A COMPARATIVE STUDY OF MERCURY SPECIATION AND THE VERTICAL MOVEMENT OF NEWLY ADDED MERCURY ACROSS THE MERCURY METHYLATION LAYER IN THREE CONTRASTING WETLANDS

A THESIS

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A Comparative Study of Mercury Speciation and the Vertical Movement of Newly Added Mercury Across the Mercury Methylation Layer in Three Contrasting Wetlands

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree

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Abstract

Extensive studies have shown that wetlands are areas of rapid methylmercury (MeHg) production, yet we are presently unable to predict the *in situ* production of MeHg in wetlands. We hypothesize that MeHg is produced primarily in a thin "mercury methylation layer" (MML) in sediments under dedicate pH, sulfide and dissolved organic matter conditions which ultimately decide mercury speciation and bioavailability to mercury methylators (e.g. sulfate reducing bacteria).

To probe the relation between MeHg production and Hg speciation, high resolution profiling was conducted to uncover detailed overlying water and porewater chemistry (e.g. Hg, MeHg, pH, DOC, sulfide, and in one instance thiols) across the MML in three contrasting wetlands (Baie St. Francois in Quebec, Delta Marsh in Manitoba and Lake 632 in Ontario). ^{202}Hg addition experiments using mesocosms were carried out in two of the wetlands (Delta Marsh and Lake 632) to further trace the development and dynamics of the MML.

Over a four-year study, a peak in MeHg was consistently observed in Baie St. Francois which affirms the existence of the MML, however there is no clear trend when comparing Hg species with the peak of MeHg across the MML. The neutral HgS^0 complex dominated Hg speciation in Baie St. Francois and Lake 632, while the three complexes HgS^0, $Hg(HS)_2$ and $HgS(HS)^-$ were found to exist in the same order of magnitude in Delta Marsh.

The ^{202}Hg addition experiment in Lake 632 at the ELA and Delta Marsh demonstrate drastic differences in the mobility of newly added Hg. Added ^{202}Hg was found to penetrate up to 24cm into the sediments within one month after addition in Delta Marsh and only a few centimeters in Lake 632 at the ELA. However, by month 11 nearly all the added ^{202}Hg was no longer found in the dissolved phase in both wetlands. The different behavior of the new added ^{202}Hg is caused mainly by the difference in productivity, bioturbation and bioirrigation in the two wetlands.

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Chapter 1. Objectives

This research project is proposed to study the mercury methylation layer (MML) in three contrasting wetlands in an attempt to determine the temporal and spatial differences in mercury speciation across the MML. This investigation has been divided into the following three sections:

I) Demonstrate the presence of the MML in the wetland Baie St. Francois, a fluvial wetland along the St. Lawrence River.

II) Compare the vertical movement of newly added Hg in the form of the stable isotope ^{202}Hg over the course of one year in two contrasting wetlands: Crescent Pond which is a Prairie wetland, and Lake 632 which is a Canadian Shield wetland .

III) Compare and contrast the speciation of Hg across the MML of the three contrasting wetlands.

The Hg dynamics and speciation results across the MML are expected to provide critical information toward further attempts to predict the production of methylmercury in natural waters, which is beyond the scope of this thesis.

Chapter 2. Introduction

Mercury (Hg), a natural occurring trace metal existing in three oxidation states (Hg(0), Hg(I), Hg(II)), is a common contaminant of concern due to its toxicity and its ability to accumulate in organisms. Once released into the environment, inorganic mercury can be transformed into the highly toxic methylmercury (MeHg) species. It is these MeHg species that bioaccumulate in organisms, and further biomagnify up the food chain resulting in top predators (such as piscivorous fish) obtaining higher than expected body burdens of MeHg (Stephens, 1995). It has been shown that the main form of Hg found in fish (>95%) is in the form of MeHg (Bloom, 1989; 1992). MeHg, at environmentally low levels, is a neurotoxin that is capable of crossing the blood-brain barrier and is the cause of Minemata Disease (Harada, 1995). MeHg is the most common contaminant of fish in Canada, with unacceptable levels occurring in all regions and territories.

