

# Difficulties in relating Cd concentrations in the predatory insect *Chaoborus* to those of its prey in nature

Marie-Noële Croteau, Landis Hare, and André Tessier

**Abstract:** Because *Chaoborus* larvae take up most of their cadmium (Cd) from food, we tested the hypothesis that Cd concentrations in this insect are directly related to those in their planktonic prey. We measured Cd in *Chaoborus* and in zooplankton collected from 24 eastern Canadian lakes varying widely in their Cd concentrations. Cd concentrations in the predator were not correlated with those in bulk zooplankton, whether separated into size fractions liable to be eaten by *Chaoborus* or not. In highly acidic lakes, Cd concentrations in *Chaoborus* did not respond to increases in zooplankton Cd because of either competition between H and Cd ions at Cd absorption sites in the predator's gut or differences in prey community composition between highly acidic and circumneutral lakes. Relationships between Cd in *Chaoborus* and in its potential prey were stronger when we used Cd concentrations for specific crustacean taxa in a mechanistic model. We conclude that predictive relationships between metal concentrations in predators and their prey are likely to be strongest if the subset of prey consumed by the predator has been characterized and if this information is used in a bioaccumulation model.

**Résumé :** Parce que les larves de *Chaoborus* tirent la plus grande partie de leur cadmium (Cd) de leur nourriture, nous avons vérifié l'hypothèse selon laquelle les concentrations de Cd chez cet insecte sont directement reliées à celles de leurs proies planctoniques. Nous avons mesuré les teneurs en Cd de *Chaoborus* et du zooplancton dans 24 lacs de l'est du Canada dont les concentrations en Cd variaient considérablement. Les concentrations de Cd chez ce prédateur n'étaient pas reliées à celles du zooplancton global, qu'il soit séparé ou non en fractions de taille susceptibles d'être consommées par *Chaoborus*. Dans les lacs très acides, les concentrations de Cd chez *Chaoborus* ne reflétaient pas les concentrations de Cd du zooplancton, soit en raison d'une compétition entre les ions H<sup>+</sup> et Cd<sup>2+</sup> aux sites d'absorption du Cd dans le tube digestif du prédateur, ou alors en raison de différences dans la composition des communautés de proies entre les lacs très acides et les lacs proches de la neutralité. Les relations entre les teneurs en Cd chez *Chaoborus* et celles de ses proies potentielles se sont avérées plus significatives lorsque les concentrations de Cd de taxons particuliers de crustacés furent utilisées dans un modèle mécanistique. En conclusion, le pouvoir prédictif des relations entre les concentrations de métaux chez les prédateurs et leurs proies sera vraisemblablement accru si les proies véritablement consommées par le prédateur ont été bien identifiées et si ces renseignements sont utilisés dans un modèle de bioaccumulation.

[Traduit par la Rédaction]

## Introduction

Industrial activities have increased the entry of trace metals into aquatic environments (Pacyna et al. 1995). To improve our ability to predict the biological impact of these pollutants and to conduct meaningful ecological risk assessments for aquatic communities, we need to better understand the accumulation of metals by organisms (Pace 2001). In aquatic systems, metals are accumulated by primary producers (St.-Cyr and Campbell 1994; Luoma et al. 1998) and then transferred to animals at higher trophic levels (Fisher and Reinfelder 1995). The diet, as opposed to water, is an

important route of metal accumulation for many animals (Schlekat et al. 2001). As a consequence, risk assessments that do not consider dietary metal exposure can underestimate animal metal concentrations and resulting effects on target species in nature (Chapman and Wang 2000). A consequence of dietary metal uptake is that metal concentrations show trends along food chains. Thus concentrations of lipophilic forms of mercury and selenium are reported to increase with increasing trophic level (Luoma et al. 1992; Cabana et al. 1994; Chen et al. 2000), whereas the results of several laboratory studies suggest that the concentrations of nonlipophilic metal forms tend to decrease along freshwater

Received 11 July 2002. Accepted 15 May 2003. Published on the NRC Research Press Web site at <http://cjfas.nrc.ca> on 15 August 2003.  
J16989

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food chains (Hatakeyama and Yasuno 1987; van Hattum et al. 1989; Croteau et al. 2001). In the field, trends in trace metal concentrations along planktonic food chains are difficult to discern because feeding relationships between organisms at various trophic levels are usually not known (Hare 1992) and because of the difficulty in obtaining sufficient biomass for measuring metals in small-sized organisms such as copepods and cladocerans. Because of this latter limitation, most investigators measure metals in bulk plankton samples (Chen and Folt 2000; Chen et al. 2000). However, bulk plankton includes a wide range of animal types, sizes, and trophic levels that could obscure trends in metal concentrations along food chains (Watras and Bloom 1992).

We set out to determine if cadmium (Cd) concentrations in the predator *Chaoborus* are related to those in zooplankton, the major source of Cd for this insect (Munger and Hare 1997; Munger et al. 1999). No previous field studies have attempted to relate Cd concentrations in a planktonic predator to those in its prey. We collected zooplankton and final instar *Chaoborus* larvae from a large number of lakes located along a Cd gradient in eastern Canada. Then we compared Cd concentrations in *Chaoborus* with those measured in either bulk zooplankton or in zooplankton subfractions separated according to size (*Chaoborus* is a gape-limited predator; Hare and Carter 1987) or taxonomic group (cladocerans and copepods). Lastly, we incorporated these measurements into a mechanistic Cd bioaccumulation model (Croteau et al. 2001, 2002) to determine if this laboratory-based model could successfully predict Cd concentrations in the predator from those measured in these various fractions of zooplankton.

