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Geochimica et Cosmochimica Acta 71 (2007) 3393–3406
Geochimica et Cosmochimica Acta
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Mercury transformations and fluxes in sediments of a riverine wetland

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Received 2 May 2006; accepted in revised form 23 April 2007; available online 24 May 2007

Abstract

Porewater samples were obtained on five occasions during spring, summer and fall by *in situ* dialysis from three sites of a large freshwater wetland situated along the St. Lawrence River. These samples were analysed for total dissolved mercury ($[Hg]_T$) and methylmercury ([MeHg]) concentrations and for complementary variables including dissolved sulfate, sulfide and elemental sulfur concentrations. Sediment cores were obtained on three occasions from one of these sites for the determination of total mercury ($\{Hg\}_T$) and methylmercury ({MeHg}) concentration as well as mercury methyltransferase (HgMT) activity profiles. {MeHg} and HgMT activity varied with time and sediment depth. The porewater $[Hg]_T$ and [MeHg] depth profiles varied with time and among sites. Modeling the porewater [MeHg] profiles with a one-dimensional reaction-transport equation allowed identification of the sediment depths where MeHg is produced or consumed, as well as an estimate of the net *in situ* MeHg production rates in the sediments. The model-predicted depths of MeHg production, as well as the sulfate concentration and the HgMT activity depth distributions are all consistent with the involvement of sulfate reducing bacteria in the production of MeHg.

1. INTRODUCTION

Methylmercury (MeHg) is a chemical Hg species that poses a potential risk to humans and aquatic animals. Its biomagnification along food chains and its potential toxicity warrant investigations to identify the biogeochemical variables that control its production and cycling in aquatic environments. Anoxic sediments are widely recognized as a major site for Hg methylation (Gilmour et al., 1992; Hammerschmidt and Fitzgerald, 2004; Hines et al., 2004), but the biogeochemical processes responsible for *in situ* MeHg production in sediments are not fully characterized.

The main inferences on the mechanism of *in situ* mercury methylation result from laboratory experiments and thermodynamic calculations, and they often point to the key role played by sulfur species. For example, it is proposed, based on pure culture or sediment Hg spike experiments, that methylation of inorganic Hg occurs via sulfate reducing bacteria (SRB) (Compeau and Bartha, 1985; Gilmour et al., 1992; King et al., 1999, 2001). Octanol-water

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doi:10.1016/j.gca.2007.04.032

partitioning measurements (Benoit et al., 1999a) and thermodynamic calculations (Benoit et al., 1999b) suggest that diffusion of the neutral species $HgS_{(aq)}$ across bacterial membranes mediates MeHg production. Measurements of Hg methylation rates following Hg addition to sediments indicate that high dissolved sulfide concentrations inhibit MeHg production (Gilmour et al., 1998). This inhibition has been explained either by a reduction in the concentration of $HgS_{(aq)}$ by shifting Hg speciation in favour of charged Hg-sulfide complexes (Benoit et al., 1999a,b, 2001), or by a decrease in dissolved or bioavailable inorganic Hg due to the precipitation of $HgS_{(s)}$ (Winfrey and Rudd, 1990). Several other laboratory studies suggest that microorganims other than SRB can be involved in the process of Hg methylation (Warner et al., 2003; Desrosiers et al., 2006; Fleming et al., 2006). Finally, abiotic formation cannot be excluded (Celo et al., 2006). Consequently, there is a need to test some of these inferences and hypotheses in natural aquatic habitats.

This study focuses on porewater and sediment chemistry of Hg in a large freshwater wetland located along the St. Lawrence River. Temperate freshwater wetlands are usually considered sites of rapid methylmercury production (Branfireun et al., 1996), although most studies have been conducted on wetlands from small boreal lakes. Wetlands of the St. Lawrence River release methyl mercury to fluvial lakes that sustain large fisheries thus a potential route of MeHg exposure to humans (Canuel et al., 2005). We determined concentration profiles of porewater and solid phase mercury and methylmercury as well as those of complementary variables including porewater sulfate, sulfide and elemental sulfur at several times and sites in the sediments of the wetland. Our extensive dataset is used to obtain *in situ*-derived information on mercury speciation, control, and net methylation rates in marsh sediments and its transport across the sediment-water interface. This initiative was part of a larger research program aimed at determining mercury inputs to and outputs from the wetland (Poissant et al., 2004a,b; Garcia et al., 2006; Zhang et al., 2006), and its transfer to neighbouring human communities (Canuel et al., 2005). It complements an earlier study on porewater chemistry in the wetland (Zhang et al., 2004), which showed that biogenic thiols could be significant Hg and MeHg ligands in nature.

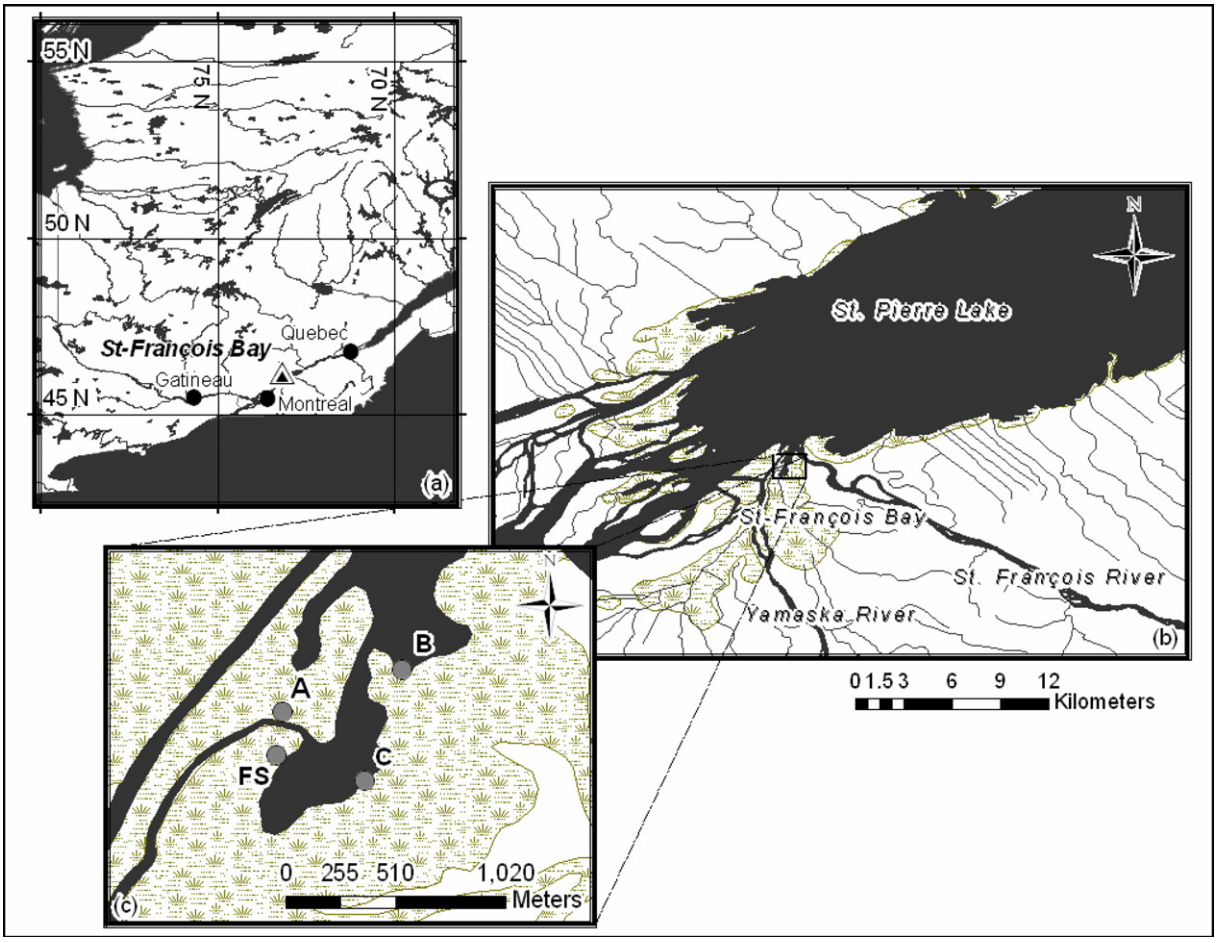

Fig. 1. Area map of the St. François Bay wetland situated on the shoreline of Lake St. Pierre along the St. Lawrence River, Quebec, Canada. Also shown with filled circles is location of sampling stations A (summer 2001), B (fall 2001) and C (spring, summer and fall 2002). FS indicates the location of the permanent station where atmospheric and water quality data were monitored.