2.1 Global Hg Cycle

The average elemental concentration of Hg in the continental crust is estimated to be 40 μg/kg (Wedepohl, 1995). From this natural pool, Hg is emitted naturally to the atmosphere by volcanic activity, forest fires, degassing from water surfaces, and weathering of rocks (Rasmussen, 1994). Major anthropogenic inputs arise from coal combustion, waste incineration and through the industrial use of Hg (Mason *et al.*, 1994). Evidence from dated soil, peat bog, and lake-sediment records indicate that anthropogenic inputs of Hg have become the major source of Hg to the atmosphere since

the onset of industrialization (Fitzgerald *et al.*, 1998). This coincides with the findings that human activities have major impacts on global cycles of trace metals (Nriagu and Pacyna, 1988). Once emitted to the atmosphere, Hg mainly exists in the highly volatile, reduced form of elemental Hg, Hg(0), which can reside in the atmosphere for up to one year (Slemr, 1992). Within this time frame, Hg can be transported around the globe, ending up thousands of miles from its original point of release. Upon coming into contact with airborne aerosols or cloud droplets, Hg(0) becomes oxidized to Hg(II) where it can then be deposited to the aquatic and terrestrial environments via dry or wet deposition.

2.2 Wetlands and the Mercury Methylation Layer

Wetlands are defined as areas where wet soils are prevalent, having a water table near or above the mineral soil for part of the year while supporting hydrophylic vegetation (Mitsch and Gosselink, 1986). These environments are critical habitats for many plants and animals whose lifecycles have evolved to depend on such areas. Wetlands are diverse in type ranging from nutrient poor fens and bogs such as those found throughout the Canadian Shield to highly productive eutrophic marshes and fens that are scattered across the Prairies. Research over the past decade has indicated that wetlands play a critical role in the biogeochemistry of Hg as being important areas of MeHg production.

St. Louis *et al.* (1994) reported yields of MeHg 26-79 times higher from wetland portions of a boreal catchment compared to the upland area of the catchment. A follow up study

conducted on four boreal forest catchments found all wetland areas of the catchments to be net sources of MeHg to downstream lakes (St. Louis *et al.*, 1996). Rivers receiving waters from wetland/forested watersheds have been found to have higher yields of MeHg when compared to rivers receiving water from watersheds comprised strictly of agricultural areas (Hurley *et al.*, 1995). Rudd (1995) reviewed data from the Experimental Lakes Area Reservoir Project (ELARP), a whole ecosystem manipulation study, and concluded that runoff from wetlands is an important source of MeHg to aquatic systems. An extensive study of Lake 632, a headwater wetland comprising an abundance of peat at the Experimental Lakes Area (ELA), northwestern Ontario, showed MeHg levels to increase threefold as water entered the hillslope margin of the wetland to where the wetland stream exited and entered a downstream lake (Branfireun *et al.*, 1996). Upon modeling the data gathered from this investigation, it was concluded that sources within the wetland catchment were responsible for the MeHg produced as opposed to other potential sources such as atmospheric deposition (Branfireun *et al.*, 1998).

Investigating the anoxic sediments of wetlands and lakes has shown that *in situ* mercury methylation is carried out mainly by sulfate-reducing bacteria (Benoit *et al.*, 2001b; Benoit *et al.*, 1999a; Benoit *et al.*, 1999b; Gilmour *et al.*, 1992). Goulet *et al.* (in preparation) measured vertical profiles of MeHg across the sediment/water interface down into the sediments of a fluvial wetland in Quebec and demonstrated the existence of a peak of dissolved MeHg that routinely appears slightly below the sediment/water interface. This peak, although found to slightly differ in depth and concentration with different seasons and sites, routinely occurred during their work in 2001 and 2002. This

MeHg peak has also been demonstrated in the sediments of a shallow polylimnic lake (Hines *et al.*, 2004) and in salt marsh sediments (King *et al.*, 2001). This layer of wetland sediments where this peak of MeHg exists will be termed the Mercury Methylation Layer (MML) throughout this thesis. The MML is a dynamic area that constantly undergoes deposition and re-suspension of dissolved elements and nutrients. Redox reactions occurring on a daily and seasonal cycle along with bioturbation and bioirrigation from the activity of benthic organisms provide the MML with a heterogeneous nature. This heterogeneity of the MML found throughout and between wetland sediments has proved challenging in studying the speciation of Hg and its bioavailability to methylating organisms.

2.3 Mercury Cycling in Wetlands

Reduction/oxidation, adsorption/desorption, precipitation/dissolution, and methylation/demethylation are major chemical processes of Hg that can take place depending on the physical, chemical and biological characteristics of the specific wetland. These processes and characteristics will dictate where and at which concentration Hg will be present at any given place and time. Within a wetland, Hg can exist as reduced, dissolved gaseous mercury Hg(0), oxidized inorganic Hg(II) complexes, or as organo-metallic Hg(II) complexes. Once Hg(II) is deposited in a wetland, a high variety of pathways are available for its cycling as depicted in Figure 1. Due to the high vapor pressure of Hg(0) ($1.7x10^{-6}$ atm at 25 °C; Stumm and Morgan, 1996), the consequences of photoreduction (Krabbenhoft *et al.*,1998; Amyot *et al.*, 1997) and

