2+}) and fed ^{109}Cd-labeled *P. subcapitata* (labeling procedure follows) having a mean Cd concentration of 218 $\pm$ 48 (SD) nmol Cd g^{-1}. In the second treatment level, copepods were treated the same except that the artificial lakewater was initially Cd-free. However, because algae lost Cd, Cd^{2+} concentrations in the second treatment level reached 1.6 $\pm$ 0.7 (SD) nmol L^{-1}. In both treatment levels (Table 1), water and algae were changed every 6 h and the pH was maintained at 5.5. After 18, 30, 48, and 54 h, copepods were removed for ^{109}Cd counting.

Experiment 4: Cd^{2+}-H^+ competition on copepods—We placed 120 *D. minutus* in each of fifteen 30-mL HDPE containers filled with artificial lakewater adjusted to pH 4.8, 5.5, or 6.4 (five containers per pH) and containing uncontaminated *P. subcapitata* (1.6×10^6 cells mL^{-1}). They were held for 24 h in the dark on a shaker to acclimate to experimental conditions. However, the containers at pH 6.4 spilled on the shaker, and thus copepods at this pH did not undergo acclimation. We then transferred the copepods to 30-mL HDPE containers filled with artificial lakewater at these same pH levels and containing a nominal [Cd^{2+}] of 4 nmol L^{-1} as well as Cd-contaminated *P. subcapitata* (description of exposure follows) in excess of the copepods' needs (1.6×10^6 cells mL^{-1}). Every 6 h, we changed the algae and exposure water and readjusted the pH as necessary. After 12, 24, 36, 60, and 72 h of Cd exposure, we removed dead *D. minutus* (if any) and measured ^{109}Cd in copepods by sieving them onto a nylon mesh, rinsing them five times in Cd-free artificial lakewater, and placing them live in counting vials. After 200 s, copepods were returned to fresh exposure medium and the experiment was continued. There was no significant difference in copepod mortality among the three pHs ($p >$ 0.5, analysis of variance [ANOVA]), suggesting their health was not influenced by ambient pH.

Experiment 5: Cd loss from copepods—To measure the loss of Cd from copepods, we transferred *D. minutus* that had been exposed to Cd at various pHs for 72 h (as described for experiment 4) to 30-mL HDPE containers filled with artificial lakewater to which an excess of uncontaminated *P. subcapitata* had been added as food; water and algae were subsequently changed every 6 h. A total of 12 containers was used, four for each pH (4.8, 5.5, or 6.4). After 0.5, 1.5, 6, and 24 h, we sieved, rinsed, and measured ^{109}Cd in live copepods, as described for experiment 4 above, and then replaced them in their containers until the next counting time. To calculate Cd concentrations in *D. minutus*, we assumed a dry weight of 1.1 $\pm$ 0.3 (SD) μg copepod^{-1} (Munger and Hare 2000). On the basis of preliminary microscopic observations on 10 copepods held without food after feeding on algae, we considered that most copepods emptied their gut of algae within 30 min, and thus we estimated the Cd loss rate constant from the time of 30 min.

^{109}Cd-labeling of phytoplankton—The green alga *P. subcapitata* was maintained in simplified bold basal medium (BBM; Starr and Zeikus 1993) at 20°C at a constant illumination of 60 μmol of photons m^{-2} s^{-1}. Simplified BBM (Table 1) contained no added ethylenediaminetetraacetic acid (EDTA) or trace metals. Cells in the late log phase were rinsed four times by centrifuging them at 6,000 $\times$ g for 4 min and resuspending them in simplified BBM. We then spiked 4×10^5 cells mL^{-1} into Cd-contaminated simplified BBM. After 4 d of growth, cell densities reached 1.2×10^6 cells mL^{-1} and they were considered to be uniformly ^{109}Cd-labeled. Cells were subsequently rinsed four times at 6,000 $\times$ g for 4 min in Cd-contaminated or Cd-free artificial lakewater (Table 1), according to the treatment, and fed to copepods. For the measurement of algal Cd, algae were collected onto 0.4-μm polycarbonate filters (Poretics, Osmonics Inc.) and rinsed with 10 mL of 10^{-2} mol L^{-1} EDTA for 10 min to remove any surface-bound ^{109}Cd. Algal growth and surface area were quantified using a particle counter (Coulter Multisizer II, Coulter Electronics).