2. SAMPLING SITES

The St. François Bay wetland (area of 16 km^2) is on the southwest shoreline of Lake St. Pierre (46°11′N, 72°54′W), a fluvial lake along the St. Lawrence River located between Montréal and Québec City, Québec, Canada (Fig. 1a), at the confluence of the Yamaska and St. François Rivers (drainage basin areas of 4780 and 10,200 km^2, respectively) with the St. Lawrence River (Fig. 1b). The water level of this wetland varies yearly and seasonally from spring flooding to summer low water-levels. Consequently, the whole wetland is not permanently flooded and part of the sediments becomes exposed to air. At a permanent field station (FS in Fig. 1c), the surface water had the following values of pH and concentrations of dissolved constituents (mean ± SD; $n = 25$ samples collected from May to October): pH 7.50 ± 0.55; Ca 826 ± 145 μM; Mg 408 ± 58 μM; Na 839 ± 287 μM; K 120 ± 90 μM; Fe 76 ± 115 μM; Mn 9.5 ± 13.9 μM; Al 0.7 ± 0.2 μM; HCO_3^- 3.5 ± 1.1 mM; Cl^- 774 ± 296 μM; SO_4^{2-} 152 ± 153 μM; P 239 ± 120 μg L^{-1}; NO_3^- 41.4 ± 46.8 μM; and DOC 11.9 ± 5.5 mg C L^{-1}. The vegetation includes the emergent macrophyte *Typha latifolia* L., and submerged macrophytes such as *Ceratophyllum demersum*, *Myriophyllum spicatum*, and *Nuphar variegatum.*

Porewaters were sampled on five occasions over 2 years at the following sites in the wetland (Fig. 1c): site A in July 2001, site B in November 2001 and site C in May, July and November 2002. Water depth varied between 1 and 2 m during the sampling periods at the three sites. Sediment cores were obtained from sites A, B and C.

3. METHODS

Clean techniques were used for the preparation of material and for sample collection. All labware and material for sampling porewater were soaked in 5–15% nitric acid, rinsed thoroughly with ultrapure water (>18 MΩ cm) and dried in a laminar flow hood; polyethylene gloves were worn for handling. The cleaned Teflon centrifuge tubes used for storing samples for the measurement of $[Hg]_T$ were filled with ultrapure water and stored in double polyethylene bags until use. Detection limits (DL) given below correspond to three times the standard deviation of blanks or of low concentration standards. In this paper, [X] and {X} refer to the concentrations of species X in the aqueous and solid phases, respectively.

3.1. Porewater sampling and analysis

Acrylic *in situ* dialysis samplers (peepers; 1-cm vertical resolution; two columns of 4-mL cells) of the type described by Carignan et al. (1985) were used to sample porewater. Although the reliability of peepers for sampling porewater was not verified specifically for Hg and MeHg, it was assessed for many other metals (Carignan et al., 1985). Moreover, Muresan et al. (2007) did not find any significant difference in $[Hg]_T$ between samples of overlying waters collected with peepers and those collected with Teflon bottles followed by filtration. To remove O_2 (Carignan et al., 1994), the peepers were kept under N_2 for a minimum of 15 days prior to rapidly filling the cells with ultrapure water and covering them with a pre-cleaned 0.2 μm nominal pore size hydrophilic polysulfone membrane (HT-200, Gelman). Once assembled, the peepers were returned under a N_2 atmosphere for at least another 5 days prior to deployment in the sediments. Generally, at sites A, B and C, 14 peepers were deployed, of which six were used to collect samples for determining the concentrations of total dissolved Hg ($[Hg]_T$), and six to collect samples for measuring the concentrations of dissolved methylmercury ([MeHg]); the remaining two peepers were used to collect samples for duplicate analyses of dissolved sulfide (ΣS(-II)), elemental sulfur (ΣS(0)), pH, Fe, Mn, Al, sulfate, inorganic carbon (DIC) and organic carbon (DOC). For the July 2002 campaign at site C, 6 additional peepers were deployed to obtain replicate samples for [MeHg] measurements; the 12 peepers required for duplicate measurements of [MeHg] were deployed within an area of about 2 m^2. Location of peepers with respect to the sediment-water interface was determined visually by scuba divers or with a boroscope. When the peepers were retrieved, procedural blanks for $[Hg]_T$, [MeHg], DOC, ΣS(-II) and ΣS(0) were prepared on site by transferring ultrapure water using the same techniques and containers as described below for the porewater samples.

Samples (40 mL) for [MeHg] determination were obtained at each 1-cm depth by piercing the membrane of six peepers with a Gilson pipette fitted with an acid-cleaned plastic tip and transferring the pooled content into new 60-mL amber high density polyethylene (HDPE) bottles to which concentrated ultrapure HCl (0.3 mL; SeaStar) was added; amber bottles were used because MeHg is sensitive to light (Sellers et al., 2001). The samples (40 mL) for $[Hg]_T$ measurements were obtained similarly from six additional peepers and the pooled content was poured into cleaned 50-mL Teflon centrifuge tubes, which were kept frozen until analyzed. Briefly, [MeHg] was determined by capillary gas chromatography (GC; Hewlett-Packard 5890; DL of 0.05 pM) coupled with cold vapour atomic fluorescence spectrometry (CVAFS; PSA Analytical, UK) as described by Cai et al. (1996). Methylmercury was preconcentrated on sulfydryl-cotton fibers followed by elution with acidic KBr and $CuSO_4$ and extraction in methylene chloride. Cai et al. (1996) reported that quantitative recoveries of MeHg (90–95%) were obtained for freshwaters and seawater samples with this method. We found that recovery of MeHg spikes in ultrapure water was always above 95% and that method and procedural blanks were below the detection limit. $[Hg]_T$ was determined by cold vapour atomic fluorescence spectrometry (AFS) using a Tekran 2600 (DL = 0.2 pM) after successive addition of BrCl, hydroxylamine and $SnCl_2$ (U.S.E.P.A., 2001). Measured $[Hg]_T$ in 50-fold diluted reference material (rain water, FP-HG77-5 and FP-HG77-2 from Environment Canada, Ottawa, Canada and ORMS-2 and ORMS-3 from National Research Council of Canada) were within 5% of the reported median values. Method and procedural blanks were below the detection limit.

Sampling of peepers and the analytical methods for the measurement of pH and concentrations of dissolved Al, Ca, Fe, Mg, Mn, sulfide, SO_4^{2-}, and organic and inorganic C are described in detail elsewhere (Alfaro-De la Torre and Tessier, 2002; Laforte et al., 2005). Samples for ΣS(0) analysis were also collected from the peepers with N_2-purged polypropylene syringes and injected through Teflon-lined septa into pre-weighted 5-mL amber glass vials containing 2 mL of ethanol, 0.4 mL of 1 M $NaNO_3$, 40 μL of tetrahydrofuran, and 10 μL of 1 M HNO_3 added in a glove box under a N_2 atmosphere to minimize contamination with oxygen (Wang et al., 1998). ΣS(0) was determined by square-wave cathodic stripping voltammetry (SWCSV; BAS-100B; DL = 0.015 μEq L^{-1}) according to the method described in Wang et al. (1998).

