Factors affecting methylmercury biomagnification by a widespread aquatic invertebrate predator, the phantom midge larvae *Chaoborus*

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A B S T R A C T

MeHg biomagnification by the phantom midge *Chaoborus* in relation to MeHg concentrations in their prey and its migratory behavior was investigated in two Canadian Precambrian Shield lakes. Three *Chaoborus* species with contrasted migratory behavior were collected in a fishless and a fish-inhabited lake. All species accumulated MeHg through their ontogenic development. In the lake inhabited by fish, all instars of *Chaoborus punctipennis* displayed a marked migratory behavior and were unable to biomagnify MeHg, whereas in the fishless lake, *Chaoborus americanus* and *Chaoborus trivittatus* biomagnified MeHg. Reduced biomagnification capacity of *C. trivittatus*, the coexisting species living with *C. americanus*, was also ascribed to a progressive vertical segregation with age. Growth dilution, amount and type of prey items or trophic position could not explain the different patterns of biomagnification. Our findings demonstrate that the most common invertebrate predator of temperate planktonic food webs can biomagnify mercury, contrarily to previous reports.

1. Introduction

Anthropogenic emission of mercury (Hg), its atmospheric transport and its deposition have increased Hg inputs in many aquatic ecosystems (Driscoll et al., 2007). Methylmercury (MeHg) is the main metal species accumulating along aquatic food webs and represents a major environmental threat for wildlife and human populations (Clarkson, 2002; Harris et al., 2007). Several key environmental parameters such as low pH, high DOC (Rencz et al., 2003; Watras et al., 1995a) and surrounding wetland areas (Guentzel, 2009; St. Louis et al., 1996) are associated with increasing bioaccumulation of MeHg in aquatic biota. Those parameters are favorable to MeHg production in-lake and/or in adjacent wetlands from microbial methylation of inorganic Hg under anoxic conditions (Fleming et al., 2006; Gilmour et al., 1992). Further, the magnitude at which MeHg is accumulated and biomagnified is strongly linked to the accumulation or dilution of MeHg by primary producers (Pickhardt et al., 2002), and to the structure of the food web (Cabana et al., 1994; Power et al., 2002). For consumers, it is essential to integrate exposure to MeHg from different feeding patterns and foraging habitats (Eagles-Smith et al., 2008; Power et al., 2002) since exposure to MeHg occurs primarily through diet.

Aquatic invertebrate predators are often important conduits of contaminants to fish and commonly have higher MeHg concentrations than their prey (Chetelat et al., 2011; Cremona et al., 2008; Mason et al., 2000). However, *Chaoborus* larvae (Insecta, Diptera), an invertebrate predator distributed worldwide, does not seem to respond to the classical pattern. This key predator seems to be inefficient in accumulating and biomagnifying MeHg (Back and Watras, 1995; Paterson et al., 1998).

Chaoborus larvae undergo four aquatic stages before their metamorphosis to pupae and emergent adults. Diet of the larvae is mainly composed of zooplankton but varies during their ontogenic development (Fedorenko, 1975a; Persaud and Dillon, 2010). Chaoborid larvae often perform diel vertical migrations in order to avoid fish predation pressure (Dawidowicz et al., 1990; Garcia and Mittelbach, 2008; McQueen et al., 1999). This migrating behavior affects their feeding activities inducing a local vertical segregation between food resource habitat and refuge habitat in the water column over day and night cycles. Such behavior may also occur to various degrees in fishless lakes and ponds for chaoborid species

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cohabiting and competing with *Chaoborus americanus*, a non-migrating species (Croteau et al., 2003a; Fedorenko, 1972; Garcia and Mittelbach, 2008).

The aim of this study was to evaluate the effect of feeding habits and diel vertical migration on MeHg accumulation and biomagnifications by *Chaoborus* species in a fishless lake and a fish-inhabited lake from the boreal region. We hypothesize that (i) chaoborids will accumulate MeHg during their ontogenic development; (ii) MeHg levels in chaoborids will be related to the contamination of prey in their crop, and (iii) MeHg biomagnification will be affected by spatial segregation between refuge habitat and food resource habitat due to differential feeding habits and migratory behavior.

2. Material and methods

2.1. Site description and sampling periods

The study was carried out in two small Precambrian Shield lakes. Lake Geai is a small bog lake (area: 0.99 ha, max depth: 6.5 m) with acidic and dystrophic waters (Table 1). Two large chaoborid species (*C. americanus* and *Chaoborus trivittatus*) were the dominant invertebrate predators in this fishless lake (Table S1) (Masson and Pinel-Alloul, 1998). Lake Croche is a typical dimictic boreal lake with a larger area (4.74 ha) and depth (max. depth: 12 m) (Table 1). Waters are circumneutral and the lake is classified as oligo-mesotrophic. This lake is characterized by the presence of planktivorous fish (Masson et al., 2004) and its chaoborid community is composed of the small species *Chaoborus punctipennis* (Table S1). Samplings of water and organisms were conducted at the site of maximum depth in each lake on three dates in early June (S1), late June (S2), and at the end of August (S3) 2009.

2.2. Water quality

At each date, we measured water temperature, dissolved oxygen, pH and conductivity (YSI 600 QS meter) to determine the vertical structure of the water column (epi-, meta- and hypolimnion strata). Triplicates of dissolved organic carbon (DOC), total phosphorus (TP) and total nitrogen (TN) were analyzed once from an integrated sample collected over the water column at the beginning of June. Sampling and analyses of water samples for DOC were conducted according to the standard methods described in Garcia and Carignan (1999).

2.3. Zooplankton

Samples for taxonomic analysis were collected using integrated vertical tows from one meter above the sediment to the surface with a 53 μm mesh size cantilevering plankton net. Zooplankton were narcotized with carbonated water and preserved in buffered 4% formaldehyde. Zooplankters in subsamples (5–20 mL) were identified to species level to calculate densities (individuals m^{-3}). Rotifer and crustacean biomass (mg of dry weight m^{-3}) was estimated based on published length–weight regressions of Malley et al. (1989) and weight biomass from previous studies (Pinel-Alloul, unpublished data).

2.4. Chaoborus

We used an image-based key www.cfb.unh.edu/cfbkey/html/index.html and taxonomic keys of Saether (1972) to identify *Chaoborus* species and instars. The instars were determined according to the length of their head capsule. Body length of each larva was also measured.

