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Refining and Testing a Trace Metal Biomonitor (*Chaoborus*) in Highly Acidic Lakes

MARIE-NOELE CROTEAU,
LANDIS HARE,* AND ANDRÉ TESSIER
Institut National de la Recherche Scientifique-Eau (INRS-EAU), Université du Québec, C.P. 7500, Sainte-Foy, Québec, Canada G1V 4C7

Biomonitor organisms can be used to estimate bioavailable contaminant concentrations provided that a reliable model exists between organism and environmental contaminant concentrations. We evaluate a recently proposed model designed to relate Cd concentrations in a proposed biomonitor, the insect *Chaoborus*, to free cadmium ion concentrations, [Cd^{2+}], in lakes. In highly acidic metal-contaminated lakes, Cd concentrations in the biomonitor tend to be depressed despite the often high Cd^{2+} concentrations in these lakes. We test the hypothesis than one or several of the cations H^+, Cu^{2+}, Ni^{2+}, and Zn^{2+}, which also tend to be present in high concentrations in such lakes, compete with Cd^{2+} for biological uptake sites and thereby reduce Cd accumulation in the biomonitor. We show that to effectively relate Cd concentrations in the biomonitor to those in its environment, only competition from H^+ needs to be taken into account, suggesting that H^+ is the major competitor with Cd^{2+} at biological uptake sites and that the competitive effects of the other metal ions can be ignored. We also show that while it is best to treat the various species of *Chaoborus* separately, species can be pooled with only moderate loss of predictive power. In contrast to Cd, *Chaoborus* larvae could not be used to monitor the concentrations of either copper or zinc because the concentrations of these essential metals appear to be regulated by the larvae.

Introduction

Mining and other industrial activities have increased the fluxes of many trace metals to aquatic ecosystems (*1*, *2*). Effective means of assessing the concentrations and the biological impact of these potentially toxic pollutants are required. Although trace metals can be monitored by the collection and analysis of water or sediment alone, such measurements are of limited use in the absence of a reliable means of relating them to metal concentrations in, or effects on, aquatic organisms (*3*). Biomonitors have been used to provide more biologically meaningful measurements of a pollutant in a particular environment (*4*). The effective use of a biomonitor depends on the development of models that permit us to relate metal concentrations in the aquatic environment to those in the organism chosen as a biomonitor.

A biomonitor should be highly tolerant to a contaminant, as opposed to a bioindicator whose sensitivity to a contaminant should allow the detection of toxic effects (*3*, *4*). A good biomonitor should also be easy to collect, widespread and abundant, tolerant of a wide range of chemical conditions, and provide sufficient tissue for contaminant analysis (*3*). In freshwater, large bivalve mollusks have been used as metal biomonitors (*5*, *6*). However, bivalves are absent from lakes of pH below about 6, and their metal concentrations could be subject to the confounding influence of a reproductive cycle (*7*). The phantom midge *Chaoborus* has recently been proposed as an alternative biomonitor (*8*). Larvae of this insect occur over a wide range of chemical conditions (e.g., pH, ionic strength), are abundant and widely distributed in lakes throughout North America (*9*, *10*), are easy to collect and identify, are able to accumulate and tolerate high concentrations of trace metals (*8*, *11*), and are not involved in reproduction.

The most important feature of an effective monitor is that a simple relationship should exist between its trace metal content and the concentration of the metal in its environment (*3*). Ideally, such a relationship should not be purely empirical. Use of a biomonitor is more likely to be reliable if the model relating organism and environmental metal concentrations is rational, that is, founded on biological and chemical principles.

Few rational models are available that relate metal concentrations in the environment to those in a biomonitor. A notable exception is the model tested in a recent study designed to relate Cd concentrations in the insect *Chaoborus* to Cd concentrations in the epilimnion of lakes (*8*). The main conclusion of this study was that the concentration of the free Cd ion, Cd^{2+}, predicts Cd concentrations in the insect, provided that the influence of other ions competing for biological uptake sites was taken into account (*8*). However, there was some doubt as to the identity of the major competing ion. On the basis of precedents in the literature, hydrogen ions, H^+, were assumed to be a more important competitor with Cd^{2+} at biological uptake sites than were free metal ions such as copper, Cu^{2+}, or zinc, Zn^{2+} (*12*). Doubt about the ion responsible for the competition can be ascribed to the fact that the few highly acidic lakes studied were all influenced by the same trace metal and acidity source, which also explains the strong correlations among [H^+], [Cu^{2+}], [Zn^{2+}], and [Cd^{2+}] for these lakes (*12*). Additional data are required if we are to resolve the ambiguity in the *Chaoborus*-Cd model and thereby improve its rational footing.