Radioactivity measurements and statistical analyses—^{109}Cd in water, algae, and invertebrates were measured by gamma counting (1480 Wallac Wizard 3, NaI[Tl] well-type counter; 60% counting efficiency for ^{109}Cd) and converted to total Cd concentrations based on the specific activities of the various Cd solutions used in our experiments. Counting

times were sufficient to yield propagated counting errors of <5%, and blanks were used to verify that samples were uncontaminated by experimental manipulations.

Differences in Cd assimilation efficiency by *C. americanus* were compared across treatments (pH 4.5, 5.5, and 6.5) and times (days 4 and 9) using a repeated-measures ANOVA. Differences in Cd concentrations in copepods were also compared using a repeated-measures ANOVA followed by Tukey's multiple comparison test. All statistical tests were performed using the computer program SAS (SAS Institute Inc.). Nonlinear regressions to determine the values of model constants were performed using the programs SPSS or SigmaPlot; mean values of measured variables were used for these determinations.

Results and discussion

Although acidic lakes have some of the highest dissolved Cd concentrations reported from freshwaters, larvae of the phantom midge *Chaoborus* that live in such lakes are reported to be low in Cd (Croteau et al. 1998; Hare and Tessier 1996, 1998). These results suggest that H ions compete with Cd ions at biological uptake sites in these highly acidic lakes. We tested for such competition at two trophic levels, that of the predator *Chaoborus* and that of its zooplanktonic prey.

Experiment 1: Cd^{2+}-H^+ competition in the gut of Chaoborus americanus—Because *Chaoborus* larvae take up Cd from their food (Munger and Hare 1997; Munger et al. 1999), we tested the possibility that competition between Cd and H ions for biological uptake sites in the digestive tract occurs in the gut of these larvae if they swallow sufficient acidic water along with their prey (Croteau et al. 2003*a*). For example, the pH of the gut of fish is reported to be influenced by the pH of their food and water (Amerio et al. 1991). By exposing *C. americanus* larvae to the same Cd concentrations in prey and water, but at various [H^+] (experiment 1), we were able to determine if this predator assimilates Cd less efficiently from prey when both it and its prey are held in acidic water. For this purpose we compared the proportion of prey Cd that was assimilated by *C. americanus*, rather than [^{109}Cd] in this predator, because the former allowed us to take into account differences in feeding rates among individuals, whereas the latter did not. We calculated Cd assimilation efficiencies (AE, percentage) using Eq. 2:

$$\mathrm{AE} = \frac{dQ_{\text{Cd-}Chaoborus}/dt}{N \times Q_{\text{Cd-prey}}} \times 100 \qquad (2)$$

where $dQ_{\text{Cd-}Chaoborus}/dt$ is the rate of change in the Cd burden of *C. americanus* (pmol d^{-1}), $Q_{\text{Cd-prey}}$ is the mean quantity (±SD, pmol) of Cd in an individual copepod (0.13 ± 0.01 [$n = 3$] for the period 0–4 d and 0.12 ± 0.01 [$n = 3$] for the period 5–9 d), and N is the mean number of copepods ingested per day; that is, 38 ± 7 ($n = 27$) for the period 0–4 d and 21 ± 7 ($n = 27$) for the period 5–9 d. We calculated AEs independently for each of the two time periods. We ignored Cd losses from the predator in Eq. 2 because Cd loss from *C. americanus* is very slow; that is, Croteau et al. (2002) reported a loss rate constant of 0.0007 d^{-1} for larvae at 14°C, a temperature similar to that of our experiment (15°C).