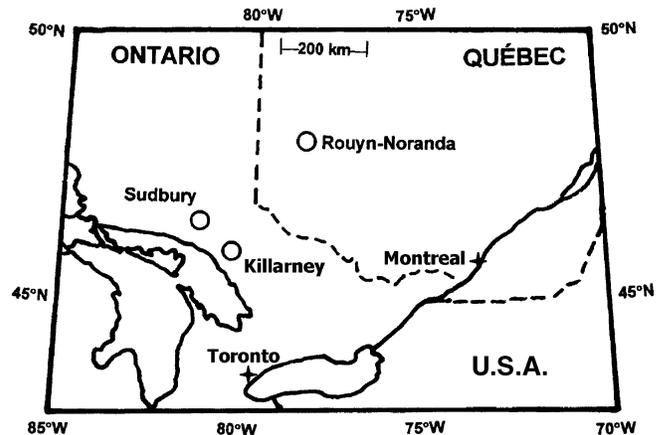
## Methods

### Collection of field samples

We collected samples of invertebrates and water between 1996 and 1998 from a series of lakes located on the Canadian Shield in the mining areas of Sudbury (Ontario) and Rouyn-Noranda (Québec) (Fig. 1, Table 1). Samples were collected only in the spring (late May to mid-June) to minimize confounding seasonal changes in *Chaoborus* Cd concentrations among sites (Hare and Campbell 1992).

*Chaoborus* larvae were collected after sunset near lake centre by hauling a 250  $\mu\text{m}$  mesh aperture plankton net horizontally in the water column of each lake (Table 2). We transported live animals to the laboratory in plastic bags filled with lake water. *Chaoborus* larvae were identified to species using the keys in Saether (1972), and final (fourth) instar larvae were selected on the basis of head capsule lengths as given in Larow and Marzolf (1970; *C. punctipennis*), Fedorenko and Swift (1972; *C. americanus*), and Parma (1971; *C. flavicans*). We chose final instars because they were the predominant instar in most spring samples, because they provide a larger mass for Cd analyses than do younger instars, and because they consume the greatest range of prey types and sizes (Hare and Carter 1987; Moore 1988). We held larvae in their original lake water for 12–24 h to eliminate their gut contents. Where numbers permitted, we placed five replicate samples of 3–15 *Chaoborus* larvae (or 1 larva per sample from Lake H el ene) on pieces of preweighed acid-washed Teflon sheeting that were frozen until Cd analysis. Although we treated larvae whole, trends in larval Cd

**Fig. 1.** Locations of our three study areas (Rouyn-Noranda, Sudbury, and Killarney) in eastern Canada.



would not have differed if we had measured Cd in only the major organ of accumulation, that is, the gut, because almost all larval Cd (~95%) is found in this organ (Croteau et al. 2001). As a corollary, because little Cd is associated with the larval exoskeleton (<5%), differences among larvae in the time since their last moult should have no influence on their Cd concentrations.

We collected other planktonic organisms just before sunset by horizontally hauling a 64  $\mu\text{m}$  mesh net near the centre of each lake; the net's path ensured that plankton was collected from all depths. Aliquots of this plankton were placed in plastic bags with lake water. Remaining bulk plankton was gently poured over a series of acid-washed nylon sieves of four mesh sizes (1000, 500, 300, and 64  $\mu\text{m}$ ); animals (mainly *Holopedium gibberum*) and debris retained by the 1000- $\mu\text{m}$  sieve were discarded. The plankton retained by each sieve was gently rinsed with filtered (64  $\mu\text{m}$ ) lake water into plastic bags for transport to the laboratory where we placed 2–6 replicate samples for each zooplankton size fraction and unsieved zooplankton (0.1–7 mg dry weight) on pieces of preweighed acid-washed Teflon sheeting that were frozen until Cd analysis. Remaining organisms were preserved in a 5% formalin solution for later identification and counting of crustacean zooplankton. In eight lakes having dense crustacean plankton communities (pH > 6.3, Table 3), we isolated copepods and cladocerans from bulk samples under a microscope for Cd measurements. Because of the large number of crustaceans (100–150) needed to make up a sample, we did not identify animals further. Individuals of each taxon were placed on preweighed acid-washed Teflon sheeting that was frozen until Cd analysis. We did not measure zooplankton Cd over several weeks before collecting *Chaoborus* (so as to integrate possible temporal changes in prey Cd) because unpublished data (Croteau) show that Cd concentrations in bulk plankton vary little over the 2 weeks that it takes *Chaoborus* larvae to achieve a steady state in their Cd concentrations in the early spring (Croteau et al. 2001, 2002). Furthermore, even if there were a drastic drop in zooplankton Cd, it would take a minimum of 3 weeks for *Chaoborus* to lose half of its Cd (Munger et al. 1999).

We collected water samples in 2–3 diffusion samplers (Croteau et al. 1998) that were suspended for 3 days in the epilimnion of each lake. On installation and retrieval dates,

**Table 1.** Study lake locations, pH, and concentrations of total dissolved organic carbon ( $C_{org}$ ), Ca, and Cd.

Region and lake	Lake code	Location	pH	[ $C_{org}$ ], mg C·L <sup>-1</sup>	[Ca], µM	[Cd], nM
Killarney, Ontario						
Terry*	TE	46°04'N, 81°17'W	4.56	7.9	37	0.9
Sudbury, Ontario						
Chief*	CH	46°22'N, 81°01'W	4.47	5.3	40	2.7
Crooked	CK	46°25'N, 81°02'W	5.07	3.6	74	4.5
Crowley	CW	46°23'N, 80°59'W	6.85	2.8	59	1.2
Daisy*	DY	46°27'N, 80°53'W	5.36	4.3	59	2.5
Forest	FO	46°23'N, 81°00'W	6.23	2.5	65	1.4
Hazen*	HA	46°25'N, 80°59'W	4.46	8.8	2237	3.3
Johnny	JH	46°26'N, 81°02'W	7.12	4.6	229	1.4
Pine*	PI	46°22'N, 81°02'W	4.30	6.0	30	3.8
Tilton	TI	46°22'N, 81°04'W	6.61	2.4	119	1.3
Rouyn-Noranda, Quebec						
Bousquet	BO	48°14'N, 78°34'W	6.35	12.1	97	0.85
Caron	CA	47°56'N, 78°58'W	7.01	11.3	288	0.89
Chevreuil*	CV	47°54'N, 79°00'W	4.50	5.2	29	2.5
d'Alembert	DA	48°23'N, 79°01'W	7.15	9.0	160	0.44
Desperiers*	DE	48°11'N, 79°09'W	5.16	5.6	42	3.2
Duprat	DU	48°20'N, 79°07'W	7.11	6.7	173	0.41
Flavrian	FL	48°18'N, 79°11'W	7.27	7.8	156	0.43
Hélène	HE	48°13'N, 79°10'W	8.05	8.9	420	0.07
Joannès	JO	48°11'N, 78°41'W	6.95	10.4	164	0.64
La Bruère	LA	48°10'N, 78°57'W	7.79	9.9	304	0.61
Marlon	MA	48°16'N, 79°04'W	7.35	8.6	138	1.18
Surimeau*	SU	48°08'N, 79°19'W	4.95	8.5	39	0.9
Turcotte*	TU	48°18'N, 79°04'W	4.83	5.4	105	19.5
Vaudray	VA	48°07'N, 78°42'W	6.65	7.8	81	0.57