Chaoborus vertical diel migration was studied at each sampling date by collecting the larvae in epi- and hypolimnetic strata during daytime (11:00 AM) and after nightfall (11:00 PM) with a cantilevering net (53 μm mesh size) equipped with a net closer trigger line. Organisms were anesthetized (carbonated water) and preserved with buffered formaldehyde (7% final concentration). The diel migration study was completed in mid-June 2010 by sampling larvae in the water column and in the upper sediment layers at noon and after nightfall in both lakes. Collections of *Chaoborus* in the water column were made every 1 m from the surface to 1 m above the sediment with a Plexiglas plankton trap of 0.125 m^3 equipped with a 53 μm mesh size plankton net. An Ekman grab ($16 \times 16 \times 16$ cm) was used to collect the *Chaoborus* larvae from the upper sediment. Sediments were then sieved through 1 mm, 700 μm and 500 μm mesh size screens. Larvae collected in the water and sediment samples were anaesthetized and then fixed with buffered 7% formaldehyde (final concentration). Daytime and nighttime densities (individuals m^{-3}) of each species and instars were estimated.

The average depths of the vertical distribution of the *Chaoborus* species were calculated according to weight mean depth (WMD) with the equation of Frost and Bollens (1992):

$$\mathrm{WMD} = \frac{\sum(n_i \times z_i \times d_i)}{\sum n_i \times z_i}$$

where n_i is the density (individuals m^{-3}) of larvae in the sample i, d_i is the midpoint of the water strata and z_i is the thickness of the strata. WMD was calculated at each sampling date (S1, S2, S3 and mid-June 2010) according to the thickness of each stratum and also based on larvae collected at each meter interval from the surface to the sediment in mid-June 2010. Depths were calculated from the water surface (which had a depth of 0 m) with increasing positive values toward the sediments.

2.5. Chaoborus crop contents

Because feeding activities occurred mainly at night, chaoborids collected at night were used for gut contents analysis at S1, S2, S3 sampling dates. Crop contents of larvae collected in mid-June 2010 were also analyzed to characterize feeding activities in relation to their spatial distribution at noon and after nightfall. Prior to fixation (7% formalin final concentration), *Chaoborus* larvae were anaesthetized with carbonated water. All instars were dissected to remove their crop after identification. Crop content was colored with rose Bengal and mounted on slides. Prey items were identified under a microscope (×200, ×400 magnifications), counted and classified among four categories: Rotifera, Copepoda, Cladocera, and phytoplankton. The number (number $crop^{-1}$) and the biomass (μg DW $crop^{-1}$) of each prey category were estimated for each crop. Phytoplankton biovolume was first estimated based on the formula of Hillebrand et al. (1999) and then converted to biomass according to Vrede et al. (1999). Zooplankton biomass of each prey category was calculated as for the biomass estimated for the zooplankton community (see Section 2.3).

2.6. MeHg analyses

Glassware for MeHg analysis was washed overnight with a mixture of 45% (v/v) concentrated HNO_3 acid and 5% HCl. Plastic wares were washed overnight with 10% HCl. All acid-washed material was triple rinsed with ultrapure water (Milli-Q, >18 Mohm cm^{-1}) and transported to the field in double plastic bags. Clean technique procedures for trace metals were used in the field and the laboratory.

To measure MeHg in water, a 20 L high density polyethylene container was filled from vertically integrated water samples collected with a PVC tube at each sampling date (S1, S2, S3) in both lakes. Preliminary tests indicated that this sampling device neither significantly contaminated nor retained Hg during transit. The water samples corresponded to the epi- and the metalimnion strata, the two layers in which chaoborids typically feed. At the laboratory, the water was filtered with an online 0.45 μm polyethersulfone filter cartridge (Polycap GW, Whatman) into acid-washed amber glass bottles. All aqueous Hg samples were preserved with HCl (0.8% final concentration; Omnitrace Ultra, VWR) at 4 °C until analysis. Water samples for MeHg were acid-distilled to remove matrix interferences, then derivatized by aqueous-phase ethylation, purged on Tenax (Tenax Corporation, Baltimore, MD, USA) and separated by gas chromatography (GC) before determination with a Tekran 2500 cold vapor atomic fluorescence spectrophotometer (CVAFS, Tekran Instruments Corporation). Procedural blanks contained 2 ± 1 pg MeHg. Analytical detection limits, estimated as three times the standard deviation of 10 blanks was 0.02 ng L^{-1} for MeHg.

Microbial communities (phytoplankton and bacteria) which corresponded in this study to suspended particulate matter (0.7 μm < SPM > 53 μm), were collected on a pre-combusted glass membrane filter (GF/F, Whatman, and 0.7 μm of porosity). Prior to the final filtration with a Teflon apparatus, a subsample of water was pre-filtered onto a 53 μm mesh size net in order to remove the zooplankton.

To measure MeHg in invertebrates, small zooplankton were collected with cantilevering plankton net of 53 μm mesh size on vertical hauls at each sampling date (S1, S2, S3). *Chaoborus* larvae and large-size zooplankton were collected with a 210 μm plankton net and poured into clean glass containers. At the laboratory, samples were sieved through 600, 500, 210 and 53 μm Nitex mesh size and each size fraction was rinsed with ultrapure water (Milli-Q, >18 Mohm cm^{-1}). Small zooplankton (mainly rotifers and nauplii of calanoids and copepods) retained on the 53 μm mesh size were collected as bulk into clean polypropylene vials and kept at −20 °C until lyophilization. The dominant species of macro-zooplankton collected at the time of the sampling were sorted live under a binocular microscope. Between 200 and 800 individuals of each dominant zooplankton species or group were sorted at least in triplicate, collected into clean vials and frozen at −20 °C. Mature stages of copepods were mainly selected. Chaoborid larvae were separated live according to their body length into two to three size categories depending on community composition and sorted by species for the third and fourth intars. *C. trivittatus* pupae and larvae going through metamorphosis to pupae were also collected in late August (S3). From 25 to 100 individuals were handpicked into clean vials in triplicates for each category and frozen at −20 °C until lyophilization.

