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Sources of organic matter and methylmercury in littoral macroinvertebrates: a stable isotope approach

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Abstract The main objective of this study was to assess organic matter (OM) and methylmercury (MeHg) sources for freshwater littoral macroinvertebrate primary consumers. The carbon and nitrogen stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$) of sources (epiphytes, macrophytes, suspended particulate matter _SPM) and of macroinvertebrate consumers were measured in a fluvial lake with extensive macrophyte beds (emergent and submerged). To determine the relative contribution of each OM source to macroinvertebrate diets we used the IsoSource model that examines all possible combinations of solutions for each source. Total and MeHg concentrations of consumers were also measured. Results show that epiphytes and macrophytes are dominant in the diet of macroinvertebrates, especially in early summer (July). In mid-summer (August), SPM constitutes a non-negligible OM source to the primary consumers. Hg concentrations were higher in epiphytes than in the other OM sources. The proportion of epiphytes in macroinvertebrate diet was positively correlated with the percentage of MeHg in their tissues. There was no relationship between SPM assimilation and Hg concentration in macroinvertebrate consumers. These results suggest that epiphytes and macrophytes constitute the main pathway of Hg bioaccumulation in littoral food webs.

Keywords Freshwater macroinvertebrates · Methylmercury · Food webs · Stable isotopes · IsoSource model

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Introduction

River-wetland systems are characterized by complex food webs, which span both terrestrial and aquatic environments (Haines and Montague 1979). The composition of organic matter (OM) in river-wetland systems is determined by the mixing of terrestrial, lacustrine, and riverine sources (Hedges et al. 1986; Albuquerque and Mozeto 1997). One of the most difficult problems in these systems is identifying the major sources of OM available to aquatic consumers. Indeed, the great temporal and spatial variability in autochthonous production and allochthonous OM in wetlands represents a major obstacle (Hedges et al. 1986). Furthermore, consumers seldom rely on a single food source during their ontogeny and a plethora of sources are potentially available to a consumer at a given time (Peterson 1999). For macroinvertebrate

primary consumers in wetlands, there are generally three groups of food sources: epiphytic algae, macrophytes (mostly submerged and emergent), and suspended particulate matter (SPM; Cummins 1973; Lamberti and Moore 1984; Frost et al. 2002).

Determining the OM source of lower trophic levels in wetlands is essential because wetlands are considered net exporters of environmental pollutants like mercury (Hg) to other systems (St. Louis et al. 1994). In wetlands, inorganic Hg is thought to be methylated by micro-organisms into methylmercury (MeHg) which is biomagnified along the food chain (Cabana and Rasmussen 1994; Sampaio da Silva et al. 2005).The methylated form of Hg is a ubiquitous neurotoxic metal and a concern for freshwater ecosystem health worldwide (Lucotte et al. 1999; Boening 2000). Important macroinvertebrate OM sources like macrophyte-epiphyte system have been described as major methylating sites (Cleckner et al. 1999; Guimarães et al. 2000; Hamelin et al. 2004). Thus, in order to better understand relationships between MeHg concentrations and dietary OM of macroinvertebrates it is necessary to assess OM sources at the base of the food chain.

In the last few decades, the use of stable isotopes in food web studies has provided a time-integrated method for assessing OM sources available to consumers (Peterson and Fry 1987; Junger and Planas 1993, 1994; Peterson 1999; Hershey et al. 2006). Until recently, most studies used two stable isotopes and a three end-member model to assess the contribution of OM sources. However, for n isotopic signatures if the number of sources is greater than $n + 1$, a unique solution establishing proportions of each OM source cannot be obtained (Phillips 2001). This means that in studies using two stable isotope signatures the maximum number of sources a given organism may feed on is three, forcing researchers to give preference to some sources and exclude others. This leads to an underestimation of the widespread omnivory of invertebrates (Zah et al. 2001), and would partially be responsible for the pooling of sources in large categories that correspond to "signals" i.e., autochthonous versus allochthonous, pelagic versus littoral (e.g., France 1995) instead of elucidating the particular proportion of each source. To deal with the multiple potential food sources, Phillips and Gregg (2003) have proposed a method that provides the range in solutions that are possible based on the constraints of mass balance. They also developed a computer program called "IsoSource" which gives a range of possible solutions. IsoSource remains routinely employed for aquatic food web partitioning (Benstead et al. 2006; Herwig et al. 2007) though Bayesian approaches to solve mixing model problems have recently been proposed (Moore and Semmens 2008 *but see* Jackson et al. 2008).

The goal of this work is to assess the contribution of the different OM sources to the diets of wetland macroinvertebrates and the relationship to diet partitioning of MeHg. We considered the following OM sources in the studied macrophyte beds: epiphyton (biofilm attached to submerged and emergent macrophytes), macrophytes (submerged and emergent), and SPM.

Methods

Study site

Our study was conducted in Lake St. Pierre, a fluvial lake of the St. Lawrence River, located in Southern Quebec, Canada (46°08′N 072°39′W). Macrophyte beds thrive in the shallow (<3 m) parts of the lake and cover 80% of the surface area (300 km^2; Vis et al. 2003). The lake presents three distinct water masses of different origins: the brown waters of the north shore, rich in dissolved organic carbon (DOC), are under the influence of the Ottawa River and other Canadian Shield tributaries and differ remarkably from the south shore waters. Tributaries from the south shore drain water from agricultural fields and carry turbid, nutrient-rich waters. Between these two water masses clear waters from the Great Lakes flow through the artificially dredged (>11 m) navigation channel, preventing the other two water masses from mixing (Vis et al. 2003).

