Influence of Calcium Concentrations on Cadmium Uptake by the Freshwater Mussel *Elliptio complanata*

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The effect of calcium ions on the uptake of cadmium by the freshwater mussel *Elliptio complanata* was studied in controlled laboratory experiments. Radioactive ^{45}Ca and ^{109}Cd were used as tracers. The experiments were designed to minimize geochemical differences between treatments so that the physiological interactions of the two ions could be studied. Uptake of Cd was correlated with concentration of Cd in the experimental tanks. However, the concentrations of Ca also had a strong impact on uptake rates. When uptake of Cd was expressed as a function of the molar ratio of Cd to Ca, greater than 96% of the variation could be explained.

Au cours d'expériences en laboratoire, on a étudié l'effet des ions calcium sur l'absorption du cadmium chez l'anodonte *Elliptio complanata*. On a utilisé du ^{45}Ca et du ^{109}Cd radioactifs comme marqueurs. Les expériences ont été faites de manière à minimiser les écarts géochimiques entre les traitements de façon à ce qu'on puisse étudier les interactions physiologiques des deux ions. On a établi la corrélation entre l'absorption de Cd et la concentration de Cd dans les bassins qui servaient aux expériences. Cependant, la concentration de Ca a un effet marqué sur les taux d'absorption. Lorsque l'absorption de Cd est exprimée en fonction de la proportion molaire de Cd par rapport au Ca, plus de 96 % de la variation peut être expliquée.

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Accumulation of cadmium (Cd) by aquatic organisms can occur either via direct uptake across body membranes or via indirect uptake by absorption from digested food in the gut tract. The relative importance of these two uptake mechanisms remains open to debate and probably varies among organisms (Abel and Bärlocher 1988; Evans and Lasenby 1993).

Of the two processes, direct uptake has been the more difficult to quantify. Direct uptake involves the transport of ionic or complexed cadmium to receptor sites, followed by transfer through an animal's membrane. In most cases, only the free metal ion is expected to interact with the binding site and the extent of binding would depend on the ion activity. Thus it is generally believed that the ionic form (aquo-ion) of most metals, including Cd, is most available to aquatic organisms (Stokes and Campbell 1985; Morel and Morel-Laurens 1981) and that the total Cd concentration in water does not provide a reliable indication of bioavailability (Laxen 1984). Variation in the activity of the free metal ion will affect the rate of its uptake. Many physicochemical factors such as redox potential, pH, sorption, chelation, complexation, precipitation, and hydrolysis may affect water Cd speciation, and therefore activity of the free ionic form.

For freshwater mussels, gills are proposed as the major site of metal uptake from solution, because of their large surface area and the immediate accumulation of any administered dose (Holwerda et al. 1989). It has been suggested that 90% of Cd uptake is by absorption from solution, and is facilitated by diffusion of $CdCl_2$ across the gills or by some type of complexation with a high molecular weight compound present on the gill surface (Carpene and George 1981).

Cadmium has no known metabolic functions and our knowledge about its transport mechanism at the molecular level across cell membranes is still very limited. Most information comes from the study of gill transport in fish (e.g., Wicklund and Runn 1988). Proteins are believed to be the pathway of substances between the intra- and extra-cellular compartments. Possible mechanisms include: passive co-diffusion of polar ions through pores along a potential gradient (Stein 1967); complexation with ligands normally used to transport nutritionally essential cations (Hunn 1985); and complexation of specific ligands with metal ions which induces a conformation change in the membrane (Pärt et al. 1985). A better membrane transport model is required to assess the toxicological and environmental importance of Cd.