Zooplankton, chaoborids and GF/F filters with suspended particulate matter were dried, ground and weighed prior to analysis. Typically around 0.8–2.5 mg of material was then digested in 2.5 mL of 4 M HNO_3 at 55 °C for 16 h. The digestate was derivatized by aqueous-phase ethylation using NaBEt4, and measured by GC-CVAFS (Tekran Instruments Corporation) based on the method of Bloom (1989).

Procedural blanks contained 1 ± 1 pg MeHg. Certified reference material (TORT-2) was analyzed every 10 samples, and recoveries averaged 148 ± 15 ng g^{-1} ($n = 24$) corresponding to $97 \pm 10\%$ of the certified value.

2.7. MeHg accumulation through ontogenic development and biomagnification of MeHg

Temporal changes in MeHg in chaoborid larvae through their growing season were calculated as the ratio between MeHg levels in instar IV collected in late August (S3) and instar III collected in late June (S2). For *C. trivittatus*, MeHg levels measured in pupae were divided by those in *C. trivittatus* IV collected at the same date in late August.

The biomagnification factor (BMF) of the larvae was calculated as: the ratio between the mean MeHg concentration in *Chaoborus* species and instar, and the estimated MeHg contamination of crop at each sampling date, respectively. The following equation was used:

$$\mathrm{BMF}_i = \frac{[\mathrm{MeHg}]_{\mathrm{Chaoborus}\ i}}{[\mathrm{MeHg}]_{\mathrm{Crop}\ i}} \quad (1)$$

where BMF_i is the biomagnification factor of the larvae (i), $[\mathrm{MeHg}]_{\mathrm{Chaoborus}\ i}$ is the mean MeHg concentration in *Chaoborus* species or instar at the date of the sampling of the larvae (i), and $[\mathrm{MeHg}]_{\mathrm{Crop}\ i}$ is its estimated MeHg concentration of the crop i.

The MeHg concentration of the crop was estimated as follow:

$$[\mathrm{MeHg}]_{\mathrm{Crop}\ i} = \frac{\sum\left(\text{Biomass of prey} \times [\mathrm{MeHg}]_{\mathrm{prey}}\right)}{\text{Total Biomass of the crop}_i} \quad (2)$$

Total crop biomass was determined from the sum of the biomass of each prey category in the crop. $[\mathrm{MeHg}]_{\mathrm{prey}}$ refers to the concentration measured in each prey category on each sampling date. Mean MeHg concentrations measured in the different taxa of Copepoda and Cladocera collected in the water column at S1, S2, and at S3 sampling dates were used as a proxy for MeHg concentration in the Copepod category and in the Cladocera category found in gut contents. Because it was not technically possible to hand-pick phytoplankton nor rotifers, their MeHg concentrations were related to the ones measured in the particulate matter size fractions of 0.7–53 μm and 53–210 μm, respectively.

2.8. $\delta^{15}N$ isotope analyses

Samples of chaoborids were freeze-dried and ground. For nitrogen isotope analysis, samples were weighed into tin cups prior to combustion in a stable isotope mass spectrometer (Isoprime100 DI). Trophic position was calculated according to the formula of Cabana and Rasmussen (1996). No evidence of different trophic levels between the different *Chaoborus* species was found (Table S4).

2.9. Statistical analyses

Because of unequal sample sizes, variation in the occurrence and diversity of sorted species (zooplankton, *Chaoborus* instars and pupae), and lack of normality of the crop content data, a nonparametric test was used. For MeHg concentrations in organisms, crop content variables (number and biomass of prey, estimated MeHg concentrations of the crop), MeHg accumulation through ontogenic development and biomagnification factor ($\log_{10}$ BMF), the Wilcoxon test was applied to compare species and instars between or within lakes. A simple linear regression was used to explore the relationship between the $\log_{10}$ mean BMF (biomagnification factor) and the amplitude of migration (ratio of weighted mean depths: $\mathrm{WMD}_{\mathrm{noon}}/\mathrm{WMD}_{\mathrm{night}}$). The Shapiro–Wilk test was used to check the normality of the data. Significance was accepted at an alpha level of $p = 0.05$.

3. Results and discussion

3.1. MeHg levels in water and planktonic food webs

Aqueous MeHg concentrations were an order of magnitude higher in Lake Geai, compared to Lake Croche (Table 1), but within the range of concentrations previously recorded in North America (Gorski et al., 2003; Roy et al., 2009; Watras et al., 1995b). As both lakes are geographically close and within similarly forested catchments with no major direct tributaries, they should be exposed to similar atmospheric deposition of Hg. It is likely that the difference in aqueous MeHg levels is related to differences in water chemistry. Lake Geai is characterized by an acid pH and twice as much dissolved organic carbon (DOC) when compared to Lake Croche (Table 1). As a consequence, MeHg concentrations measured in various components of the plankton food web in Lake Geai were higher than in Lake Croche (Table 2). MeHg concentrations were twice as high in small particulate matter (0.7 μm < SPM < 53 μm) and 1.5–3 times higher in Copepoda and Cladocera, respectively.

Low pH is known to increase bacterial bioavailability of Hg (II) (Kelly et al., 2003), whereas high DOC is related to its increased mobility (Garcia and Carignan, 1999; Watras et al., 1998). Moreover, the geomorphologic characteristics of Lake Geai are favorable to anoxia in deep water layers. Anoxic layers represented at least two third of the water column in mid-spring in Lake Geai but never exceeded one-half of the water column in Lake Croche over the summer season (Table 1). It is well established that microbial MeHg production generally occurs under anoxic and suboxic conditions (Morel et al., 1998).

3.2. Diel vertical migration of Chaoborus larvae

At night, the highest densities of chaoborids were always recorded in the epi- and metalimnion in both lakes (Fig. 1, Table S2, supplement information). During daytime, *Chaoborus* species in Lake Geai stayed in the upper limnetic water layers (1–4 m) whereas in Lake Croche, they moved to the deepest water layers (6–10 m) and up to 99% of the instars were found in the sediments, during the day (Figs. 1 and 2).

Significant differences between day and night WMDs were detected for the coexisting instar IV of *C. trivittatus* in Lake Geai and for instars III and IV of *C. punctipennis* in Lake Croche (Fig. 1). Daytime WMD increased significantly ($p < 0.05$) with the age for *C. trivittatus* (Fig. 1) whereas *C. americanus* stayed in upper limnetic strata through ontogenic development.