Sampling was carried out between 0 and 1.5 m depth, at the summer macrophytes biomass peak in July and August 2004 at two stations, Girodeau Island (GIR) near the north shore and Baie St. François (BSF) on the south shore. July and August were chosen for sampling because it is during this period that macroinvertebrate productivity is greatest and that all potential OM sources are available to consumers (Cremona et al. 2008a). Both sites were covered in submerged and emergent aquatic macrophytes. GIR macrophyte cover consisted mostly of *Scirpus fluviatilis* (Torr.; emergent) and *Potamogeton perfoliatus*

(L.; submerged) in July and *S. fluviatilis* and *Elodea canadensis* Rich. (submerged) in August while BSF was covered with *Typha angustifolia* L. (emergent) and *Myriophyllum spicatum* L. (submerged) in July and *T. angustifolia* and *Ceratophyllum demersum* L. (submerged) in August. Because tissue turnover rates are rapid for small sized invertebrates during summer high temperature conditions, consumers and OM sources were sampled within a time scale of 1 week (McIntyre and Flecker 2006).

Sampling of macroinvertebrate consumers

A modified Downing box (Downing and Rigler 1984), with an increased capacity of 13 L and a handheld aquatic net were used to sample macroinvertebrates. Invertebrates were separated from their substrate by vigorous shaking. Content was sieved on a 500 μm net and transferred to Nalgene™ jars filled with lake water. In the laboratory, organisms were identified and grouped together according to taxon composition, determined in general to the family level (Pennak 1953; Clarke 1981; Merritt and Cummins 1996). When the number of individuals collected for a given taxon was insufficient for stable isotope analysis, organisms from a higher taxonomic level were grouped together. According to some recent studies (Kaehler and Pakhomov 2001; Jardine et al. 2005) gut clearing only marginally influences consumer stable isotope ratios, thus organisms were not gut cleared.

Sampling of OM sources

Macrophytes and epiphytes

Dominant submerged and emergent macrophytes were sampled at each site. In the strata from the surface to a depth of 60 cm, nine field replicates of both macrophyte types and of their associated epiphytes were sampled using 0.68 L Pac-man boxes (Downing and Rigler 1984, modified by C. Vis, Parks Canada, Cornwall, Ontario). Once in the laboratory, epiphytes were separated from macrophytes by mechanical shaking (9 min in a Red Devil® paint shaker, method previously tested for removing epiphytes without destroying algal cells). Two aliquots/field replicates of the epiphyte suspension were filtered through pre-combusted and pre-weighted GF/C filters and then kept frozen until analysis.

SPM

During summer, SPM in Lake St. Pierre represents a mixture of allochthonous detritus with a non-negligible fraction of autochtonous matter (Caron et al. 2008). Integrated water samples were collected for SPM using an electric pump equipped with a 210 μm filter followed by a 64 μm filter. The pre-filtered water was then treated by ultrafiltration using a Pellicon filter system by Millipore® with a 0.45 μm Durapore® membrane. The particles from 0.45 to 0.64 μm making up the SPM were collected and concentrated down to a volume of 1 L. The samples were then transferred to four 250 ml Nalgene® bottles and stored in a freezer until analysis.

Isotopes and Hg analyses

All samples were frozen at −80°C, a preservation method little susceptible to alteration of sample isotope ratios (Ponsard and Amlou 1999). The macroinvertebrate samples contained between 3 and 100 individuals. Gastropods shells and opercula which may influence the bulk $\delta^{13}C$ signature value (Kaehler and Pakhomov 2001) were manually removed prior to analysis. Samples were then freeze-dried for 24 h and grounded manually with a 10% HCl rinsed glass rod. For the epiphytes, filters were dried in an oven at 40°C until constant weights were achieved. Carbon to nitrogen atomic ratios (C/N), an indicator of nutritive value (the lower the more nutritive), was measured in OM sources by a Carlo-Erba™ at the Geochemistry Geodynamics Research Center (GEOTOP-UQÀM-McGill).

Isotopic analyses were performed with an Isotopic Resolution Micromass™ mass spectrometer. Isotope ratios were expressed in parts per thousand (‰) relative to the reference material following the standard equations (Verardo et al. 1990):

$$\delta^{13}C = \left[\left({}^{13}C/{}^{12}C_{sample}/{}^{13}C/{}^{12}C_{standard}\right) - 1\right] \times 1000 \quad (1)$$

$$\delta^{15}N = \left[\left({}^{15}N/{}^{14}N_{sample}/{}^{15}N/{}^{14}N_{standard}\right) - 1\right] \times 1000 \quad (2)$$

For C, this reference is Vienna Pee Dee Belemnite (VPDB) and for N, it is atmospheric nitrogen (N_2). Repeated analyses of an internal standard ($n = 3$ for each group of 20–50 samples) resulted in typical precision of ±0.1‰ for $\delta^{13}C$ and ±0.2‰ for $\delta^{15}N$.

Total Hg concentrations ([THg]) were obtained with cold vapor fluorescence atomic spectrometry (CVFAS; Bloom 1989) with a detection limit of 1 ng g^{-1}. Methymercury concentrations ([MeHg]) were analyzed using Bloom (1989) method modified by Pichet et al. (1999). Inorganic Hg was calculated by the difference between [THg] and [MeHg] in ng g^{-1} dry weight (DW). More details about Hg analyses in invertebrates can be found in Cremona et al. (2008b).

Data treatment

At each station (BSF, GIR) and for each sampling month (July, August), five OM sources were considered as available to invertebrate consumers: submerged macrophytes, emergent macrophytes, epiphytes growing on submerged macrophytes, epiphytes growing on emergent macrophytes, and SPM. Emergent and submerged macrophytes and their epiphytes were considered separately because of differences in $\delta^{13}C$ signatures (Peterson and Fry 1987; Keough et al. 1998). The isotopic value for each combination (OM sources and consumer) was computed using IsoSource software (Phillips and Gregg 2003). All possible contributions of each source combination (0–100%) were examined using specified small (1%) increments with a tolerance value starting at 0.05‰. If mixture isotopes were outside the polygon delineated by the food end members, the tolerance value was incrementally increased by 0.05‰ up to 0.2‰. We reported the range as the 1st to the 99th percentile of solutions of source contribution as recommended by Phillips and Gregg (2003).

