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Thiols in wetland interstitial waters and their role in mercury and methylmercury speciation

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Abstract

Organic sulfhydryl compounds, or thiols, are ligands that strongly complex class B metals such as Hg and methylmercury (MeHg). We determined the concentration profiles of five low-molecular-weight thiols (cysteine, thioglycolic acid, glutathione, *N*-acetyl-L-cysteine, and 3-mercaptopropionic acid) in sediment interstitial waters, at a vertical resolution of 1 cm, in three contrasting freshwater and brackish wetlands in Canada. All five thiols were detected in the porewaters, with concentrations ranging from nanomolar to submicromolar. In one of the wetlands (Baie St. Francois) the profiles of Hg and MeHg were also obtained at the same vertical resolution. Thermodynamic calculations revealed that at these levels thiols play a negligible role in inorganic Hg speciation in sediment interstitial waters, but they can dominate the MeHg speciation. Consistent with recent findings that intracellular MeHg in fish is dominated by MeHg–thiol complexes, this suggests that thiols also play a significant role in MeHg speciation in the extracellular environment.

There is increasing evidence suggesting that the methylation of Hg in the aquatic environment occurs primarily in the oxic-sulfidic boundary layer (e.g., hypolimnetic and sediment interstitial waters) where sulfate-reducing bacteria (SRB) are present (Compeau and Bartha 1985; Benoit et al. 1999; King et al. 2001). Since Hg^{2+} forms strong complexes with reduced sulfur species that can be abundant in this layer, extensive studies have been carried out to identify the role of sulfide (Gilmour et al. 1992; Benoit et al. 1999) and polysulfides (Jay et al. 2002) in determining the Hg speciation and bioavailability to SRB. However, no study has been reported on the role of organic sulfides or thiols (RSH; e.g., cysteine, glutathione) in Hg speciation. This ignorance of thiols in the extracellular environment is rather surprising, as thiols are known to play important roles in binding and detoxifying intracellular Hg (Westoo 1967; Taylor and Carty 1977; Miura and Clarkson 1993). Harris et al. (2003) recently confirmed that methylmercury (MeHg) in fish is indeed dominated by MeHg-RSH complexes.

Nanomolar to millimolar levels of thiols have been commonly reported in seawater (Matrai and Vetter 1988; Tang et al. 2000*a*; Al-Farawati and van den Berg 2001) and marine sediment interstitial waters (Luther et al. 1986; Kiene and Taylor 1988; MacCrehan and Shea 1995). They can be formed via a variety of pathways, including (1) microbial deamination of sulfur-containing amino acids (Bird and Moir 1972; Salsbury and Merricks 1975; Mopper and Taylor 1986), (2) microbial degradation of dimethylsulfoniopropionate (DMSP) (Shea and MacCrehan 1988) produced by marine algae and halophytic plants (Yoch 2002), and (3) abiotic Michael addition reaction between sulfide or polysulfides and unsaturated organic compounds (Mopper and Taylor 1986; Vairavamurthy and Mopper 1987, 1989). Although their biogeochemical significance in the early diagenesis of organic matter, sulfur (Aizenshtat et al. 1995; Boulegue et al. 1982), and trace metals such as Cu (Shea and MacCrehan 1988; Leal and Van Den Berg 1998) has been increasingly documented in marine sediments, little is known about the nature, distribution, and biogeochemical roles of thiols in freshwater systems.

Here we report the distribution of five low-molecular-weight thiols across the sediment–water interface, at a vertical resolution of 1 cm, in three freshwater and brackish wetlands in Canada, and examine their role in determining Hg and MeHg speciation. We carried out the study at wetlands because they are known contributors of MeHg to downstream lakes and waters (St. Louis et al. 1994; Bran-

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fireun et al. 1998). The three wetlands chosen are markedly different in geology and hydrology (Canadian Shield, Prairies, and fluvial, respectively) and differ over orders of magnitude in pH, ionic strength, and nutrient levels. The results from this study are thus expected to be representative of a large range of wetlands.

Materials and methods

Analytical method for thiols—The thiols in sediment interstitial waters were analyzed by high-performance liquid chromatography (HPLC) with fluorescence detection after being derivatized with a fluorogenic reagent ammonium-7-fluorobenzo-2-oxa-1,3-diazole-4-sulfonate (SBD-F) (Imai et al. 1983; Tang et al. 2000*b*). The technique was based mainly on the procedure described by Tang et al. (2000*b*) with several modifications (see Results and Discussion) to optimize the performance.

Chemicals: All the chemicals used were of American Chemical Society (ACS) grade or higher, unless otherwise specified. L-cysteine (CYS), thioglycolic acid, glutathione (GSH), *N*-acetyl-L-cysteine (NAC), 3-mercaptopropionic acid (3-MPA), *N*-cysteinylglycine, *N*-γ-glutamylcysteine, ethylenediaminetetraacetic acid (EDTA; SigmaUltra grade), SBD-F (ammonium salt), sodium tetraborate (SigmaUltra grade), and sodium acetate (SigmaUltra grade) were purchased from Sigma. Tris(2-carboxyethl)phosphine hydrochloride (TCEP) was obtained from Pierce. Acetic acid and methanol (HPLC grade) were purchased from Fisher. The peat humic acid standard (1S103H) and fulvic acid standard (2S103F) were purchased from the International Humic Substances Society (IHSS).

Stock solutions of thiol standards (1.0 mmol L^{-1}) were prepared in 5.0 mmol L^{-1} acetate buffer (pH 4.0) containing 0.1 mmol L^{-1} EDTA; EDTA was added to complex some trace metals to minimize the formation of metal–thiol complexes to improve the derivatization efficiency (Mopper and Delmas 1984). Individual and mixed working standards (5–1000 nmol L^{-1}) were freshly prepared from the stock solutions by dilution with the same EDTA-containing acetate buffer before derivatization. Deoxygenated deionized water (Milli-Q Element, Millipore; 18.2 MΩcm) was used for the preparation of all the thiol solutions; it was prepared by boiling the water followed by purging with N_2 until it cooled down to room temperature.

Preconcentration: The thiol standards and samples were preconcentrated 10-fold to improve the detection limit. In brief, 1 ml of thiol standard or sample was added to a 1.5-ml polypropylene microcentrifuge tube and covered with the cap, into which five to six holes were pierced. The tubes were then placed in a low-temperature freezer (model 86C, Forma Scientific) at −79°C overnight, followed by drying in a lyophilizer (Virtis) at −35°C and 125 millitorr overnight. The freeze-dried samples were then taken out from the lyophilizer, to which 100 μl of deionized water was added and mixed thoroughly.

Derivatization: Ten microliters of 10% TCEP was added to 90 μl of a preconcentrated standard or sample in a 1.5-ml polypropylene microcentrifuge tube. After the reduction at room temperature for 30 min, 100 μl of SBD-F solution (1 mg ml^{-1}, prepared in 2 mol L^{-1} potassium borate buffer, pH 9.5) and 200 μl of 2 mol L^{-1} potassium borate buffer (pH 10.5) containing 5 mmol L^{-1} EDTA were added. The derivatization reaction was carried out at 60°C in an incubator (Isotemp 145D, Fisher Scientific) for 60 min. The derivatized samples were then transferred to HPLC vials for analysis.