microbial activity (Mason *et al.*, 1995), most Hg(II) deposited to natural waters undergoes reduction to Hg(0) and is lost once again to the atmosphere (Fitzgerald *et al.*, 1994; Mason *et al.*, 1994). The remaining oxidized Hg(II) becomes complexed with various ligands present in the system depending on redox conditions; the free mercuric ion, Hg^{2+}, has been found to exist in such minute levels it is deemed insignificant. Within oxic waters, Hg(II) is found complexed predominantly with the inorganic ligands such as Cl^-, OH^-, as well as with organic ligands (generally termed as dissolved organic carbon or DOC). Once transported downwards into anoxic waters near or below the sediment/water interface, these Hg(II) complexes are transformed in two ways . Reduction can occur again via microbial activity to Hg(0) and revolatilized to the atmosphere. For the remaining Hg(II) species, reduced sulfur compounds (e.g., inorganic sulfide, organic sulfides or thiols, polysulfides) present in the anoxic system will out compete the previous ligands producing a variety of Hg-sulfide complexes. Hg^{2+} is classified as a class B metal ion or soft Lewis acid; its deformed electron sheath results in a higher polarity and thus a high affinity for sulfide that is a soft Lewis base (Stumm and Morgan, 1996). Even at trace levels (nM), sulfide will out compete other ligands which exist in higher concentrations and will bond with Hg(II). Upon binding with sulfide ligands, Hg(II) can precipitate out of solution as HgS(s) (amorphous or cinnabar) and become deposited within the sediment whereupon the sediments act as a sink for Hg.

As stated earlier, it is also in anoxic porewater where it has been accepted that *in situ* mercury methylation is carried out by sulfate-reducing bacteria (Benoit *et al.*, 2001; Benoit *et al.*, 1999a; Benoit *et al.*, 1999b; Gilmour *et al.*, 1992). Once produced, MeHg

can be demethylated by bacteria (Winfrey and Rudd, 1990), it can remain in the sediments were the sediments act as a sink, or it can diffuse upwards and into the water column. In the water column, MeHg can either be demethylated via photodegradation (Sellers *et al.*, 1996) or transported downstream to other water bodies such as lakes. It is in these lakes where MeHg enters the lower levels of the food chain where it bioconcentrates (Mason *et al.*, 1996), and further biomagnifies up the food chain (Watras and Bloom, 1992).