To reduce the correlations among potential competitors for Cd uptake sites, we sampled water and *Chaoborus* larvae from nine highly acidic lakes in two geographical areas (Sudbury, Ontario, and Rouyn-Noranda, Québec). The lakes chosen in these areas are all influenced by metal smelters, but emissions from these smelters vary in their relative concentrations of trace metals and acid-producing substances. With this additional data set, our intent is to determine whether H^+, Cu^{2+}, Ni^{2+}, or Zn^{2+} ions should be included in the rational model to relate Cd concentrations in water to those in the biomonitor *Chaoborus*.

Study Areas

Water samples and insect larvae were collected between May 27th and June 13th 1996 from nine acidic lakes (pH 4.30−5.36) located on the Precambrian Shield in the mining areas of Sudbury (Ontario) and Rouyn-Noranda (Québec) (Table 1). Cadmium concentrations measured in early spring *C. punctipennis* larvae are likely to be representative of those collected during the winter months as well (*13*). Lakes were selected on the basis of their low pH, their expected high concentrations of trace metals (Table 2) and the presence of

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* To whom correspondence should be addressed; fax: (418) 654-2600; e-mail: landis@inrs-eau.uquebec.ca.

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TABLE 1. Location of the Lakes Sampled in the Present Study

lake	region	location
Chief	Sudbury, Ontario	46°22′ N, 81°01′ W
Daisy	Sudbury, Ontario	46°27′ N, 80°53′ W
Hazen	Sudbury, Ontario	46°25′ N, 80°59′ W
Pine	Sudbury, Ontario	46°22′ N, 81°02′ W
Terry	Killarney, Ontario	46°04′ N, 81°17′ W
Chevreuil	Rouyn-Noranda, Québec	47°54′ N, 79°00′ W
Desperiers	Rouyn-Noranda, Québec	48°11′ N, 79°09′ W
Surimeau	Rouyn-Noranda, Québec	48°08′ N, 79°19′ W
Turcotte[a]	Rouyn-Noranda, Québec	48°18′ N, 79°04′ W

[a] Manipulated lake.

the phantom midge *Chaoborus punctipennis* (Diptera, Insecta). All of the lakes have been subject to inputs of acidic and trace metal rich materials from nearby Ni and Cu (Sudbury; *2*, *14*) or Cu, Pb, and Zn (Rouyn-Noranda; *15*) mining and smelting operations. One of the lakes in the Rouyn-Noranda region, Lake Turcotte, was chosen to be representative of systems impacted by direct anthropogenic manipulations, including liming and the dumping of mining residues.

Materials and Methods

To minimize inadvertent trace metal contamination, labware and water-sampling materials were soaked in 15% nitric acid and rinsed in ultrapure water (Milli Q system water; >18 MΩ cm^{-1}) prior to use. Water samples were collected in the epilimnion using in situ diffusion samplers (216 × 72 × 12 mm; Gelman HT-200 membrane) similar to those described by Carignan et al. (*16*). Samplers (8 compartments per sampler; 4 mL volume each) were filled with ultrapure water and sealed individually in clean plastic bags prior to placement in the lakes. Three diffusion samplers were suspended 1 m above the bottom in the epilimnion of each lake. The samplers were retrieved after 3 days and sampled immediately (3 days is considered to be sufficient for peeper equilibration; Tessier and De Vitre, unpublished results).

Samples (1 mL) for organic and inorganic carbon determinations were removed with a syringe from 1 compartment in each peeper and injected through a septum into pre-evacuated and prewashed glass tubes. From the same compartment, a sample (1.5 mL) for dissolved SO_4 and Cl analyses was collected with a plastic-tipped pipet and injected into prewashed microcentrifuge tubes (1.5 mL; high-density polyethylene, HDPE). Samples for trace metal (Cd, Cu, Ni, and Zn) and major cation (Ca, Mg, Na, and K) analysis were removed from the seven remaining compartments in each peeper by piercing the peeper membrane with a pipet fitted with an acid-cleaned tip. These samples were injected into prewashed and preacidified (53 μL of 1.35 N Anachemia HNO_3) HDPE bottles (4 mL capacity). One of these samples was used for dissolved Ca, Mg, Na, and K analyses; the remaining six were kept for trace metal analysis. On two different days, pH was measured with a portable pH meter (Hanna instruments, Micro processor model HI9024/HI9025) in water collected at the depth of the peepers using a Van Dorn sampler.