We assumed a 0.4‰ fractionation for $\delta^{13}C$ carbon isotopes per trophic level for all organisms (Post 2002). For $\delta^{15}N$, the 3.4‰ source to consumer enrichment has been questioned for invertebrates. Recent studies showed that the fractionation value is probably lower (Zah et al. 2001; Anderson and Cabana 2005), especially for primary consumers (McCutchan et al. 2003). We thus applied a fractionation of 2.2‰ for herbivorous organisms (feeding on emergent and submerged macrophytes and their respective epiphytes; McCutchan et al. 2003). The mean 2.3‰ fractionation reported by McCutchan et al. (2003) for mixed diet organisms was applied to SPM, as SPM may contain plant material and microorganisms. At each station and for a given month, the values of the potential diet sources were generally significantly different (Tukey HSD, $p < 0.05$) with a few exceptions. At BSF, the signatures of the epiphytes growing on *M. spicatum* and *T. angustifolia* in July were merged according to the a priori aggregation method (Phillips et al. 2005) because they were not significantly different (Tukey HSD, $p > 0.05$). We did the same for the epiphytes on *C. demersum* and *T. angustifolia* in August. As recommended by Phillips and Gregg (2003) for graphical representation, mean as well as minimum and maximum proportions for each source were used. Isotopic signatures and Hg concentrations of organisms between sampling months and stations were compared by ANOVA followed by Tukey's honestly significant differences (HSD) multiple comparison tests when more than two groups were being compared (SAS Institute Inc. 1991; α set at 5%). Relationships between organic matter source proportions and other variables were calculated using Pearson pair-wise correlations (Legendre and Legendre 1998) and simple linear regressions.

Results

C/N ratios and isotopic signatures of OM sources and primary consumers

OM sources

C/N atomic ratios varied among OM source categories. Epiphytes presented the lowest ratios, SPM and submerged macrophytes were intermediate and emergent macrophytes the highest. Epiphyte C/N ratios varied from 8.5 for epiphytes collected on *S. fluviatilis* at GIR in August to 14.8 for epiphytes on *T. angustifolia* at the BSF station in August (for macrophytes, the C/N ratio varied between 30 and 129 for emergent macrophytes, and between 11.5 and 27.7 for submerged macrophytes, Table 1). The C/N ratios of the SPM were comprised between 12.4 and 24. Temporal variations in C/N ratios were observed for all sources. For the two emergent macrophyte species and for epiphytes on emergent and submerged macrophytes, C/N ratios increased up to four times between July and August (Table 1). On the other hand, C/N ratios for SPM and submerged macrophytes decreased between July and August; however, the macrophyte species collected in July were not always the same ones found in August.

Table 1 Mean ± SE of $\delta^{13}C$ and $\delta^{15}N$ signatures (‰), C/N atomic ratios, and THg and MeHg concentrations (ng g^{-1} for organisms, ng L^{-1} for SPM) of organic matter sources at Baie St. François (BSF) and Girodeau Island (GIR) in July and August

Organic matter sources	$\delta^{13}C$	$\delta^{15}N$	*n*	C/N	*n*	[THg]	[MeHg]	*n*
Macrophytes								
T. angustifolia (BSF, July)	−27.7 ± 0.4	5.8 ± 0.4	3	29.9	1	2.6 ± 0.1	3.0	3
T. angustifolia (BSF, August)	−27.4 ± 0.4	6.5 ± 1	2	129.4	1	1.4	0.8 ± 0.1	3
M. spicatum (BSF, July)	−20.7 ± 0.5	7.4 ± 0.4	2	27.7	1	12.3 ± 0.8	2.9 ± 0.1	3
C. demersum (BSF, August)	−31.3 ± 0.4	12.5 ± 1	2	11.5	1	17.2 ± 0.9	1.0 ± 0.1	3
S. fluviatilis (GIR, July)	−27.6 ± 1.1	5.4 ± 0.3	3	46.3	1	2.3	0.6	3
S. fluviatilis (GIR, August)	−28 ± 0.1	4.5 ± 0.3	2	71.6	1	1.9	0.9	3
P. perfoliatus (GIR, July)	−16.5 ± 1.1	8.1 ± 0.3	3	23.6	1	5.8 ± 0.3	1.3 ± 0.1	3
E. Canadensis (GIR, August)	−14.7 ± 0.1	7.7 ± 0.3	2	16.2	1	8.1 ± 0.3	1.4	3
Epiphytes growing on:								
T. angustifolia (BSF, July)	−24.8 ± 0.2	9.8 ± 0.1	18	9.8 ± 0.1	18	32.8 ± 1.3	7.4 ± 0.1	3
T. angustifolia (BSF, August)	−28 ± 0.2	12.2 ± 0.6	6	14.8 ± 0.5	5	50.1 ± 1.5	8.3 ± 0.1	3
M. spicatum (BSF, July)	−24.1 ± 0.3	9.8 ± 0.3	6	7.4 ± 0.2	6	40.6 ± 1.6	9.7	3
C. demersum (BSF, August)	−27.5 ± 0.2	12.9 ± 0.6	6	9.4 ± 0.4	6	58 ± 0.1	4.3	3
S. fluviatilis (GIR, July)	−22 ± 0.3	6.9 ± 0.1	36	8.5 ± 0.3	18	137.2 ± 1.2	4.2 ± 0.2	3
S. fluviatilis (GIR, August)	−20 ± 0.2	8.4 ± 0.2	6	9.2 ± 0.2	6	148.1 ± 1.6	2.6 ± 0.1	3
P. perfoliatus (GIR, July)	−15.3 ± 0.6	6.7 ± 0.1	12	9.6 ± 0.4	6	109.5 ± 5.2	4.2 ± 0.1	3
E. Canadensis (GIR, August)	−13.1 ± 0.7	7.8 ± 0.2	6	10.9 ± 0.2	6	63.7 ± 1.6	3.9 ± 0.1	3
SPM (BSF, July)	−17.7	4.6	1	23.9 ± 0.4	3	1	0.14	3
SPM (BSF, August)	−23.9	7.1	1	12.4 ± 0.4	3	0.8	0.2	3
SPM (GIR, July)	−11.3	4.8	1	17.7 ± 2.8	3	0.13	0.01	3
SPM (GIR, August)	−15.6	4.7	1	12.4 ± 0.3	3	0.35 ± 0.1	0.02	3

At each station and for each month, carbon isotopes provided good discrimination (at least >2‰; Fig. 1) among aquatic OM primary producers. Differences in $\delta^{15}N$ were narrower compared to carbon $\delta^{13}C$ (>1‰). Epiphytes on emergent plants had a distinct signature in relation to their substrate (>4‰ for $\delta^{13}C$ and >2‰ for $\delta^{15}N$). Differences between $\delta^{13}C$ of epiphytes sampled on emergent and on submerged plants were conspicuous. The $\delta^{13}C$ of epiphytes from emergent plants were at least >6‰ lower in relation to the epiphytes from submerged plants (Fig. 1c, d).