3.2. Sediment sampling and analyses

Sediment cores were obtained at site C (one in May 2002, and two in both July and November 2002), close to the peepers, with butyrate tubes (9.5 cm diameter) for the measurement of sedimentary Hg methyltransferase (HgMT) activity and methylmercury ({MeHg}) and total mercury ($\{Hg\}_T$) concentrations. The sediment cores were extruded and sliced in the field at 1-cm intervals. These sediment samples were placed in plastic bags, immediately frozen with liquid N_2 and transported to the laboratory in a cooler containing dry ice, where they were stored at −80 °C. Separate cores were obtained also at sites A, B and C for the measurement of sediment porosity upon freeze-drying of the weighed wet sediment.

The frozen sediment samples were thawed, and subsamples were used for the analysis of Hg methyltransferase (HgMT) activity. In this assay, it is assumed that the Hg methylation pathway is similar to that of methionine enzymatic synthesis, where a methyl group is transferred from methyl-tetrahydrofolate (MeTHF) to homocysteine to form methionine. It is hypothesized that Hg competes with homocysteine for the methyl group and accidentally produces MeHg (Siciliano and Lean, 2002). Conducting the HgMT assay required first the extraction of enzymes from sediment using a method modified from Ogunseitan (1997). Sediment (~3 g) was mixed with 6 mL of extraction buffer (20 mM Tris–Cl; pH 7.4; 1 mM dithiothreitol; 1 mM phenylmethylsulfonyl fluoride), vortexed for 30 s, and sonicated (3 min; 195 W), and the supernatant was collected after centrifugation (10 min; 7000*g*; 4 °C). Sterile glycerol (1:10 by volume) was added to this enzyme extract that was then frozen at −20 °C for later analysis (Siciliano and Lean, 2002) by the Bradford assay (Koch, 1994).

Mercury methyltransferase (HgMT) activity was estimated from the production of THF in the presence of Hg (MeTHF + Hg = THF + MeHg) using a procedure very similar to that described by Drummond et al. (1995) for assessing methionine synthase activity. To the enzyme extract (containing 1–10 μg of proteins) were added: 80 μL of 1 M potassium phosphate buffer at pH 7.2, 338 μL of sterile distilled water, 40 μL of dithiothreitol (500 mM), 4 μL of 3.8 M S-adenosyl-methionine (SAM) and either 10 μL of 100 μg/L $HgCl_2$ or 10 μL of distilled water (control; see below). The addition of hydroxocobalamine (500 μM; 80 μL) generated hydrogen peroxide and made the assay solution anaerobic. After 5 min of incubation, 48 μL of 250 μM MeTHF were added, and the mixture was incubated for 20 min at 22 °C in the dark, which produced THF. In order to measure THF, the reaction was quenched by the addition of 200 μL of 5 M HCl in 60% formic acid and THF was transformed to methenyltetrahydrofolate by heating at 80 °C for 10 min. Methenyltetrahydrofolate was determined by absorbance at 350 nm and values compared with known standards. Since THF production is not specific to MeHg formation, mercury specific methyltransferase (HgMT) activity was assessed by comparing methyltransferase activity in the presence of 10 μL of 100 μg/L $HgCl_2$ to that in a control assay where only 10 μL of distilled water was added. One unit of HgMT activity (U g^{-1} of sediment) was defined as the formation of 1 nmol of tetrahydrofolate in response to the addition of 1 ng of Hg to the reaction vessel.

MeHg in sediments was extracted and analysed by GC-AFS (Cai et al., 1997), as described in detail in (Holmes and Lean (2006); absolute DL = 0.05 pmol). The remaining thawed sediments were freeze-dried and analysed for total Hg by pyrolysis (Milestone AMA 254; DL = 5 pmol g^{-1}). Recoveries of MeHg from the BCR-580 certified reference sediment (Commission of the European Communities, Belgium) and of total Hg from the certified material MESS-3; National Research Council of Canada) were 85–90%. Recoveries of Hg and MeHg spikes were within 90–95% and method blanks contained neither detectable inorganic Hg nor MeHg.

4. RESULTS

4.1. Dissolved concentration profiles

Figs. 2 and 3 depict the depth distributions of porewater $[Hg]_T$, [MeHg], pH, [Mn], [Fe], $[SO_4^{2-}]$, ΣS(-II) and ΣS(0) obtained at sites A–C. The presence of numerous concentration gradients above the sediment-water interface (e.g., Fig. 2a and i, Fig. 3j, k, p, and q) indicates that the overlying water at the sampling sites was likely stagnant.

Mercury methylation is a process that has been closely associated to sulfate reduction. Fig. 2 and upper panels of Fig. 3 show that $[SO_4^{2-}]$, which was constant in the overlying water, decreased sharply with depth in porewater, indicating that sulfate was reduced below the sediment-water interface at sites A and B and in May at site C. The presence of measurable concentrations of sulfide and elemental sulfur only in porewater at these sites and dates is consistent with this scenario. In contrast, middle and bottom panels of Fig. 3 show low $[SO_4^{2-}]$ just above the sediment-water interface and little or no $[SO_4^{2-}]$ gradient in porewaters, indicating that sulfate reduction occurred above the sediments-water interface at site C in July and November. Relatively elevated ΣS(-II), ΣS(0), [Fe] and [Mn] in the overlying water at this site and time periods are all consistent with this scenario.

At sites A, B and C, $[Hg]_T$ were 4–20 pM in the overlying water; the concentrations remained similar (Fig. 2a,