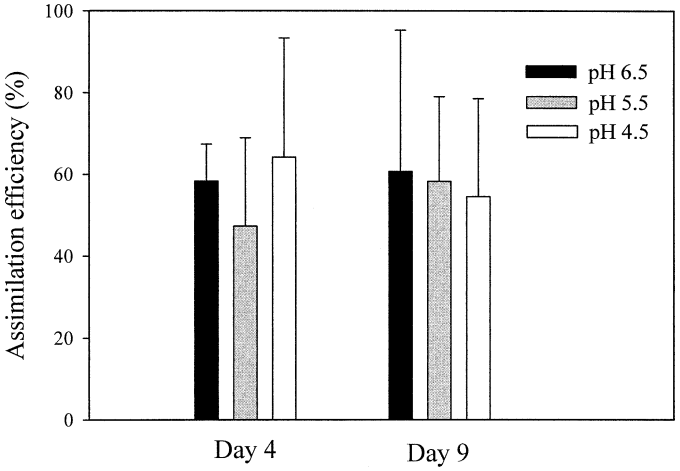

Fig. 1. Efficiency (mean + SD, $n = 9$) with which larvae of *C. americanus* assimilated Cd from their prey (the copepod *D. minutus*) for two time periods (0–4 and 5–9 d) and its relationship to the pH of ambient water.

The efficiency with which *C. americanus* larvae assimilated Cd from their copepod prey did not vary with the pH of ambient water (Fig. 1; $p > 0.05$, ANOVA), and this over a two orders of magnitude range in [H^+]. The mean (± 95% confidence interval [CI]) AE of 58% ± 5% for *C. americanus* feeding on the calanoid copepod *D. minutus* at 15°C is identical to that of 58% reported by Croteau et al. (2002) for this *Chaoborus* species feeding on calanoid copepods at 5°C. Although Croteau et al. (2002) also reported a corresponding AE for animals held at 14°C (18%), they questioned the reliability of this value because the *C. americanus* larvae they used were near pupation. Our results suggest that their AE value at 14°C is indeed underestimated and that *C. americanus* larvae assimilate Cd with equal efficiency at 5°C and 14°C.

Experiment 2: Gut pH of C. americanus—To explain the lack of competition between Cd and H ions in the gut of *C. americanus*, we determined if its gut pH varies with ambient pH by holding 10 larvae in two pH-sensitive dyes without prey. After 3 h, all individuals had ingested some ambient water because the contents of both the pharynx (the anterior portion of the gut in which prey are crushed) and the intestine were colored by the dye. At an ambient pH of 4.5, lacmoides dye in the gut was dark blue, suggesting a gut pH above 6.5. At an ambient pH of 9, alizarin yellow in the gut was yellow, suggesting a pH below 8. Thus, the gut pH of *C. americanus* larvae lies between 6.5 and 8 and appears to be little influenced by the pH of the ambient water. Circumneutral to weakly acidic gut pHs have been reported for a variety of aquatic invertebrates (Hare 1992; Ahrens and Lopez 2001). Control over gut pH explains why Cd assimilation by *C. americanus* (experiment 1) was not influenced by the [H^+] of ambient water. We conclude that H ions do not

compete with Cd for uptake sites in the gut of *Chaoborus*, but that such competition occurs at a lower level in the food chain leading to this predator.

Experiment 3: Relative importance of water and food as Cd sources for copepods—We reasoned that if copepods take up Cd from water this should result in competition between Cd and H ions at Cd-uptake sites on these crustaceans. To determine if water is a Cd source for copepods, we exposed *D. minutus* to the same Cd concentrations in food (the alga *P. subcapitata*) but to different Cd concentrations in water at constant pH. If we consider *D. minutus* to be a single compartment, temporal changes in its Cd concentrations, $d[\mathrm{Cd}]_{\mathrm{copepod}}/dt$, is the difference between Cd influx from water and food and physiological Cd efflux (Thomann 1981; Munger et al. 1999); that is,