Insect larvae were collected after sunset by hauling a 250 μm plankton net horizontally in the water column of each lake until a sufficient number of *Chaoborus* was obtained. Insects were maintained at field temperatures and transported to the laboratory in plastic bags filled with lake water. In the laboratory, *Chaoborus* larvae were sorted according to species (*17*) and left for 12−24 h in 1 L HDPE bottles filled with 64 μm-filtered lake water to eliminate their gut contents. Where numbers permitted, five replicate samples of 10−15 similar-sized fourth instar larvae of each species were placed on acid-washed, preweighed Teflon pieces in acid-washed microcentrifuge tubes and frozen at −4 °C until analysis.

Analyses

Total dissolved metal concentrations were measured by flameless atomic-absorption spectrophotometry (AAS) (Varian model Spectra AA-30, graphite furnace Varian model GTA-96 for Zn; THGA graphite tube atomizer, Perkin-Elmer model Simaa 6000 for Cd, Cu, and Ni). Certified reference water samples (Riverine water reference material 1643d, NRCC) were also analyzed for metals during each analytical run and measured trace metal concentrations were within the certified range for each element. Major cation concentrations were measured by flame AAS (Varian model Spectra AA-20). Concentrations of SO_4 and Cl were measured by ion chromatography (Dionex AutoIon, system DX300). Dissolved inorganic carbon concentrations were obtained by gas chromatography (Hewlett-Packard GC 5890 Series 2) and dissolved organic carbon concentrations with a Technicon Auto-Analyzer by persulfate-UV oxidation, followed by conductometric determination of the CO_2 released.

TABLE 2. Mean Concentrations of Cd in the Various *Chaoborus* Species ($[Cd]_{Chaoborus}$) (*punct.* = *C. punctipennis*; *alb.* = *C. albatus*; *flav.* = *C. flavicans*; *amer.* = *C. americanus*) and Dissolved Constituents in Lake Water as Well as Lake pH

	$[Cd]_{Chaoborus}$ (μg g^{-1})				(nM)				$[C_{org}]$	(μM)		
lake	*punct.*	*alb.*	*flav.*	*amer.*	[Cd]	[Cu]	[Zn]	[Ni]	(mg L^{-1} C)	[Ca]	[Mg]	pH
Sudbury Area												
Highly Acidic Lakes Sampled by Hare and Tessier (*8*)												
Crooked	1.4 ± 0.2	1.6 ± 0.4			7.1	1674	372	4907	2.5	77	44	4.58
Clearwater	4.0 ± 0.5				4.9	637	346	2550	3.4	137	57	4.79
Wavy	2.5 ± 0.4				2.2	263	223	1132	3.1	52	30	4.62
Highly Acidic Lakes Sampled in this Study												
Pine	3.9 ± 1.1	3.4 ± 0.2			3.8	552	165	2158	6.0	30	18	4.30
Hazen	1.1 ± 0.1		1.6 ± 0.1	2.6 ± 0.4	3.3	306	173	2058	8.8	2237	1222	4.46
Chief	1.5 ± 0.1				2.7	280	169	1664	5.3	40	26	4.47
Terry	1.9 ± 0.1				0.9	19	176	98	7.9	37	23	4.56
Daisy	2.8 ± 0.3				2.5	212	116	2210	4.3	59	51	5.36
Rouyn-Noranda Area												
Highly Acidic Lakes Sampled in this Study												
Chevreuil	1.5			3.0 ± 0.5	2.5	20	162	23	5.2	29	19	4.50
Turcotte	3.1 ± 0.1			9.9 ± 0.8	19.5	388	4764	28	5.4	105	41	4.83
Surimeau	2.7 ± 0.2		1.3 ± 0.1		0.9	25	76	16	8.5	39	17	4.95
Desperiers	1.1 ± 0.2		2.8 ± 0.4		3.2	39	250	15	5.6	42	18	5.16

TABLE 3. Basic Equations Used to Described Cd Bioaccumulation According to the Free-Ion Activity Model

According to the free-ion activity model (FIAM), the Cd concentration in a unicellular organism, $[\mathrm{Cd}]_{\mathrm{organism}}$, is given by

$[\mathrm{Cd}]_{\mathrm{organism}} = k_1\,[\equiv\mathrm{XCd}]$ (1)

where $[\equiv\mathrm{XCd}]$ is the concentration of uptake sites on a membrane that are occupied by Cd and k_1 is a proportionality constant. Reaction of these uptake sites with Cd, other metals (M = Cu, Zn, Ca) and H^+ can be described by the following (pseudo)equilibrium reactions:

$\mathrm{Cd}^{2+} + \equiv\mathrm{X} = \equiv\mathrm{XCd};\ K_{\mathrm{Cd}} = [\equiv\mathrm{XCd}]/([\mathrm{Cd}^{2+}][\equiv\mathrm{X}])$ (2)

$\mathrm{M}^{2+} + \equiv\mathrm{X} = \equiv\mathrm{XM};\ K_{\mathrm{M}} = [\equiv\mathrm{XM}]/([\mathrm{M}^{2+}][\equiv\mathrm{X}])$ (3)

$\equiv\mathrm{XH} = \equiv\mathrm{X} + \mathrm{H}^+;\ K_{\mathrm{a}} = ([\equiv\mathrm{X}][\mathrm{H}^+])/[\equiv\mathrm{XH}]$ (4)

where $[\equiv\mathrm{X}]$, $[\equiv\mathrm{XM}]$, and $[\equiv\mathrm{XH}]$ are the concentrations of free sites, and those occupied by M and H, respectively.

The mass balance equation for sites is

$[\equiv\mathrm{X}]_{\mathrm{T}} = [\equiv\mathrm{X}] + [\equiv\mathrm{XH}] + [\equiv\mathrm{XCd}] + [\equiv\mathrm{XM}]$ (5)

Depending on the element competing with Cd for uptake sites, $[\mathrm{Cd}]_{\mathrm{organism}}$ can be best described by the following expressions.

-**No competition by H^+ or M^{2+}** (combine eqs 1, 2, and 5; neglect $[\equiv\mathrm{XH}]$, $[\equiv\mathrm{XCd}]$, and $[\equiv\mathrm{XM}]$ in eq 5):

$[\mathrm{Cd}]_{\mathrm{organism}} = F\,[\mathrm{Cd}^{2+}]$ (6)

where $F = kK_{\mathrm{Cd}}[\mathrm{X}]_{\mathrm{T}}$

-**Competition by H^+ alone** (combine eqs 1, 2, 4, and 5; neglect $[\equiv\mathrm{XCd}]$ and $[\equiv\mathrm{XM}]$ in eq 5):

$[\mathrm{Cd}]_{\mathrm{organism}} = F\,[\mathrm{Cd}^{2+}]/([\mathrm{H}^+] + K_{\mathrm{a}})$ (7)

where $F = kK_{\mathrm{Cd}}K_{\mathrm{a}}[\mathrm{X}]_{\mathrm{T}}$

-**Competition by M^{2+} alone** (combine eqs 1, 2, 3, and 5; neglect $[\equiv\mathrm{XH}]$ and $[\equiv\mathrm{XCd}]$ in eq 5):

$[\mathrm{Cd}]_{\mathrm{organism}} = F\,[\mathrm{Cd}^{2+}]/(1+K_{\mathrm{M}}[\mathrm{M}^{2+}])$ (8)

where $F = kK_{\mathrm{Cd}}[\mathrm{X}]_{\mathrm{T}}$

-**Competition by both H^+ and M^{2+}** (combine eqs 1, 2, 3, 4, and 5; neglect $[\equiv\mathrm{XCd}]$ in eq 5):

$[\mathrm{Cd}]_{\mathrm{organism}} = F\,[\mathrm{Cd}^{2+}]/(K_{\mathrm{a}} + [\mathrm{H}^+] + K_{\mathrm{a}}K_{\mathrm{M}}[\mathrm{M}^{2+}])$ (9)

where $F = kK_{\mathrm{a}}K_{\mathrm{Cd}}[\mathrm{X}]_{\mathrm{T}}$

It should be noted that if cadmium accumulation by a consumer is mainly from its food, i.e.,

$[\mathrm{Cd}]_{\mathrm{consumer}} = k_2[\mathrm{Cd}]_{\mathrm{organism}}$ (10)

then the Cd concentration in the consumer could also appear to follow the FIAM, i.e., $[\mathrm{Cd}]_{\mathrm{organism}}$ could be replaced by $[\mathrm{Cd}]_{\mathrm{consumer}}$ in eqs 6–9. Following the same logic, equations could be developed for any number of trophic levels (*12*).