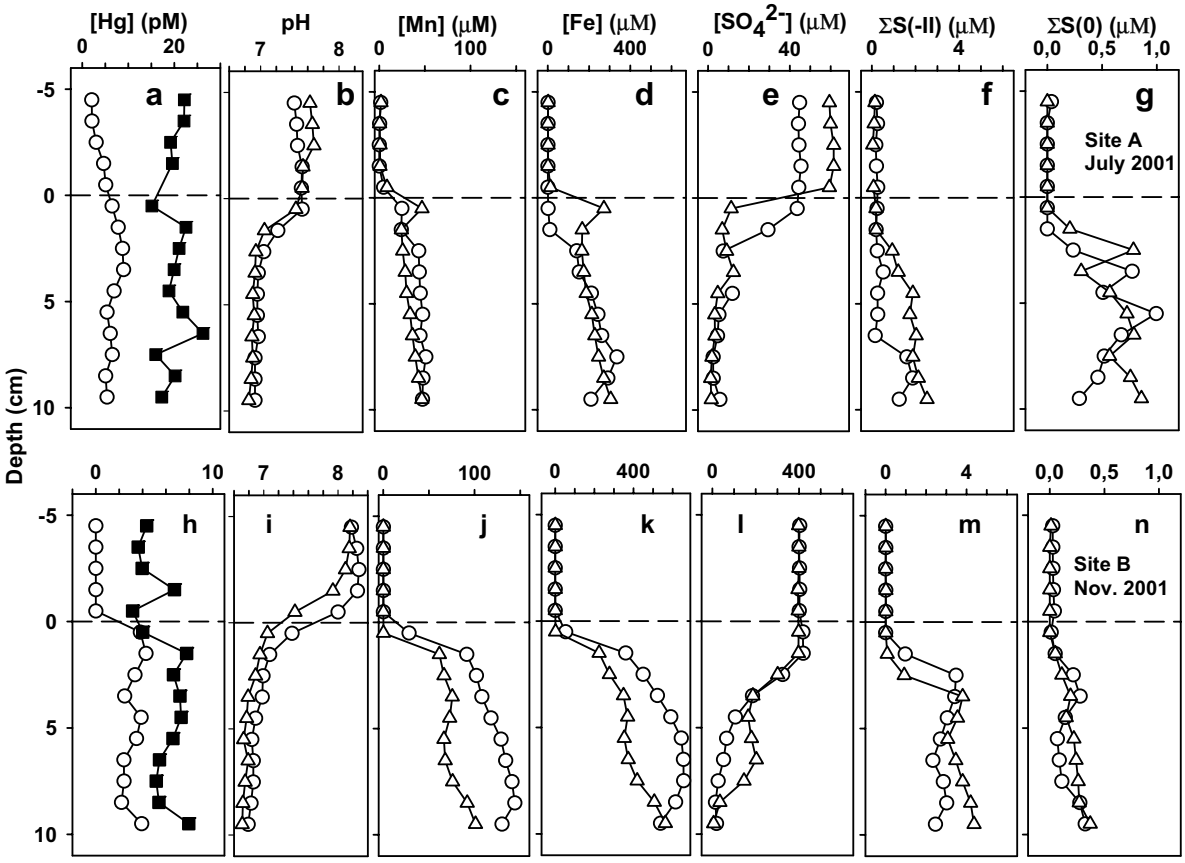

Fig. 2. Porewater profiles of (a and h) methylmercury (empty circles) and total Hg (filled squares), (b and i) pH, (c and j) manganese, (d, k) iron, (e and l) sulfate, (f and m) sulfide and (g and n) elemental sulfur at site A (July 2001; upper panels) and at site B (November 2001; lower panels) of the St. François Bay wetland. The horizontal dotted lines represent the sediment-water interface. The empty circles and triangles are for replicate profiles.

Fig. 3h and o) or were slightly higher (Fig. 2h) or lower (Fig. 3a) in the porewater. These concentrations are in the range of those reported in porewater from several sites in the San Francisco Bay—Delta (10–50 pM; Choe et al., 2004), in the Patuxent River estuary (10–40 pM; Benoit et al., 1998), in Lavaca Bay (50–100 pM; Bloom et al., 1999) and in the Everglades (10–75 pM; Gilmour et al., 1998).

Dissolved MeHg concentrations at sites A–C (mean ± SD = 3.5 ± 1.8 pM; range of <0.05–9 pM; Figs. 2 and 3) were in the range of those reported in porewaters of the Lavaca Bay, USA (0–100 pM; Bloom et al., 1999), the San Francisco Bay—Delta, USA (0–60 pM; Choe et al., 2004), the Saguenay Fjord, Canada (0–50 pM; Gagnon et al., 1996), the Barn Island Salt Marsh, USA (0–45 pM; Langer et al., 2001), a seepage lake in Northern Minnesota (0.5–8 pM; Hines et al., 2004), the Florida Everglades, Florida, USA (1–4 pM; Gilmour et al., 1998) and the Patuxent River estuary, USA (0.2–1 pM; Benoit et al., 1998). The average (±SD) proportion of total dissolved Hg in the methylated form was 27 ± 19%, with a range of 1–95%; higher proportions of MeHg were observed when SO_4^{2-} was consumed in the porewaters (29 ± 12% at site A; 52 ± 16% at site B; 35 ± 27% in May at site C) than when it was not (16 ± 7% and 19 ± 7% at site C in July and November, respectively). Proportions of total dissolved Hg in the form of MeHg varying between <0.5% and 120% have been reported (Bloom et al., 1999; Ullrich et al., 2001; Choe et al., 2004; Hines et al., 2004) for porewaters from other aquatic systems. If allowance is made for the precision on the position of the sediment-water interface, all [MeHg] profiles, except those obtained in July at site C (Fig. 3h), indicate a diffusion of MeHg from the sediments to the overlying water. The two [MeHg] profiles obtained within an area of about 2 m^2 in July at site C (open circles and triangles in Fig. 3h) are consistently flat and the replicate [MeHg] show similar values. This good reproducibility indicates that the methodology used for sampling and analysis of MeHg was adequate and that higher concentrations measured at other times and sites were probably real and not artefacts associated with sampling and handling procedures. Spatial variability in [MeHg] was attenuated since each [MeHg] was an average value from six pooled peepers.

4.2. Solid phase concentration profiles

Total sedimentary Hg concentrations ($\{Hg\}_T$) at site C (Fig. 4) varied between 120 and 270 pmol g^{-1}. This range of $\{Hg\}_T$ values is comparable to those reported for

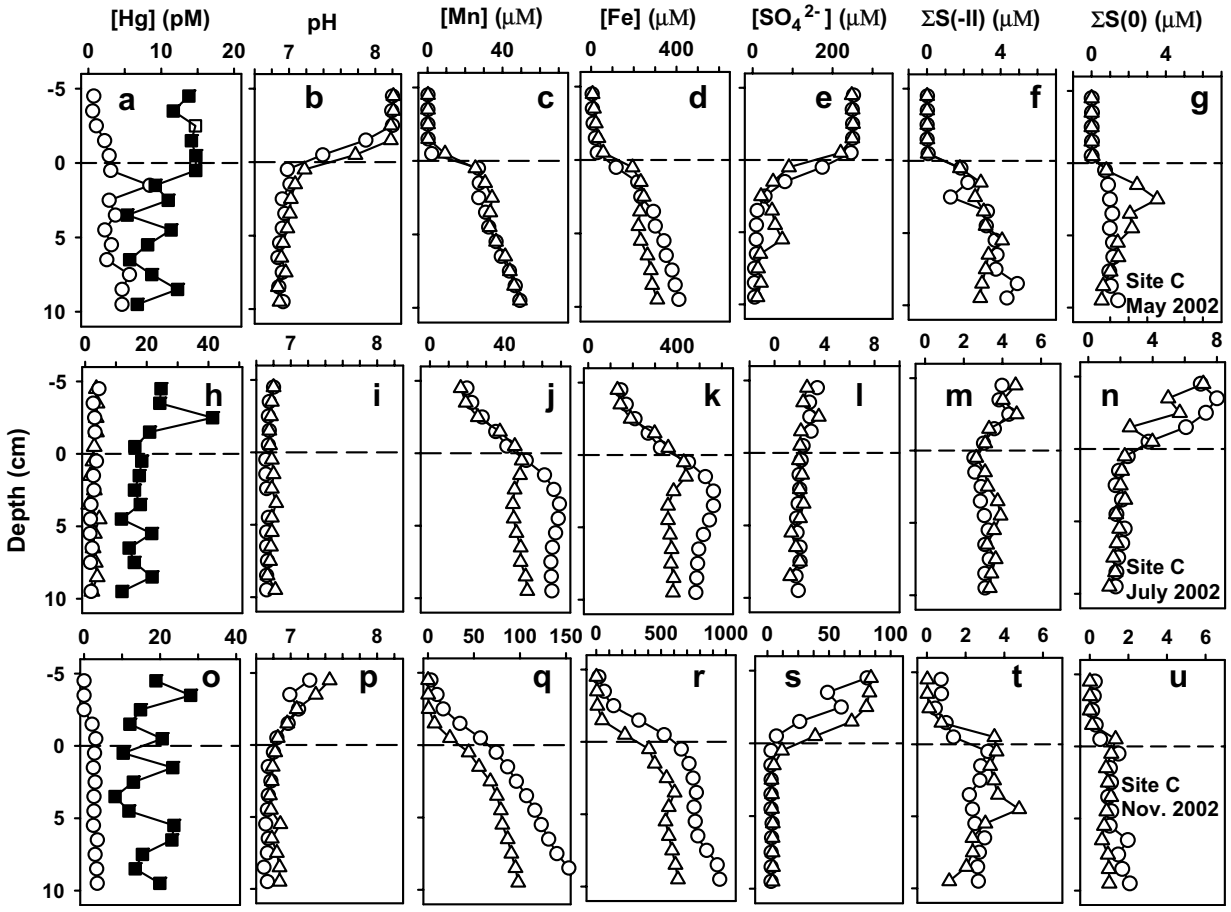

Fig. 3. Porewater profiles of (a, h, and o) methylmercury (empty circles and triangles) and total Hg (filled squares), (b, i, and p) pH, (c, j, and q) manganese, (d, k, and r) iron, (e, l, and s) sulfate, (f, m, and t) sulfide and (g, n, and u) elemental sulfur at site C of the St. François Bay wetland in May (upper panels), July (middle panels) and November (lower panels) 2002. The horizontal dotted lines represent the sediment-water interface. The empty circles and triangles are for replicate profiles.

relatively uncontaminated sediments, e.g., at sediment depths corresponding to pre-industrial periods in a seepage lake (~250 pmol g^{-1}; Hines et al., 2004), in Adirondack lakes (~1 nmol g^{-1}; Heit et al., 1981), in San Francisco Bay (300 ± 50 pmol g^{-1}; Hornberger et al., 1999) and in the Strait of Georgia (~300 pmol g^{-1}; Johannessen et al., 2005). The partition coefficient of total Hg ($K_D^{Hg} = \{Hg\}_T/[Hg]_T$) at site C ranged from 6×10^3 to 34×10^3 L kg^{-1} with an average value (±SD) of $16 \pm 7 \times 10^3$ L kg^{-1}.