The signatures of OM in Lake St. Pierre encompassed a wide range of isotopic values. $\delta^{13}C$ extended roughly over 20‰, with the most depleted C signature observed for the submerged macrophyte *C. demersum* at BSF in August (−31.3 ± 0.4‰) and the most enriched for SPM at GIR in July (−11.3‰, Table 1; Fig. 1b, c). The $\delta^{15}N$ varied between 7 and 15‰, the lowest average was 4.5 ± 0.3‰ for emergent *S. fluviatilis* at GIR in August, while the highest average signature was measured in epiphytes growing on *C. demersum* at BSF in August (12.9 ± 0.6‰). The spatial distribution of $\delta^{13}C$ signatures indicated that OM sources were more depleted in BSF than in GIR, particularly in August, while the reverse was observed for $\delta^{15}N$ values. Temporal variations were found in SPM signatures at the two stations, $\delta^{13}C$ and $\delta^{15}N$ shifted by −5 and 2.3‰ respectively, in BSF and by −4 and 2‰ in GIR from July to August.

Primary consumers

After correction for fractionation, a majority of taxa fell within the polygons delineated by the OM source end members, suggesting that the main OM sources had successfully been sampled at each station and each month (Fig. 1). Signatures of both isotopes differed less between macroinvertebrate taxa than between the OM sources. The $\delta^{13}C$ signatures ranged from −27.1‰ in Prosobranchia to −13.6‰ in Pulmonata and the $\delta^{15}N$ from 6.9‰ in Baetidae to

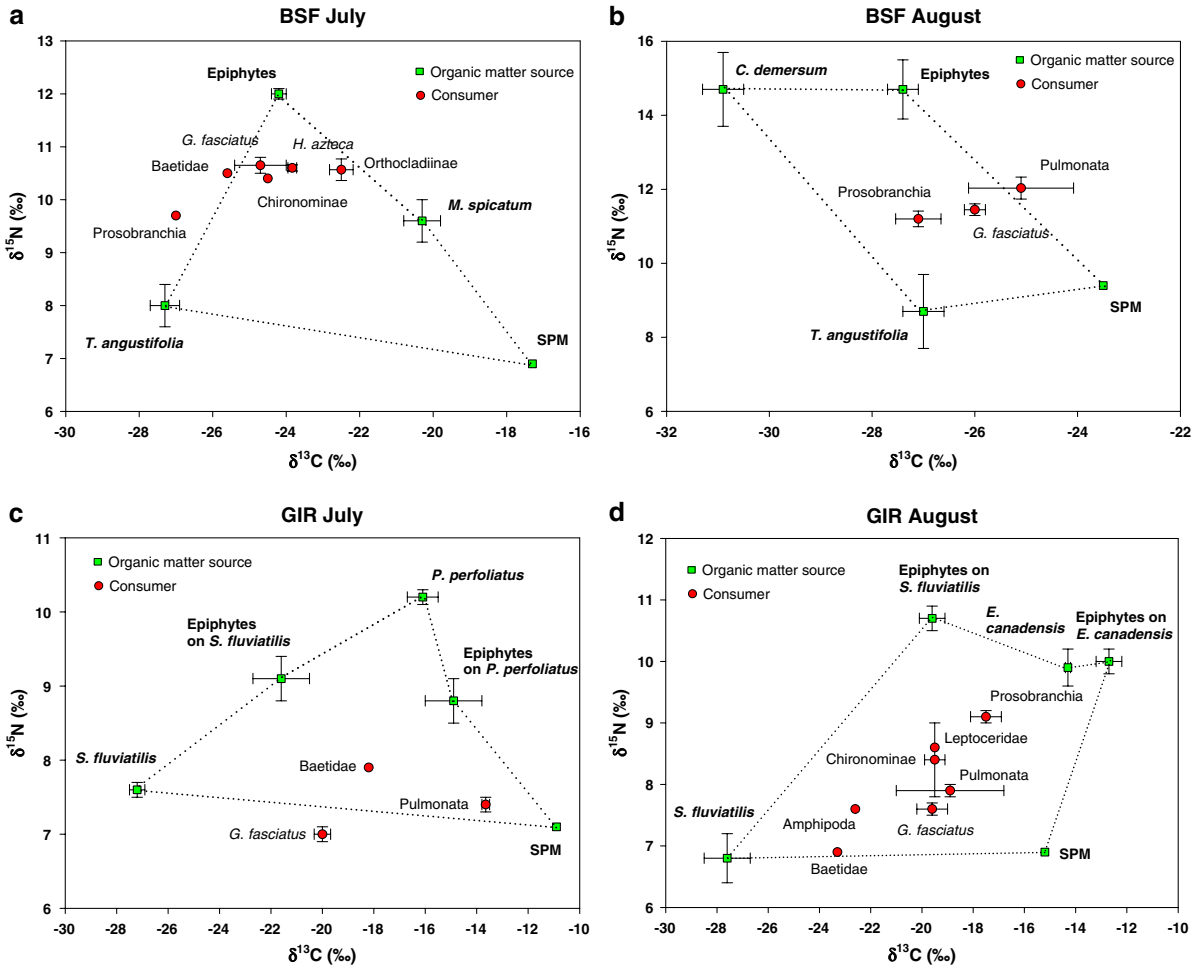

Fig. 1 Mean ± SE of stable isotope ratios (δ^{15}N vs. δ^{13}C) of OM sources (*squares*) and macroinvertebrate primary consumers (*circles*) from Lake St. Pierre. **a** BSF station in July 2004 **b** in August 2004 **c** GIR station in July 2004 **d** in August 2004. Source ratios are corrected by +0.4‰ for δ^{13}C, and +2.2‰ (epiphytes, macrophytes) or +2.3‰ (SPM) for δ^{15}N because of fractionation by consumers. *Dotted lines* represent mixing polygon limits