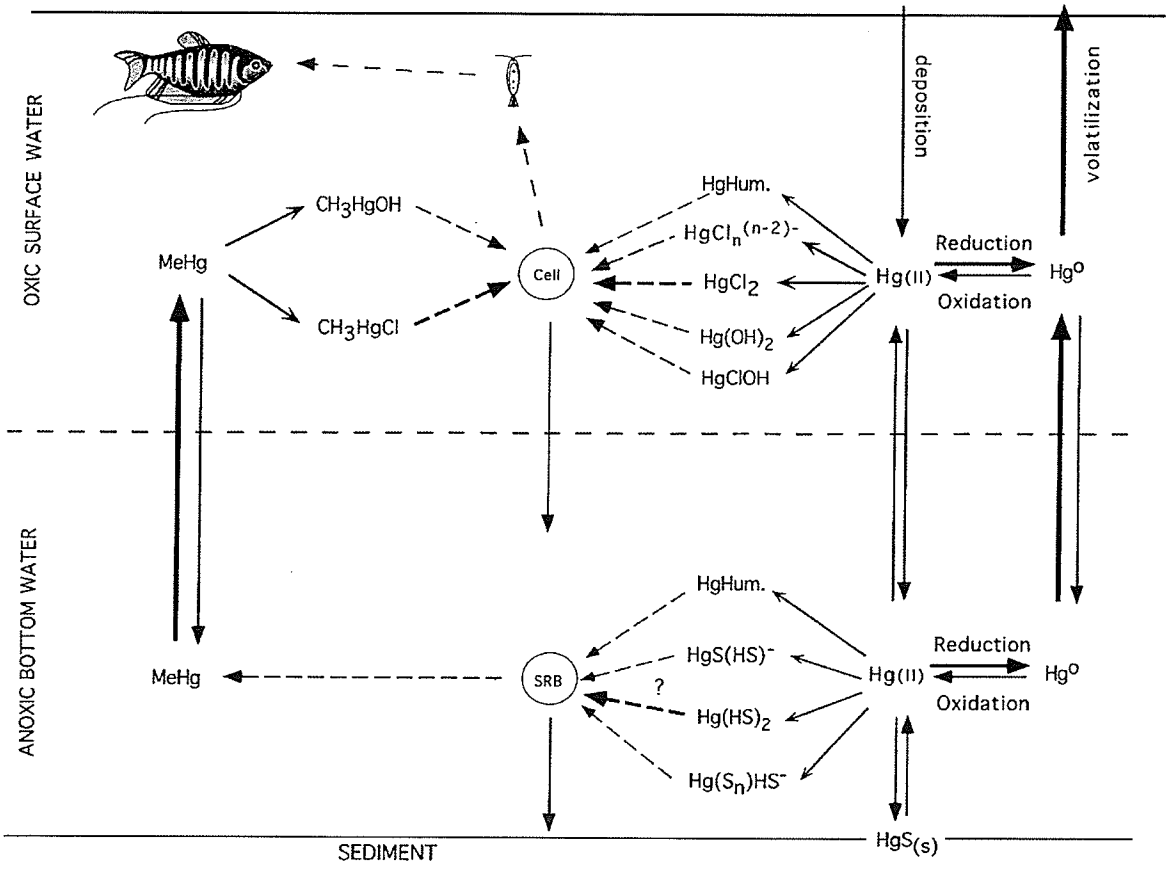

Figure 1. Biogeochemical cycle of Hg in natural waters. Reprinted from Morel *et al.* (1998). SRB = Sulfate-reducing bacteria.

2.4 Bioavailability and Mercury Speciation

The bioavailability of a metal is defined as the fraction of a metal in a matrix that can be taken up by an organism which in turn can trigger a biological effect (Stein *et al.*, 1996). The ability to determine the composition of a metal within a matrix provides much more information than simply the total concentration of a metal, which gives little indication of the potential biological hazards from occurring (Tessier and Campbell, 1987). Chemical speciation is defined as a specific form of an element defined as to its isotopic composition (e.g., ^{196}Hg, ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg, ^{204}Hg), the present oxidation state (e.g., Hg(0), Hg (I), Hg(II)), and the present complex or molecular structure (e.g., $HgCl_2$, HgClOH, HgS) (Templeton *et al.* 2000). Stumm and Morgan (1996) state that the physiological, ecological and toxicological effects of a metal can depend on the metal's speciation, not only the total concentration.

Due to the different behavior of metals, the pathway for a metal to become bioavailable to biota has been proven to be diverse. The Free Ion Activity Model (FIAM) predicts the bioavailability of a metal is based on the ability of the free aquo ion of the metal to form surface complexes on the exterior of a cell membrane (Morel and Hering, 1993; Campbell, 1995). For example, the Cu^{2+} aquo ion has been found to follow the FIAM in its toxicity to a freshwater crustacean (Stumm and Morgan, 1996). However, the FIAM cannot explain the bioavailability of metals such as Hg whose free aquo ion exists in minute concentrations relative to other species. In cases such as these, the investigation

must turn to examining the bioavailability of the various metal species that are formed in natural systems (Benoit et al. 1999a, 1999b; Golding *et al.* 2002).

2.5 DOC as a Hg-Complexing Ligand

The bioavailability of Hg-DOC complexes is poorly understood. While some studies have revealed positive relationships between DOC and MeHg production (Fjeld and Rognerud, 1993; Driscoll *et al.*, 1994), others have reported negative relationships (Barkay *et al.*, 1997; Miskimmin *et al.*, 1992). This begs the question as to what role Hg-DOC complexes play with respect to the bioavailability of Hg.

DOC is the dissolved fraction of organic carbon in natural waters. A small percentage of DOC is composed of identifiable compounds such as carbohydrates and amino acids, while the remaining 50% (Leenheer and Croue, 2003) to 80% (Ravichandran, 2004) are defined as humic substances. Humic substances are complex mixtures of aromatic and aliphatic hydrocarbon structures with a variety of functional groups (e.g., amide, carboxyl, hydroxyl, ketone, thiol). Humic substances are further divided into humic acids, which precipitate at pH=1, and fluvic acids which remain in solution at pH=1 (Stumm and Morgan, 1996). The composition of humic substances is site specific since humic substances arise from allochthonous sources (e.g., leaves, trees and animal excrement from the watershed) and autochthonous sources (e.g., algal cells, zooplankton and animal excrement from within the water body). This site-specific diversity in

structure and composition in humic substances has been challenging in determining the role Hg-DOC complexes play in determining Hg bioavailability (Chapman *et al.*, 1998).