The Windermere Humic Aqueous model (WHAM 1.0, *18*) was used to estimate trace metal ion concentrations. Model parameters for "Model V", a component of WHAM 1.0, are semiempirical in nature because they were determined by Tipping and Hurley (*19*) from fitting laboratory metal titration data performed under conditions (ionic strength, metal:ligand ratios, etc.) that can differ from those observed in natural systems. In particular, the ratio of metal:ligand can influence binding constant values (*20*), and these ratios tend to be higher in laboratory experiments than in nature. Concentrations of fulvic and humic acids required as input data to the WHAM computer code were estimated from our measurements of dissolved organic carbon ($[\mathrm{C}_{\mathrm{org}}]$) by assuming that humic substances (HS) contain 50% carbon; the ratio of humic to fulvic acids (HA:FA) is 1:9; and all dissolved organic carbon is present as humic substances. The ratio HA:FA is reported to vary little in lakes (between 1:8.5 and 1:9; *21*), that is, FA appear to dominate dissolved HS. Elemental analysis of lake FA indicates that they contain between 42 and 52% C (*20, 22*), whereas HA contain between 53 and 59% C (*20*). Given the fact that FA dominate HS, the value of 50% C used herein is reasonable.

Frozen *Chaoborus* larvae were dried in a lyophilizer. Dried samples were weighed on their Teflon piece with a Mettler ME30 electronic microbalance and digested in a Teflon vial with concentrated nitric acid (Aristar; approximately 100 μL mg^{-1} tissue) in an autoclave at 120 °C for 3 h. Certified reference material (lobster hepatopancreas, TORT-1, NRCC) was submitted to the same digestion procedure during each analytical run. Trace metal concentrations measured in TORT-1 were within the certified range for each element. After dilution, cadmium, copper, and zinc concentrations were analyzed by flameless AAS (Varian Spectra AA-30). Minimum Cd values in digested larvae (1 μg L^{-1}) greatly exceeded those in procedural blanks (0.00 ± 0.01 μg L^{-1}).

Results and Discussion

The free ion activity model, as presented in Table 3, postulates that the concentration of metal taken up by an organism is proportional to the concentration of the free ionic form of the metal to which it is exposed, i.e., $[\mathrm{M}^{z+}]$. However, Hare and Tessier (*8*) reported that Cd concentrations in the insect *C. punctipennis*, $[\mathrm{Cd}]_{C.punct.}$, were not significantly related ($p > 0.1$) to free Cd ion concentrations in lake water. The lack of a correlation between $[\mathrm{Cd}]_{C.punct.}$ and $[\mathrm{Cd}^{2+}]$ was ascribed to the influence of a few highly acidic lakes (pH < 5.5) in which $[\mathrm{Cd}]_{C.punct.}$ was low (<4 μg g^{-1}) and $[\mathrm{Cd}^{2+}]$ was high compared to less acidic lakes (*8*). Data from the large number of highly acidic lakes sampled in the present study (Table 2) confirm the trend toward low Cd bioaccumulation (<4 μg g^{-1}) in lakes of low pH (<5.5). Reduced Cd bioaccumulation in acidic lakes has been reported for several other types of organisms including algae (*23*) and crustaceans (*24*).

TABLE 4. Pearson Correlation Coefficients between the Concentrations of H^+, Cd^{2+}, Cu^{2+}, Zn^{2+}, and Ni^{2+} in the Data Set of Hare and Tessier (*12*; in Parentheses) and in Our Combined Data Sets; $n = 14$ for Hare and Tessier's $[\mathrm{Ni}^{2+}]$ Data or $n = 23$ for Their Data on Other Metals

	$[\mathrm{Cd}^{2+}]$	$[\mathrm{Cu}^{2+}]$	$[\mathrm{Zn}^{2+}]$	$[\mathrm{Ni}^{2+}]$
$[\mathrm{Cu}^{2+}]$	(0.89)^a 0.43^a			
$[\mathrm{Zn}^{2+}]$	0.97^a 0.93^a	(0.81)^a 0.13		
$[\mathrm{Ni}^{2+}]$	(0.98)^a 0.32	(0.93)^a 0.88^a	(0.95)^a 0.01	
$[\mathrm{H}^+]$	(0.84)^a 0.36^a	(0.76)^a 0.39^a	(0.90)^a 0.15	(0.81)^a 0.47^a

^{a} $p < 0.05$.