**Note:** Water samples were collected from the epilimnion of each lake using in situ diffusion samplers.

\*Data from Croteau et al. (1998).

we measured the pH in water samples collected with a van Dorn bottle at the depth of the diffusion samplers.

### Analyses

To minimize inadvertent trace metal contamination, we soaked all labware and sampling material in 15% nitric acid and rinsed them in ultrapure water (>18 MΩ cm) before use. We measured total dissolved Cd concentrations by flameless atomic absorption spectrophotometry (AAS; THGA graphite tube atomiser, model SIMAA 6000 (Perkin-Elmer, Boston, Mass.)). Certified reference riverine water samples (National Research Council of Canada (NRCC); SLRS-4, National Institute of Standards and Technology, 1643d) were analyzed during each analytical run and measured trace metal concentrations were within the certified range. We measured calcium by flame AAS (Spectra AA-20; Varian Instruments, Walnut Creek, Calif.) and dissolved organic carbon using a total organic carbon analyser (TOC-5000A; Shimadzu, Columbia, Md.).

*Chaoborus* larvae and zooplankton samples were freeze-dried (FTS Systems, Stone Ridge, N.Y.), weighed on a microbalance (ME30; Mettler-Toledo, Columbus, Ohio), and digested in concentrated nitric acid (100 µL·mg<sup>-1</sup> dry weight (DW); Aristar grade). For invertebrate samples collected in 1996 and 1997, we carried out digestions in thick-walled, screw-cap Teflon vials in an autoclave at 120°C for 3 h. Cooled digested samples were diluted to volume with ultrapure water. For samples collected in 1998, we conducted

digestions at room temperature in 4-mL HDPE vials for 7 days. Hydrogen peroxide (40 µL·mg<sup>-1</sup> DW) was added 24 h before final dilution with ultrapure water (760 µL·mg<sup>-1</sup> DW). Cadmium values were comparable for the two digestion methods used. We submitted samples of similar weight of a certified reference material (lobster hepatopancreas, TORT-1, NRCC) to the same digestion procedure during each run. Cadmium concentrations in animal digests were analyzed by flameless AAS (Varian, Spectra AA-30); Cd concentrations measured for TORT-1 were within the certified range and the recovery of Cd in spiked samples was within 10% of the amount added. The software SYSTAT 10 (Systat Software, Richmond, Calif.) was used for all statistical analyses. We used linear regressions to relate Cd concentrations in *Chaoborus* to those in its potential prey, as well as to relate Cd measurements in zooplankton to those in lake water. Comparisons of Cd concentrations among plankton size fractions were made by *t* tests.

### Results and discussion

*Chaoborus* larvae are reported to take up Cd mainly from the zooplankton that they consume as prey (Munger and Hare 1997; Munger et al. 1999). Thus we might expect that at steady-state Cd concentrations in this predator,  $[Cd]_{Chaoborus}$  would be directly related to those in its prey,  $[Cd]_{zooplankton}$ , that is,

**Table 2.** Mean Cd concentrations ( $\mu\text{g}\cdot\text{g}^{-1}$  dry weight;  $\pm$  standard deviation) in bulk and size-fractionated zooplankton samples, as well as in various *Chaoborus* species from our study lakes.