Reduced sulfur functional groups (thiols) are the main binding site of Hg(II) to DOC (Haitzer *et al.*, 2002; Xia *et al.*, 1999). In what can be referred to as uncontaminated natural waters, Hg(II) can exist in concentrations up to 100pM or 20ng/L (Morel *et al.*, 1998). With a DOC concentration of 5mg/L containing only 1% sulfur sites with 20% (a conservative value) being in the form of reduced sulfur (Xia *et al.*, 1999), the amount of reduced sulfur is present in the order of 8n M. This value is 3 orders of magnitude greater than the natural abundance of Hg(II) in natural waters. Oxygen containing functional groups (e.g., -OH, -COOH) within DOC also show an affinity for binding to Hg(II); however, giving the large number of strong binding sites available from the presence of reduced sulfur groups, oxygen functional groups are less likely to be potential binding sites for Hg(II). If the water body in question is acidic, the DOC molecules will be less negatively charged which reduces the overall binding sites available for Hg(II) to bind. However, again with the thiol functional groups so numerous in comparison to Hg(II), Hg-DOC complexes will be readily formed.

Presently there are no analytical methods that can unambiguously determine Hg-DOC complexes in a water sample. Therefore, the amount of Hg that binds to DOC in natural waters can only be determined through the use of thermodynamic stability constants that are incorporated into predictive models such as the Humic Ion-Binding Model (Tipping, 1998). A thermodynamic constant in this case is the overall formation constant (K) for

the binding of DOC to Hg under specified conditions such as pH and salinity (Stein *et al.*, 1996). Thermodynamic constants for Hg-DOC complexes are in constant evolution in the literature and span over 26 orders of magnitude. While older values published are above the order of 10^{28}, the most recent studies indicate that the actual values are in the order of $10^{22} - 10^{28}$ (Ravichandran, 2004). The difficulties in determining accurate stability constants arise from the heterogeneity nature of DOC from different natural waters, the difficulty in working with trace levels of Hg that are representative of natural systems (pM), and the sensitivity of the analytical methods that are used to separate the Hg-DOC complexes.

DOC can decrease the bioavailability of Hg(II) in wetlands by forming Hg-DOC complexes and carrying Hg(II) further downstream to lakes that are less efficient at methylating Hg . Evidence shows that DOC is a strong complexing ligand with Hg(II) in oxic waters where free sulfide is not present. However, in waters where free sulfide is present, even at trace amounts (nM), Hg speciation will be dominated by sulfide complexes causing Hg-DOC complexes to be insignificant or negligible (Benoit *et al.*, 2001a; Dyrseen and Margareta, 1991).

2.6 Sulfide as a Hg-Complexing Ligand

Sulfide, a soft base, is produced in a variety of environments and is present in anoxic waters such as hypolimnectic waters and sediment porewaters in lakes and wetlands (Zhang and Millero, 1994). Hydrogen sulfide, H_2S, is a weak diprotic acid and is present as different species depending on the pH of the system:

$$H_2S = H^+ + HS^- \; ; \; K_{a1}$$

$$HS^- = H^+ + S^{2-} \; ; \; K_{a2}$$

where pK_{a1} is 7.02 (Chen and Morris, 1972) and pK_{a2} is >> 14 (Schoonen and Barnes, 1988). Below pH of 7.02, H_2S is the main sulfide species and above pH 7.02, HS^- is the main sulfide species. Due to the extremely high pK_{a2} value, S^{2-} is never a dominant aqueous species.

Present mainly as sulfate (SO_4^{2-}) in the water column, oxidized sulfur species can settle down to the anoxic sediments where the following reaction mediated by sulfate-reducing bacteria termed *dissimilatory sulphate reduction* takes place:

$$SO_4^{2-} + 2(CH_2O) + 2H^+ = H_2S + 2CO_2 + 2H_2O$$

where CH_2O refers to organic matter. It is this reaction that is responsible for a peak in sulfide that is often seen millimeters to centimeters beneath the sediment/water interface (Wang and Chapman, 1999). The newly derived sulfide can diffuse upwards into oxic waters, where it is oxidized to sulfate allowing it to act again as a terminal electron acceptor for the above reaction. Diffusion downward into the sediment is another pathway it can take where it often forms sparingly soluble complexes with metal ions such as Fe^{2+} and Mn^{2+} and acts as a sink.

Hg(II), a soft acid, when present will readily bind to sulfide in anoxic waters. Although the stability constants of Hg-sulfide complexes are controversial, it is generally agreed that the stability constants found for Hg-sulfide complexes are a higher order of magnitude than for other possible ligands such as DOC, thus sulfide when present, will dominate Hg speciation (Benoit *et al.*, 1999b). Sulfide concentrations across the MML in wetland sediments can range from submicromolar levels in low productive wetlands to millimolar levels in highly productive, brackish wetlands (DeVries and Wang, 2003). The concentration of sulfide along with the pH of the system will dictate the specific complexes that are present in the system. Benoit *et al.* (1999a; 2001b) demonstrate that at low sulfide levels (μM), neutral Hg-sulfide species are dominant such as $HgS^0_{(aq)}$. While at higher sulfide levels (mM), charged Hg-sulfide species are predominant such as HgS_2^{2-}. Thus different wetlands will be host to a wide variety of Hg-sulfide complexes.