Reduced Cd accumulation in *C. punctipennis* larvae from highly acidic lakes was ascribed to competition between Cd^{2+} and H^+ for biological uptake sites (*8*). By taking this competitive effect into account (eq 7 in Table 3), Cd concentrations in the insect were reliably predicted from free Cd and H ion concentrations in lakes (*8*). However, the pivotal role of the hydrogen ion is questionable because H^+ concentrations were strongly correlated with those of other cations (Table 4) that could also act as competitors for Cd uptake sites. Because of these strong correlations, Cd concentrations in *C. punctipennis* can be explained equally well by using, for example, Cu^{2+} ($r^2 = 0.92$, $p < 0.001$) rather than H^+ ($r^2 = 0.86$, $p < 0.001$) as a competitor for biological uptake sites (eq 8 in Table 3). The strong correlations among hydrogen and metal ions in the Hare and Tessier (*8, 12*) data set can be ascribed to the fact that the small number of highly acidic lakes they sampled are approximately equidistant from the same anthropogenic source of metal and acid-producing emissions (Table 2). By adding to their data set values from highly acidic lakes in mining regions differing in their relative H^+ and trace metal concentrations, we weakened the correlations among potential competitors for Cd uptake sites (Table 4). The combined data set should allow us to determine whether H^+ or metal ions such as Cu^{2+}, Ni^{2+}, or Zn^{2+} are the major competitors with Cd for biological uptake sites.

Considering the pooled data set, Cd concentrations in *C. punctipennis* larvae are not significantly related to free Cd ion concentrations ($p = 0.65$; Figure 1A) unless highly acidic lakes are ignored ($p < 0.001$, $r^2 = 0.92$, regression not shown). If we presume that H^+ is the major competitor for Cd at biological uptake sites (eq 7 in Table 3) and if we ignore the manipulated lake in our data set (Lake Turcotte), we obtain a strong ($r^2 = 0.78$) and significant ($p < 0.001$) linear relationship between Cd concentrations in the insect and its environment (Figure 1B). If we include the data point for the manipulated lake, the regression remains significant ($p < 0.001$) but is weaker ($r^2 = 0.58$). We chose to remove the manipulated lake in subsequent data analysis because of its clear position as an outlier [for comparative purposes data points for this lake are, however, represented on all figures by the asterisk (*)]. The presence of mining residues probably explains the high concentrations of Zn (an order of magnitude higher than any other lake) and Cu (much higher than other lakes in its region) in this lake (Table 2). Although we are not yet able to explain the lower than predicted Cd concentrations in *C. punctipennis* from this lake, we note that fourth instar larvae were the lightest of any collected (0.1 versus a mean of 0.3 mg $individual^{-1}$) and that a major food group exploited by this species (copepod and cladoceran microcrustaceans; *25*) was conspicuous by its near absence (Croteau, unpublished material). Low *Chaoborus* body weights have been reported to result from reductions in microcrustacean populations (*26*), and microcrustacean communities are reported to be sensitive to trace metals such as Cd (*27*−*29*). These observations suggest that a lack of planktonic prey, from which *Chaoborus* larvae obtain most of their Cd (*11*), could be limiting to the growth and Cd accumulation of larvae in this manipulated lake.

The weakened correlations among Cu^{2+}, Zn^{2+}, Ni^{2+}, and H^+ in the combined data set (Table 4), allowed us to determine which of these ions is likely to be the superior competitor with Cd ions for biological uptake sites. Cadmium concentrations in *C. punctipennis* were better explained by a model with H^+ as a Cd^{2+} competitor (eq 7 in Table 3; Figure 1B), as opposed to models with either Cu^{2+}, Zn^{2+}, or Ni^{2+} as a Cd^{2+} competitor (eq 8 in Table 3; M = Cu, Figure 1C; M = Zn, Figure 1D; M = Ni, $r^2 = 0.01$). Furthermore, the addition of either Cu^{2+} or Zn^{2+} as a second competitor along with H^+ (eq 9 in Table 3), provided little further increase in predictive power ($r^2 = 0.80$ and 0.79, respectively; regressions not shown) over that afforded by H^+ alone ($r^2 = 0.78$, $n = 31$, Figure 1B). Similarly, adding Ni^{2+} as a cocompetitor with H^+ had no effect on predictive power ($r^2 = 0.75$, $n = 23$, regression not shown). The results of our study suggest that a simple formulation of the bioaccumulation model (eq 7 in Table 3) that considers competition by H^+ but ignores competition from other trace metals (eqs 8 and 9 in Table 3) is the preferred choice. Such simplicity is advantageous if one wishes to use the biomonitor as a practical environmental tool to either rank lakes in terms of their bioavailable Cd concentrations or to estimate $[Cd^{2+}]$.