Higher daytime WMDs in fish-inhabited Lake Croche than in fishless Lake Geai are consistent with earlier reports of fish avoidance behavior in these larvae (Garcia and Mittelbach, 2008). Deeper WMDs with age for *C. punctipennis* in Lake Croche are due to increased body length, which results in increased vulnerability toward fish predators (Lagergren et al., 2008; Voss and Mumm, 1999). In Lake Geai, absence of predation pressure and the strong constraint imposed by anoxia below a depth of 3 m (Table 1) may explain why both sympatric *Chaoborus* species always inhabited the pelagic strata (Figs. 1 and 2). However, a diel migration was still detected in this lake, with *C. trivittatus* IV occupying deeper water layers during the day (Fig. 1). Greater spatial segregation with age between sympatric species in fishless lakes likely reduced interspecific predation pressure on food resource and allowed *C. americanus* and *C. trivittatus* to coexist (Fedorenko, 1975b).

3.3. Feeding activity and diet of Chaoborus

Diel vertical migration affected feeding activities of chaoborids differently in the two lakes (Fig. 2). Chaoborids in Lake Geai continued to feed during the day with 16–83% of the larvae having prey in their crop (Fig. 2, A). However, feeding activities were still higher at night than during daytime with at least 63% of individuals with prey in their crop. In contrast, less than 3% of the individuals collected at noon had food in their crop in Lake Croche. The ascent of the larvae at night in the epi- and metalimnion was associated with an increase of individuals with prey in their crop, with values ranging from 0% to 100% (Fig. 2, B).

Prey biomass in crops ranged from 0.5 μg to 2.7 μg DW. *C. trivittatus* III and IV significantly fed ($p < 0.05$) on a greater biomass of prey than the other instars, with approximately 2.5 μg of dry weight per crop (Table 3). Day and night feeding activities confirmed that the amount of food in crops of *C. trivittatus* was at least twice as high as for the one reported for *C. americanus* of similar body size (Fig. 3, Table 3 and Table S1). No significant differences of biomass in crop were observed between instars of

Table 1
Location, area, maximum depth, limit of anoxic layer, water chemistry, and dissolved MeHg concentrations in Lake Croche and Lake Geai.

Lake	Area (ha)	Z_{max} (m)	Upper limit (m) of anoxic water layers			pH	DOC ($mg\,L^{-1}$)	TP ($\mu g\,L^{-1}$)	TN ($\mu g\,L^{-1}$)	DMeHg ($ng\,L^{-1}$)
			S1	S2	S3					
Geai 45°59′46″N 73°59′35″W	0.99	6.5	3	3	2	5.45	7.8 ± 0.53	22.58 ± 0.59	537.2 ± 17.7	0.48 ± 0.2
Croche 45°59′30″N 74°00′50″W	4.74	12	10	8.5	6.5	6.7	3.42 ± 0.18	5.97 ± 0.94	287.9 ± 32.5	0.04 ± 0.02

ha, hectare; S1 (early June), S2 (late June), S3 (late August), sampling dates; m, meter depth; DOC, dissolved organic carbon; TP, total phosphorus; TN, total nitrogen; DMeHg, dissolved methylmercury; ±SD.

C. americanus (except for *C. americanus* III) in Lake Geai and of *C. punctipennis* in Lake Croche (Table 3) in spite of the smaller body size of *C. punctipennis* (Table S1).

In both lakes, crustaceans were the dominant prey in terms of biomass for all instars and species of *Chaoborus*. Cladocerans and copepods represented 30–75% and 23–68% of crop biomass, respectively. Even though Lake Geai was less diverse in terms of large crustacean species compared to Lake Croche (Table S3, supplement information), similar prey types were recorded in chaoborid crops. Chaoborids were somewhat selective in their diet, since the distribution of cladoceran biomass in crop did not fully reflect the crustacean community structure in the water column (Table S3). Indeed, in Lake Geai copepods generally dominated zooplankton biomass whereas in the circumneutral Lake Croche cladocerans generally prevailed. Chaoborid diet also included phytoplankton and rotifers, but those groups represented less than 2% of crop biomass, except for *C. punctipennis*, for which rotifers reached 6.3% (Table 3). The relative homogeneity of diet among chaoborid species was further corroborated by isotopic $\delta^{15}N$ signatures of the fourth instar of each chaoborid species (Table S4, supplement information). All species occupied a similar trophic position that ranged between 2.51 ± 0.05 in Lake Geai and 2.87 ± 0.18 in Lake Croche.

Our results indicate that chaoborids performing important vertical migration in the presence of fish concentrate their feeding activities during nighttime in the epilimnion. In the fishless lake, Lake Geai, chaoborids tended to eat during the whole day, although they still ate more at night, a pattern consistent with studies of Fedorenko (1972) and Croteau et al. (2003a). Migrating chaoborids of large and small sizes (*C. trivittatus* and *C. punctipennis*) had higher crop biomass at night than non-migrating species (*C. americanus*), probably to compensate for their daytime starvation.

3.4. MeHg concentrations in crops and in zooplankton

Mean MeHg concentrations in *Chaoborus* crops ($[MeHg]_{crop}$) ranged from 32 to 51 $ng\,g^{-1}$ for the different instars of *C. punctipennis* in Lake Croche, and were consistently lower than for *C. americanus* and *C. trivittatus* in Lake Geai, where they ranged from 70 to 106 $ng\,g^{-1}$ (Table 3). Cladocerans were the most contaminated planktonic group, with MeHg levels ranging from 83 to 85 $ng\,g^{-1}$ in Lake Croche and 142–243 $ng\,g^{-1}$ in Lake Geai (Table 2). Since they also constituted the main component of crop biomass for all instars in both lakes (except for *C. americanus III*), they represent the main conduit of MeHg contamination for *Chaoborus* in these systems. Indeed the least contaminated chaoborid crop in Lake Geai was associated with *C. americanus III*, because it contained 68% of copepods (per weight) and 30% of cladocerans. Similarly, in Lake Croche, *C. punctipennis* III ingested less biomass of prey and fed on less biomass of cladocerans than the IV instar (Table 3). As a result, $[MeHg]_{crop}$ was significantly ($p < 0.05$) higher in the older instar.