Region and lake	Plankton Cd concentrations				<i>Chaoborus</i> species Cd concentrations			
	Bulk	500–1000 $\mu\text{m}$	300–500 $\mu\text{m}$	64–300 $\mu\text{m}$	<i>punctipennis</i>	<i>albatus</i>	<i>flavicans</i>	<i>americanus</i>
Killarney, Ontario								
Terry*	0.72 $\pm$ 0.02 (3)	0.64 $\pm$ 0.06 (3)	0.50 $\pm$ 0.03 (3)	1.0 $\pm$ 0.1 (3)	1.9 $\pm$ 0.1 (5)	NP	NP	NP
Chief*	1.2 $\pm$ 0.3 (3)	INS	2.7 $\pm$ 0.2 (3)	0.45 $\pm$ 0.02 (3)	1.5 $\pm$ 0.1 (4)	NP	NP	NP
Crooked†	1.4 $\pm$ 0.3 (3)	3.9 $\pm$ 3 (2)	1.1 $\pm$ 0.2 (2)	0.46 $\pm$ 0.01 (3)	3.2 $\pm$ 0.3 (5)	3.8 (1)	4.0 $\pm$ 0.4 (2)	4.0 $\pm$ 0.2 (5)
Crowley†	4.1 $\pm$ 0.5 (4)	3.8 $\pm$ 0.5 (3)	1.5 $\pm$ 0.2 (4)	2.2 $\pm$ 0.3 (3)	10.9 $\pm$ 0.3 (5)	NP	8.6 $\pm$ 0.5 (5)	NP
Daisy*	2.8 $\pm$ 0.4 (3)	INS	2.1 $\pm$ 0.01 (3)	3.4 $\pm$ 0.01 (3)	2.8 $\pm$ 0.3 (5)	NP	NP	NP
Forest†	2.5 $\pm$ 0.7 (4)	6.2 $\pm$ 0.4 (4)	3.4 $\pm$ 0.1 (4)	1.2 $\pm$ 0.1 (4)	8.3 $\pm$ 0.4 (5)	NP	4.9 $\pm$ 0.2 (5)	NP
Johanny†	3.4 $\pm$ 0.5 (3)	1.5 $\pm$ 0.3 (2)	1.0 $\pm$ 0.4 (2)	4.0 $\pm$ 0.3 (3)	11.9 $\pm$ 0.8 (5)	NP	NP	13.6 $\pm$ 0.9 (5)
Hazen*	2.2 $\pm$ 0.1 (3)	INS	3.4 $\pm$ 0.9 (3)	2.0 $\pm$ 0.1 (3)	1.1 $\pm$ 0.1 (5)	NP	1.6 $\pm$ 0.1 (5)	2.6 $\pm$ 0.4 (5)
Pine*	2.1 $\pm$ 0.1 (3)	INS	1.3 $\pm$ 0.1 (3)	2.3 $\pm$ 0.1 (3)	3.9 $\pm$ 1.1 (3)	3.4 $\pm$ 0.2 (5)	NP	NP
Tilton†	3.7 $\pm$ 0.3 (4)	0.81 $\pm$ 0.1 (3)	0.46 $\pm$ 0.04 (3)	1.5 $\pm$ 0.3 (4)	7.6 $\pm$ 1.0 (5)	NP	6.6 $\pm$ 0.6 (5)	NP
Rouyn-Noranda, Quebec								
Bousquet†	2.0 $\pm$ 0.1 (4)	0.95 $\pm$ 0.03 (4)	0.72 $\pm$ 0.4 (4)	0.34 $\pm$ 0.03 (4)	2.0 $\pm$ 0.2 (5)	NP	3.0 $\pm$ 0.4 (5)	NP
Caron	3.9 $\pm$ 0.5 (4)	1.0 $\pm$ 0.1 (4)	0.36 $\pm$ 0.1 (4)	0.24 $\pm$ 0.06 (4)	5.6 (1)	NP	4.8 $\pm$ 0.4 (5)	NP
Chevreuil*	2.1 $\pm$ 0.2 (3)	INS	0.43 $\pm$ 0.06 (3)	0.37 $\pm$ 0.05 (5)	1.5 (1)	NP	NP	3.0 $\pm$ 0.5 (5)
d'Alembert†	8.1 $\pm$ 1.1 (4)	4.3 $\pm$ 0.4 (4)	2.7 $\pm$ 0.1 (4)	1.1 $\pm$ 0.04 (4)	2.3 (1)	NP	1.7 $\pm$ 0.1 (5)	NP
Desperiers*	3.9 $\pm$ 0.3 (5)	5.3 $\pm$ 0.5 (5)	2.3 $\pm$ 0.03 (5)	1.4 $\pm$ 0.1 (5)	1.1 $\pm$ 0.2 (5)	NP	2.8 $\pm$ 0.4 (5)	NP
Duprat†	2.2 $\pm$ 0.1 (4)	1.7 $\pm$ 0.2 (4)	0.96 $\pm$ 0.04 (4)	0.36 $\pm$ 0.01 (4)	1.4 $\pm$ 0.1 (5)	NP	1.3 $\pm$ 0.2 (5)	NP
Flavrian§	3.6 $\pm$ 0.2 (3)	7.0 $\pm$ 0.1 (4)	1.8 $\pm$ 0.2 (3)	1.5 $\pm$ 0.1 (4)	NP	1.3 $\pm$ 0.1 (5)	NP	NP
Hélène	ND	ND	ND	ND	0.13 $\pm$ 0.02 (3)	0.18 $\pm$ 0.04 (3)	NP	NP
Joannès	5.0 $\pm$ 0.1 (4)	3.4 $\pm$ 0.3 (4)	3.1 $\pm$ 0.2 (4)	0.69 $\pm$ 0.02 (4)	NP	NP	3.6 $\pm$ 0.2 (5)	NP
La Bruère†	3.2 $\pm$ 0.3 (4)	2.5 $\pm$ 0.1 (4)	2.5 $\pm$ 0.1 (4)	0.44 $\pm$ 0.04 (4)	1.7 $\pm$ 0.1 (5)	NP	NP	NP
Marlon†	43 $\pm$ 14 (4)	41 $\pm$ 3 (4)	28 $\pm$ 2 (4)	1.8 $\pm$ 0.2 (4)	5.6 $\pm$ 0.6 (4)	4.9 $\pm$ 0.5 (4)	NP	NP
Surimeau*	33 $\pm$ 2 (5)	8.5 $\pm$ 1.8 (6)	2.3 $\pm$ 0.3 (5)	29 $\pm$ 3 (5)	2.7 $\pm$ 0.2 (5)	NP	1.3 $\pm$ 0.1 (3)	NP
Turcotte*	2.7 $\pm$ 0.2 (3)	INS	2.0 $\pm$ 0.1 (2)	4.1 $\pm$ 1.2 (3)	3.1 $\pm$ 0.1 (5)	NP	NP	9.9 $\pm$ 0.8 (5)
Vaudray†	3.0 $\pm$ 0.5 (4)	4.2 $\pm$ 0.3 (4)	3.3 $\pm$ 0.3 (4)	0.39 $\pm$ 0.05 (4)	2.9 $\pm$ 0.1 (2)	NP	4.5 $\pm$ 0.3 (5)	NP

**Note:** All zooplankton samples (including *Chaoborus*) were collected on a single date over a short time period (late May to mid-June) in the spring. Sample numbers are given in parentheses. INS, insufficient number of organisms; NP, not present; ND, not determined.

\**Chaoborus* data from Croteau et al. (1998).  
 †*Chaoborus* data from Croteau et al. (2001).  
 ‡*Chaoborus punctipennis* data from Croteau et al. (2002).  
 §*Chaoborus* data from Croteau et al. (2002).

**Table 3.** Mean Cd concentrations ( $\mu\text{g}\cdot\text{g}^{-1}$ ;  $\pm$  standard deviation) in copepods and cladocerans, as well as the ratio of their densities in the 64- to 300- $\mu\text{m}$  size fraction of plankton collected from lakes in the region of Rouyn-Noranda, Quebec.