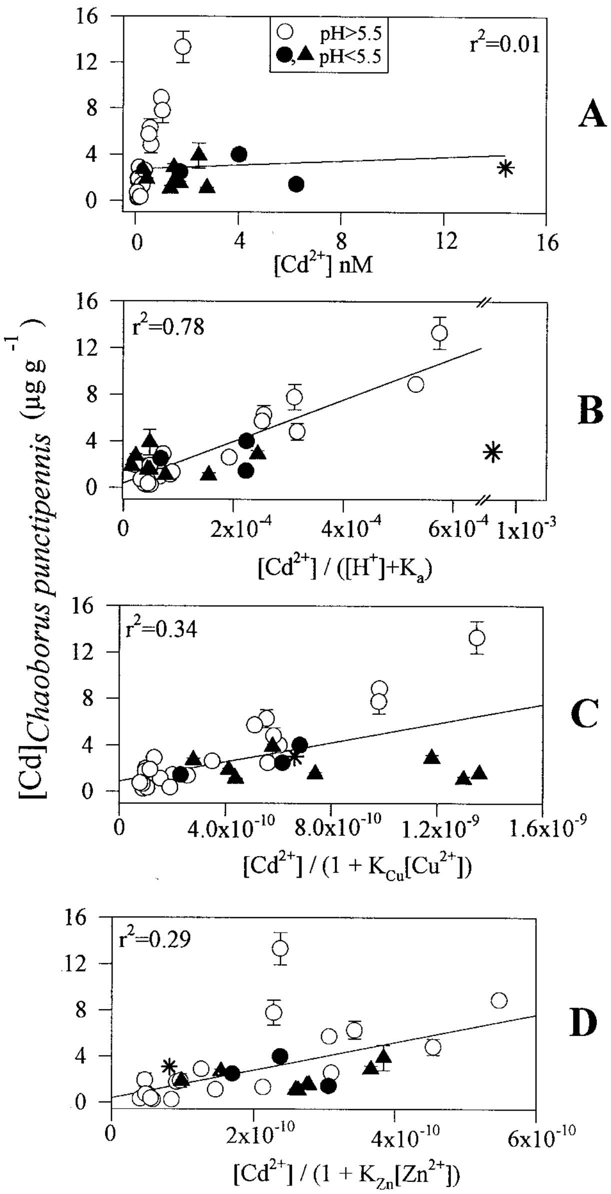

FIGURE 1. Relationships between mean (±SD, μg g^{-1} dry weight) Cd concentrations in larvae of the insect *Chaoborus punctipennis* and (A) computed free Cd ion concentrations ($[Cd^{2+}]$) in lake water, (B) $[Cd^{2+}]$ normalized for hypothesized competition between hydrogen and free Cd ions for biological uptake sites (eq 7 in Table 3), (C) $[Cd^{2+}]$ normalized for hypothesized competition between free Cu and Cd ions for biological uptake sites (eq 8 in Table 3), (D) as in panel C, except that free Cu ions are substituted by free Zn ions; solid symbols, pH <5.5; open symbols, pH >5.5; circles, data from ref *12*; triangles, lakes in this study. The value (±SE) of the slope (=*F* in eq 7 in Table 3) and the *y*-intercept of the regression in panel B are 18 000 ± 1700 μg g^{-1} and 0.37 ± 0.35 μg g^{-1}, respectively, whereas $K_a = 8 \times 10^{-7}$ mol L^{-1}. The manipulated lake (*) was not taken into account in the regressions in panels B, C, and D.

In terms of model simplification, it would also be advantageous if all *Chaoborus* species could be treated together as opposed to developing a relationship for each taxon. Not identifying larvae to species would be advantageous for routine biomonitoring because specific determinations usually require a microscope, while a generic identification can be made in the field. We combined results for *C. punctipennis* with our less extensive data sets for three other species collected from the study lakes (i.e., *C. ameri-*

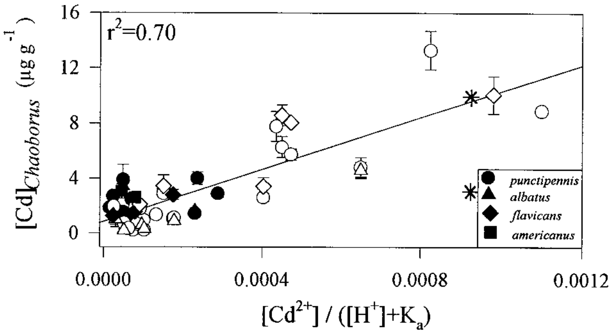

FIGURE 2. Relationships between mean (±SD, μg g^{-1} dry weight) Cd concentrations in larvae of various species of the genus *Chaoborus* and [Cd^{2+}] normalized for hypothesized competition between hydrogen and free Cd ions for biological uptake sites (eq 7); solid symbols, pH $<$5.5; open symbols, pH $>$5.5. The value (±SE) of the slope ($=F$ in eq 7) and the *y*-intercept of the regression are 9500 ± 880 μg g^{-1} and 0.87 ± 0.29 μg g^{-1}, respectively, whereas $K_a = 8 \times 10^{-7}$ mol L^{-1}. The manipulated lake (*) was not taken into account in the regression.