12‰ in Pulmonata (Table 2). Similar to OM sources, the majority of invertebrate taxa present at both stations tended to be more depleted in δ^{13}C and more enriched in δ^{15}N at BSF compared to GIR (ANOVA, *Gammarus fasciatus* in July $p < 0.05$ for δ^{13}C, $p < 0.001$ for δ^{15}N; *G. fasciatus* in August $p < 0.0001$ for δ^{13}C and δ^{15}N; Prosobranchia in August $p < 0.001$ for δ^{13}C, $p < 0.0001$ for δ^{15}N; Pulmonata in August $p = 0.15$ for δ^{13}C, $p < 0.001$ for δ^{15}N). Monthly variations in N signatures were observed, with δ^{15}N increasing from July to August while δ^{13}C varied insignificantly (ANOVA, *G. fasciatus* at BSF $p = 0.29$ for δ^{13}C, $p < 0.05$ for δ^{15}N; *G. fasciatus* in GIR $p = 0.57$ for δ^{13}C, $p < 0.01$ for δ^{15}N).

Contributions of the different OM sources

There was a high variability in the contributions of OM sources to consumer diet (Table 3). The majority of OM in invertebrate diets came from epiphytes and macrophytes, with a minor influence of SPM. At BSF, epiphytes (July) and epiphytes–macrophytes (August) constituted the bulk of the diet of primary consumers with a minimum contribution never lower than 52%. At GIR, the invertebrates shifted from a mixed diet in July to a more epiphytes–macrophytes oriented diet in August. Taxonomic differences were generally unconspicuous. Within the Gastropoda class, Pulmonata exhibited a more detritivorous diet compared

Table 2 Average ± SE of $\delta^{13}C$ and $\delta^{15}N$ signatures (in ‰), total and methylmercury concentrations ([THg], [MeHg] in ng g^{-1} DW) of macroinvertebrate primary consumers at BSF and GIR in July and August

Macroinvertebrate taxon	$\delta^{13}C$	$\delta^{15}N$	[THg]	[MeHg]	*n*
Baetidae (BSF, July)	−25.6	10.5	46	28	1
Baetidae (GIR, July)	−18.2	7.9	45	31	1
Baetidae (GIR, August)	−23.3	6.9	191	61	1
Chironominae (BSF, July)	−24.5	10.4	60	45	1
Chironominae (GIR, August)	−19.5 ± 0.4	8.4 ± 0.6	77.5 ± 11.5	55 ± 18	2
Orthocladiinae (BSF, July)	−22.5 ± 0.3	10.5 ± 0.2	39.3 ± 1.6	28.3 ± 1.2	3
G. fasciatus (BSF, July)	−24.7 ± 0.7	10.6 ± 0.1	58 ± 3	49 ± 1	2
G. fasciatus (BSF, August)	−26 ± 0.2	11.4 ± 0.1	62.5 ± 9.2	47.5 ± 3.3	4
G. fasciatus (GIR, July)	−20 ± 0.3	7 ± 0.1	60 ± 3.7	51.7 ± 4.7	4
G. fasciatus (GIR, August)	−19.6 ± 0.6	7.6 ± 0.1	75.4 ± 8.9	64.6 ± 8.1	7
H. azteca (BSF, July)	−23.8 ± 0.1	10.6 ± 0.1	46 ± 4.7	38 ± 4	3
Amphipoda (GIR, August)	−22.6	7.6	181	131	1
Prosobranchia (BSF, July)	−27	9.7	88	50	1
Prosobranchia (BSF, August)	−27.1 ± 0.4	11.2 ± 0.2	111.9 ± 6.7	53.7 ± 4.6	8
Prosobranchia (GIR, August)	−17.5 ± 0.6	9.1 ± 0.1	34.7 ± 16.2	25 ± 8	2
Pulmonata (BSF, August)	−25.1 ± 1	12 ± 0.3	74.3 ± 14.9	32.7 ± 5.9	2
Pulmonata (GIR, July)	−13.6 ± 0.1	7.4 ± 0.1	51.5 ± 4.5	22 ± 7	2
Pulmonata (GIR, August)	−18.9 ± 2.1	7.9 ± 0.1	91 ± 2	64 ± 4	2
Leptoceridae (GIR, August)	−19.5	8.6	39	32	1

to Prosobranchia. Insects (Baetidae, Leptoceridae, Chironominae, and Orthocladiinae) were generally insignificantly more phytophageous than other classes.

Hg concentrations of sources and consumers

For epiphytes and macrophytes, THg concentrations ranged between 1.4 ng g^{-1} DW (*T. Angustifolia*, BSF, August) to 148 ng g^{-1} DW (epiphytes on *S. fluviatilis*, GIR, August). Generally, epiphytes exhibited concentrations at least one order of magnitude higher than macrophytes. SPM had low concentrations throughout the study (0.13–1 ng L^{-1}). MeHg concentrations varied between 0.6 and 3 ng g^{-1} DW in macrophytes to 2.6–8.3 ng g^{-1} DW in epiphytes. In SPM, MeHg concentrations were low again (0.01–0.14 ng L^{-1}).