At the same site, {MeHg} profiles (Fig. 4b, e, h) showed an upward increase from ~1–2 to ~4–8 pmol g^{-1}; the highest {MeHg} values were found in May (Fig. 4b). The range of {MeHg} is similar to that reported for the sediments of a seepage lake (1–8 pmol g^{-1}; Hines et al., 2004). The partition coefficient of methylmercury ($K_D^{MeHg} = \{MeHg\}/[MeHg]$) at site C ranged from 0.4×10 to 2.6×10^3 L kg^{-1} with an average value (±SD) of $1.0 \pm 0.5 \times 10^3$ L kg^{-1}. Sediment K_D^{MeHg} values reported in the literature vary over a wide range: for example, in San Francisco Bay, between ~30 L kg^{-1} and $\sim 1.0 \times 10^5$ L kg^{-1} (Choe et al., 2004), depending on sampling site and season. Replicate cores obtained in July and November (Fig. 4) showed differences in $\{Hg\}_T$ and {MeHg} profiles, indicating some heterogeneity of the sediments.

At site C, methyltransferase (HgMT) activity reached 1130 U g^{-1} close to the sediment surface and decreased with depth in May (Fig. 4c), whereas it remained relatively constant with depth at values below 350 U g^{-1} in July (Fig. 4f) and November (Fig. 4i). These results suggest that Hg-specific methylation activity was occurring in May, particularly in the top 3 cm, but little or no methylation occurred in July and November. The HgMT activities reported here are 4–10 times larger than values (50–300 U g^{-1}) reported by Siciliano and Lean (2002) for ombrotropic bog soils. The lower values for the bog soils are not surprising given that their acidity limits their productivity compared to minerotrophic wetlands such as that of the St. François Bay (Mitsch and Gosselink, 2000).

5. DISCUSSION

5.1. Speciation of dissolved inorganic Hg and Methylmercury

The speciation programs Windermere Humic Aqueous Model (WHAM 6.0; Tipping, 2002) and ECOSAT (Keizer

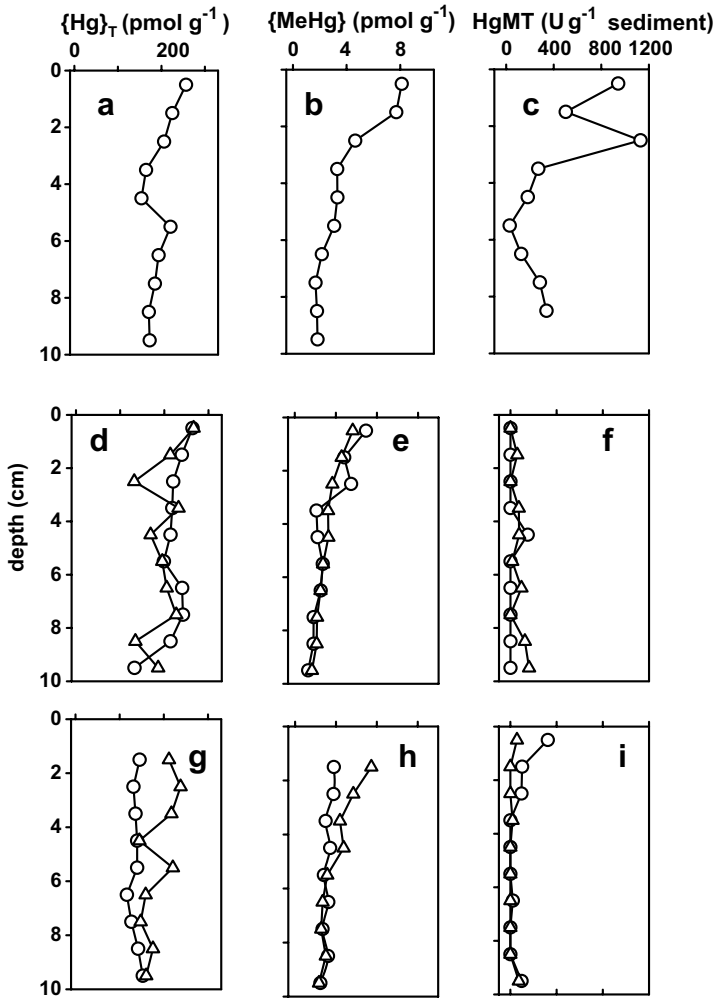

Fig. 4. Depth concentration profiles of (a, d, and g) total Hg ($\{Hg\}_T$), (b, e, and h) methyl mercury ({MeHg}) and (c, f, and i) Hg methyltransferase activity (HgMT), in sediment cores obtained at site C of the St. François Bay wetland in May (panels a–c), July (panels d–f) and November (panels g–i) 2002. Different symbols refer to replicate cores.

and van Riemsdijk, 2002) comprising the Non-Ideal Competitive Adsorption (NICA; Milne et al., 2003) model to take into account complexation by humic substances were used to calculate the speciation of inorganic Hg ($[Hg]_I = [Hg]_T - [MeHg]$) and MeHg at sites A, B and C for which a complete set of data exists. The measured pH and $[Hg]_I$, [MeHg], ΣS(-II), ΣS(0), [Al], [Fe], [Mn] and major ions, as well as estimates of humic (HA) and fulvic (FA) acid concentrations were inputs to the speciation codes. To estimate [HA] and [FA], we assumed that DOC contained 50% C (Buffle, 1988) and that all DOC was humic substances with a ratio of [FA]:[HA] of 9:1 (Malcolm, 1985). The WHAM 6 and ECOSAT thermodynamic databases were updated with the equilibrium constants for the systems Hg-sulfide-polysulfide and MeHg-sulfide given in Table 1, and dissolved elemental sulfur ($S(0)_{(aq)}$) and $MeHg^+$ were added as components in the codes. The formation constants recommended by Powell et al. (2005) for Hg complexation with OH^-, Cl^- and CO_3^{2-} were also used to update the WHAM 6 and ECOSAT databases. The formation constants of Hg complexes with FA and HA used in the calculation were those given in the thermodynamic databases of WHAM 6.0 (Tipping, 2002) and ECOSAT (NICA model; Milne et al., 2003).

Figs. 5 and 6 depict the speciation of dissolved inorganic Hg at sites A, B and C estimated with WHAM 6. The major Hg species are complexes with humic substances, polysulfides (mainly HgS_nOH^-) and sulfide (mainly $HgS_{(aq)}$), depending on the relative concentrations of humic substances, ΣS(0) and ΣS(-II). The WHAM 6 code predicts that inorganic Hg should be predominantly bound to humic substances (>99%) when dissolved sulfide and elemental sulfur are below the detection limits, and that polysulfide (HgS_nOH^-) and sulfide ($HgS_{(aq)}$) complexes should dominate Hg speciation (>99%) when ΣS(-II) and ΣS(0) reach about 0.5 μM. The speciation code WHAM 6 also indicates that MeHg should be present essentially (nearly 100%) as the species $MeHgS^-$ (data not shown).