Polysulfides (S_x^{2-} where x = 2-6) are also prevalent where sulfide is found. These chain sulfide compounds are formed by sulfide reacting with elemental sulfur or can exist as intermediates during the biological oxidation or reduction of sulfur (Jay *et al.*, 2000). Paquette and Helz (1997) state that Hg-polysulfide complexes most likely exist along an anoxic/oxic boundary layer such as that of a wetland's sediment/water interface. It is here where redox reactions are taking place, thus maintaining an environment where elemental sulfur would exist. Hg-polysulfide complexes exist predominantly as charged species (Jay *et al.*, 2002), with neutral Hg-polysulfide complexes comprising an insignificant minor fraction (Jay *et al.*, 2000).

Thiols, the organic form of sulfide, are also found to exist in the dissolved phase across the MML and down into the sediments of wetlands (this study and Zhang *et al.*, 2004). Thiols are formed biotically from microbial deamination of sulfur-containing amino acids (Salsbury and Merricks, 1975) and microbial degradation of dimethylsulfoniopropionate (Shea and MacCrehan, 1988) which is a product from marine algae (Yoch, 2002). Thiols are also formed abiotically via the Michael addition reaction involving sulfide or polysulfides and unsaturated organic compounds (Appathurai and Mopper, 1987). Although thiols have been reported in seawater (Al-Farawati and van den Berg, 2001) and marine sediment porewater (Kiene and Taylor, 1998), little is known regarding the biogeochemistry of thiols in freshwaters such as wetland porewater (Zhang et al., 2004).

2.7 Mercury Uptake Mechanisms by Methylating Bacteria

Earlier studies have demonstrated that MeHg inputs (dry and wet deposition) into natural waters and abiotic production of MeHg within natural water bodies cannot account for the high levels of MeHg present within the biota of these aquatic ecosystems (Fitzgerald *et al.*, 1991). This has led researchers to show that MeHg is produced mainly within the water body itself, mainly in the intracellular environment of sulfate reducing bacteria (SRB) (Gilmour *et al.*, 1991; Compeau and Bartha, 1985). At the present time, no consensus has been reached on the relationship between mercury speciation and the methylmercury produced by these methylating bacteria. Benoit et al. (1999a; 1999b; 2001a) suggested that the neutral Hg species, particularly HgS^0, are the most bioavailable

species for Hg methylation, whereas Golding *et al.* (2002) reported that all the charged and neutral Hg species are bioavailable for methylation.

Passive diffusion of a lipophilic compound across a lipid bilayer has been demonstrated as a viable pathway for a compound to gain entrance into a cell. Gutknecht (1981) demonstrated that neutral Hg species were able to diffuse across artificial phospholipid membranes. Mason *et al.* (1996) showed using diatoms, a unicellular algae comprising a lipid bilayer, that passive diffusion of the neutral $HgCl_2$ complex readily entered the cytoplasm of the cell. Benoit *et al.* (2001b) conducted a study where they grew the SRB *Desulfobulbus propionicus* over a wide range of sulfide concentrations. Results showed a strong correlation between MeHg production and sulfide levels that promoted the neutral HgS species to be the dominant Hg species present. These data suggested passive diffusion across the lipid bilayer of the neutral $HgS^0_{(aq)}$ species as a valid pathway for Hg to gain entrance into the cell.

Within the lipid bilayer that makes up the bacteria cell wall are numerous transport proteins that are responsible for transporting micronutrients, macronutrients and ions across the biological membrane into the intracellular environment of the bacteria. This has led researchers to determine the possibility of facilitated transport of charged Hg(II) complexes into the cell of bacteria. Two bioengineered bacteria termed "bioreporters", have recently been developed (Golding *et al.*, 2002) that upon Hg(II) entering the cell, proteins that are responsible for light production are synthesized. The light produced (measured with a scintillation counter) is directly proportional to the amount of Hg(II)

that has entered the cell. Using these two bacteria (*Vibrio anguillarum* and *Escherichia coli*), Golding *et al.* (2002) demonstrated that the diffusion of the neutral Hg(II) complexes into the cell differed under aerobic and anaerobic conditions. If the lipophillic neutral Hg(II) complexes are indeed the most bioavailable Hg species, there should not have been a difference in the uptake whether or not oxygen was present. The study further shows under anaerobic conditions that the uptake of Hg(II) was dependent of the total concentration of Hg, and not proportional to the abundance of neutral Hg species as was found in a study using diatoms (Mason *et al.*, 1996).