$$\frac{d[\mathrm{Cd}]_{\mathrm{copepod}}}{dt} = \underbrace{k_{uw}[\mathrm{Cd}^{2+}]}_{\text{Cd influx from water}} + \underbrace{k_{uf}[\mathrm{Cd}]_f}_{\text{Cd influx from food}} - \underbrace{k_e[\mathrm{Cd}]_{\mathrm{copepod}}}_{\text{Cd efflux}} \quad (3)$$

where $[\mathrm{Cd}]_{\mathrm{copepod}}$ and $[\mathrm{Cd}]_f$ are the Cd concentrations (nmol g^{-1}) in copepods and their algal food, respectively; k_{uw} (L g^{-1} d^{-1}) and k_{uf} (d^{-1}) are rate constants for Cd uptake from water and food, respectively; and k_e (d^{-1}) is the rate constant for Cd loss from the copepod. Because $[\mathrm{Cd}^{2+}]$ and $[\mathrm{Cd}]_f$ were maintained constant during the course of the experiments, integrating this equation yields

$$[\mathrm{Cd}]_{\mathrm{copepod}} = \left(\frac{k_{uw}[\mathrm{Cd}^{2+}] + k_{uf}[\mathrm{Cd}]_f}{k_e}\right)(1 - e^{-k_e t}) + [\mathrm{Cd}_0]_{\mathrm{copepod}} e^{-k_e t} \quad (4)$$

where $[\mathrm{Cd}_0]_{\mathrm{copepod}}$ is the initial Cd concentration in the copepods.

Because there were significantly higher Cd concentrations in copepods that had been exposed to the higher $[\mathrm{Cd}^{2+}]$, but at the same pH and $[\mathrm{Cd}]_f$ (Fig. 2; $p < 0.05$, ANOVA), we conclude that these crustaceans must take up some Cd from water. To quantify the relative importance of water and food as Cd sources for this copepod, we proceeded as follows. We first estimated k_e by fitting the following equation

$$[\mathrm{Cd}]_{\mathrm{copepod}} = [\mathrm{Cd}_0]^{\mathrm{efflux}}_{\mathrm{copepod}} e^{-k_e t} \quad (5)$$

to the data from our Cd-efflux experiment at pH 5.5 (experiment 5) by nonlinear regression; Eq. 5 was obtained by integrating Eq. 3 and assuming that Cd uptake from food and water were negligible during the Cd-efflux experiment. $[\mathrm{Cd}_0]^{\mathrm{efflux}}_{\mathrm{copepod}}$ is the Cd concentration in copepods at time 30 min of the efflux experiment (*see Methods*). The value of k_e obtained is given in Table 2, and a comparison of experimental and modeled data is shown in Fig. 3. We then determined the value of the rate constants for Cd influx from water, k_{uw}, and from food, k_{uf}, (Table 2) by substituting in Eq. 4 the value of k_e and our data for the ''high'' and ''low'' Cd treatment levels and then resolving the two resulting equations to obtain the values of these rate constants. Knowing the values of the three rate constants (Table 2), we used Eq. 3 to estimate the relative proportions of Cd that copepods took up from water ($k_{uw}[\mathrm{Cd}^{2+}]$ term) and from their algal food ($k_{uf}[\mathrm{Cd}]_f$ term) at the high-Cd treatment level; that is, in which algae and copepods were exposed to the same $[\mathrm{Cd}^{2+}]$ (as would be the case in nature). It revealed that the majority of the Cd taken up by copepods came from water (63% ± 10% [SD]), which suggests that direct competition between Cd^{2+} and H^+ at uptake sites on these crustaceans is likely in highly acidic lakes. Because these animals also take up a substantial proportion of their Cd from food (37% ± 9%), lakewater pH could also indirectly influence their Cd concentrations because their algal food is likely to be lower in Cd in highly acidic lakes (Campbell and Stokes 1985) and algal community structure is likely to be influenced by lakewater pH (Nicholls et al. 1992).