The second largest component of crop biomass, the copepods, had MeHg levels corresponding to 38–52% of MeHg levels of cladocerans (Table 2). MeHg content of phytoplankton (SPM) and rotifers had a limited impact on the overall concentration of MeHg in *Chaoborus* crops because of their limited contribution to the total biomass of prey.

Cladocerans have been identified as important planktonic conveyor of MeHg (Back and Watras, 1995). Since MeHg levels vary in prey items and between seasons, and since prey selectivity does not tightly reflect prey composition, our study suggests that prey contamination cannot be simply derived from MeHg levels in bulk zooplankton (Croteau et al., 2003b). The relative contribution of prey types in the crop must be taken into account.

Table 2
MeHg ($ng\,g^{-1}$) concentrations in suspended particulate matter (phytoplankton) in size class 0.7–53 μm, in rotifers (SPM in size class 53–210 μm) and in sorted zooplankton species at June (S1 and S2) and late August (S3) sampling dates. Number in bold are mean concentrations for major prey types.

	Lake Geai*		Lake Croche**	
	June (S1, S2)	Late August (S3)	June (S1, S2)	Late August (S3)
Phytoplankton (0.7 μm < SPM < 53 μm)	**60.75 ± 20.17**	**62.17 ± 3.55**	**29.87 ± 13.56**	**33.12 ± 3.87**
Rotifera (53 μm < SPM < 210 μm)	**64.16 ± 3.16**	**20.92 ± 7.83**^{a,g}	**18.10 ± 4.48**	**20.88 ± 2.78**^{d}
Copepoda, cyclopoida	_	**123.05 ± 24.83**^{b}	**60.33 ± 5.60**	**47.08 ± 7.87**^{e}
Copepoda, calanoida	**54.29 ± 9.08**	**128.97 ± 24.17**^{b,h}	**36.82 ± 20.67**	**39.11 ± 20.64**^{e}
Aglaodiaptomus leptopus	54.29 ± 9.08	128.97 ± 24.17	_	_
Leptodiaptomus minutus	_	_	61.50 ± 4.46	51.32 ± 22.62
Epischura lacustris	_	_	22.01 ± 2.54	26.89 ± 10.29
Cladocera	**141.85 ± 22.82**	**243.26 ± 58.77**^{c,i}	**85.35 ± 25.22**	**83.02 ± 18.61**^{f}
Diaphanosoma sp.	156.77 ± 11.40	_	_	_
Daphnia sp.	119.48 ± 12.46	_	84.55 ± 21.18	85.15 ± 17.31
Holopedium gibberum	_	253.26 ± 58.77	114.12 ± 15.44	101.35 ± 18.15

(*, **) indicate a significant difference ($p < 0.05$) of MeHg concentrations in prey resources between lakes. (a, b, c, d, e, f) different letters indicate a significant difference ($p < 0.05$) of MeHg concentrations between zooplanktonic groups within a lake. (g, h, i) different letters indicate a significant difference ($p < 0.05$) of MeHg concentrations between June and late August within a taxonomic group of the lake.

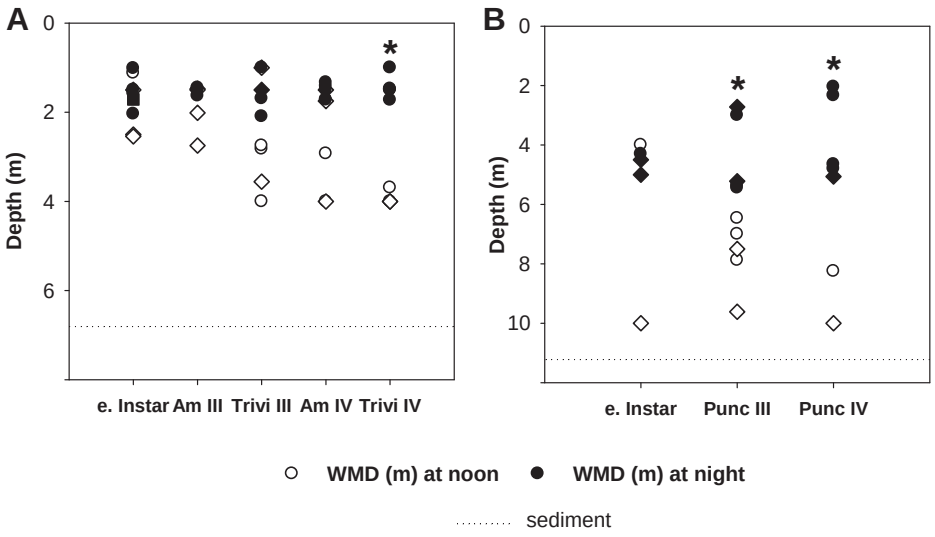

Fig. 1. Averaged weight mean depth (WMD) of *Chaoborus* species and instars at noon (open symbols) and at night (closed symbols) in the water column without sediment calculated for each sampling date (S1, S2, S3) (circles) and in the water column including sediment (diamonds) during the sampling of mid-June 2010 in Lake Geai (A) and in Lake Croche (B). WMD (daytime and at nighttime) was calculated with the equation of Frost and Bollens (1992). Standard deviation is shown and (*) indicates a significant difference ($p < 0.05$) between WMD at noon and at night for a given species or instar. Legend: e. Instar, early instar (instars I and II); III, Instar III; IV, Instar IV; Am, *C. americanus*; Trivi, *C. trivittatus*; Punc, *C. punctipennis*.

3.5. MeHg contamination of Chaoborus species

MeHg concentrations in *C. americanus* and *C. trivittatus* instars ($[MeHg]_{Chaoborus}$) in Lake Geai were 3.0–16.6 times higher than in *C. punctipennis* instars in Lake Croche (Fig. 4A–C). $[MeHg]_{Chaoborus}$ in Lake Geai chaoborids presented a marked seasonal pattern between spring (S1, S2) and summer (S3). MeHg concentrations increased, exceeding 300 ng g^{-1} by the end of August whereas levels remained in the range of 20 ng g^{-1} for *C. punctipennis* in Lake Croche. Instars of *C. americanus* and of *C. punctipennis* of different ages but sampled on the same date did not present significant difference of MeHg concentration (except *C. americanus* III at spring, S2). In contrast, *C. trivittatus* IV always had a lower ($p < 0.05$) level of MeHg than the early instars sampled on the same date. Instar IV (Trivi IV*) undergoing metamorphosis to pupal stage was also a major stage of MeHg depuration for *C. trivittatus*, with a loss in MeHg concentration of 73%.