It has been reported that water hardness inhibits Cd uptake in many freshwater organisms (Wicklund and Runn 1988; Abel and Bärlocher 1988). How this happens is unknown. There are two possible mechanisms by which major cations such as Ca and Mg could affect Cd uptake. At the geochemical level, major cations can compete for binding sites on organic and inorganic ligands causing an increase in Cd^{2+} activity (Campbell and Evans 1987). At the organism level, it has been suggested that Ca can act directly in one of several ways, thereby depressing the heavy-metal uptake. Competition for binding sites on the membrane between Ca and Cd ions could alter Cd uptake (Hunn 1985), as could gill permeability changes induced by altered Ca concentrations (Rogers and Beamish 1983; Pärt et al. 1985). Of these hypotheses, the competition mechanism is the most

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popular and widely accepted. Stated simply, the process involves the accidental uptake of Cd across the membrane through Ca uptake channels.

There is some evidence to support the competition mechanism. For example, Cd^{2+} can be substituted for Ca^{2+} on Ca-binding proteins (Suzuki et al. 1985; Flik et al. 1987). Also, Nilsson et al. (1986) suggested that Cd and Ca utilize a similar transport mechanism in pancreatic Beta-cells.

Identification of the potential for Ca control of Cd uptake is important from an environmental management perspective. If Cd uptake by aquatic invertebrates is largely controlled by available Ca concentrations in lakewater, then we should be able to predict those systems in which we would expect elevated body burdens of Cd in organisms. If, on the other hand, the observed effects of the presence of Ca on Cd burdens in organisms (Campbell and Evans 1991) is largely a geochemical phenomenon, then prediction of organisms' concentrations will be much more dependent on subtle geochemical differences between systems and prediction will be much more difficult.

In this study, we have examined the role of Ca in Cd uptake in a controlled laboratory experiment using the freshwater mussel *Elliptio complanata*. This approach allows us to separate geochemical and physiological influences and to examine more precisely the role of Ca in Cd uptake across the gill membrane.

Methods

Over 1000 mussels were collected from a sandy sediment area (water depth ~2–3.5 m on the south shore of Deer Bay, Harvey Township, Peterborough County, Ontario (Longitude 78°26′ Latitude 44°28′) during the late summer of 1988. This site was chosen because both the Otonabee River (which provided the water for the laboratory experiments) and Deer Bay are located on the Trent Waterway system, and therefore, have similar water chemistry characteristics (Table 1).

The mussels were chosen by shell length in order to control size variation as much as possible (±1.5 cm), because mussel size has a strong negative correlation with trace metal burdens. Jones and Walker (1979) found that larger mussels (with heavier tissue weights) from the same area, regardless of age and sex, had lower metal concentrations than did smaller mussels (with lower tissue weight). Since shell length of mussels is positively correlated with tissue weight (Fischer 1988), variation in shell length (as the only measurable parameter when a mussel is alive) was minimized to eliminate variation in tissue weight and perhaps metal uptake.

Preliminary experiments (Y. Wang and R.D. Evans, unpublished data) revealed that the mussel shells were scavengers of most of the free Cd ions in the water. More than 90% of Cd lost from water was adsorbed onto the shells. Thus, a problem arose in trying to maintain a stable Cd concentration in the water throughout an experiment. To counteract this problem, before the experiment started, the shells of all mussels were stripped of their periostracum and any attached mosses, and polished to provide reasonably smooth surfaces. They then were coated with two layers of acrylic nail polish. After the shells had been coated, these mussels were left sitting in 0.45 μm filtered water for 2 d to evacuate their guts, preventing adsorption of Cd from the water by feces excreted during the experimental period. This treatment had no apparent effect on the organisms as survival post treatment of at least 2 yr in tanks has been observed. Mortality during the experiment was zero. The treatment containers were large plastic pails which had a water volume of 19 liters. Preliminary experiments indicated that this volume was necessary to ensure that during the experiment Cd and Ca concentrations in the water remained at a level not less than 90% of the initial concentrations (Fig. 1).