For macroinvertebrate consumers, THg concentrations ranged between 35 ng g^{-1} DW in Prosobranchia, to 191 ng g^{-1} DW in Baetidae (Table 2). MeHg concentrations ranged between 25 ng g^{-1} DW in Prosobranchia and 131 ng g^{-1} DW in Amphipods. The percentage of MeHg relative to THg in primary consumers ranged from 32% in Baetidae in August to 86% in some *G. fasciatus*. THg and MeHg concentrations were more elevated in August than in July (test effect model followed by ANOVA, $p < 0.05$), while they did not differ between stations (ANOVA, $p > 0.05$). The mean adjusted concentrations of THg increased from 64 ± 8 ng g^{-1} DW in July to 89 ± 7 ng g^{-1} DW in August while MeHg concentrations only rose from 45 ± 4 to 56 ± 4 ng g^{-1} DW during the same period of time. Thus, MeHg/THg diminished in macroinvertebrates from July to August.

Relationships between OM sources and Hg concentrations

There were significant relationships between OM sources calculated from IsoSource outputs and Hg concentrations in macroinvertebrate primary consumers (Table 4). The proportion of macrophytes in the diet was correlated with THg concentrations (Pearson correlation coefficient = 0.71, $p < 0.002$) and MeHg concentrations, but with a lower correlation coefficient ($r = 0.56$, $p < 0.05$). The proportion of epiphyte contribution to the consumer diet was not correlated with MeHg concentrations ($p = 0.19$), but was correlated positively with MeHg/THg ($r = 0.59$, $p = 0.01$)

Table 3 Range of organic matter source contributions for macroinvertebrate consumers (in %) using the IsoSource mixing model (mean value in brackets)

	Organic matter sources					
	E.Sub.[a]	E.Eme.[b]	E.Sub. + E.Eme.[c]	M.Sub.[d]	M.Eme.[e]	SPM[f]
BSF July						
Baetidae	NS	NS	NS	NS	NS	NS
Chironominae	–	–	52–63 (57.8)	0–17 (8.4)	28–31 (29.6)	0–9 (4.2)
G. fasciatus	–	–	60–67 (63.5)	0–11 (5.4)	27–30 (28.6)	0–6 (2.5)
H. azteca	–	–	53–69 (61.6)	0–27 (13.2)	17–20 (18.5)	0–14 (6.7)
Orthocladiinae	–	–	41–70 (56.1)	0–51 (25)	3–8 (5.8)	0–27 (13.1)
Prosobranchia	NS	NS	NS	NS	NS	NS
GIR July						
Baetidae	0–39 (12.3)	0–36 (11.7)	–	0–21 (6.5)	21–39 (32.1)	26–44 (37.5)
G. fasciatus	NS	NS	NS	NS	NS	NS
Pulmonata	0–15 (5)	0–16 (4.7)	–	0–9 (2.3)	0–14 (11.6)	72–80 (76.5)
BSF August						
G. fasciatus	–	–	6–42 (24.5)	0–31 (14.9)	0–27 (12.7)	32–63 (47.9)
Prosobranchia	–	–	0–42 (21.7)	0–37 (17.7)	24–58 (41)	1–39 (19.6)
Pulmonata	–	–	0–52 (25.2)	0–82 (40.1)	7–30 (19.1)	11–20 (15.6)
GIR August						
Amphipoda	0–26 (8.3)	0–21 (6.6)	–	0–27 (8.5)	52–65 (59.6)	9–28 (17)
Baetidae	0–3 (0.9)	0–3 (0.9)	–	0–2 (0.6)	64–66 (65.3)	31–34 (32.3)
Chironominae	0–51 (16.7)	0–41 (13.3)	–	0–51 (17.2)	20–45 (34.6)	4–40 (18.3)
G. fasciatus	0–24 (8)	0–20 (6.4)	–	0–26 (8.3)	28–40 (35.4)	35–53 (42)
Prosobranchia	0–65 (21.9)	0–55 (20.5)	–	0–75 (25.3)	0–30 (17.5)	0–42 (14.7)
Pulmonata	0–35 (11.2)	0–28 (8.9)	–	0–35 (11.4)	20–37 (29.8)	28–53 (38.6)
Leptoceridae	0–54 (18)	0–46 (15.4)	–	0–59 (19.7)	18–45 (34.2)	0–37 (12.7)

NS no solution

[a] Epiphytes growing on submerged macrophytes

[b] Epiphytes growing on emergent macrophytes

[c] Mix of epiphytes growing on submerged and emergent macrophytes (C and N isotopic signatures not significantly different)

[d] Submerged macrophytes

[e] Emergent macrophytes

[f] Suspended particulate matter

and negatively with THg ($r = -0.59$, $p < 0.05$). No relationship was found between Hg concentrations in primary consumers and assimilated SPM ($p > 0.05$). The proportion of epiphyte contribution in the macroinvertebrate diet was positively correlated with the MeHg concentrations in epiphytes (Epiphytes assimilated $= -3.28 + 6.57[\text{MeHg}]_{\text{epi}}$, $r^2 = 0.58$, $p < 0.0005$, $n = 16$). There was no relationship between the proportions of macrophyte contribution to the consumer diet and MeHg concentration in macrophytes ($p = 0.16$) nor with SPM and MeHg concentration in SPM ($p = 0.19$).

Discussion

Contribution of the OM sources to macroinvertebrate diet

In contrast to the study by Herwig et al. (2007) conducted in the floodplain of the Mississippi River, the majority of consumer signatures in the St. Lawrence River fell within the mixing polygon defined by the potential OM sources. However, for a few consumers there was no feasible solution with the OM sources we employed, even when the mass balance

Table 4 Pearson pair-wise correlations of percentage of food source contributions and different forms of Hg concentrations (in ng g^{-1} DW) of macroinvertebrate primary consumers in Lake St. Pierre

Row	THg	MeHg	MeHg/THg	Epiphytes	Macrophytes	SPM
THg	1					
MeHg	0.78 (**0.0003**)	1				
MeHg/THg	−0.49 (0.054)	0.07 (0.80)	1			
Epiphytes	−0.59 (**0.015**)	−0.35 (0.19)	0.59 (**0.01**)	1		
Macrophytes	0.71 (**0.002**)	0.56 (**0.02**)	−0.29 (0.28)	−0.37 (0.15)	1	
SPM	0.05 (0.86)	−0.09 (0.74)	−0.37 (0.16)	−0.71 (**0.002**)	−0.39 (0.13)	1