The speciation estimated for inorganic Hg depends, however, on the code used as well as on the presence/absence of auxiliary data such as dissolved Al in the input files. For example, the same calculation carried out for

Table 1
Equilibrium constants ($T = 25$ °C) and corresponding reactions relevant for calculation of Hg speciation in this study

Reaction	Log K	References
$HgS_{(s,cinn)} + H^+ = Hg^{2+} + HS^-$	−39.1 ($I = 0.0$)	Martell et al. (2003)
	−36.7 ($I = 0.7$)	Paquette and Helz (1995)
$HgS_{(s,cinn)} + HS^- = HgS_2^{2-} + H^+$	−13.0 ($I = 0.3$)	Jay et al. (2000)
$HgS_{(s,cinn)} + HS^- = HgS_2H^-$	−4.5 ($I = 0.3$)	Jay et al. (2000)
$HgS_{(s,cinn)} + HS^- + H^+ = Hg(SH)_2$	1.0 ($I = 0.3$)	Jay et al. (2000)
$HgS_{(s,cinn)} = HgS_{(aq)}$	−9.3 ($I = 0.3$)	Jay et al. (2000)
$HgS_{(s,cinn)} + HS^- + (n-1)/4S_{8(rhom)} = Hg(S_n)_2^{2-} + H^+$	−11.7 ($I = 0.3$)	Jay et al. (2000)
$HgS_{(s,cinn)} + (n-1)/8\ S_{8(rhom)} + H_2O = HgS_nOH^- + H^+$	−15.7 ($I = 0.3$)	Jay et al. (2000)
$MeHg^+ + HS^- = MeHgS^- + H^+$	7.0 ($I = 0.1$)	Martell et al. (2003)
$2MeHg^+ + HS^- = (MeHg)_2S + H^+$	23.3 ($I = 0.1$)	Martell et al. (2003)
$3MeHg^+ + HS^- = (MeHg)_3S^+ + H^+$	30.3 ($I = 0.1$)	Martell et al. (2003)
$1/4S_{8(rhom)} + HS^- = S_3^{2-} + H^+$	−12.5 ($I = 0$)	Boulègue and Michard (1978)
$3/8S_{8(rhom)} + HS^- = S_4^{2-} + H^+$	−9.52 ($I = 0$)	Boulègue and Michard (1978)
$1/2S_{8(rhom)} + HS^- = S_5^{2-} + H^+$	−9.47 ($I = 0$)	Boulègue and Michard (1978)
$5/8S_{8(rhom)} + HS^- = S_6^{2-} + H^+$	−9.66 ($I = 0$)	Boulègue and Michard (1978)
$H_2S_3 = HS_3^- + H^+$	−4.6 ($I = 0$)	Schwarzenbach and Fischer (1960)
$HS_3^- = S_3^{-2} + H^+$	−7.9 ($I = 0$)	Schwarzenbach and Fischer (1960)
$H_2S_4 = HS_4^- + H^+$	−4.2 ($I = 0$)	Schwarzenbach and Fischer (1960)
$HS_4^- = S_4^{-2} + H^+$	−6.7 ($I = 0$)	Schwarzenbach and Fischer (1960)
$H_2S_5 = HS_5^- + H^+$	−3.9 ($I = 0$)	Schwarzenbach and Fischer (1960)
$HS_5^- = S_5^{-2} + H^+$	−6.1 ($I = 0$)	Schwarzenbach and Fischer (1960)
$H_2S_6 = HS_6^- + H^+$	−3.41 ($I = 0$)	Schwarzenbach and Fischer (1960)
$HS_6^- = S_6^{-2} + H^+$	−5.63 ($I = 0$)	Schwarzenbach and Fischer (1960)
$1/8\ S_{8(rhom)} = S(0)_{(aq)}$	−6.82 ($I = 0$)	Boulègue (1978)

Reported ionic strength (I) is given in parentheses.

inorganic Hg speciation, but with the code ECOSAT, results in a stronger complexation of mercury with humic substances than shown in Figs. 5 and 6, i.e., according to ECOSAT, the proportions of Hg complexes with humic substances should be higher (and those of Hg-sulfide and Hg-polysulfide complexes lower) than those predicted by WHAM 6. The discrepancies between WHAM 6 and ECOSAT depend mainly on how the heterogeneity of humic substance binding sites is treated in the two codes, as explained in detail in Tipping (2002). Briefly, model VI, which is included in the code WHAM 6 to take into account interactions with humic substances, uses a number of discrete binding sites for metals, including a small number of artificially-introduced "strong" (high affinity) sites, to treat heterogeneity of humic substances whereas NICA, which plays a similar role to model VI in the code ECOSAT, uses continuous functions. Removal of Al concentrations from the input file affects marginally the output of ECOSAT, but considerably that of WHAM 6, because this metal competes efficiently with Hg for the "strong" sites. In the absence of Al, WHAM 6 predicts a stronger complexation of mercury with humic substances than does ECOSAT. It should be noted that, although the strength of Hg complexation with humic substances varies between the two codes, the relative concentrations of the inorganic complexes of inorganic Hg remain the same.

Prediction of Hg speciation depends also on the thermodynamic database used in the calculation. Whereas the reported formation constants for the Hg complexes with OH^-, Cl^- and CO_3^{-2} are believed to be reliable (Powell et al., 2005), those with humic substances, sulfide and polysulfides remain uncertain. The Hg-FA and Hg-HA complexation constants included in the WHAM 6 and ECOSAT databases were obtained by extrapolation techniques, because of the absence of reliable experimental data (Tipping, 2002). As for the equilibrium constants for the formation of Hg-sulfide and polysulfide complexes (see Table 1), we retained the consistent set proposed by Jay et al. (2000), although we recognize some potential problems with these thermodynamic data. For example, the formation constant for the complex $HgS_{(aq)}$ was not determined experimentally, but was estimated by using the formation constants for $CdS_{(aq)}$ and $ZnS_{(aq)}$ and extraction constants for the dithizone-carbon tetrachloride system (Dyrssen, 1988). In addition, the formation constants for the complexes HgS_2^{2-}, HgS_2H^- and $Hg(SH)_2$ were averages of literature values rounded to the nearest 0.5 log unit (Benoit et al., 1999b). Lastly, the formation of mixed-complexes (e.g. DOC-Hg-SH), which may increase the overall Hg binding by organic matter (Glaus et al., 1995; Miller et al., 2007), is not considered in the speciation calculations because no thermodynamic data are available.

The possibility that pure solid phases such as cinnabar (Winfrey and Rudd, 1995) could control porewater Hg concentrations was examined by comparing the solubility product ($HgS_{(s,cinn)}$; $K_{sp} = 10^{-39.1}$; Table 1) and the ion activity product (IAP = $(Hg^{2+})(HS^-)/(H^+)$, where () indicates activities) as obtained with WHAM 6. The results show that the porewaters are undersaturated with respect to cinnabar by 2–4 orders of magnitude. Similar calculations reveal that the porewaters are also undersaturated with $HgO_{(s)}$ by about 30 orders of magnitude. It thus appears