The radioisotopes ^{109}Cd and ^{45}Ca were used as tracers for the uptake of stable Cd and Ca in mussel tissue. Specific activity was varied by adjusting stable Cd and Ca concentrations. Knowledge of the specific activity of each element in the organisms, at the outset and at the conclusion of an experiment, allows calculation of the uptake rates of the stable elements. Cadmium concentrations ranging from 4 to 120 μg/L and Ca concentrations ranging from 43 to 130 mg/L were used. A five by five matrix design was employed to produce 25 different ratios of Cd to Ca. The experiment was repeated using a smaller number of ratios, resulting in a total of 31 different ratios of Cd to Ca ranging from 0.04 to 2.7 μg Cd/mg Ca. In addition, one control tank containing five mussels but no radioisotopes or stable Cd or Ca was used to monitor Mg, Cl, O_2, temperature, conductivity, and pH throughout the 72 h experiment.

Otonabee river water (Table 1) was filtered through a polypure 60 μm and 0.45 μm double filter system prior to use in the experiments. Stable $CdCl_2 \cdot 2\frac{1}{2}H_2O$ (Mallinckrodt) was used for its high solubility ($\log K_{sp} = 2.6$) producing mostly Cd^{2+} which should be the most bioavailable chemical form of this element. Changes in Cl^- concentration from lowest to highest treatment from addition of $CdCl_2$ were less than 1% of the total concentration and less than analytical variability in Cl measurements. Thus it is unlikely that changing Cl concentrations throughout the treatments affected uptake of Cd (Jackim et al. 1977; Fischer 1986). Because much higher concentrations of Ca were added, stable $Ca(NO_3)_2 \cdot 4H_2O$ (Fisher Scientific) was used to alter Ca concentrations. The addition of nitrate to the tanks should not affect Cd uptake. Each tank contained ^{109}Cd and ^{45}Ca activities of 35 and 1005 Bq/mL, respectively.

Preliminary experiments (Wang 1990) indicated that aeration was necessary to maintain dissolved oxygen levels and to prevent the formation of a concentration gradient within the tanks. Since Cd speciation is pH dependant, pH and conductivity were closely monitored as well. Concentrations of Cd, Ca, Cl, and Mg as well as dissolved O_2, pH, temperature, and conductivity were measured initially in all tanks. Water samples were taken at 12-h intervals from each tank for Cd and Ca while samples from the non-radioactive control tank only were measured for all other parameters at the same intervals. Ca and Mg concentrations were measured by flame atomic absorption spectrophotometry and Cd concentration was performed by Zeeman flameless atomic absorption spectrophotometry. Water samples for these elements were kept in polyethylene vials at 4°C until analyzed. Cl was determined using an ion specific electrode, oxygen using a dissolved oxygen meter, pH using a pH meter and conductivity and temperature using a combined meter. All of these parameters were measured in situ.

Five randomly selected mussels were placed in each of the 25 treatment tanks for 72 h, following which they were removed and killed. The soft tissues were removed from the shell, dried at 90°C for 24 h then ground into powder before further analysis. Shells were air dried and their weights and lengths were measured. Radioactivity was measured in each of the five organisms from each tank.

Activity of ^{45}Ca was measured by liquid scintillation counting (Beckman LS7000). A 2-mL water sample was mixed with

TABLE 1. Water chemistry for Otonabee river water used in experimental tanks and Deer Bay from which mussels were collected. All measurements have units of mg/L except for Cd which has units of μg/L. Values from this study for Otonabee water indicate mean and standard deviation for measurements taken during the 72 h of the experiment in the control tank containing five mussels but with no radioactive or stable Cd or Ca added.

	Otonabee River	Deer Bay[d]
Calcium	30.0 ± 2.06	26.5
Cadmium	0.2 (D.L.)[a]	0.02
Chloride	7.5 ± 0.5	
Magnesium	4.0 ± 1.12	
pH	7.9 ± 0.25	7.5
conductivity	190 ± 10.7	
oxygen	9.8 ± 1.3	9.0
temperature	22 ± 1	21[e]
Sodium	3.7[b]	
Potassium	0.7[b]	
Sulphate	7.9[b]	
Nitrate	0.01[c]	

[a]Detection Limit.
[b]Measured by Ontario Ministry of Environment, 1987.
[c]Measured by Ontario Ministry of Environment, 1976.
[d]From Campbell (1987).
[e]Measured at the same time of year as the present study.