Lake	[Cd] <sub>copepods</sub>	[Cd] <sub>cladocerans</sub>	Abundance ratio copepods:cladocerans
Bousquet	0.99 $\pm$ 0.1 (3)*	0.89 (1)	1.2
d'Alembert	3.1 $\pm$ 0.4 (3)*	4.2 $\pm$ 0.5 (4)	11
Duprat	1.6 $\pm$ 0.1 (3)*	1.3 $\pm$ 0.05 (2)	20
Flavrian	2.0 (1)*	5.5 $\pm$ 0.4 (3)	3.3
Hélène	1.1 $\pm$ 0.2 (5)*	0.99 $\pm$ 0.4 (3)	1†
Joannès	2.9 $\pm$ 1 (4)	0.75 $\pm$ 0.2 (2)	31
La Bruère	2.9 $\pm$ 0.5 (3)*	1.6 $\pm$ 0.3 (3)	54
Marlon	23 $\pm$ 2 (2)	20 $\pm$ 2 (3)	8.2
Vaudray	5.8 $\pm$ 0.2 (3)*	3.2 $\pm$ 0.4 (2)	26

**Note:** Sample numbers are given in parentheses.

\*Data from Croteau et al. (2002).

†We assumed a 1:1 ratio because Cd concentrations in cladocerans and copepods from this lake were not significantly different.

$$(1) \quad [\text{Cd}]_{\text{Chaoborus}} = F_{\text{Chaoborus-zooplankton}} \times [\text{Cd}]_{\text{zooplankton}}$$

where  $F_{\text{Chaoborus-zooplankton}}$  is a proportionality constant.

### Bulk plankton as prey

We tested eq. 1 with data from 23 lakes in which we measured Cd in *Chaoborus* larvae and in bulk plankton (Table 2); rotifers (mainly *Keratella*) were the numerically dominant animal group in these plankton samples (Fig. 2a, inset). There were no significant relationships ( $P > 0.05$ ) between Cd concentrations in bulk zooplankton and those in *Chaoborus* larvae, regardless of whether data were pooled at the generic level (i.e., *Chaoborus*, not shown) or treated on a species by species basis (e.g., *C. punctipennis*; Fig. 2a). Furthermore, no significant relationships emerged if we excluded two high-Cd values for zooplankton (lakes MA and SU in Fig. 2a and Table 2). This lack of a relationship between Cd concentrations in the predator and in bulk samples of potential prey does not signify that eq. 1 is invalid, but is more likely a consequence of the fact that the predator does not consume all sizes and types of prey in bulk zooplankton. This postulate is supported by the fact that Cd concentrations in *Chaoborus* substantially exceeded those in bulk zooplankton in several circumneutral lakes (open symbols in Fig. 2a) in the Sudbury area (Table 2), whereas Cd concentrations in invertebrate predators should be lower than those in their prey (Hatakeyama and Yasuno 1987; van Hattum et al. 1989; Croteau et al. 2001).

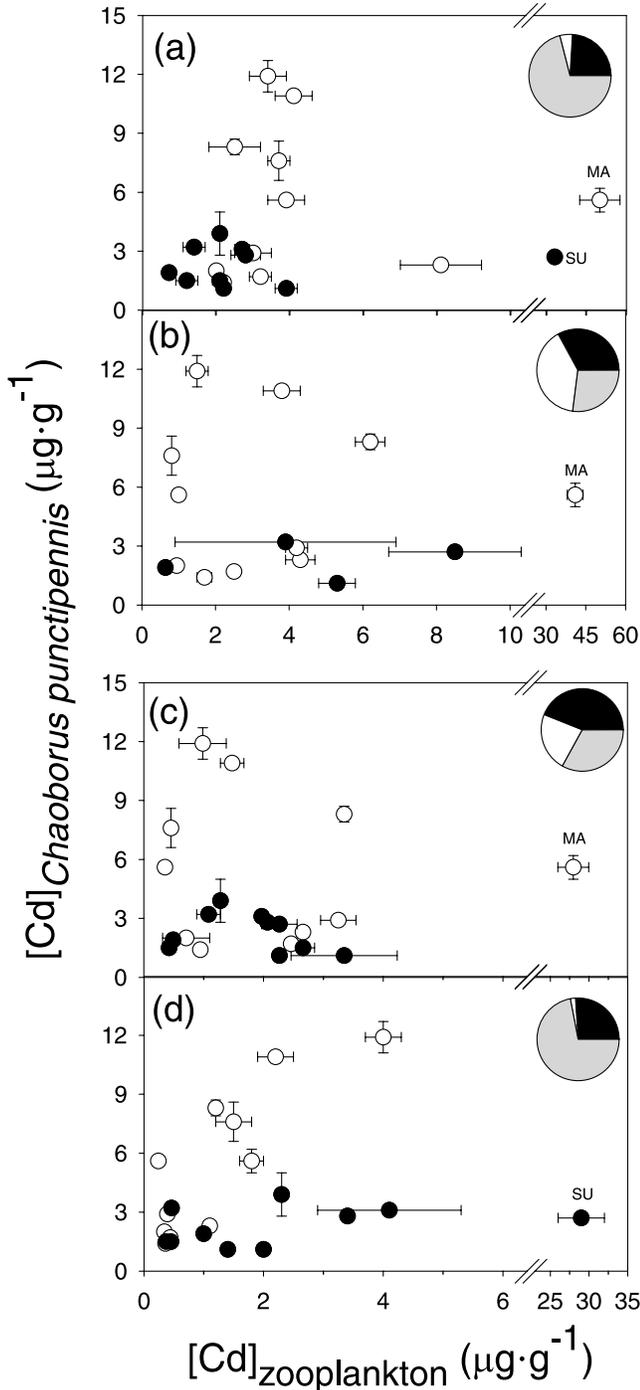
### Size fractions of zooplankton as prey