We estimated the ability of *Chaoborus* larvae to accumulate MeHg during the growing season by the ratio of $[MeHg]_{instars\ IV}$ measured in late August to $[MeHg]_{instars\ III}$ in late spring for each species. These ratios were of 2.04 ± 0.18 for *C. americanus*, 1.47 ± 0.45 for *C. trivittatus* and 1.39 ± 0.27 for *C. punctipennis*, respectively (Fig. 4D). *C. americanus* accumulated significantly ($p < 0.05$) more MeHg during the growing season than the other species. Ratios higher than 1 indicate that all instars accumulated MeHg more rapidly than they accumulated biomass from somatic growth, during the growing season.

As chaoborid species in both lakes shared similar trophic positions (Table S4), food chain length could not explain the greater contamination of the chaoborid larvae in Lake Geai. Higher concentration of MeHg at each level of the planktonic food web in this lake was most likely linked to higher aqueous concentration of MeHg. In fact, MeHg levels in instars from Lake Geai followed the same pattern as MeHg in their prey during summer. All chaoborid

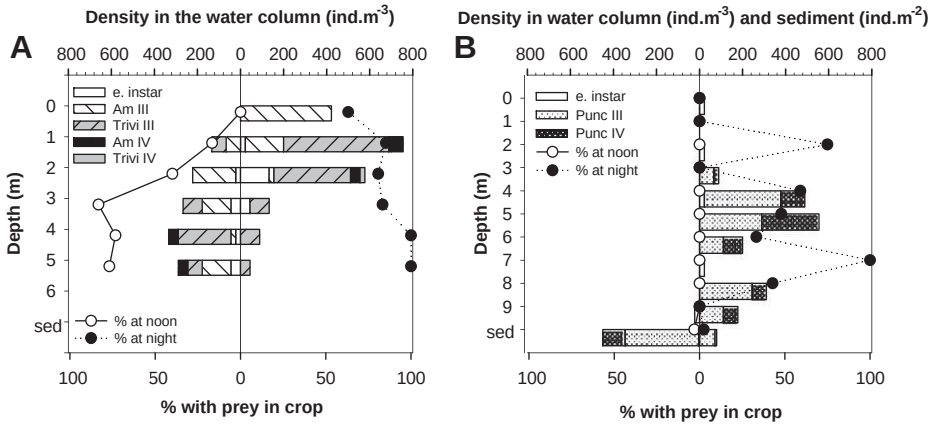

Fig. 2. Lines indicate the percentages of *Chaoborus* recorded in mid-June 2010 with at least one prey item in their crop at noon (open circles) and at night (black circles) at each 1-m interval in the water column and in sediment in Lake Geai (A) and in Lake Croche (B). Horizontal bars represent the densities in the water column (m^{-3}) and in the sediment (m^{-2}) for each species and instar collected at noon (left side) and at night (right side). Legend: e. Instar (Instars I and II), early instar; III, Instar III; IV, Instar IV; Am, *C. americanus*; Trivi, *C. trivittatus*; Punc, *C. punctipennis*.

Table 3
Diet of *Chaoborus* species based on the analysis of crop contents collected at S1, S2 and S3 sampling dates. Total number of crops analyzed with content, number of prey per crop, total biomass of prey per crop, and biomass and percentage of each prey type category (Rotifera, Copepoda, Cladocera, and Phytoplankton) recorded for each instar and chaoborid species are presented. Mean quantity (pg DW) of prey and mean concentrations (ng DW g^{-1}) of MeHg in crops over the study and at each sampling date are shown.

Chaoborus species	Lake Geai					Lake Croche	
	Early instar	*C. americanus*		*C. trivittatus*		*C. punctipennis*	
Instar	Instar II	Instar III	Instar IV	Instar III	Instar IV	Instar III	Instar IV
No. of crops analyzed	56	65	53	93	43	194	143
Number of prey $crop^{-1} \pm SD$							
Total	2.58 ± 2.74	1.76 ± 1.32	4.07 ± 6.18	6.96 ± 5.48	5.90 ± 5.01	8.36 ± 9.81	5.98 ± 11.71
Biomass of prey (μg DW) $crop^{-1} \pm SD$							
Total	1.03 ± 1.24	0.486 ± 0.595	1.04 ± 1.17	2.675 ± 3.68	2.44 ± 2.90	0.555 ± 1.20	1.29 ± 1.88
Rotifera	0.010 ± 0.017	0.009 ± 0.009	0.018 ± 0.017	0.036 ± 0.032	0.026 ± 0.03	0.035 ± 0.04	0.005 ± 0.01
Copepoda	0.398 ± 0.588	0.329 ± 0.434	0.40 ± 0.56	0.63 ± 0.53	0.85 ± 1.04	0.170 ± 0.36	0.318 ± 0.42
Cladocera	0.618 ± 0.978	0.147 ± 0.516	0.61 ± 1.05	2.00 ± 3.65	1.56 ± 2.79	0.346 ± 1.20	0.966 ± 1.88
Phytoplankton	0.000	0.000	0.0006 ± 0.004	0.000	0.000	0.004 ± 0.11	0.005 ± 0.01
Biomass of prey (%) $crop^{-1}$							
Rotifera	1.0	1.9	1.8	1.3	1.1	6.3	0.39
Copepoda	38.8	67.7	38.8	23.7	34.8	30.7	24.54
Cladocera	60.2	30.4	59.3	75.0	64.1	62.4	74.56
Phytoplankton	0.0	0.0	0.1	0.0	0.0	0.6	0.41
Estimated quantity and concentration of MeHg in crops							
Mean quantity (pg)	0.17 ± 0.24	0.05 ± 0.10	0.14 ± 0.21	0.42 ± 0.70	1.28 ± 2.65	0.04 ± 0.10	0.10 ± 0.17
Mean concentration (ng g^{-1})	105.72 ± 77.21	69.91 ± 51.39	96.24 ± 86.48	103.28 ± 62.97	103. ± 66.00	31.9 ± 21.70	50.72 ± 25.88
S1	103.65 ± 32.86	–	89.1 ± 46.63	82.14 ± 47.27	66.36 ± 42.41	26.9 ± 18.49	48.71 ± 20.74
S2	100.95 ± 32.86	66.92 ± 29.62	82.6 ± 42.65	95.57 ± 42.35	92.14 ± 44.07	35.88 ± 24.9	53.42 ± 29.12
S3	111.5 ± 87.76	73.19 ± 68.17	112 ± 106.26	138.72 ± 74.01	141.37 ± 85.6	33.55 ± 20.5	50.33 ± 26.69

NB, number; DW, dry weight; S1, S2, S3, sampling dates.

species accumulated MeHg through their growth and concentrations recorded in Lake Geai were higher by about 45–16.7 times than those previously reported for *Chaoborus* species (Back and Watras, 1995).