18 mL of Aqueous Counting Scintillant (ACS-Amersham), and allowed to sit in complete darkness for 48 h in order to quench light excitation prior to counting. Ground, dry tissue (0.1 g) was dampened with 0.2 mL of distilled water followed by 2 mL of NCS tissue solubilizer (quaternary ammonium base in toluene, Amersham). The capped vials were kept in a 50°C sand bath for 24 h or until all of the tissue was digested. Finally, 10 mL of Organic Counting Scintillant (OCS, Amersham) was introduced. All samples were corrected for color quenching using Nitromethane (CH_3NO_2) colour standards. Activities of ^{109}Cd in the water and tissue samples were determined by gamma spectroscopy using a well-type NaI detector. Aliquots of 0.5 mL of water and 0.2 g of tissue were counted in a uniform geometry with no prior sample preparation. Preliminary tests indicated that there was no interaction between the Cd and Ca isotopes in either counting procedure.

Results and Discussion

Many factors other than available ion concentrations can affect both the geochemistry of a system and the physiology of the organisms, and thus ultimately affect trace metal uptake rates. Many of the geochemical variables were eliminated by the experimental design. For example, the same minimal concentration of dissolved organic carbon (DOC ~1 mg/L, R.D. Evans, unpublished data) was present in each experimental tank. Preliminary experiments were used to determine the most important variables which might alter organism physiology during the experiments. These were found to be water temperature, pH, dissolved oxygen (DO), and Mg concentrations (Wang 1990). Because of the large volume of water relative to the number of mussels, these parameters did not vary significantly over the course of the experiments (Table 1).

The Ca and the Cd were added as compounds chosen to keep the largest amounts as the free ionic forms. The chemical

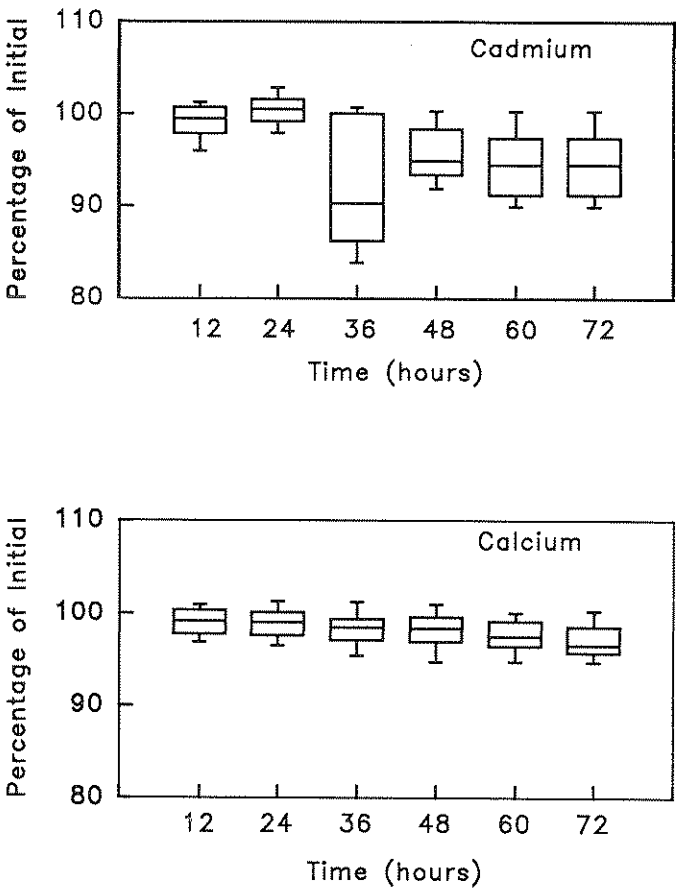

FIG. 1. Changes in Cd and Ca concentration through time expressed as a percentage of the initial concentration. Boxes show mean, 25th, and 75th percentile for 25 experimental tanks. Whiskers are 5 and 95 percentiles of data.

speciation model MinteqA2 (version 3.11, available from the U.S. E.P.A. Centre for Exposure Assessment Modeling, Athens, Georgia) was used to calculate the theoretical speciation in each experimental container. One limitation to these calculations is that the model (like all equilibrium models) does not have good partition coefficients for DOC. Since DOC concentrations were low, the estimates of free metal concentrations will be only slightly high. Free Ca^{2+} was constant at 97.5% ± 0.2 of total concentration (independent of concentration) while the percentage of total Cd predicted to be in the free Cd^{2+} form varied between 40.6 and 46.0%. The proportion of free Cd increases with increasing Ca concentration primarily due to competition for the carbonate ligand to which most of the remaining Cd is bound.