Because *Chaoborus* larvae are limited by the gape of their mouth to prey below a certain size (Hare and Carter 1987), we fractionated bulk plankton samples into three size classes. The largest size fraction (500–1000  $\mu\text{m}$ ) would be unavailable to fourth instars of our small-sized study species, *C. punctipennis* and *C. albatus* (mouth gapes  $\approx$  450  $\mu\text{m}$ ; Moore 1988), and available only in part to the large-sized species, *C. flavicans* (mouth gape  $\approx$  630  $\mu\text{m}$ ; Stenson 1980) and *C. americanus* (mouth gape  $\approx$  700  $\mu\text{m}$ ; Fedorenko 1975). Prey in the two smaller size fractions (300–500 and 64–300  $\mu\text{m}$ ) should be available to all of these *Chaoborus* species. Crustaceans (copepods and cladocerans) dominated numerically

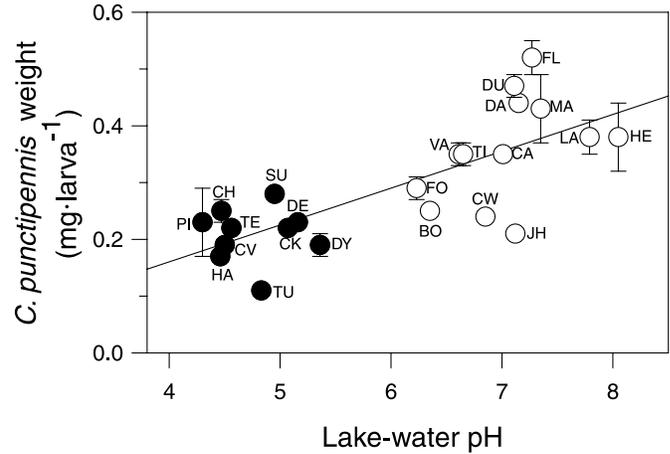
the larger size fractions (Figs. 2b–2c, insets), whereas rotifers dominated the smallest size fraction (Fig. 2d, inset). Again, there were no significant relationships ( $P > 0.05$ ) between Cd concentrations in any zooplankton size fraction and those in *Chaoborus* at either the genus (not shown) or the species level (e.g., *C. punctipennis*; Figs. 2b–2d). In addition, no significant relationships emerged when we excluded extreme zooplankton values (MA and SU in Figs. 2b–2d).

This lack of correlation is especially evident for the highly acidic lakes (Fig. 2, solid circles) in which Cd concentrations in *Chaoborus* are consistently low in spite of large differences in zooplankton Cd among these lakes. One possible explanation for this trend is that if *Chaoborus* larvae take in sufficient lake water to influence midgut pH (unknown at present), then hydrogen ions could outcompete Cd ions from prey at biological uptake sites in the predator's gut leading to lower Cd concentrations in larval tissues. Competition between hydrogen and Cd ions has been invoked as an explanation for the generally low Cd concentrations measured in *Chaoborus* larvae from highly acidic lakes (Croteau et al. 1998). A second possible explanation for the uniformly low Cd concentrations in *Chaoborus* from highly acidic lakes is that there is a scarcity of the crustacean prey preferred by *Chaoborus* in these lakes (Havens and Hanazato 1993; Locke et al. 1994). In such lakes, *Chaoborus* larvae would be limited to feeding largely on rotifers, which, given their small mass, would result in reduced prey ingestion rates and low predator mass for a given body length (Havens and Hanazato 1993). Reduced predator mass with decreasing pH is supported by data for final instar *C. punctipennis* from a large number of lakes (Fig. 3,  $P < 0.001$ ,  $r^2 = 0.57$ ). Feeding studies on *C. punctipennis* in one of our highly acidic lakes (Turcotte, pH 4.83) confirm that final instar larvae have a small mass (Fig. 3, TU), feed largely on rotifers (Croteau et al. 2003), and are low in Cd in spite of the high dissolved Cd concentrations in this lake (Tables 1 and 2; Croteau et al. 1998). Because *Chaoborus* in highly acidic lakes have a smaller range of prey types from which to choose (Havens and Hanazato 1993; Locke et al. 1994), our measurements of Cd in zooplankton from acidic lakes are likely to be more representative of prey actually eaten by *Chaoborus* than are those on zooplankton from circumneutral lakes. This appears

**Fig. 2.** Relationships between mean ( $\pm$  standard deviation;  $\mu\text{g}\cdot\text{g}^{-1}$  dry weight) Cd concentrations in final instar larvae of *Chaoborus punctipennis* and (a) samples of bulk zooplankton  $>64\ \mu\text{m}$ , (b) the 500- to 1000- $\mu\text{m}$  zooplankton size fraction, (c) the 300- to 500- $\mu\text{m}$  zooplankton size fraction, and (d) the 64- to 300- $\mu\text{m}$  zooplankton size fraction (solid symbols, pH  $< 5.5$ ; open symbols, pH  $> 5.5$ ). Each point corresponds to a particular lake (MA, Marlon; SU, Surimeau). Inset pie graphs illustrate the mean relative numbers of major zooplankton groups for all lakes: solid, copepods; shaded, rotifers; open, cladocerans.