HPLC analysis: After derivatization, thiols were analyzed on a Shimadzu HPLC system (LC-10AD VP) equipped with a system controller (SCL-10A VP), a degasser (DGU-14A), an auto injector (SIL-10A), a fluorescence detector (RF-10A XL), and the Class-VP Chromatography Data software. In brief, 100 μl of the derivatized standard or sample passed through a C_{18} guard column. The thiol–SBD adducts were then separated in a reversed-phase octadecyl-silane (ODS) column (Hypersil, 250 mm × 4.6 mm, particle size 5 μm). Mobile phase A was a 0.1 mol L^{-1} acetate buffer (pH 5.0) and mobile phase B was methanol, complementary to phase A (A% = 100% − B%). The two mobile phases were filtered through a 0.45-μm membrane before use. The separation was carried out at room temperature using a flow rate of 1.0 ml min^{-1}. The fluorescence detector was set to 385 nm in the excitation and 515 nm in the emission mode.

Porewater sampling—The field sampling was carried out at three contrasting wetlands in Canada (Table 1). Forster's Bay is a eutrophic, brackish prairie wetland in Delta Marsh on the south shore of Lake Manitoba, Manitoba, characterized with a high pH (8.55) and conductivity (1830 μS cm^{-1}) of the surface water. Lake 632 is an oligotrophic Canadian shield wetland in the Experimental Lakes Area, northwestern Ontario, characterized with a slightly acidic pH (6.0) and very low conductivity (16 μS cm^{-1}) of the surface water. Baie St. Francois is a eutrophic fluvial wetland in Lake St. Pierre, a fluvial lake of the St. Lawrence River, Quebec. The pH and conductivity of the surface water of Baie St. Francois are intermediate between those of Forster's Bay and Lake 632.

Dialysis samplers (peepers) of the type described by Carignan et al. (1985) were used to collect sediment interstitial waters at a vertical resolution of 1 cm. Each peeper consisted of a 30 × 15 × 1 cm Plexiglas plate in which 0.6 × 7.0 × 0.6 cm compartments spaced 1 cm center to center were machined, a 0.2-μm hydrophilic polysulfone membrane (HT-200, Gelman) to cover the bottom plate, and a 0.2-cm Plexiglas cover sheet with windows matching the cells on the bottom plate. The Plexiglas components of the samplers were acid-washed in a solution of 5% HNO_3 for >24 h, and kept under a N_2 atmosphere for >15 d before filling the cells with Milli-Q Element deionized water and covering them with the membrane. It is critical to remove O_2 from the Plexiglas to avoid its slow release into the sampler compartments during in situ equilibrium, as this can significantly alter the shape of the profiles of redox-sensitive species such as sulfide and thiols (Carignan et al. 1994). The assembled peepers

Table 1. Comparison of the three wetlands.

	Forster's Bay*	Lake 632‡	Baie St. Francois§
Location	98°24′W, 50°11′N	93°48′W, 49°41′N	74°17′W, 46°06′N
Maximum depth (m)	0.5	1.2	<3
Surface area (km^2)	0.44†	8.6×10^{-3}	16.5
Surface water property:			
Conductivity at 25°C ($\mu S\ cm^{-1}$)	1,830 (1,650–2,020)	16 (14–33)	301 (205–420)
Alkalinity (mg $CaCO_3\ L^{-1}$)	303 (262–388)	2.6 (0.6–9.1)	
pH	8.55 (8.5–8.6)	6.0 (4.9–6.6)	7.0 (5.3–8.1)
SO_4^{2-} (mg L^{-1})	164 (147–182)	1.8 (0.05–2.99)	39.2
DOC (mg L^{-1})	17.6 (16.5–17.1) (TOC)	12.3 (1.9–30.5)	12.2 (7.8–19.0) (TOC)
DO (mg L^{-1})			5.6 (1.2–10.9)
Total P ($\mu g\ L^{-1}$)	<50	4.0 (2–15) (TDP)	239 (64–626)

* Based on the monitoring data at one station for July–September 1994 ($n=3$) (Goldsborough 1994).
† The surface area of the entire Delta Marsh is 180 km^2, with a maximum depth of about 3 m (G. Goldsborough, pers. comm.).
‡ Based on the monitoring data at the center station for 1992–1997 ($n=178$) (S. Page, pers. comm.).
§ Based on the monitoring data at one station for May–August 2002 ($n=18$) (L. Poissant, pers. comm.). The exceptional pH range at this site might be caused by the diurnal and seasonal changes in photosynthesis, as the site is highly eutrophic.

were replaced under a N_2 atmosphere again for 7 d before placement in the sediment.

Three peepers were deployed about 20 cm apart at each of the three wetlands during August–November 2003. After 3 weeks of equilibration, the peepers were retrieved individually from the sediment and sampled immediately for different constituents. Samples (3 ml) for inorganic sulfide $\Sigma[H_2S]$ were collected with polypropylene syringes in less than 2 min and injected through Teflon septa into N_2-purged amber glass vials containing the Cline reagents (Cline 1969). The concentrations of the Cline reagents were 5.4 mmol L^{-1} *N*,*N*-dimethyl-*p*-phenylenediamine (DMPD) and 5.5 mmol L^{-1} $FeCl_3$ (40 μl of each) for samples from Lake 632 and Baie St. Francois and 0.21 mol L^{-1} DMPD and 0.22 mol L^{-1} $FeCl_3$ (40 μl of each) for samples from Forster's Bay, due to the different concentrations of sulfide in these wetlands. Samples for thiols (2 ml) and dissolved organic carbon (DOC) (2 ml) were collected with polypropylene syringes and injected through Teflon septa into N_2-purged amber glass vials. Samples (1 ml) for pH measurements were taken using 1-ml syringes measured for pH within 10–30 min in the field with an Orion pH meter and pH electrode (model 290).

Additional peepers were deployed in Baie St. Francois at the same time to obtain samples for total Hg (Hg_T) and total MeHg ($MeHg_T$). Samples (16 ml; by combining water from two peepers at each depth) for Hg_T were obtained by piercing the peeper membrane with an Eppendorf pipette fitted with acid-cleaned plastic tip and transferred into precleaned Teflon bottles containing 80 μl concentrated ultrapure HCl (Optima, SeaStar). Samples (16 ml; by combining water from the other two peepers at each depth) for $MeHg_T$ were collected in a similar way into amber high-density polyethylene bottles containing 80 μl of concentrated ultrapure HCl. The Hg_T and $MeHg_T$ bottles were prepared in metal-free Class 10–1000 clean room and clean room enclosures at the Ultra-Clean Trace Element Laboratory (UCTEL) of the University of Manitoba. The bottles were doubly bagged before and after the sampling and the ''clean hands, dirty hands'' technique (St. Louis et al. 1994) was strictly followed during the sampling.