Both the Ca and Cd concentrations in the experimental tanks remained essentially constant throughout the 72-h experiment. The variation through time (Fig. 1) was small and probably a result, primarily, of large scale dilutions required to reduce concentrations to a suitable analytical range. None of the slopes of the regressions between water concentration and time were significantly different from zero (t-test, $p = 0.05$, $n = 7$) for Ca, and all but one were not significantly different from zero for Cd.

There is almost no loss of Cd from marine clams during a depuration period of 12 d (Klumpp and Burdon-Jones 1982) and in fresh water mussels over weeks of depuration (D. Maley

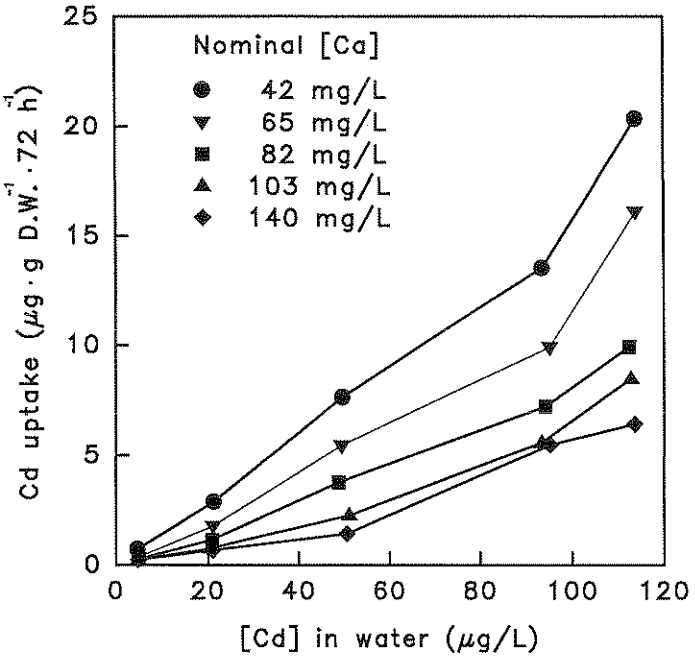

FIG. 2. Cd uptake by *E. complanata* as a function of total Cd in the experimental tanks, separated according to nominal Ca concentrations. Each point represents the average uptake for five individuals from that treatment.

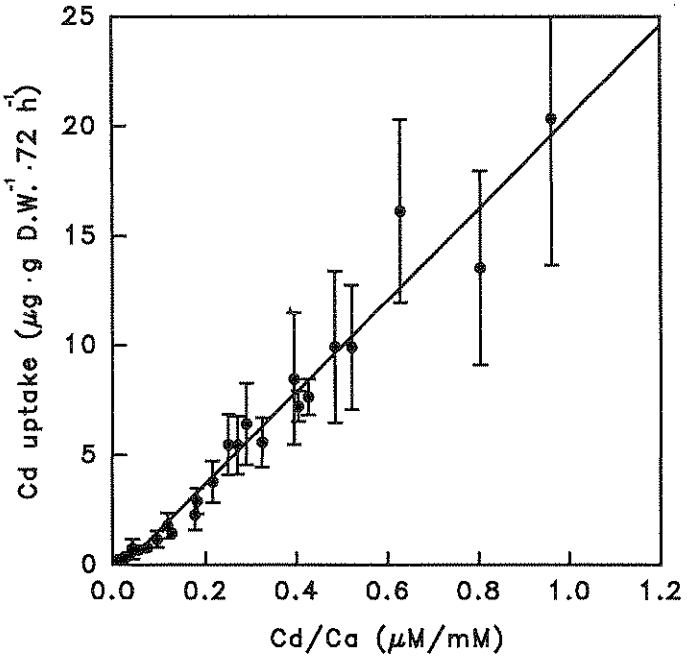

FIG. 3. Cd uptake by *E. complanata* as a function of the molar ratio of Cd to Ca. The equation for the least-squares regression line is given in the text.