Metamorphosis to pupal stage was a major stage of MeHg depuration (Fig. 4D). These results contrast with the trends reported for some others dipterans for which an increase in the concentration of MeHg was observed between larvae and prepupae (Sarica et al., 2005) or between larvae and adults (Bartrons et al., 2007; Chételat et al., 2008). Differences in feeding regime or growth development between taxa could explain part of these discrepancies. Alternately, physiological differences in metal distribution could also be involved, since metamorphosis to pupae induces major histolysis and biosynthesis of tissues (Arrese and Soulages, 2010; Boggs, 2009).

3.6. MeHg biomagnification by chaoborids

C. americanus and *C. trivittatus* in Lake Geai biomagnified MeHg. MeHg concentration in *C. americanus* (III and IV) and *C. trivittatus* (III and IV) were in the range of 3.7–5.8 times and of 1.4–2.5 times higher than in their crop, respectively. In contrast, MeHg levels in *C. punctipennis* represented between 40% and 90% of those of their crop (Fig. 5). Early and third instars had higher mean BMFs than fourth instars (Fig. 5); this pattern was significant ($p < 0.05$) for *C. trivittatus IV* and *C. punctipennis IV* but not for *C. americanus IV*. In addition, a general relationship was observed in which the ratio of weighted mean depths of chaoborids between day and night was inversely related to the biomagnification factor (R^2 adjusted = 0.37, $F = 12.18$, $p < 0.0026$, $n = 20$). This trend suggests that chaoborids which migrated the least were the best MeHg biomagnifiers.

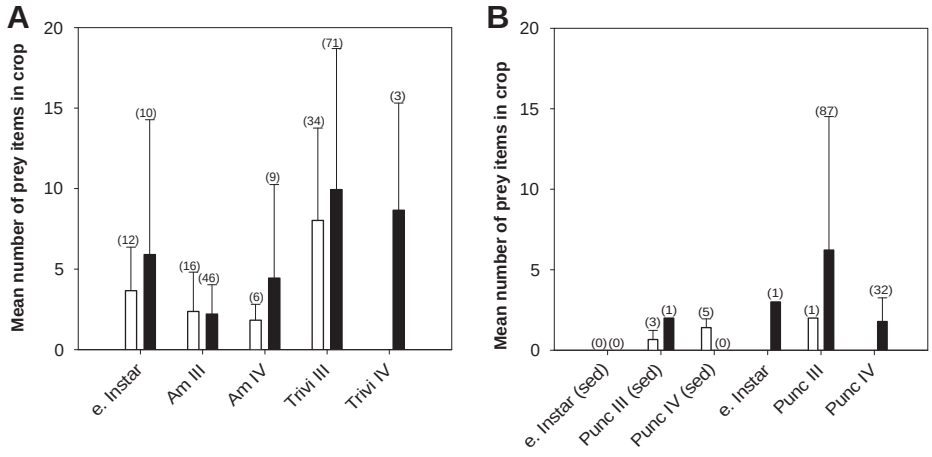

Fig. 3. Mean number of prey items (±SD) counted in each crop containing food of *Chaoborus* instars and species collected in mid-June 2010 in Lake Geai (A) and in Lake Croche (B) at day (white bars) and night (black bars). The number of crops with prey is indicated in parenthesis. Legend: e. Instar (Instars I and II), early instar; III, Instar III; IV, Instar IV; Am, *C. americanus*; Trivi, *C. trivittatus*; Punc, *C. punctipennis*; (sed), sediment.