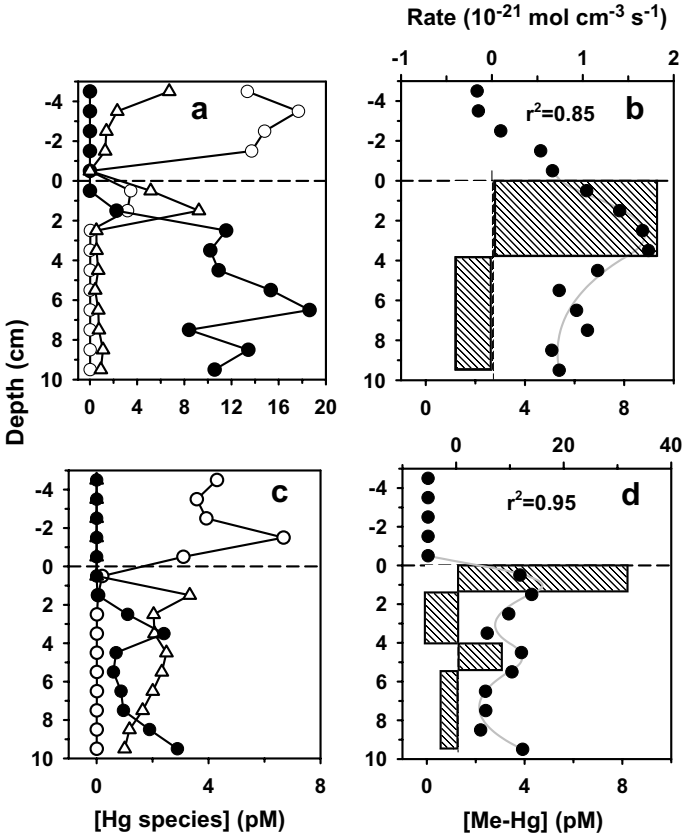

Fig. 5. Porewater profiles of (a and c) the major inorganic Hg species (empty circles, empty triangles and filled circles are for Hg-humic substances, $HgS_{(aq)}$ and HgS_5OH^- complexes, respectively), and (b and d) comparison of predicted and measured porewater MeHg concentrations at sites A (upper panels) and B (lower panels) of the St. François Bay wetland. The horizontal broken line indicates the sediment-water interface. In panels b and d, the grey line following the experimental points (filled circles) is the best fit line according to the code PROFILE (r^2 values indicated) and the black line enclosing hatched zones represents the MeHg net consumption/production rates, with zero value indicated by a dashed vertical line.

that porewater inorganic Hg concentrations in the wetland are not controlled by dissolution/precipitation equilibrium of a distinct Hg mineral.

5.2. Net methylation rates

The diagenetic equation for a solute such as MeHg that is in adsorptive equilibrium with sediment solids can be written, if porosity and adsorption are assumed to be constant with depth (Berner, 1980):

$$\frac{\partial \phi[\mathrm{MeHg}]}{\partial t} = \left(\frac{\phi D_s}{1+K}\right)\frac{\partial^2[\mathrm{MeHg}]}{\partial x^2} + \left(\frac{1}{1+K}\right)\left(\sum \phi R^{\mathrm{MeHg}}_{\mathrm{dissolved}} + m\sum R^{\mathrm{MeHg}}_{\mathrm{adsorbed}}\right) \quad (1)$$

where ϕ is sediment porosity, x is depth (cm; positive downward), D_s is the effective diffusion coefficient of MeHg species in sediment (cm^2 s^{-1}), K ($= 1000\,\mathrm{m}\,K_D/\phi$) is a dimensionless adsorption coefficient, m (g cm^{-3} of whole sediment) is the dry bulk density and $R^{\mathrm{MeHg}}_{\mathrm{dissolved}}$ (mol cm^{-3} of porewater s^{-1}) and $R^{\mathrm{MeHg}}_{\mathrm{adsorbed}}$ (mol g^{-1} s^{-1}) are the net rates of processes other than adsorption that produce (positive values of R) or consume (negative values of R) dissolved and particulate MeHg, respectively. Eq. (1) assumes that transport of MeHg in porewater is only by diffusion and that transport by advection, bioturbation or bioirrigation is negligible.

If we assume that the net methylation rate ($R_{\mathrm{methylation}}$; mol cm^{-3} of whole sediment s^{-1}) is given by:

$$\sum \phi R^{\mathrm{MeHg}}_{\mathrm{dissolved}} + m\sum R^{\mathrm{MeHg}}_{\mathrm{adsorbed}} = R_{\mathrm{methylation}} \quad (2)$$

Eq. (1) becomes at steady-state:

$$\frac{\partial}{\partial x}\left(\phi D_s \frac{\partial[\mathrm{MeHg}]}{\partial x}\right) = R_{\mathrm{methylation}} \quad (3)$$

We used the computer code PROFILE (Berg et al., 1998) to solve Eq. (3) numerically for $R_{\mathrm{methylation}}$. As input to the code, we used our measured values of ϕ, the [MeHg]-depth profiles and a value of D_s estimated as follows. We assumed that $D_s = \phi^2 D_w$ (Berg et al., 1998), where D_w is the tracer diffusion coefficient of the MeHg species present in the porewater (CH_3HgS^-, according to the speciation calculation carried out with the code WHAM 6). Since the D_w value of the species CH_3HgS^- has never been measured, we used an estimate of 8×10^{-6} cm^2 s^{-1} at 25 °C, on

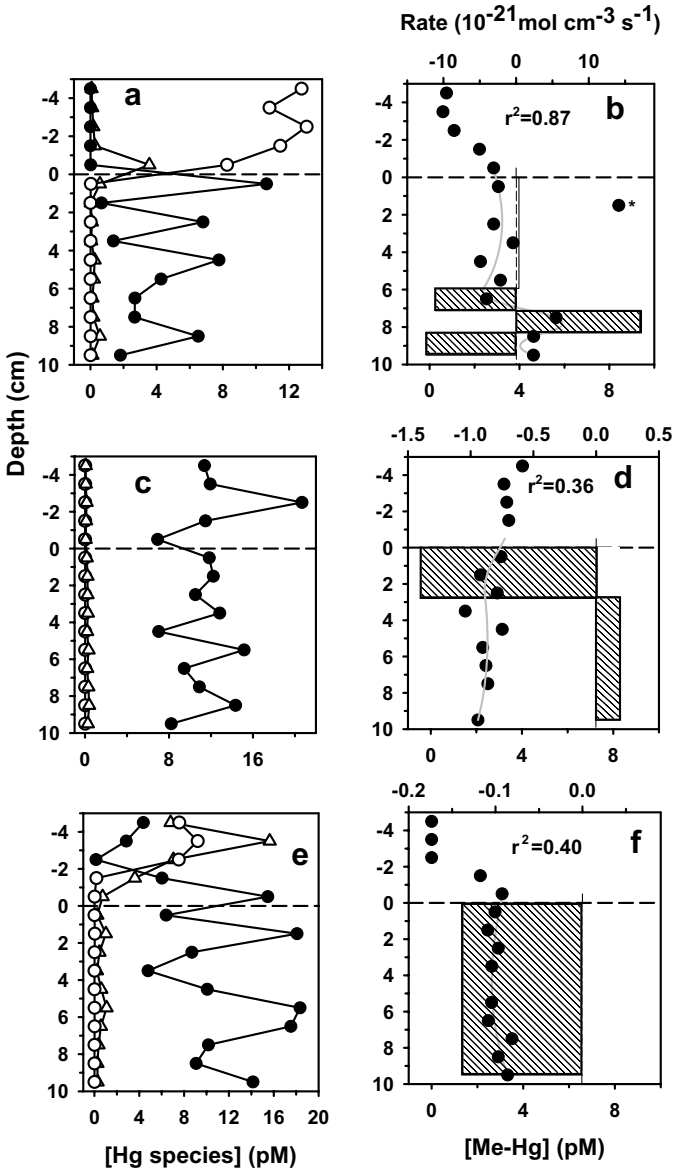

Fig. 6. Porewater profiles of (a, c, and e) the major inorganic Hg species (empty circles, empty triangles and filled circles are for Hg-humic substances, $HgS_{(aq)}$ and HgS_5OH^- complexes, respectively), and (b, d, and f) comparison of predicted and measured porewater MeHg concentrations at site C of the St. François Bay wetland in May (upper panels), July (middle panels) and November (lower panels) 2002. The horizontal broken line indicates the sediment-water interface. In panels b, d and f, the grey line following the experimental points (filled circles) is the best fit line according to the code PROFILE (r^2 values indicated) and the black line enclosing hatched zones represents the MeHg net consumption/production rates, with zero value indicated by a dashed vertical line. The asterisk indicates a data point not considered in the data analysis.

the basis that this species behaves similarly to other singly charged anionic species of comparable mass (Boudreau, 1997). This D_w value at 25 °C was corrected for *in situ* temperature with the Stokes–Einstein equation (Boudreau, 1997).