Significant correlations (p) are presented in bold characters

tolerance value was raised (as recommended by Phillips and Gregg 2003) up to 0.2‰. The absence of solution for some taxa is consequently more related to a missing OM source than to a mixing model issue. For the organisms that fell outside the polygon because of their low $\delta^{15}N$ signatures, this is probably caused by the assimilation of sources depleted in ^{15}N we did not sample like terrestrial coarse organic matter (i.e., leaves and terrestrial plant detritus; Vannote et al. 1980). Among the OM sources considered, epiphytes and macrophytes made the greatest contributions to the diets of Lake St. Pierre macroinvertebrate primary consumers. SPM also constituted a non-negligible food source, although mostly in August when SPM was enriched in N (lower C/N ratio) indicating a greater content of autochthonous material.

The IsoSource model output data indicated that there was a considerable variation in the contribution of each source for a given taxon, often from 0 to 50% or more. This large range in contributions is due to the array of possible solutions calculated by the IsoSource model (Phillips and Gregg 2003). At some stations, for instance at BSF in July, the range is narrower, as indicated by the proximity of primary consumers and epiphytes signatures. On the other hand, at GIR in August, the signatures of the consumers are in the middle of the mixing polygon and thus are roughly equidistant from several organic matter sources. In this latter case, additional information is needed to discriminate among food provenances. Knowledge of the consumer organic matter preferences as well as of the palatability of the sources could permit a narrowing of the range of possible solutions given by the IsoSource model. Most taxa of littoral macroinvertebrate primary consumers in our study are mainly opportunistic feeders (Clarke 1981; Jacobsen and Sand-Jensen 1995; Merritt and Cummins 1996; Tate and Hershey 2003). These organisms have fairly unspecialized mouth parts, such as the chewing mandibles of amphipods, chironomids, mayflies, caddisflies, or the radulae of gastropods that can collect detritus and algae in the periphytic biofilm as well as macrophyte tissue (Clarke 1981; Carlsson and Brönmark 2006). Thus, they could potentially feed on any of the food sources if their nutritional quality was similar.

The C/N ratios are indicators of the nutritional quality of the food sources (Tuchman et al. 2003). The large range illustrates the considerable differences in the nutritional quality of the available OM sources and could influence selective feeding by primary consumers. For example, the C/N ratio of decaying *T. angustifolia* at BSF in August was 130, nearly ten times the ratio for epiphytes growing on this macrophyte species. Herbivores are thus very unlikely to use this OM source once they have scraped the epiphyte film covering it. Macroinvertebrate herbivores usually assimilate the food source with greater nutrient content when different sources are available (Rincón and Martínez 2006). On the other hand, when the C/N ratios of macrophytes and epithytes are very close to each other, macroinvertebrates might potentially feed on both OM sources, though assimilating macrophytes with a lower efficiency. This was probably the case, for example, for *C. demersum*, epiphytes, and SPM that had similar C/N ratios (11.5, 9.4, and 12.4, respectively) in August at BSF. Thus, N-rich particulate matter could also constitute a substantial dietary resource for macroinvertebrates. As suggested by the $\delta^{13}C$ and $\delta^{15}N$ values of the OM sources and nutritional quality (C/N ratios), macroinvertebrates at BSF appeared to switch

from an epiphyte diet in July to a mix of epiphytes, submerged macrophytes and SPM in August.

At GIR, however, OM preferences could not be as clearly established. C/N ratio rankings of the sources (epiphytes < SPM < submerged macrophytes < emergent macrophytes) were to some extent similar to what had been observed at BSF. In spite of C/N ratios indicating that substrate vascular plants had slightly less nutritional quality than the epiphytes (C/N = 10.9 for the epiphytes and 16.2 for the plant) invertebrates may have included *E. canadensis* in their diet. At GIR in August, the macroinvertebrate herbivores may also have ingested *S. fluviatilis* (C/N ratio = 71) as a dietary resource despite the high C/N ratio. If this plant is not considered in the diet, no other OM source could explain the $\delta^{13}C$ signatures below −19‰ found in consumers at this station in August.

The contribution of macrophytes to aquatic herbivorous invertebrate diets seems surprising since they are generally considered to be of lower nutritional quality compared to algae (Kitting et al. 1984; Brönmark 1989; Suren and Lake 1989). But several studies report invertebrates grazing upon live (Sheldon 1987; Jacobsen and Sand-Jensen 1995; Elger and Willby 2003; Elger and Lemoine 2005; Carlsson and Brönmark 2006) and decaying (James et al. 2000) aquatic vascular plants. Carlsson and Brönmark (2006) observed individuals of the herbivorous snail *Pomacea canaliculata* grazing on the soft parts of macrophytes from the inside out after having penetrated the plant via a decaying spot in the fibrous cuticle. At our sampling sites we observed the same behavior in amphipods and some chironomid taxa. These organisms derive protection from predators and decreased competition with other herbivores for food in exchange for a less nutritive, but plentiful OM source (Merritt and Cummins 1996). This trade-off may be more common in late summer (August) when competition for food is high since grazer populations are very well established and the amount of periphytic algae is reduced by overgrazing (Cattaneo 1983).

It has been demonstrated that ^{15}N trophic fractionation values can vary significantly according to the diet quality (Webb et al. 1998). Organisms feeding on low quality, high C/N ratio food sources can be enriched up to 5‰ relative to diet. These physiological properties would cause some modifications in the mixing polygon. The $\delta^{15}N$ signature of poorly nutritive sources like emergent macrophytes would be moved further from the consumers, especially at GIR in August. Thus, considering that fractionation values are held constant and are assumed invariant regardless of nutritional quality probably causes an overestimation of macrophytes and other low-quality OM source contributions. Unfortunately, IsoSource must be parameterized with using single values for $\delta^{13}C$ and $\delta^{15}N$ of OM sources and consumers, this issue is thus difficult to address. Very recently, Bayesian approaches have been tested and appear very promising, though their behavior is still discussed (Jackson et al. 2008).