All the samples and blanks were stored at 4°C in the dark and transported to the laboratory, where samples for sulfide were analyzed within 48 h, samples for Hg_T and $MeHg_T$ were kept frozen at −20°C, and samples for thiols were kept frozen at −79°C until analysis.

Porewater analysis—Thiols were analyzed using the optimized HPLC method after SBD-F derivatization, as described above. $\Sigma[H_2S]$ was determined on a Cary 50 UV–visible spectrophotometer at 670 nm (Cline 1969). DOC was analyzed using the high-temperature combustion method (APHA method 5310B) on a Shimadzu TOC-5000A carbon analyzer. Hg_T was analyzed at UCTEL using cold vapor atomic fluorescence spectroscopy (U.S.EPA 2002) on a Tekran 2600 Hg analyzer. The Hg_T concentration in the Milli-Q Element deionized water and in the SeaStar HCl was analyzed to be 0.05 ng L^{-1} and 4.2 ng L^{-1}, respectively. $MeHg_T$ was determined in the Laboratory of the University of Ottawa (D. Lean) using capillary gas chromatography coupled with atomic fluorescence spectroscopy as described by Cai et al. (1996). The detection limits for Hg and MeHg were 0.1 and 0.6 ng L^{-1} in a 15-ml water sample, respectively.

Results and discussion

Optimization of the analytical method for thiols—Thiols are redox-sensitive and may exist in reduced (RSH) or oxidized (RSSR′) forms in natural waters, depending on the redox condition. In this study, a reducing agent, TCEP, was used to cleave the disulfide bond and convert the oxidized forms to the reduced thiols at room temperature. Therefore, the thiol concentrations reported in this study are the sum of reduced and oxidized forms, although a previous study found that thiols in intertidal sediments are dominated by the reduced form (Mopper and Taylor 1986). Whereas tri-*n*-butylphosphine (TBP) has been previously used as the reducing agent (Mopper and Taylor 1986; Tang et al. 2000*b*), TCEP

is much less malodorous and we found it is as effective as TBP in the reaction.

On the basis of Tang et al. (2000*b*) and our preliminary testing, the following conditions were used throughout this study:

$$\mathrm{RSSR'} \xrightarrow[30\ \mathrm{min}]{\mathrm{TCEP}} \mathrm{RSH} + \mathrm{R'SH} \quad (1)$$

$$\mathrm{RSH(R'SH)} + \mathrm{SBD\text{-}F} \xrightarrow[60°\mathrm{C}]{\mathrm{pH}\ 9.5} \mathrm{RS\text{-}SBD(R'S\text{-}SBD)} + \mathrm{HF} \quad (2)$$

The thiol–SBD adducts were found to be stable for a week in a 4°C refrigerator.

Several mobile phases have been used in separating low-molecular-weight thiols; these include acetonitrile (Mopper and Delmas 1984), methanol (Mopper and Delmas 1984), and trifluoroacetic acid (Tang et al. 2000*b*). Under the experimental conditions of this study, we found that methanol was the most effective mobile phase and that the pH of the mobile phase was critical, as it affects the distribution of thiol–SBD adducts between the mobile and stationary phase. The optimal separation was achieved when a 0.1 mol L^{-1} acetate buffer (pH 5.0) was used as the ''A'' phase and the methanol as the ''B'' phase with the following gradient elution profile (A% = 100% − B%): 0–10 min, isocratic 0% B; 10–15 min, 0–20% B; 15–20 min, isocratic 20% B; 20–30 min, 20–100% B; 30–35 min, isocratic 100% B; 35–40 min, 100–0% B; 40–45 min, isocratic 0% B.

Typical chromatograms of a mixed thiol standard and a wetland porewater sample are shown in Fig. 1. Figure 2 shows typical calibration curves for the thiols; typical detection limits (determined by analyzing eight replicates of the lowest standard solution and calculate at a confidence level of 99%) are in the range of 10–20 nmol L^{-1}.

Standard solutions of inorganic sulfide (prepared from Na_2S), humic acid (prepared from the IHSS peat humic acid standard), and fulvic acid (prepared from the IHSS peat fulvic acid standard) were also analyzed following the same procedure. No measurable fluorescence signals were observed, suggesting that sulfide and humic substances do not interfere with the thiol measurements.

Thiols in sediment interstitial waters—The distributions of thiols across the sediment–water interface in the three wetlands are shown in Figs. 3–5. All the five thiols were found to be ubiquitous in both the overlying waters and sediment porewaters. Their concentrations ranged from nanomolar to submicromolar, which were one to three orders of magnitude lower than those reported in intertidal sediment porewaters (e.g., Shea and MacCrehan 1988; MacCrehan and Shea 1995), but much higher than those in surface coastal waters (e.g., Al-Farawati and Van Den Berg 2001). Many of the profiles shown in the figures are highly structured with pronounced peaks, which are caused by a variety of complicated chemical (e.g., redox reactions) and biological (e.g., bioturbation, and bioirrigation) processes. To our knowledge, Figs. 3–5 represent the first depth profiles of thiols in sediment interstitial waters from freshwater and brackish waters, although depth profiles of several thiols in marine sediment interstitial waters have been previously reported (MacCrehan and Shea 1995; Shea and MacCrehan 1988). Whereas MacCrehan and Shea (1995) and Shea and MacCrehan (1988) collected the marine interstitial water samples by sectioning and filtering precollected sediment cores, the porewater profiles in this study were obtained by in situ dialysis, which not only was less prone to cross-contamination and artifacts (Carignan et al. 1985), but also yielded higher vertical resolution (1 cm).

One of the major findings from Figs. 3–5 is the lack of covariation between the concentrations of thiols and sulfide. Although sulfide concentration in the sediment interstitial waters of Forster's Bay (up to 126.6 μmol L^{-1}) was much higher than those in Lake 632 (up to 10.2 μmol L^{-1}) and Baie St. Francois (up to 4.9 μmol L^{-1}), the concentrations of the thiols in the three wetlands were in the same order of magnitude. Correlation analyses did not reveal any significant correlation between thiols and sulfide (H_2S, HS^-, or both) for any or all of the sites. No significant correlation was observed between the thiols and DOC either.

The origin of thiols in freshwater sediments has been poorly studied. Extensive studies are, however, available for marine sediments. One of the major pathways for the formation of thiols in marine sediments is the abiotic reaction between H_2S and unsaturated bonds in sedimentary organic matter (Mopper and Taylor 1986; Vairavamurthy and Mopper 1987, 1989). 3-MPA in marine sediments, for instance, is formed predominantly by the Michael addition of H_2S to acrylic acid (Vairavamurthy and Mopper 1987); a likely source of acrylic acid in marine sediments is DMSP from marine algae and plants (Yoch 2002). If this abiotic pathway dominates, one would expect that the concentration of 3-MPA would correlate to the concentration of sulfide and acrylic acid (Vairavamurthy and Mopper 1989). The lack of a covariation between thiols and sulfide in the wetland sediment interstitial waters in this study suggests either that the abiotic pathway is less important, or that the abiotic reaction is limited by the nature and concentration of unsaturated organic matter (e.g., acrylic acid). Although the latter might be the case in Forster's Bay where the sulfide concentration was sufficiently high, the fact that no correlation was observed between thiols and sulfide even in the wetlands where sulfide concentration was very low (e.g., <10 μmol L^{-1} in Lake 632 and Baie St. Francois) suggests that the thiols in the wetland sediments are unlikely to be controlled by the abiotic process. This is not surprising, as terrestrial and freshwater sediments contain only minute amounts of DMSP compared to marine systems (Kiene 1996). Kinetic studies also suggested that the rate of the Michael addition reaction between sulfide and unsaturated organic matter is favored at high ionic strength such as in marine and hyposaline waters (Vairavamurthy and Mopper 1989).