Table 2 and Figs. 5 and 6 display the zones and net rates of MeHg production or consumption ($R_{methylation}$), as provided by the code PROFILE. Interestingly, it clearly shows, for the upper zone (zone labeled No 1 in Table 2), a net production of MeHg (positive values of $R_{methylation}$) when sulfate was reduced in sediments, i.e., at site A, B and in May at site C (Fig. 2e, l and Fig. 3e), and a net consumption of MeHg (negative values of $R_{methylation}$) when sulfate was not reduced in sediments, i.e., in July and November at site C (Fig. 3l and s). This *in situ*-based approach to obtain net Hg methylation rates from porewater MeHg profiles offers an alternative to the approach where laboratory-incubated sediments are spiked with radiogenic or isotopically enriched Hg.

5.3. Mechanism of Hg methylation in sediments

It is generally assumed that methylation of inorganic Hg is carried out in anoxic sediments by sulfate reducing bacteria (SRB) (Ullrich et al., 2001). This inference is largely

Table 2
Production zones, net Hg methylation rate, and diffusive flux of MeHg across the sediment-water interface

Site (date)	Zone number and (depth interval) (cm)	$R_{methylation}$ 10^{-21} mol cm^{-3} s^{-1}	J_D^{MeHg} 10^{-21} mol cm^{-2} s^{-1}
A (July 2001)	1 (0–3.8)	1.8	−4.9
	2 (3.8–9.5)	−0.4	
B (November 2001)	1 (0–1.4)	31.9	−28.5
	2 (1.4–4.1)	−5.9	
	3 4.1–5.4)	8.6	
	4 (5.4–9.5)	−3.0	
C (May 2002)	1 (0–5.9)	0.39	−0.9
	2 (5.9–7.1)	−11.1	
	3 (7.1–8.3)	17.2	
	4 (8.3–9.5)	−12.4	
C (July 2002)	1 (0–2.7)	−1.4	3.3
	2 (2.7–9.5)	0.19	
C (November 2002)	1 (0–9.5)	−0.14	0.6

based on the observations that laboratory incubation of sediments spiked with sulfate leads to an increase in MeHg production which is concomitant with SO_4^{2-} consumption and that Hg methylation is inhibited by the addition of specific inhibitors of SRB (Compeau and Bartha, 1985; Gilmour et al., 1992; King et al., 1999; King et al., 2001). To date, few field studies have demonstrated directly the role of SO_4^{2-} levels and SRB in Hg methylation (Branfireun et al., 1999). Comparison of porewater [MeHg] profiles (Fig. 5b and d, Fig. 6b,d, and f) with the corresponding [SO_4^{2-}] profiles (Fig. 2e, l, e, l, s) and with the sediment {MeHg} (Fig. 4b,e,h) and HgMT activity (Fig. 4c, f, i) profiles allow direct field observations to be made on the implication of sulfate and SRB as well as on other features of MeHg production and cycling.

Fig. 5b, d and Fig. 6b show that MeHg was produced in the porewater just below the sediment-water interface and that the zones of net MeHg production, predicted by the code PROFILE, occurred at depths corresponding with sulfate consumption (Fig. 2e, l and e). Consistently, in cases where there was no sulfate consumption in the sediments (Fig. 3l and s), no net MeHg production is observed (Fig. 6d and f). The activity of Hg methyltransferase (HgMT) measured at site C was much higher in the top 4 cm in May (Fig. 4c), i.e., when a net MeHg production was observed (Fig. 6b), than in July (Fig. 4f) or November (Fig. 4i) when no net MeHg production was noticed (Fig. 6d and f). The HgMT assay compares the depletion of MeTHF in the presence and absence of Hg. Increased use of MeTHF in the presence of Hg is assumed to be an indication of the Hg-specific methylation activity in the sediment. All of these observations consistently indicate that sulfate reduction by SRB was involved in MeHg production in the wetland sediments.

Fig. 4 shows that substantial {MeHg} remained at site C in July (Fig. 4e) and November (Fig. 4h) even if [SO_4^{2-}] was very low and sulfate reduction (Fig. 3l and s) and net Hg methylation were not observed (Fig. 6d and f). These observations are all consistent with a low rate of MeHg demethylation in the anoxic sediments of the wetland. These field results contrast with rapid MeHg turnover (1.7 d) reported for lake sediments as measured in spike experiments (Hintelmann et al., 2000).

As a final point, the commonly accepted paradigm is that MeHg production by SRB is modulated by the species $HgS_{(aq)}$, an uncharged species that can diffuse passively through the cellular membrane (Benoit et al., 1999a,b). Fig. 5a and c show a peak in $HgS_{(aq)}$, which occurred approximately at depths where net MeHg production was predicted (Fig. 5b and d), suggesting that the species $HgS_{(aq)}$ was involved in the methylation process. In contrast, in May at site C, when net Hg methylation rate was about an order of magnitude lower, a $HgS_{(aq)}$ peak (Fig. 6a) does not appear at depths where net MeHg production was predicted (Fig. 6b); at these depths, predicted Hg speciation was dominated by Hg polysulfide complexes, with low $HgS_{(aq)}$ concentrations. Thus, the observation of a low $R_{methylation}$ value at this site and date is consistent with the report by Jay et al. (2002) that Hg-polysulfide species are not available for methylation.

5.4. Fluxes of MeHg

The estimated values of MeHg diffusive flux across the sediment-water interface (J_D^{MeHg}; mol cm^{-2} s^{-1}), as calculated with Fick's first law by the code PROFILE, are shown in Table 2 for all sites in the wetland and for all dates. The values of J_D^{MeHg} (3.3 to -28.5×10^{-21} mol cm^{-2} s^{-1}) show a large variation among sites and dates. The diffusive flux values fall within the wide range of values (-0.001×10^{-21} mol cm^{-2} s^{-1} to -2900×10^{-21} mol cm^{-2} s^{-1}) reported for a salt marsh as well as for other aquatic systems (Gill et al., 1999; Langer et al., 2001; Choe et al., 2004; Hines et al., 2004; Hammerschmidt and Fitzgerald, 2006). Our results indicate that MeHg production in the sediments of the wetland leads to its diffusion into the overlying water (negative values of the fluxes in Table 2); this finding is consistent with the reports for other aquatic systems (Gill et al., 1999; Langer et al., 2001; Choe et al., 2004; Hammerschmidt and Fitzgerald, 2006), but at odds with that of Gagnon et al. (1996) for the Saguenay Fjord. The latter study suggest that MeHg is produced in sediments, but that MeHg is actively being demethylated in the presence of Fe oxyhydroxides in the top oxic sediment layers and does not diffuse out of the sediments. However, such differences may depend on local conditions.

6. CONCLUSIONS

Extensive field measurements of several variables, namely Hg and S species as well as Hg methyltransferase depth-distributions, in porewater and solid phase of the wetland sediments indicate that sulfur chemistry and SRB bacteria are important in controlling Hg methylation. For the first time, modeling of porewater [MeHg] allows field estimates of net Hg methylation rates to be made. Our field

results suggest also that the demethylation rate of MeHg is low and that MeHg can persist in anoxic sediments of the wetland.

ACKNOWLEDGMENTS

Funding is acknowledged from the Natural Sciences and Engineering Research Council of Canada through the Collaborative Mercury Research Network (COMERN). R.R.G was financially supported by post-doctoral scholarships from the Natural Sciences and Engineering Research Council of Canada and by the Fonds québécois de la recherche sur la nature et les technologies. We thank A. Chappaz, L. Rancourt and R. Rodrigue for their technical assistance and B. Boudreau, C. Gobeil and Y. Gratton for their constructive comments. This manuscript benefited from thorough reviews by three anonymous reviewers and A. Mucci.

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Associate editor: Alfonso Mucci