Kelly *et al.* (2003) conducted further studies with these bacteria by measuring light production at decreasing pH and at natural Hg concentrations. NaCl was added to some assays to increase the concentration of neutral Hg complexes while other assays did not receive the NaCl addition to maintain a combination of charged and neutral Hg complexes. No significant difference in light production was seen, indicating that both charged and neutral Hg complexes were gaining entrance to the cell.

Although the experiments using the bioreporter bacteria are not methylating bacteria (i.e., SRB), they do indicate that Hg can be introduced into the cell of a bacterium by some degree of regulatory control. This is a recent finding in comparison to the theory of passive diffusion as being the main route for Hg to gain access to the intracellular environment of bacteria. The accuracy of predictive models that state that neutral species are the only bioavailable Hg species available to methylating bacteria (Benoit *el al.*,

1999b) is questionable based on the recent findings that both neutral and charged Hg complexes may both be bioavailable forms of Hg.

2.8 Field Sampling Techniques for Trace Hg Levels in Interstitial Porewaters

Methods for sampling porewaters have been extensively investigated. Challenges arise due to the sensitive redox conditions present at the sediment/water interface, the temperature differences across this interface, and the limited ability to obtain a high enough vertical resolution required for the specific investigation as one moves down into the sediments of the water body. When studying the distribution of trace metals within sediments, contamination of the sample during collection becomes a pressing issue. With Hg being extremely prone to contamination, this warrants a discussion of the popular techniques available for porewater collection for Hg analysis.

The process of squeezing described by Reeburgh (1967) uses gas pressure to slowly extract porewater by compressing the collected sediments thus forcing the water through filters where the water is collected in a sample bottle. By using ultra pure gas (e.g., N_2 or Ar) for the exerted pressure, and diligently cleaning the squeezing apparatus with acid before each use, cross contamination of the sample can be avoided. However, several artifacts can be introduced during the process. Upon exposing the sample to an external pressure, it is very likely that unknown quantities of Hg-containing colloids can be forced to pass through the filter and mixed with the dissolved fraction (Wang and Tessier, 1999).

In addition, the filter may adsorb a significant fraction of the dissolved Hg. Temperature change during squeezing is another concern.

Centrifugation is another popular method for separating porewater from sediment. Upon collecting a sediment sample, samples can be sectioned from the core and placed into a centrifuge to separate the interstitial water. The supernatant is then filtered with a syringe separated by an in-line filter (Elderfield *et al.*, 1981). This process is found to require a high degree of operator training and the sample is susceptible to oxidation (Mason *et al.*, 1998). During the process of centrifugation, the sample is again exposed to unnatural pressure caused by the centrifuge. Loss to the filter can also occur. Concentrations of dissolved Hg using this method again may not be representative to the natural system.

Dialysis samplers or "peepers" are plastic (e.g., Plexiglas) samplers that operate by equilibrating ultra-pure water contained within the sampler and outside sediment porewater; the ultra-pure water and sediment porewater are separated by a dialysis membrane (Hesslein, 1976; Carignan et al., 1985). This method is advantageous in that once placed into the sediments, the dissolved Hg is collected *in situ* by diffusion across the membrane over a period of weeks. This slow process of diffusion does not exert any outside force to separate the dissolved Hg from the particulate Hg present within the porewater. The *in situ* process also maintains the natural redox and temperature conditions at the sampling depth. This method is hindered from the possibilities of incomplete equilibration, bacterial degradation of the membrane and the time consuming

process of preparation and potential contamination arising during preparation and sampling (Carignan *et al.*, 1985).

Porewater is found to be a key exposure route for benthic organisms that are associated with sediments (Chapman *et al.*, 2002), hence when sampling for Hg in porewater, one wants to obtain as accurate results as possible. Mason *et al.* (1998) compared different sampling methods and concluded that centrifugation was more reliable than squeezing or dialysis samplers when determining Hg porewater concentrations, with larger sample volumes collected as being the primary benefit using this method. However, only *in situ* dialysis samplers can truly determine the dissolved fraction of Hg due to the natural diffusion of dissolved Hg across the membrane within the porewater and maintain the in situ Hg speciation information.

All the above methods suffer from a limited vertical resolution, with a maximum resolution of about 1cm. Since biogeochemical processes across the sediment/water interface and MML often differ at millimeter scales, there is a pressing need to increase spatial resolution while profiling this environment. The technique of diffusive gradients in thin films (DGT) can obtain resolutions of <0.4mm for many trace metals (Davison et al., 2000) and sulfide (Devries and Wang, 2003), however no reliable method has been developed for Hg hence it was not used in this study.