Instead, the thiols observed in the wetland sediment interstitial waters are more likely produced biotically, which usually involves microbial degradation of sulfur-containing amino acids and, where present, DMSP. Microbial pathways for the formation of cysteine, glutathione, and 3-MPA have been well documented in laboratory cultures and in marine sediments (Bird and Moir 1972; Salsbury and Merricks 1975; Kiene and Taylor 1988). Thiols in surface coastal wa-

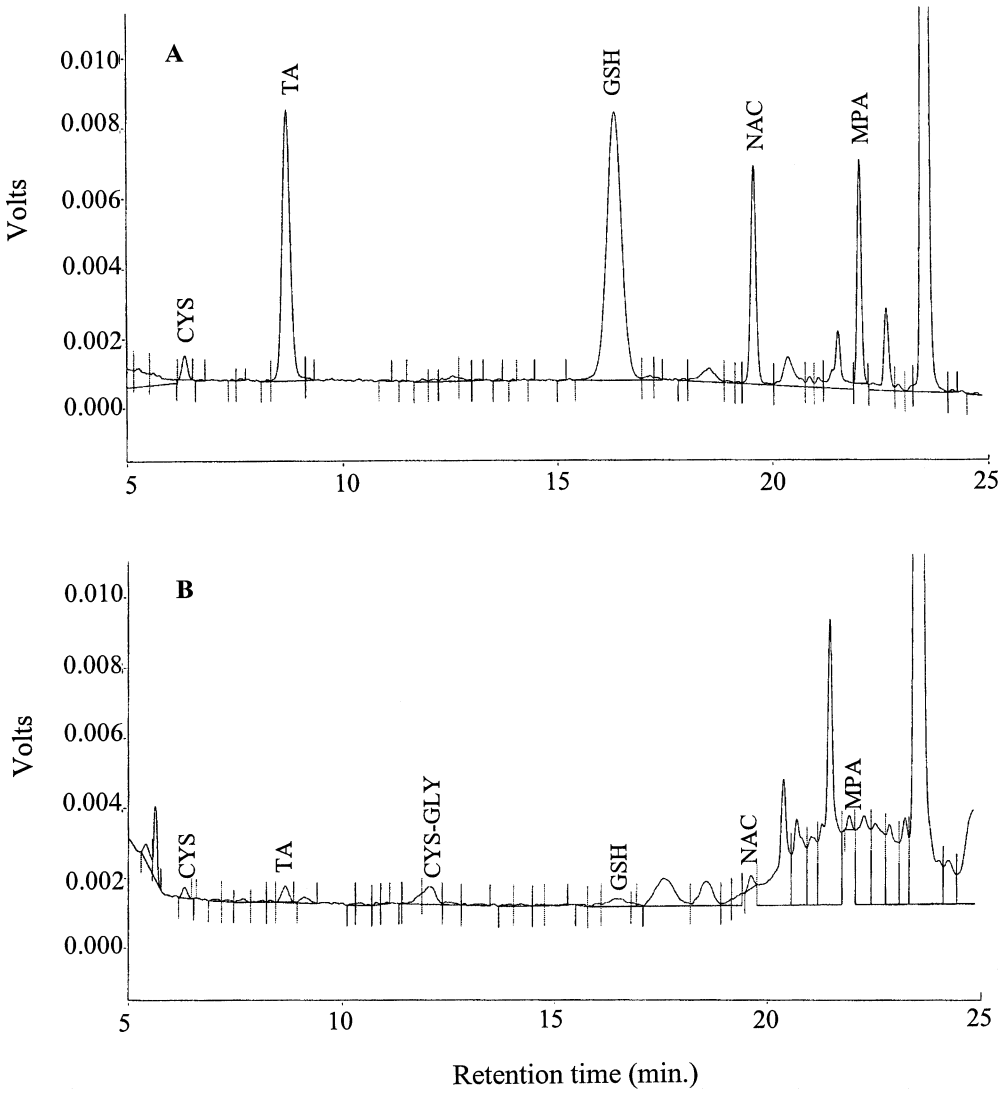

Fig. 1. Typical chromatograms for (A) a 100 nmol L^{-1} mixed standard solution of thiols, and (B) a porewater sample from Baie St. Francois after 10-time preconcentration. CYS: cysteine; TA: thioglycolic acid; GSH: glutathione; NAC: *N*-acetyl-L-cysteine; MPA: 3-mercaptopropionic acid; Cys-Gly: *N*-cysteinylglycine (Cys-Gly).

ters have also been attributed to marine phytoplankton (Al-Farawati and Van Den Berg 2001; Matrai and Vetter 1988).

Figures 3–5 also revealed the variability in the profile shapes of thiols in different wetlands. At Forster's Bay (Fig. 3), despite a very broad and evident peak of sulfide at a depth of 10–20 cm below the sediment–water interface, the peaks of thiols were not very well developed; small peaks (cysteine) or subpeaks (glutathione, *N*-acetyl-L-cysteine) were apparent at the sediment–water interface. At Lake 632 (Fig. 4), broad peaks were observed for glutathione and 3-MPA. In particular, 3-MPA showed a very evident, broad peak at a depth of 5–20 cm below the sediment–water interface, with a maximum concentration of 116 nmol L^{-1} having occurred at a depth of 11.5 cm. At Baie St. Francois (Fig. 5), very sharp peaks were observed for cysteine, thioglycolic acid, and 3-MPA just below the sediment–water interface and for glutathione at 4.5 cm below the sediment–water interface, which will be discussed further.

In addition to the five thiols studied, a new peak that was not present in the mixed standards appeared consistently in the wetland porewater samples at a retention time of approximately 12 min (Fig. 1B). On the basis of the analysis of several possible thiols and peptides, this peak was likely caused by the peptide *N*-cysteinylglycine; its concentration was estimated to be in the range of 0.02–0.1 nmol L^{-1}. The presence of *N*-cysteinylglycine suggests that the cysteine measured in this study includes both the free cysteine and cysteine in the form of *N*-cysteinylglycine, although the latter is negligible compared with the free cysteine.