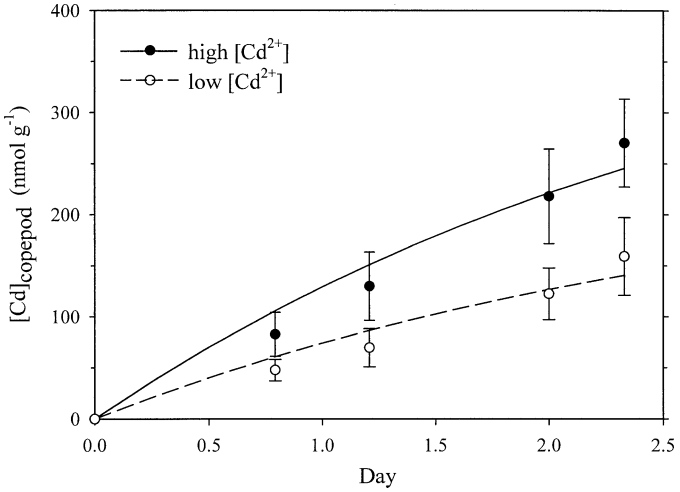

Fig. 2. Temporal changes in Cd concentrations (nmol g^{-1} dry weight) in the copepod *D. minutus* (mean ± SD, $n = 5$) exposed at pH 5.5 to Cd in both food and in water at either high or low $[\mathrm{Cd}^{2+}]$.

Experiment 4: Cd^{2+}-H^+ competition on copepods—Planktonic crustaceans in metal-contaminated acidic lakes are reported to contain little Cd in spite of the high Cd concentrations measured in surrounding waters (Yan et al. 1990; Croteau et al. 2003*a*,*b*). To determine if competition between Cd and H ions for uptake sites on these crustaceans could explain their low Cd concentrations in acidic lakes (as well as those in their predators), we exposed the copepod *D. minutus* to controlled Cd concentrations in water and food but at three different $[H^+]$. Cadmium concentrations in copepods held at pH 4.8 were significantly lower ($p < 0.05$, ANOVA) than those held at pH 5.5 (Fig. 4), which would be expected if Cd^{2+}-H^+ competition was occurring. In contrast, copepods held at pH 6.4 did not differ in their Cd concentrations from those held at pH 4.8 ($p > 0.05$, ANOVA; Fig. 4). This anomaly is partly explained by the fact that copepods held at pH 6.4 were exposed to lower $[\mathrm{Cd}^{2+}]$ (2.8 nmol L^{-1}) than those held at pH 4.8 (4.4 nmol L^{-1}) and 5.5 (3.9 nmol L^{-1}). However, it should be noted that, as a precaution, we did not use the Cd-uptake data obtained for copepods held at pH 6.4 to evaluate the impact of H^+-Cd^{2+} competition as they ate little during the initial part of the experiment (0–6 h time period, verified microscopically) because they had not

Table 2. Values (±SD) for model parameters describing Cd accumulation by the copepod *Diaptomus minutus*.

pH	$[H^+]$ (μmol L^{-1})	$[Cd^{2+}]$ (nmol L^{-1})	$[Cd]_{algae}$ (nmol g^{-1})	k_e (d^{-1})	k_{uw} (L g^{-1} d^{-1})	k_{uf} (d^{-1})	K_a (μmol L^{-1})	φ (μmol g^{-1} d^{-1})
Experiment 3: Relative importance of water and food as Cd sources for copepods								
5.5 (n=4)	3	Low Cd: 1.6±0.7	218±48	0.34±0.08 (n=5)	19.2±0.7 (n=5)	0.26±0.02 (n=5)		
		High Cd: 5±0.8	218±48					
Experiment 4: Cd^{2+}-H^+ competition on copepods								
5.5±0.06 (n=30)	3	3.9±0.1 (n=4)	134±20 (n=10)	0.34*	19.2*	0.62±0.04 (n=5)	7.4±0.8	200±3
4.8±0.04 (n=30)	16	4.4±0.2 (n=4)	134±20 (n=10)	0.34±0.13 (n=5)	8.7±1 (n=5)	0.62†	7.4±0.8	200±3
6.4±0.09 (n=30)	0.3	2.8±0.3 (n=3)	134±20 (n=10)	0.45±0.12 (n=5)	26.3±3	0.62†	7.4±0.8	200±3

* Assumed to be the same as the value shown for experiment 3 above.
† Assumed to be the same as the value shown for k_{uf} at pH 5.5 in experiment 4.