Freshwater Institute, Winnipeg, personal communication). We conducted preliminary experiments to measure depuration (Wang 1990). Six mussels were placed in each of 25 different tanks containing a similar range of Cd:Ca ratios as those used in the uptake experiments. From each tank three mussels were measured directly after uptake while three were allowed to depurate for 5 d in clean water. No significant differences were found in activity levels between the two groups (*t*-test, $p < 0.0001$, $n = 25$). The procedure of Cd transfer from the soft tissue to the shell is very slow and temperature dependent (R.D. Evans, unpublished data). No detectable internal transfer of Cd to the shells occurred over the 72 h experimental period and our surface treatment of the exterior of the shell precluded external adsorption. Thus, it can be assumed that Cd removed from the water by the mussels was located in the soft tissues.

Uptake of Cd in each treatment group was calculated for the 72-h period from the determined activity levels and the known Cd specific activities. Uptake ranged from approximately 0.2 to 20 μg · g dry weight^{-1} · 72 h^{-1} (Fig. 2). The Cd accumulation in the mussels' soft tissue, after 72 h of exposure, increased with an increase in the water Cd levels. At an approximately constant concentration of Ca, the Cd uptake is approximately linear with concentration of Cd in the water. This supports the theory that one important variable in Cd uptake is the concentration of free Cd^{2+} ions.

In Fig. 2, the data have been separated on the basis of nominal concentrations of Ca in the tanks. It is apparent that Ca concentrations in the water have a significant influence on Cd uptake by the mussels (confirmed by two-way analysis of variance) which can be observed at all Cd concentrations, and which can not be explained by differences in geochemical speciation. Cd uptake in soft tissues is positively correlated with the Cd concentration in the water but has a negative linear relationship with water Ca levels. Therefore the water Ca level depresses Cd uptake in mussel soft tissue. Similar results have been reported in field studies (O'Shea and Mancy 1978; Campbell 1987; Stephenson 1986). It is apparent from the analysis of these data that water concentrations of both Cd and Ca are needed to predict the Cd uptake in mussel soft tissue.

Given the significance of both variables, we related Cd uptake in mussels to the ratio of Cd to Ca in the water (Fig. 3). There is a very strong positive linear relationship defined by the equation:

$$(1)\quad \text{Cd uptake} \cdot \text{g dry wt}^{-1} \cdot 72\ \text{h}^{-1} = 0.029 \cdot \text{Cd/Ca} - 0.46$$
$$R^2 = 96.2\% \qquad p \le 0.0001 \qquad n = 25.$$

Using data corrected for speciation (i.e., predicted free Cd^{2+} rather than total Cd) did not change the observed relationship. The less extensive experiment using six different treatments produced results with no significant difference from those of the original five-by-five experiment (non-parametric Mann–Whitney *U* test, $t = 1.4 < 1.96 = t_{0.05}$). Neither was there any significant difference between regression slopes ($t = 0.36 < 2 = t_{0.05}$), or regression intercepts ($t = 1.40 < 2 = t_{0.05}$) for the two experiments. The variation in Cd uptake within each treatment tank (vertical bars in Fig. 3) was mainly due to variation in the size of the mussels, with uptake rate inversely related to mussel dry weight.