Terrestrial OM inputs, illustrated partly in our study by SPM signature (Caron et al. 2008), represented a smaller dietary resource for macroinvertebrate primary consumers in Lake St. Pierre. This is in agreement with a recent study by Clapcott and Bunn (2003), which reported low contributions of C_4 plants to aquatic food webs, despite their widespread distribution and production. Thus, the high density of corn fields in the Lake St. Pierre watershed could have a lower than expected contribution to consumer assimilated diets. This finding implies that the invertebrate food web in Lake St. Pierre mostly depends on autochthonous OM. Nevertheless, differences in $\delta^{15}N$ ratios between the south (BSF) and north (GIR) shore stations indicated that terrestrial inputs did influence the wetland. Organisms from the south shore tended to have a higher $\delta^{15}N$ signature, not because of their consumption of terrestrial detritus but because of the probable uptake by primary producers of inorganic, ^{15}N enriched nitrogen exported by the watershed. This isotopic signal then appears to be propagated through the entire lake food web (Anderson and Cabana 2005). Differences in plant productivity (greater in the southern water masses) could also explain these discrepancies. Indeed, the combination of greater plant productivity and decrease of runoff during the summer months could lead to a limitation of the inorganic nitrogen pool and thus an increase of ^{15}N in primary producers (Hudon and Carignan 2008).

Links between Hg concentrations and OM sources

The IsoSource model has been employed in recent food web studies (Hall-Aspland et al. 2005; Benstead et al. 2006; Gerardo Herrera et al. 2006; Herwig et al. 2007), but the utilization of IsoSource to approach the issue of contaminant transfer in food webs is far less common. In our study, we found a correlation between the mean

contributions of some OM sources and the concentrations of THg and MeHg in consumers. However, the use of mean values output of IsoSource must be approached with caution as recommended by Phillips and Gregg (2003). Macrophytes seem to be a source of both inorganic Hg and MeHg to invertebrates. However, a stronger relationship between assimilation of macrophytes with THg than with MeHg suggests that macrophytes are more a source of inorganic than organic (Me-) Hg. Our results showed that macrophytes contain low concentrations of both inorganic and methylated mercury forms. It is thus unlikely that macrophytes are the main source of MeHg for invertebrate primary consumers.

It seems dubious that SPM contribute to the bioaccumulation of MeHg in macroinvertebrates for three reasons. First, it is less assimilated by invertebrates than epiphytes and macrophytes. Second, the MeHg concentrations in SPM are very low (Caron et al. 2008), much lower in fact than those of the other OM sources. Third, we found no relationships between SPM proportion in consumer diet and Hg concentrations in the consumers.

On the other hand, epiphytic biofilms seem to be an important source of MeHg for macroinvertebrate herbivores. It is suggested by the positive relationship between the proportion of epiphytes assimilated by macroinvertebrates and (1) the MeHg/THg ratio (2) MeHg concentrations in epiphytes. These relationships, combined with the high MeHg concentrations in epiphytes, higher indeed than the two other potential MeHg sources, suggest that the MeHg in invertebrates comes mostly from epiphytes. Recent studies suggest that Hg transfer in littoral primary consumers seems to be only marginally linked to macrophyte consumption and more dependent on the fluctuations of methylation rates in epiphyte communities. In fact, high methylation rates have been observed in epiphytes (Cleckner et al. 1999; Mauro et al. 2004). The newly generated MeHg could then be transferred to higher trophic levels as has been experimentally demonstrated by Branfireun et al. (2005). Our findings support the growing corpus of evidence on the importance of a biofilm-mediated transfer of MeHg in freshwater systems (Hamelin et al. 2004; Desrosiers et al. 2006).

Some studies have found that the natural abundance of δ^{15}N proved to be a reliable tool for predicting the transfer of Hg in pelagic and benthic food webs (Cabana and Rasmussen 1994, 1996; Allen et al. 2005). These models did not seem to apply to predict Hg concentrations in macroinvertebrates from wetlands. In our study, because we only studied the lower trophic levels, δ^{15}N variability reflected OM source choice of herbivores in the IsoSource mixing polygon more than it did prey–predator relationships. In other words, the δ^{15}N signal of primary consumers represents the variability of the food web "baseline" for stable isotope studies of food webs (Vander Zanden et al. 1997; Post 2002). Therefore, when only primary consumers are considered, δ^{15}N signatures are better indicators of the source of OM than trophic level. When the δ^{13}C was combined with δ^{15}N as a two-dimensional OM source indicator in the IsoSource mixing model, significant relationships were observed between Hg contamination (THg, MeHg, MeHg/THg) and the output variables from the IsoSource model.

Conclusion

Our results stress the significance of primary producers as the point of entry for MeHg contamination in the aquatic food web, and macroinvertebrates as potential vectors of MeHg transfer to organisms at higher trophic levels. We have demonstrated that invertebrate primary consumers in Lake St. Pierre marshes rely mainly on autochthonous OM sources, especially epiphytes and aquatic macrophytes. Macroinvertebrates were nevertheless able to assimilate suspended particulate matter or even decaying macrophytes when epiphytes were less abundant. These findings are in agreement with a growing number of studies recognizing the importance of littoral organic matter in sustaining freshwater food webs (Wetzel 1979; Vadeboncoeur et al. 2002; Bertolo et al. 2005; Hershey et al. 2006; Vander Zanden et al. 2006). In a broader perspective, this study is linked to previous research emphasizing the importance of wetlands as privileged sites for MeHg production and trophic transfer in ecosystems. Our findings could eventually contribute to the debate on wetland restoration and maintenance, particularly in a place like Lake St. Pierre which provided more than half of the 1,000 tons of fish commercially harvested in Québec fresh waters in 2004.[1]

[1] http://www.mapaq.gouv.qc.ca/Fr/Peche/Profil/pecheaquaculture/pechecommerciale

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