Fig. 3. Loss of Cd over time from the copepod *D. minutus* (means ± SD, n = 6) that had been previously exposed to Cd in both water and food at a pH of 4.8, 5.5, or 6.4 and then placed in Cd-free water and offered Cd-free prey at these same pHs. Model curves were generated using Eq. 5 (*see text for explanation*).

been properly acclimated to experimental conditions (*see Methods*). Copepods at the two lower pHs were properly acclimated and had their guts filled with algae throughout the experiment, as verified microscopically.

To model the impact of H ions on Cd uptake by copepods, we thus proceeded as follows. We first estimated the value of k_{uf} through fitting, by nonlinear regression, Eq. 4 to our Cd-uptake data from experiment 4 at pH 5.5; for this fitting exercise, we used in Eq. 4 the values of k_e and k_{uw} that were determined at the same pH in experiment 3 (Table 2). It should be noted that we did not retain the k_{uf} value obtained

Fig. 4. Temporal changes in Cd concentrations in the copepod *D. minutus* (means ± SD, n = 6) exposed to Cd in both water and its algal food at a pH of 4.8, 5.5, or 6.4. Model curves were generated using Eq. 4 (*see text for explanation*). For the model curve at pH 6.4, $[Cd_0]_{copepod}$ was taken as the value measured at 0.63 d to avoid the initial period during which copepods did not feed to satiation (*see Methods*).

at pH 5.5 from experiment 3 because copepods used in experiments 3 and 4 were collected 2 months apart; physiological differences between the two groups could have influenced their ingestion rates (an important component of k_{uf}). We also assumed that this k_{uf} value does not vary with pH and we used it for calculations at all pHs. We based this assumption on the fact that copepod ingestion rates did not appear to vary with pH because their guts were always full during our experiments (apart from an initial lag in feeding at pH 6.4; *see Methods*). An additional assumption was that H^+-Cd^{2+} competition does not occur in the gut of copepods such that Cd assimilation efficiency (also a component of k_{uf}) is not influenced by ambient pH (*see experiment 1*).

Knowing k_{uf} and k_e (by fitting Eq. 5 to the Cd-efflux data at pH 4.8; Table 2) we estimated, in a second step, the value of k_{uw} at pH 4.8 by nonlinear regression using Eq. 4 and our experimental values measured at this pH. The various rate constants are given in Table 2, from which we confirm that k_{uw} varies with pH. It has been shown (Croteau et al. 1998) that to take H^+-Cd^{2+} competition into account, k_{uw} should be expressed as

$$k_{uw} = \frac{\varphi}{[H^+] + K_a} \tag{6}$$

where φ is a proportionality constant specific to Cd and the copepod that we studied. Using our values of k_{uw} at pH 4.8 and 5.5, we solved Eq. 6 for K_a and φ (Table 2). Our estimate of K_a (7.4×10^{-6} mol L^{-1}) is close to those reported for Cd binding sites on fish gills (4×10^{-6} mol L^{-1}; Cusimano et al. 1986) and *Chaoborus punctipennis* (1.9×10^{-6} mol L^{-1}; Hare and Tessier 1996).

The model curves generated using our estimated values for all constants in Eq. 4 closely fit our experimental values for $[Cd]_{copepod}$ at pHs 4.8 and 5.5 (Fig. 4), suggesting that the model is adequate for describing H^+-Cd^{2+} competition at Cd uptake sites on *D. minutus*. Using Eqs. 3 and 4, we estimate that $[Cd]_{copepod}$ would attain 90% of its steady state value on day 7. We also used Eq. 4 to describe $[Cd]_{copepod}$ at pH 6.4, and Eq. 6 to estimate the value of k_{uw} at this pH (Table 2). The model curve also fit our data for $[Cd]_{copepod}$ at this pH (Fig. 4), although our data for $[Cd]_{copepod}$ at pH 6.4 was not used to extract either the rate constants or K_a, which supports the robustness of the model for describing $[Cd]_{copepod}$.