Since the geochemistry of our systems was controlled and quite constant throughout the experiments, it is reasonable to assume that the major factor affecting Cd uptake is the Cd : Ca ratio. This observation supports the most popular theory of Ca regulation of Cd uptake in aquatic organisms — that Cd and Ca enter organisms through the same type of channel or binding sites on the cell membrane without preference. Because of this, both Cd and Ca in water are able to compete for these sites. Apparently, a higher ratio of Cd:Ca in water provides Cd with a

greater possibility for adsorption onto the binding sites, which results in higher Cd accumulation in the tissue. These binding sites may exist for the uptake of group IIb metals in general. It would be illustrative to conduct similar tests with mixed-metal systems.

The significance of the ratio of Cd to Ca on Cd uptake in aquatic organisms has been noted in longer-term exposures in natural systems. For example, Stephenson (1986) observed the effect of Ca on the Cd uptake in a freshwater amphipod, *Hyalella azteca*, in some central Ontario lakes where the water Ca level ranged from 1.3 to 2.9 mg/L (soft water) and the Cd from 2 to 587 ng/L. In that study, the Cd accumulation in the amphipod had a strong positive linear relationship with the molar ratio of Cd to Ca ($\log(\text{Cd/Dry Wt}) = 0.832 \log(\text{Cd/Ca}) + 0.602$; $n = 51$, $R^2 = 74\%$, $p < 0.001$). Campbell (1987) studied *E. complanata* in 11 lakes in south-central Ontario with a range of water hardness including some located on the Trent water system, where the present study was carried out. Lake water concentrations of Ca and Cd ranged from 2.48 to 34.29 mg/L and 20 to 150 ng/L, respectively, and the ratio of Cd to Ca explained 86% of the variance in concentrations of Cd in the mussels.

Both of the above studies indicated that Cd accumulation in freshwater invertebrates has a strong relationship with the ratio of Cd to Ca in water column. However, both studies examined organisms from a range of lakes, making it hard to exclude the influence of other lake water variables (such as water DOC, $[O_2]$, temperature, pH and the interaction of other metals), and to separate geochemical and physiological processes. Our controlled laboratory experiment avoids the influence of most of these factors showing that the ratio of Cd to Ca in the water column is an important variable at the physiological level. Thus in lakes which are similar in all other factors, those with the highest Cd to Ca ratios will exhibit the highest Cd body burdens in its biota. Of course, true inter-lake variability will reflect the influences of geochemical differences as well. While this study was not designed to address the relative importance of geochemical versus physiological variables, it does stress the significance of the Cd to Ca ratio at the physiological level. It may be possible to separate the respective influences of each by comparison of our results with field studies. However, it is clear that any attempt to build a predictive model of Cd uptake in aquatic organisms with an uptake mechanism similar to *E. complanata* will require a component to reflect the importance of the Cd to Ca ratio.

The data also suggest an explanation for the relationship often found between Cd concentrations in organisms and lakewater pH (e.g., Campbell and Evans 1991). Cadmium and Ca tend to be inversely correlated in that soft, low pH waters will have relatively lower Ca concentrations and higher Cd concentrations than hard higher pH waters. For example, in Campbell's study this inverse relationship between Ca and Cd concentrations yields Cd:Ca ratios which vary between 0.002 and 15 μM Cd/mM Ca if all of the Cd and Ca were present in an available form. Thus the range of ratios found in nature may exceed that of our experiments. One interesting observation is that the biological potential for uptake of Cd would appear from our experiments to be much larger than that actually observed in natural populations. At a ratio of 1 μM/mM, uptake in our experiments was approximately 5 $\mu g\ Cd \cdot g\ dry\ weight^{-1} \cdot d^{-1}$. Such an uptake rate would quickly exceed even the highest concentrations found in similar systems (Campbell and Evans 1991). There are two possible explanations for this. First our experiments were conducted only in hard water systems. It is unlikely that the highest ratios which we used would be found ever in hard water systems except in very contaminated situations. Thus organism interaction with Cd may be different in soft water systems. More likely, however, is the possibility that geochemical factors control metal speciation such that only a small percentage of the total metal present in any aquatic system is in a biologically available form. This study indicates clearly the need for more work in which both the geochemical and physiological influences on Cd uptake by aquatic organisms is monitored.

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