Mercury and methylmercury speciation in thiolic waters—Figure 6A shows the profiles of Hg_T and $MeHg_T$

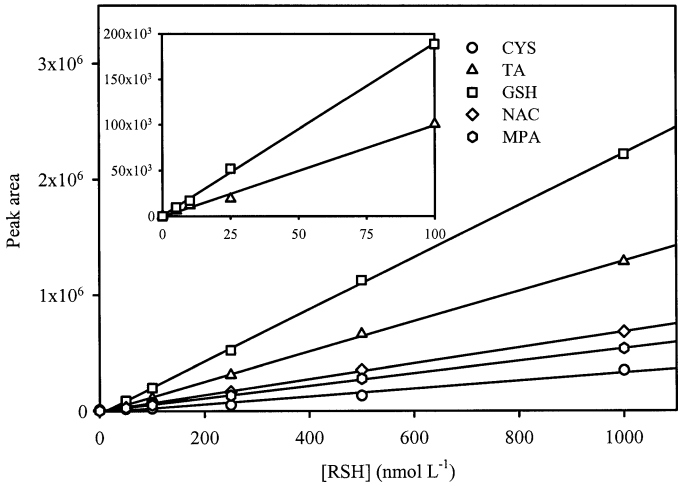

Fig. 2. Typical calibration curves of thiols.

across the sediment–water interface in Baie St. Francois, which were obtained at the same site and time as those for thiols, pH, and sulfide. The Hg_T concentration was very high at the site, ranging from 218 to 833 pmol L^{-1}. The $MeHg_T$ concentration fluctuated between 10–20 pmol L^{-1} and peaked at 45.1 pmol L^{-1} at 4.5 cm below the sediment–water interface. This $MeHg_T$ peak has been consistently observed during a multiyear study at the site starting in 2001 (as part of the Collaborative Mercury Research Network) (Tessier and Wang, unpubl. data), although the peak concentration and location vary. The presence of this $MeHg_T$ peak may result from in situ MeHg production at this depth, with subsequent diffusion to the overlying water or downward to deeper layers of the sediments.

Inorganic Hg and MeHg speciation in the overlying water and sediment interstitial water across the sediment–water interface was calculated using the speciation code WHAM (Version 6.0; Natural Environmental Research Council, UK) (Tipping 1998). The thermodynamic constants for major Hg and MeHg complexes are listed in Table 2; other constants were taken from Tipping 1998 and NIST 2003. It should be noted that although Table 2 represents the ''best available''

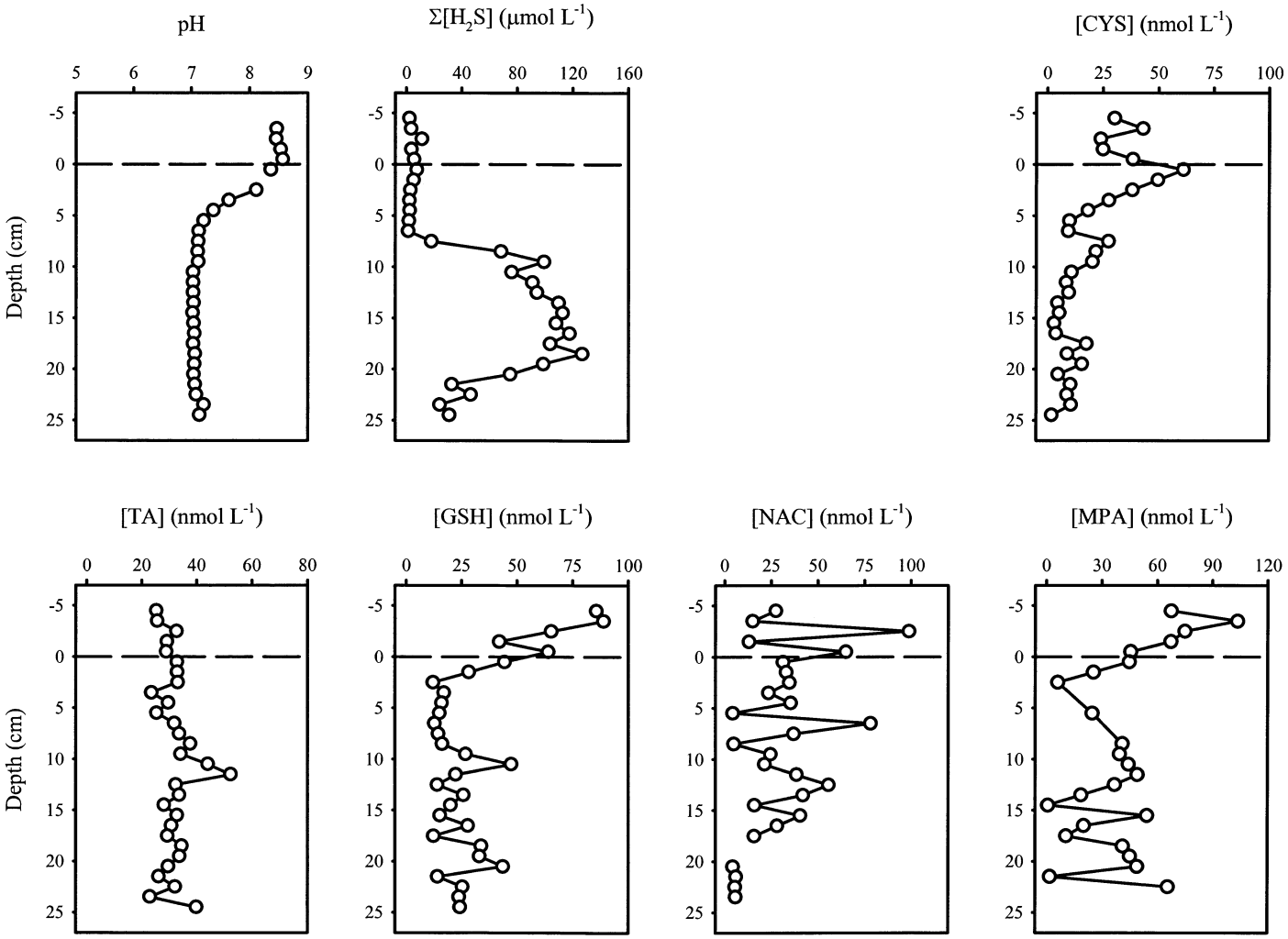

Fig. 3. Porewater profiles of pH, sulfide, and thiols in Foster's Bay.