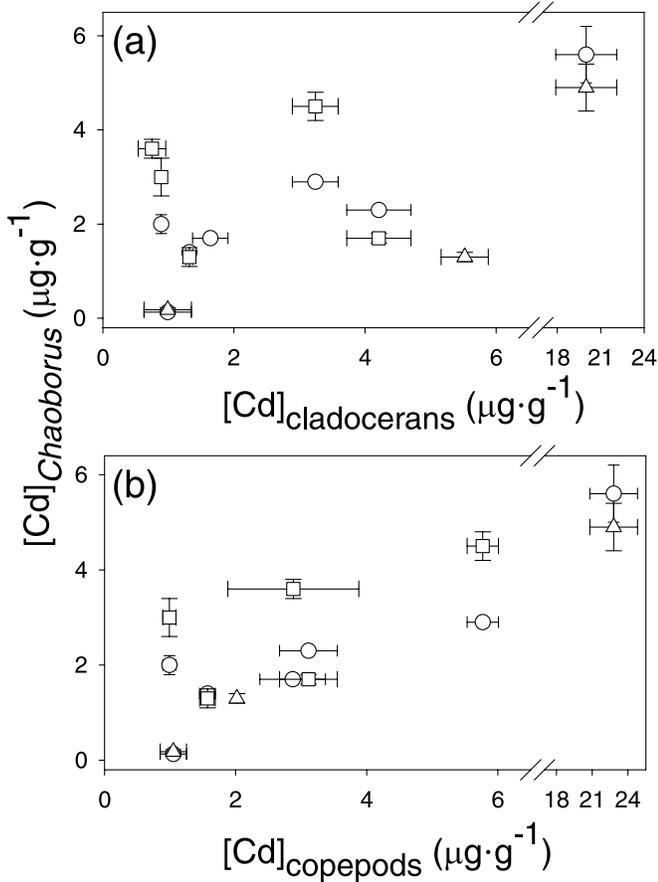
**Fig. 3.** Mean weight of final instar larvae of *Chaoborus punctipennis* ( $\pm$  standard deviation;  $\text{mg dry weight}\cdot\text{larva}^{-1}$ ) as a function of lake-water pH (solid symbols, pH  $< 5.5$ ; open symbols, pH  $> 5.5$ ). Each point corresponds to a particular lake: BO, Bousquet; CA, Caron; CH, Chief; CK, Crooked; CV, Chevreuil; CW, Crowley; DA, d'Alembert; DE, Desperiers; DU, Duprat; DY, Daisy; FL, Flavrian; FO, Forest; HA, Hazen; HE, H el ene; JH, Johnny; JO, Joann es; LA, La Bru ere; MA, Marlon; PI, Pine; SU, Surimeau; TE, Terry; TI, Tilton; TU, Turcotte; VA, Vaudray.



to be the case because Cd concentrations in *Chaoborus* from some circumneutral lakes greatly exceeded those in zooplankton (which should not be the case; Hatakeyama and Yasuno 1987; van Hattum et al. 1989; Croteau et al. 2001), whereas Cd concentrations in *Chaoborus* from highly acidic lakes did not. Although we reason above in terms of the types of prey and their rates of ingestion, a lower efficiency of Cd assimilation from rotifers than from crustaceans could also potentially contribute to the lower Cd concentrations in *Chaoborus* from highly acidic lakes (Reinfelder et al. 1998; Croteau et al. 2001); at present there is no information on assimilation efficiencies of these elements by predators from rotifers that we could use to judge the importance of this variable.

We also used our size-fraction data to test the supposition that zooplankton size fractions represent different trophic levels, with increasing animal size being equated to increasing trophic level (Chen and Folt 2000; Chen et al. 2000). If this were the case, then we would expect that Cd concentrations should decline with increasing animal size because concentrations of this metal decrease with increasing trophic level (Hatakeyama and Yasuno 1987; van Hattum et al. 1989; Croteau et al. 2001). Our data do not support this idea because Cd concentrations in the smallest size fraction were significantly lower ( $P < 0.05$ ) than those in the two larger size fractions in 12 of 17 lakes from which we obtained all size fractions (Table 2). Knowledge of feeding habits would also argue against such a trend; the widespread microcrustacean *Daphnia*, although mainly herbivorous, is larger than most omnivorous planktonic microcrustaceans (Dodson and Frey 1992). To unambiguously demonstrate trophic trends in metal concentrations, we suggest using measurements of stable N-isotope ratios to compare the relative trophic heights of purported food chain links (France et al. 1996).

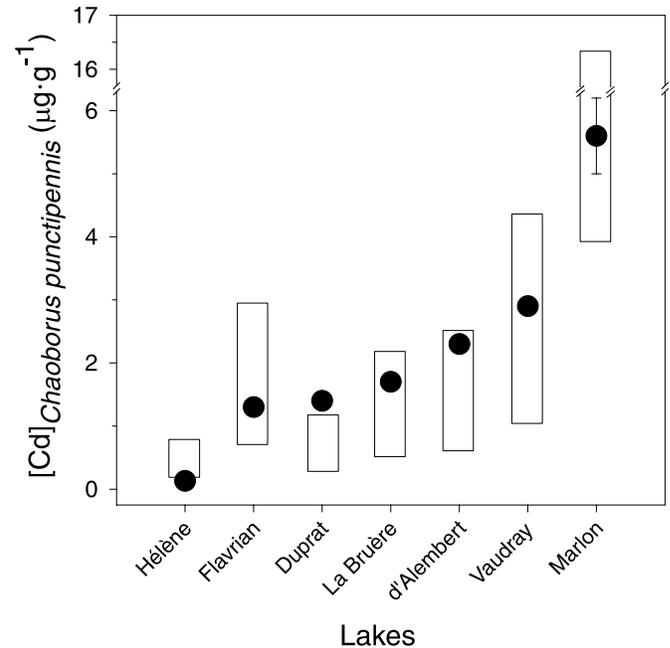
**Fig. 4.** Relationships between mean ( $\pm$  standard deviation;  $\mu\text{g}\cdot\text{g}^{-1}$  dry weight) Cd concentrations in final instar larvae of three *Chaoborus* species and their potential (a) cladoceran or (b) copepod prey.  $\circ$ , *C. punctipennis*;  $\triangle$ , *C. albatus*;  $\square$ , *C. flavicans*.