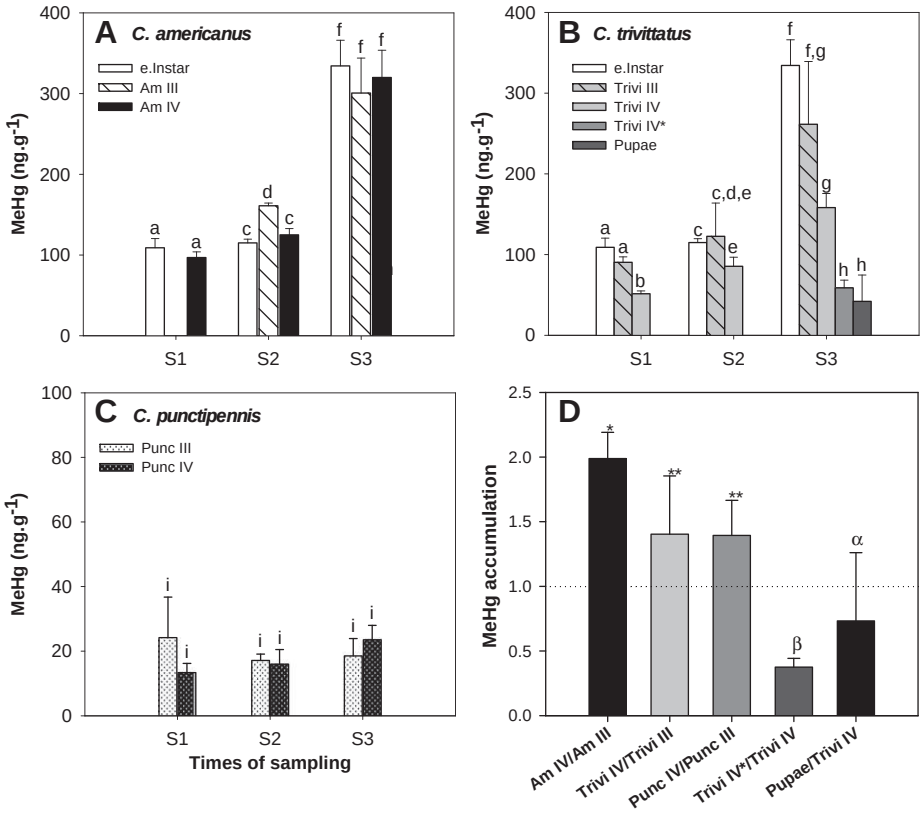

Fig. 4. Mean concentrations (±SD) of MeHg (ng g^{-1}) in instars of each *Chaoborus* species in Lake Geai (A, B) and in Lake Croche (C) at each sampling date (S1, S2, S3). Different letters (a, b, c, d, e, f, g, h, i) indicate a significant difference ($p < 0.05$) among species and instars within and between lakes. (D) MeHg accumulation through ontogenetic development of each species calculated based on the ratio between fourth instar collected in late August and the third instar collected at the end of June ($[MeHg]_{instarIV}$ S3/$[MeHg]_{instarIII}$ S2). Different numbers of stars indicate a significant difference ($p < 0.05$) between species. MeHg accumulation between different metamorphic stages collected in late August is also provided for *C. trivittatus*. α and β indicate a significant difference ($p < 0.05$) between metamorphic stages. Legend: e. Instar, early instar; III, Instar III; IV, Instar IV; Am, *C. americanus*; Trivi, *C. trivittatus*; Punc, *C. punctipennis*; Trivi IV*, instar of *C. trivittatus* IV* passing through pupal stage; Pupae, pupal stage.

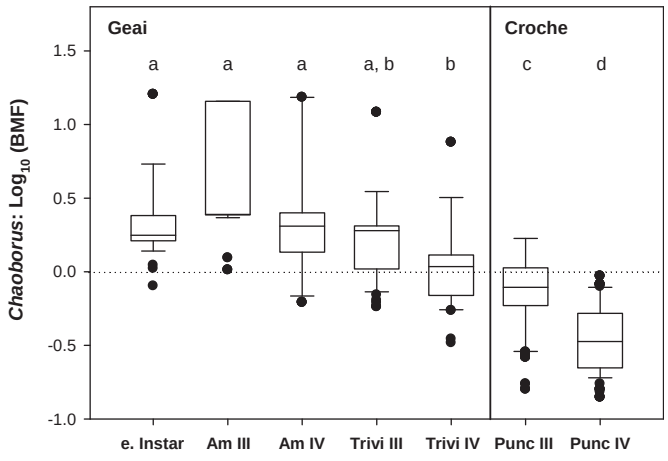

Fig. 5. Range of Log_{10} biomagnification factors (BMFs) of *Chaoborus* larvae in Lake Geai and in Lake Croche over S1, S2, S3 sampling dates. From each crop analyzed (summarized in Table 3), each BMF was calculated based on the log of the ratio between the mean concentration of MeHg measured in each *Chaoborus* species and instars and the specific concentration of MeHg estimated in each crop contents collected at the same date. Top and bottom horizontal lines of the box indicate the 75th and 25th percentiles. The horizontal line in the box represents median. Closed circles indicate extreme data. Different letters show significant differences ($p < 0.05$) in log_{10} (BMF) between instars. Legend: e. Instar, early instar; III, Instar III; IV, Instar IV; Am, *C. americanus*; Trivi, *C. trivittatus*; Punc, *C. punctipennis*.

Decrease in BMF during ontogenic development was unexpected, since accumulation increases as a function of age in many aquatic species (McIntyre and Beauchamp, 2007). This ontogenic BMF decrease was particularly significant in the case of *C. punctipennis* (Fig. 5) and may be related to an increase in diel migration with age. *C. punctipennis* was also the worst biomagnifier among all studied species, likely because it lived in fish-inhabited Lake Croche and therefore performed the longest vertical migrations (Fig. 1). Similarly, spatial vertical segregation with age reduced biomagnification capacities of *C. trivittatus* in the fishless Lake Geai, as opposed to *C. americanus*, although both species were able to feed over day and night (Figs. 2 and 3). Surprisingly, *C. americanus* which accumulated the most MeHg ingested the lowest quantities of prey relative to its size (Table 3, Table S1).

Previous studies have also shown that diel vertical migration affects the feeding activities of chaoborids (Croteau et al., 2003b; Fedorenko, 1975b; McQueen et al., 1999). Maintenance of higher feeding rate of *C. trivittatus* has been proposed as an energetic bonus in order to compensate for food depletion and lower metabolism in cold water in hypolimnion during the day (Fedorenko, 1972). In the case of Cd contamination in an experimental context, higher ingestion rates decreased Cd assimilation efficiency of *C. punctipennis*, probably due to shorter residence time of food in the gut (Munger and Hare, 2000). In addition, with a same daily ingestion rate of food, Croteau et al. (2002) observed that low temperatures (5 °C) can also affect Cd assimilation efficiency of food of the small size *C. punctipennis* but not of *C. americanus*.

We propose that the onset of diel vertical migration causes segregation between refuge habitat and resource habitat, restrain dwelling chaoborids to cooler temperatures which affects their feeding activities. Both higher ingested amounts of prey at night in upper limnetic strata and cooler temperature during the day in lower limnetic strata could reduce assimilation efficiency of MeHg.

This study is the first to report that *Chaoborus* species are able to biomagnify MeHg along food webs (Fig. 5). Differences in biomagnification potential between species could neither be linked to a shift in diet nor to a variation of trophic position in or among lakes: species and instars fed on similar prey category (Table 3) and occupied a similar trophic level according to $\delta^{15}N$ stable isotope analysis (Table S4). Rather, our data (Figs. 1 and 5 and Table 3) support the concept that biomagnification capacities of *Chaoborus* species are affected by the magnitude of diel vertical migration. Behavioral processes which can affect feeding activities and/or food assimilation are still poorly investigated in lower trophic level of pelagic food web. Our findings emphasized that they should not be underestimated in order to improve our understanding of transfers and pathways of MeHg at the base of pelagic food webs.

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Appendix A. Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.envpol.2012.02.003.

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