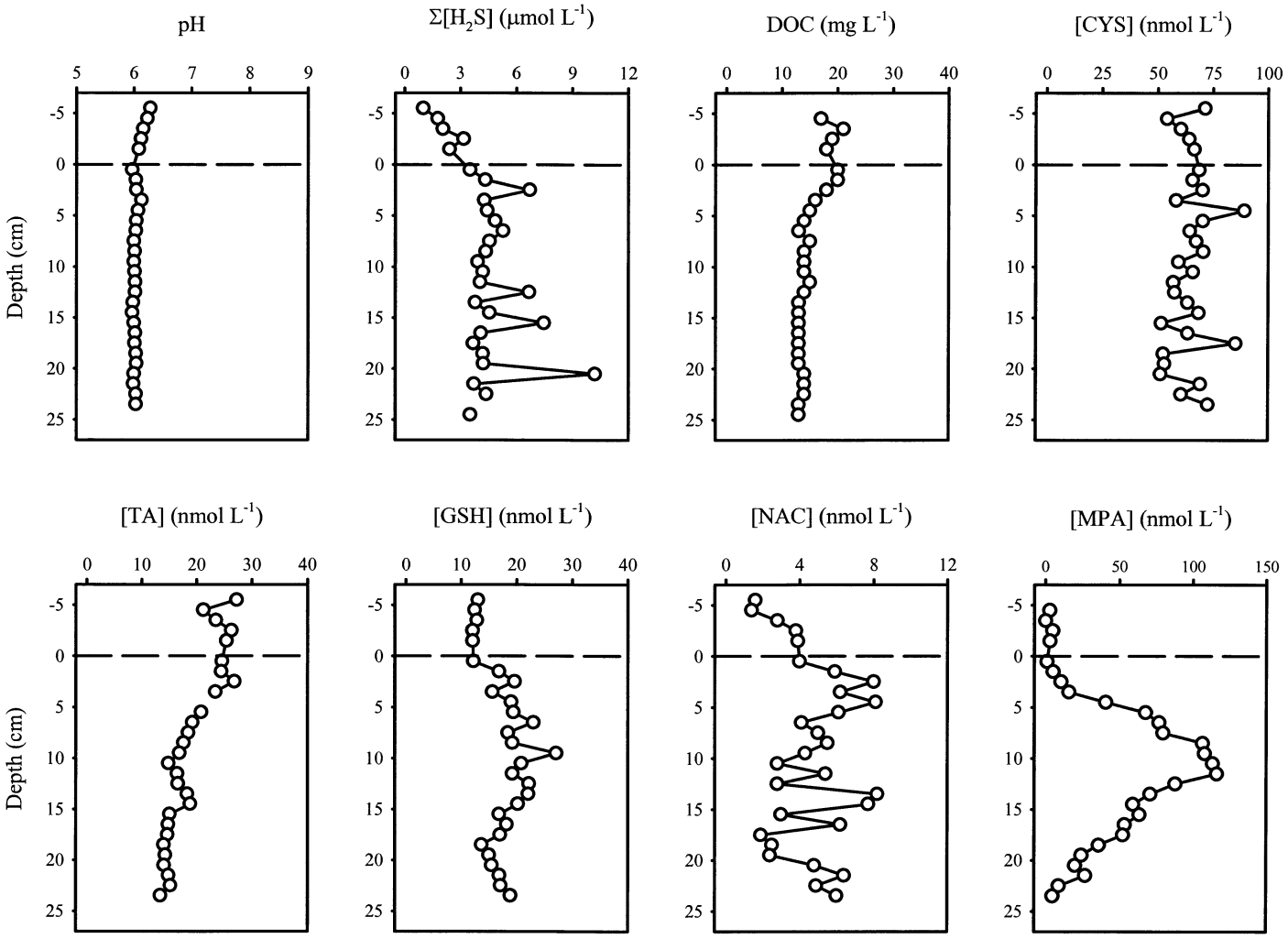

Fig. 4. Porewater profiles of thiols in Lake 632.

dataset for Hg and MeHg complexes, they by no means are all accurate and complete. For example, 3-MPA and *N*-acetyl-L-cysteine were not included in the speciation calculation simply because the formation constants for their complexes with Hg or MeHg are not available. To be consistent with previous modeling exercises (Benoit et al. 1999), we included the neutral HgS^0 species in the calculation, but it is important to note that the formation constant for this species has never been experimentally determined; the constant was simply extrapolated from formation constants for CdS^0 and ZnS^0 (Dyrssen 1989; Dyrssen and Wedborg 1991). We also did not include dissolved organic matter (DOM) in our calculation because of great uncertainties in quantifying Hg and MeHg complexes with DOM (Hintelmann et al. 1997); preliminary calculations using the two different versions of WHAM (v. 1.0 and v. 6.0), for example, yielded very different results. There is evidence, however, that at low metal concentrations DOM binding to Hg (Drexel et al. 2002) and MeHg (Hintelmann et al. 1997) occurs primarily at the ''strong binding sites'' that are presumably thiolic. Hintelmman et al. (1997) estimated that the concentration of such thiolic binding sites of DOC in lake waters is at subnanomolar level, which would be negligible compared with the free thiol concentrations measured in the wetland waters. Note also that the results from thermodynamic modeling represent the chemical composition of a system at thermodynamic equilibrium that may not be attained in nature because of kinetic constrains.

The calculation results are shown in Figs. 6B–D. The ion activity product (IAP = $\{Hg^{2+}\}\{HS^-\}/\{H^+\}$) is very close to the solubility product (K_{sp}) of cinnabar, suggesting that the Hg in the wetland porewater is slightly undersaturated or saturated with respect to $HgS_{(s,\ cinnabar)}$ in the sediments. This is consistent with observations in other ecosystems such as the Florida Everglades (Benoit et al. 1999), suggesting the importance of solid-phase precipitation or sorption as a major control on total dissolved Hg concentrations. Speciation calculations indicated that the inorganic Hg speciation is dominated by Hg–sulfide complexes (Fig. 6C). Thiols play a negligible role in inorganic Hg speciation (too low to be shown in Fig. 6C) because of their relatively low concentrations and low binding affinities to Hg when compared with sulfide.