The results of our laboratory experiments suggest that *Chaoborus* itself is not the site at which Cd^{2+}-H^+ competition occurs because this predator does not take up Cd from water (Munger et al. 1999) and because it is able to maintain its gut pH circum-neutral in highly acidic water. However, our results suggest that copepods, a common prey for *Chaoborus* larvae, do take up some of their Cd from water and that H ions compete at Cd uptake sites on these crustaceans. We can now ask the question, is the expected Cd^{2+}-H^+ competition on zooplankton in acidic lakes sufficient to explain the reduced $[Cd]_{Chaoborus}$ reported for such lakes? To answer this question, we first used Eq. 1 to calculate the expected difference in $[Cd]_{Chaoborus}$ for larvae of this insect living in lakes at pH 5.5 and 4.8. For this calculation, we used a value of 1.7 μmol L^{-1} for K_a, as reported by Hare and Tessier (1998) for the genus *Chaoborus* as a whole, and we assumed that $[Cd^{2+}]$ is constant at these two pHs so that we could consider the influence of $[H^+]$ alone. The results of this calculation suggest that Cd concentrations in *Chaoborus* collected from a lake at pH 5.5 should be 360% of those taken from a lake at pH 4.8.

Having estimated the expected increase in $[Cd]_{Chaoborus}$ from lakes at pH 4.8 and 5.5, we determined the magnitude of the effect that Cd^{2+}-H^+ competition on zooplankton alone is likely to have on the *Chaoborus* steady state Cd concentration, $[Cd]^{ss}_{Chaoborus}$. For this purpose, we used Eq. 7

$$[Cd]^{ss}_{Chaoborus} = \frac{AE \times IR \times [Cd]_{copepod}}{k_e + k_g} \tag{7}$$

where AE is the efficiency with which *Chaoborus* larvae assimilate Cd from their prey, IR is the rate at which this predator ingests prey, and k_g is the rate constant for growth by the predator (Croteau et al. 2002). This equation assumes that *Chaoborus* larvae take up Cd from prey alone (Munger and Hare 1997; Munger et al. 1999). To estimate steady state $[Cd]_{copepod}$ at pH 4.8 and pH 5.5 for use in Eq. 7, we combined Eq. 3 (where $(d[Cd]_{copepod})/dt = 0$) with Eq. 6 and used the values for the rate constants given in Table 2 (assuming that $[Cd]_{algae}$ and $[Cd^{2+}]$ are constant). This calculation, made with the additional assumption that the other terms in Eq. 7 (AE, IR, k_e, and k_g) do not vary between pH 4.8 and 5.5, indicates that Cd^{2+}-H^+ competition on zooplankton alone should result in values for $[Cd]_{Chaoborus}$ at pH 5.5 that are 140% those at pH 4.8.

The substantial difference between the expected increases in Cd values for *Chaoborus* from lakes at pH 4.8 and 5.5 (360%) and the increase in values that would be expected if Cd^{2+}-H^+ competition occurred only on copepods consumed by *Chaoborus* (140%) suggests that Cd^{2+}-H^+ competition on planktonic algae is the main reason that $[Cd]_{Chaoborus}$ are suppressed in acidic lakes. Indeed, metal concentrations in planktonic algae are reported to be reduced at low pH (Campbell and Stokes 1985). Such competition at the level of algae would lead to low [Cd] in zooplankton from acidic lakes, as has been reported by Croteau et al. (2003*b*). We suggest that detailed studies are needed to quantify the influence of $[H^+]$ on metal accumulation by phytoplankton if we are to fully explain the reduced Cd bioaccumulation reported for planktonic animals living in acidic lakes.

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Received: 29 April 2005
Accepted: 3 November 2005
Amended: 14 November 2005