canus, C. flavicans, and *C. albatus*; Table 2) and found that the generic model was almost equivalent in predictive power to that developed for *C. punctipennis* alone (a loss of 8% of the explained variability, Figure 2). Despite this encouraging result, we suggest that the generic model be used with caution until further data on species other than *C. punctipennis* become available. From a practical point of view, spring nocturnal sampling for *Chaoborus* is suggested, both because larvae are all of the same instar at this time of year and because they can be collected in the water column at night thus obviating the laborious task of separating them from the sediment.

It would broaden the use of the biomonitor if *Chaoborus* larvae could be used to evaluate the levels of other potentially toxic trace metals such as Cu or Zn. However, despite the extremely large range in calculated Cu^{2+} and Zn^{2+} concentrations in our study lakes, insect Cu and Zn concentrations varied little among lakes (Figure 3), and there was no significant relationship between aqueous and insect Cu or Zn concentrations ($p > 0.1$ for both metals). Our results for Cu and Zn suggest that *Chaoborus* larvae are not an appropriate biomonitor for these essential trace metals. Regulation of Cu or Zn or both is reported to be common to many aquatic invertebrates (*30*, *31*).

Since the predator is reported to take up little of its Cd directly from water (*11*), the biological uptake sites at which competition between Cd^{2+} and H^{+} occurs are likely to be found, not on the predator, but on its prey or on other organisms at some lower level in the food chain (eq 10 in Table 3). Taking into account differences in prey communities among lakes could provide a means to reconcile the apparently anomalous position of the manipulated lake in our data set. A better understanding of Cd uptake from food, including Cd concentrations in various prey types, Cd assimilation efficiencies, and prey consumption rates, could also help to explain the differences in Cd concentrations measured among the various species of *Chaoborus* (Table 2) and those reported to occur among larvae collected in various seasons (*13*). The importance of Cd uptake from prey also implies that we cannot reject the possibility that unknown effects of acidity on prey community density or composition could also influence predator [Cd].

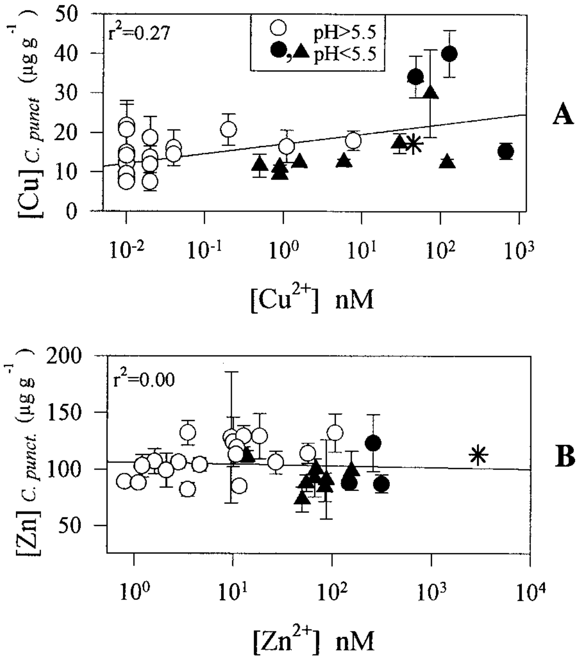

FIGURE 3. Relationships between mean (±SD, μg g^{-1} dry weight) Cu (A) or Zn (B) concentrations in larvae of the insect *Chaoborus punctipennis* and computed free Cu ion concentrations ([Cu^{2+}]) or computed free Zn ion concentrations ([Zn^{2+}]); solid symbols, pH $<$5.5; open symbols, pH $>$5.5; circles, data from ref *12*; triangles, lakes in this study. The manipulated lake (*) was not taken into account in the regressions.

The results of our research suggest that *Chaoborus* larvae could be used as effective biomonitors of Cd concentrations in even highly Cd-contaminated lakes from which sensitive taxa might have been eliminated by Cd toxicity. If Cd concentrations in this tolerant biomonitor can be related to toxic effects on Cd-sensitive taxa, then *Chaoborus* larvae could also eventually be used as an indirect bioindicator of toxic effects.

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