Although thiols play a negligible role in inorganic Hg spe-

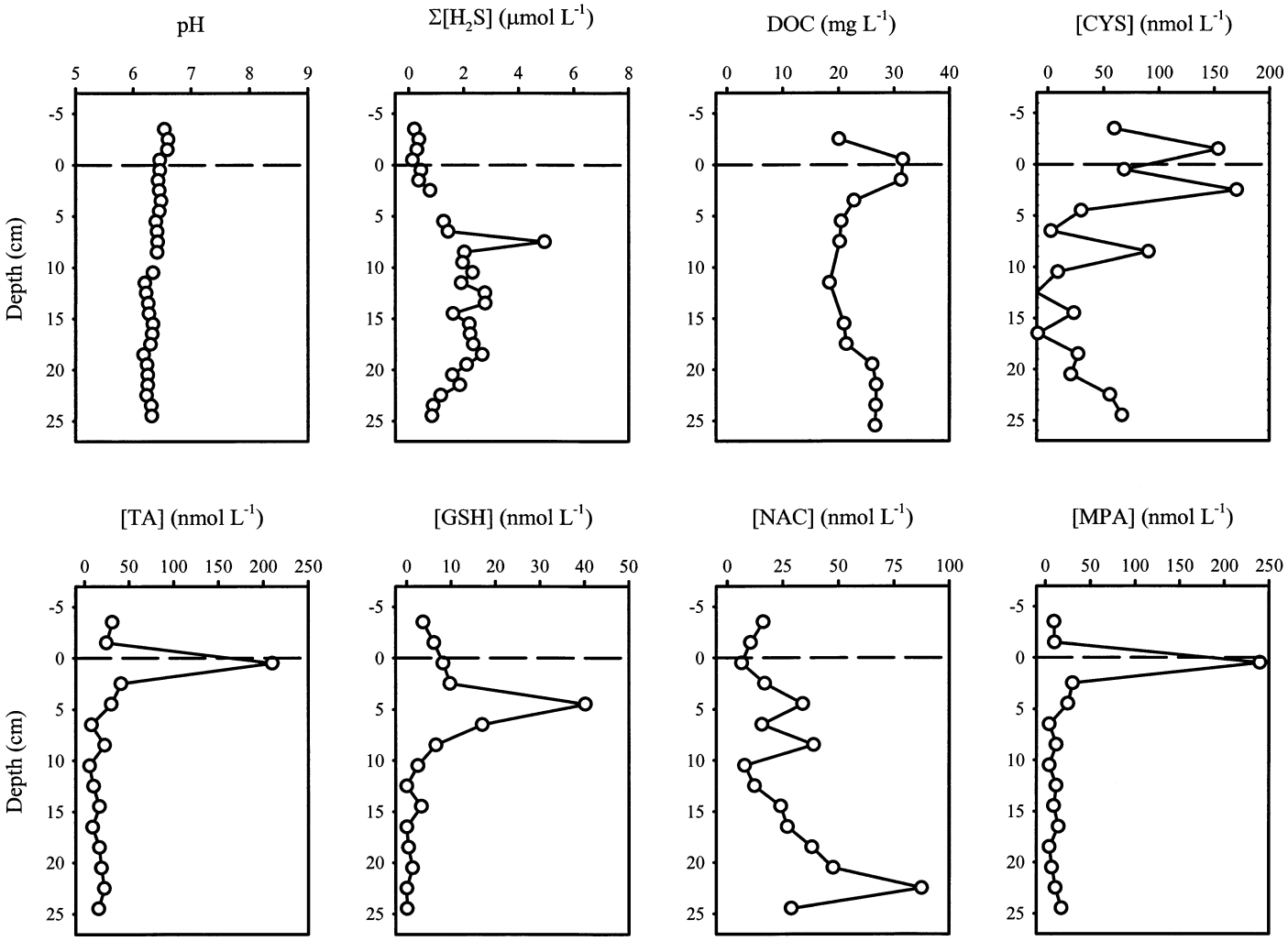

Fig. 5. Porewater profiles of thiols in Baie St. Francois.

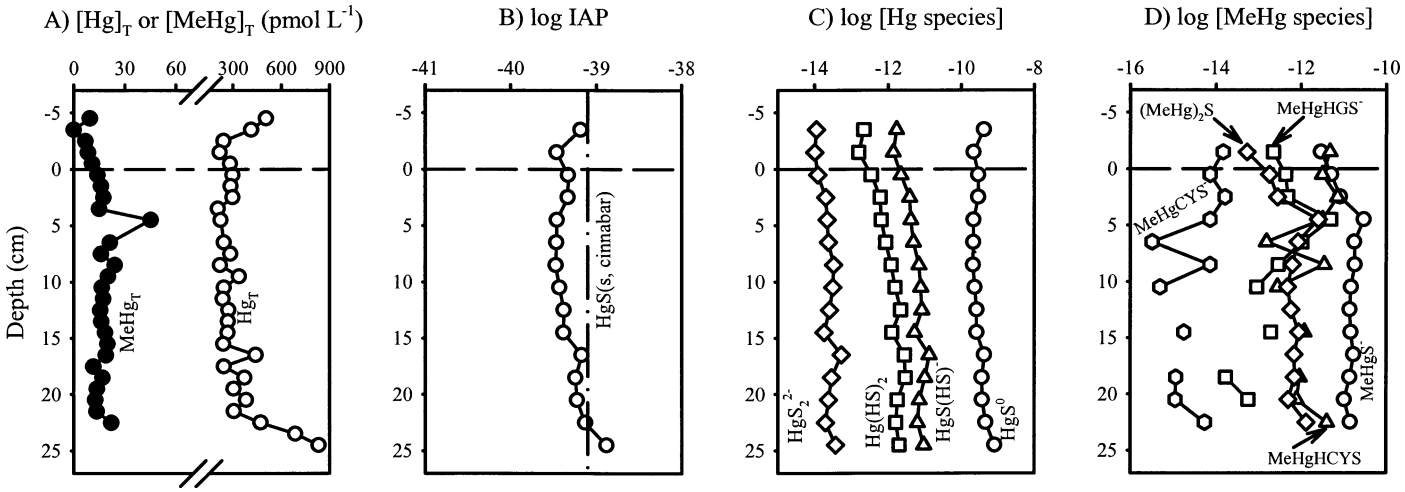

Fig. 6. Porewater profiles of (A) Hg_T and $MeHg_T$, (B) ion activity product, (C) inorganic Hg, and (D) MeHg speciation in Baie St. Francois.

Table 2. Thermodynamic constants used in the speciation calculations (t=25°C; I=0).