### Crustacean zooplankton as prey

Sieving zooplankton into size categories suitable for *Chaoborus* ingestion is inefficient because it likely traps some zooplankton on their side, that is, according to their length rather than their width. However, because *Chaoborus* larvae orient captured prey so as to swallow them head-on, it is likely that prey width is a better determinant of successful ingestion than is prey length (Moore and Gilbert 1987). This could explain in large part the lack of relationships that we found between Cd concentrations in *Chaoborus* and those of size-fractionated zooplankton prey (Figs. 2b–2d). To better isolate prey of a suitable size and prey types known to be consumed by *Chaoborus* (Swüste et al. 1973; Moore 1988), we hand-picked suitable-sized cladocerans and copepods from a subset of our study lakes in which numbers of large-bodied crustaceans were sufficient to permit their removal by hand and their aggregation into samples of sufficient biomass for Cd analyses (Table 3, circumneutral lakes). Although Cd concentrations in cladocerans and copepods were directly related ( $P < 0.05$ ) to those of *Chaoborus* (Figs. 4a–4b), these relationships depend on the high Cd values in animals from a single lake (Lake Marlon, Table 3), which are in turn a consequence of the higher total dissolved Cd concentrations

**Fig. 5.** Measured Cd concentrations (mean  $\pm$  standard deviation;  $\mu\text{g}\cdot\text{g}^{-1}$  dry weight) in final instar larvae of *Chaoborus punctipennis* collected from various lakes ( $\bullet$ ) compared with model predictions for Cd concentrations in this predator at 5 and 22°C (bars). Error bars are too small to be visible for all lakes except Lake Marlon. Predicted Cd values for *C. punctipennis* were estimated for each temperature using eq. 2 and the parameters AE, IR,  $k_g$ ,  $k_e$  (obtained from Croteau et al. 2002), and  $[\text{Cd}]_{\text{Food}}$  (abundance weighted mean of Cd concentrations measured in copepods and cladocerans from each lake). Upper and lower edges of bars correspond to model predictions at 22 and 5°C, respectively. Data for Lake Flavrian are for the closely related species *Chaoborus albatus*.



in this circumneutral lake compared with those of the other lakes in Fig. 4 (Table 1). To better determine if our Cd measurements in these prey types could be used to predict those in the predator, we incorporated these measurements into a mechanistic bioaccumulation model (Croteau et al. 2001, 2002) to determine if the laboratory-derived constants in such a model would permit prediction of  $[\text{Cd}]$  in *Chaoborus* collected in nature. Steady-state Cd concentrations in the predator,  $[\text{Cd}]_{\text{Chaoborus}}_{\text{ss}}$ , can be predicted (Croteau et al. 2001, 2002) from Cd concentrations in its planktonic food,  $[\text{Cd}]_{\text{Food}}$ , given temperature-specific values for the efficiency with which the predator assimilates this metal (AE), the rate at which it ingests its prey (IR), as well as rate constants for Cd loss ( $k_e$ ) and larval growth ( $k_g$ ), that is,

$$(2) \quad [\text{Cd}]_{\text{Chaoborus}}_{\text{ss}} = \frac{\text{AE} \times \text{IR} \times [\text{Cd}]_{\text{Food}}}{k_e + k_g}$$

Using values of AE, IR,  $k_e$ , and  $k_g$  estimated for *C. punctipennis* by Croteau et al. (2002) along with our values for Cd in potential crustacean prey from each lake (based on mean Cd concentrations in copepods and cladocerans weighted for their relative densities in the size fraction available to the predator (64–300  $\mu\text{m}$ ; Table 3)), we predict Cd concentrations in this predator for the extremes of temperature that it

is likely to encounter in nature (5 and 22°C). Because Cd concentrations in this predator are reported to be inferior to those of its prey (Munger et al. 1999; Croteau et al. 2001, 2002), we excluded data for one lake (Bousquet) in which predator Cd concentrations exceeded those measured in planktonic crustaceans (Table 3). To verify that this assumption is justified, we used the bioaccumulation model described in eq. 2 to determine under what circumstances  $[Cd]_{\text{Chaoborus}} / [Cd]_{\text{prey}}$  exceed 1; all such scenarios imply the use of biologically unrealistic values of at least one model constant (AE, IR,  $k_e$ , or  $k_g$ ). The results of these model simulations suggest that if Cd concentrations in bulk or size-fractionated zooplankton are lower than those in *Chaoborus*, then it is likely because these gross prey assemblages include a majority of individuals that are not a major component of this predator's diet.

Measured predator Cd values fall close to or within model predictions for most lakes (Fig. 5), suggesting that this model provides a rough tool for predicting Cd concentrations in *C. punctipennis* from those in its crustacean prey. Identifying prey below groupings such as "copepods" would likely improve model predictions further. For example, we measured substantial differences in the Cd concentrations (means  $\pm$  95% confidence interval (CI) in  $\mu\text{g}\cdot\text{g}^{-1}$ ) of two calanoid copepods collected from one of our study lakes (L. Caron), that is, *Limnocalanus macrurus* ( $2.9 \pm 0.1$ ), which is too large to be eaten by *Chaoborus*, and *Diaptomus* sp. ( $5.5 \pm 1.0$ ), which are eaten by this insect. When we used values for Cd in bulk or size-fractionated prey in eq. 2, measured data points usually fell outside of the range expected from model predictions (figures not shown), suggesting that finer prey taxonomic resolution will aid in predicting predator Cd.

In conclusion, a relationship between Cd concentrations in *Chaoborus* and those in its prey is to be expected given that the Cd in *Chaoborus* comes entirely from its prey (Munger and Hare 1997; Munger et al. 1999). However, our attempts to relate metal concentrations in this planktonic predator to those in its prey met with mixed success; although we observed no relationship between Cd concentrations in *Chaoborus* and those in bulk or size-fractionated zooplankton samples, a positive relationship was observed between Cd concentrations measured in specific prey taxa and those predicted in *Chaoborus* by a bioaccumulation model. We suggest that predictions of metal concentrations between adjacent links in food chains, as well as trends in metal concentrations along food chains, are likely to be unreliable if they are based on metal measurements in samples of poorly identified bulk prey and if there is a lack of knowledge of the prey actually consumed by the predator living in planktonic communities composed of different mixtures of prey types. Field measurements of metals in well-defined planktonic food chains are needed to better understand metal trophic transfer in nature.

## Acknowledgements

Funding was provided by the Natural Sciences and Engineering Research Council of Canada, the Metals In The Environment Research Network (contribution number 34), Ontario Power Generation, the Mining Association of Canada, Human Resources Development Canada, and the Quebec Fonds de Recherche sur la Nature et les Technologies.

Technical assistance by M.G. Bordeleau, J. Bernier, M.-R. Doyon, P. Fournier, S. Fournier, P. Marcoux, R. Savard, N. Simard, S. St-Pierre and R. Rodrigue is acknowledged.

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