Reaction	log K	Reference
$HgS(s, cinnabar)+H^+=Hg^{2+}+HS^-$	−39.1	NIST 2003
$Hg^{2+}+H_2O=Hg(OH)^++H^+$	−3.4	NIST 2003
$Hg^{2+}+2H_2O=Hg(OH)_2+2H^+$	−6.17	NIST 2003
$2Hg^{2+}+H_2O=Hg_2OH^{3+}+H^+$	−3.3	NIST 2003
$MeHg^++H_2O=MeHgOH+H^+$	−4.53	NIST 2003
$2MeHg^++H_2O=(MeHg)_2OH^++H^+$	−2.11*	NIST 2003
$Hg^{2+}+Cl^-=HgCl^-$	7.3	NIST 2003
$Hg^{2+}+2Cl^-=HgCl_2$	14.0	NIST 2003
$Hg^{2+}+3Cl^-=HgCl^{3-}$	15.0	NIST 2003
$Hg^{2+}+4Cl^-=HgCl_4^{2-}$	15.6	NIST 2003
$Hg^{2+}+Cl^-+H_2O=HgClOH+H^+$	4.3	Drexel et al. 2002
$MeHg^++Cl^-=MeHgCl$	5.45*	NIST 2003
$H_2S=H^++HS^-$	−7.02	NIST, 2003
$Hg^{2-}+HS^-=HgHS^-$	22.29†	Benoit et al. 1999
$Hg^{2+}+2HS^-=Hg(HS)_2$	40.39†	Benoit et al. 1999
$Hg^{2+}+HS^-=HgS^0+H^+$	29.8†	Benoit et al. 1999
$Hg^{2+}+2HS^-=HgS(HS)^-+H^+$	34.6†	Benoit et al. 1999
$Hg^{2+}+2HS^-=HgS_2^{2-}+2H^+$	25.51†	Benoit et al. 1999
$MeHg^++HS^-=MeHgS^-+H^+$	7.0‡	NIST 2003
$2MeHg^++HS^-=(MeHg)_2S+H^+$	23.52‡	NIST 2003
$3MeHg^++HS=(MeHg)_3S^++H^+$	30.52‡	NIST 2003
$CYS^{2-}+H^+=HCYS^-$	10.74	NIST 2003
$CYS^{2-}+2H^+=H_2CYS$	19.10	NIST 2003
$CYS^{2-}+3H^+=H_3CYS^+$	20.80	NIST 2003
$Hg^{2+}+CYS^{2-}=HgCYS$	15.28*	NIST 2003
$MeHg^++CYS^{2-}=MeHgCYS^-$	16.90*	NIST 2003
$MeHg^++CYS^{2-}+H^+=MeHgHCYS$	26.07*	NIST 2003
$GS^{3-}+H^+=HGS^{2-}$	10.17*	NIST 2003
$GS^{3-}+2H^+=H_2GS^-$	19.25*	NIST 2003
$GS^{3-}+3H^+=H_3GS$	22.96*	NIST 2003
$GS^{3-}+4H^+=H_4GS^+$	25.04*	NIST 2003
$Hg^{2+}+GS^{3-}=HgGS^-$	27.32*	NIST 2003
$Hg^{2+}+GS^{3-}+H^+=HgHGS$	34.04*	NIST 2003
$Hg^{2+}+GS^{3-}+2H^+=HgH_2GS^+$	37.24*	NIST 2003
$Hg^{2+}+GS^{3-}+H_2O=HgOHGS^{2-}+H^+$	16.68*	NIST 2003
$Hg^{2+}+2GS^{3-}=Hg(GS)2^{4-}$	34.06*	NIST 2003
$Hg^{2+}+2GS^{3-}+H^+=HgH(GS)2^{3-}$	43.94*	NIST 2003
$Hg^{2+}+2GS^{3-}+2H^+=HgH_2(GS)_2^{2-}$	54.50*	NIST 2003
$Hg^{2+}+2GS^{3-}+3H^+=HgH_3(GS)_2^-$	57.92*	NIST 2003
$MeHg^++GS^{3-}=MeHgGS^{2-}$	16.66*	NIST 2003
$MeHg^++GS^{3-}+H^+=MeHgHGS^-$	26.35*	NIST 2003
$MeHg^++GS^{3-}+2H^+=MeHgH_2GS$	30.01*	NIST 2003
$TA^{2-}+H^+=HTA^+$	10.61	NIST 2003
$TA^{2-}+2H^+=H_2TA$	14.25	NIST 2003
$Hg^{2+}+2TA^{2-}=Hg(TA)2_2^{2-}$	43.8§	NIST 2003
$MeHg^++TA^{2-}=MeHgTA^-$	17.34*	NIST 2003
$MeHg^++TA^{2-}+H^+=MeHgHTA$	21.41*	NIST 2003

* Recalculated from 1 = 0.1 mol L^{-1} using the Davis equation.
† Recalculated from 1 = 0.3 mol L^{-1} using the Davis equation.
‡ 20°C.
§ I = 1.0 mol L^{-1} (not corrected to I=0).

ciation, Fig. 6D reveals that they can play a significant role in MeHg speciation. About 50% of the total MeHg near the sediment–water interface is in the form of MeHg–cysteine complexes (mainly MeHgHCYS), with the remaining 50% in the form of MeHg–sulfide complexes (mainly $MeHgS^-$); the concentrations of the free $MeHg^+$, MeHgCl, and MeHgOH are negligible (too low to be shown in Fig. 6D). To our knowledge this is the first report on the importance of MeHg-RSH complexes in extracelluar environments, which coincides with the recent finding that MeHg-RSH complexes are also the dominant intracellular MeHg species in fish (Harris et al. 2003). A comparison of Figs. 5 and 6A also revealed some degrees of covariation between $MeHg_T$ and glutathione; both peaked at similar depths in the sediments. Whether this covariation is consistent and ubiquitous warrants further investigation.

We further estimated MeHg speciation as a function of the thiol concentration under the following conditions rele-

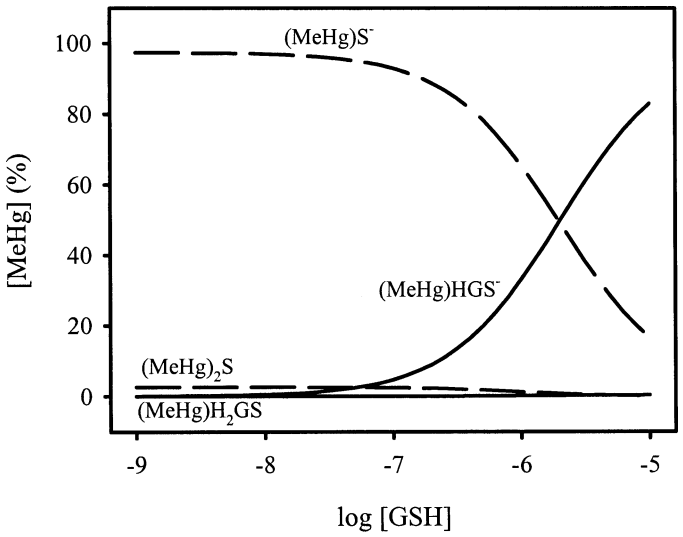

Fig. 7. MeHg speciation as a function of the concentration of glutathione under the following conditions: pH = 6.5, Σ [H_2S] = 5 μmol L^{-1}, I = 0.1 mol L^{-1}.

vant to the sediment porewaters of Baie St. Francois and Lake 632: pH = 6.5, Σ [H_2S] = 5 μmol^{-1}, I = 0.1 mol L^{-1}. Figure 7 shows the result using glutathione as an example, which indicates that MeHg-GSH complexes become important when [GSH] $>$ 10 nmol L^{-1} and will dominate the MeHg speciation when [GSH] $>$ 1 μmol L^{-1}. Since calculations with other thiols (e.g., cysteine and thioglycolic acid) yielded similar results, MeHg speciation in the porewater is expected to be dominated by MeHg-RSH complexes when the total RSH concentration exceeds 1 μmol L $^{-1}$. This is generally the case in wetlands, as shown in Figs. 3–5.

The finding that MeHg in both the intracellular (Harris et al. 2003) and extracullelar (this study) can be dominated by MeHg-RSH complexes raises critical questions on the sources, bioavailability, and uptake routes of MeHg. For example, are MeHg-RSH complexes in the sediment interstitial waters excreted from in vivo MeHg-RSH complexes (as a means of detoxification of MeHg) or formed in vitro between $MeHg^+$ (e.g., produced by SRB) and RSH? At present, MeHgCl is the most commonly used MeHg surrogate species in MeHg bioavailability and toxicity studies. Since MeHg in sediment porewaters is dominated by MeHg–sulfide and MeHg-RSH complexes (Figs. 6D and 7) instead of the free $MeHg^+$ ions or MeHgCl, it is critical to evaluate the bioavailability and toxicity of MeHg–sulfide and MeHg-RSH complexes in both extracellular and intracellular environments. Whereas lipophilic MeHg species such as MeHgCl are generally thought to readily cross the biological membrane (Gutknecht 1981; Mason et al. 1996) and hence are bioavailable, the bioavailability of MeHg–thiol complexes remains a timely scientific debate (Ekino et al. 2004; Hudson and Shade 2004; Stern 2004).

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