Chapter 3. Materials and Methods

3.1 Field Sites

The field study of this thesis was conducted at three contrasting wetlands: Crescent Pond of Delta Marsh, Lake 632 of the Experimental Lakes Area, and Baie St. Francois. These three wetlands are markedly different in geology and hydrology (Prairie, Canadian Shield and fluvial, respectively) and differ over orders of magnitude in pH, ionic strength, sulfide, DOC, and nutrient levels.

Delta Marsh is a prairie marsh comprising a surface area of 180 km^2 located 70 km north west of Winnipeg in Manitoba. Situated along the south shore of Lake Manitoba, Delta Marsh is a highly productive eutrophic marsh. Crescent pond ($50^0$11'N, $98^0$24'W) is a small littoral isolated pond within Delta Marsh with *Typha* and *Phragmites* as the dominate emergent vegetation encircling the pond (Hann, 1995). Crescent Pond was chosen as our field site due to its isolation from the rest of the marsh, restricted access and the availability of historical data.

The Experimental Lakes Area (ELA), a freshwater research center located on the Canadian Shield, is located 270 km East of Winnipeg in northwestern Ontario. Lake 632 ($49^0$41'N, $93^0$48'W) of ELA is an oligotrophic fen surrounded by a raised peat mound dominated by various *Sphagnum* species, shrubs such as Labrador tea (*Ledum groenlandicum*), and black spruce (*Picea mariana*) (Branfireun *et al.* 1996). As with

Crescent Pond, Lake 632 was chosen as a field site based on its isolation, restricted access and the availability of historical data.

Baie St. Francois is a fluvial wetland located 90km northwest of Montreal, Quebec. Located at the mouth of two tributaries, the Yamaska and St. Francois rivers, Baie St. Francois ($46^0$06'N, $74^0$17'W) is the largest (16.5 km^2) of many wetlands that surround Lake St. Pierre which is a portion of the St. Lawrence River. Baie St. Francois is presently the site of the St. Lawrence River Case Study of the NSERC Collaborative Mercury Research Network of Canada (COMERN). This fluvial wetland was added early in this project thus enabling us to study a third type of wetland into the overall comparison of the MML in wetland sediments. Table 1 compares the three wetlands physically and chemically.

Table 1. Comparison of the three wetlands.

	Cresent Pond *	Lake 632 +	Baie St. Francois +
Maximum depth (m)	0.85	1.2	<3
Surface area (km^2)	$0.05x10^{-3}$	$8.6x10^{-3}$	16.5
Surface water property:			
Conductivity at 25°C ($\mu S\ cm^{-1}$)	1529 (1140-1790)	16 (14-33)	301 (205-420)
pH	8.33 (8.06-8.60)	6.0 (4.9-6.6)	7.0 (5.3-8.1)
SO_4^{2-} ($mg\ L^{-1}$)	116 (79.2-160)	1.8 (0.05-2.99)	39.2
DOC ($mg\ C\ L^{-1}$)	28.2 (19.2-42.0)	12.3 (1.9-30.5)	12.2 (7.8-19.0) (TOC)
Total P ($ug\ L^{-1}$)	356 (121-714)	4.0 (2-15) (TDP)	239 (64-626)

* (Hann 1995, Goldsborough 1987)

+ (Zhang et. al. 2004)

3.2 Mesocosm Study

To test the effect of different levels of Hg, sulfide, pH, DOC, and thiols on Hg speciation across the MML of contrasting wetlands, we decided to conduct a ^{202}Hg addition experiment using enclosures. The design involved 5 enclosures (1.5m diameter) deployed at both Lake 632 and Crescent Pond. One enclosure acted as the control (no addition of ^{202}Hg). Two enclosures received a one-time dosage of 5 times the annual deposition of Hg and the other two enclosures received a one-time dosage of 10 times the annual deposition of Hg. Duplicates of the five times and ten times treatments were decided upon to test the reproducibility of the results. The natural annual deposition rate of Hg at the ELA is found to be $14\mu g/m^2$/year (St. Louis *et al.* 1993). Since no such information is available at Delta Marsh, we decided to accept the ELA value for the natural annual deposition of Hg at Delta Marsh, as Delta Marsh is not that far away from ELA and is void of any major industry that may be a source of Hg to the wetland. No enclosures were deployed at Baie St. Francois.

3.2.1 Mesocosm Deployment

Mesocosms used for the ^{202}Hg addition experiment were polyethylene tubes of 1.5 m diameter, 2.4 m long and ¼ inch thick. For the Crescent Pond deployment, five

mesocosms were first washed with the water from the pond, and then deployed in the center of the western basin at the end of May 2003. This area of the pond was found to have the deepest water depth. The five mesocosms were set up in the shape of a "U" surrounding a sampling platform with the overlying water within the enclosures open to the sediments beneath. Water depth within the mesocosms ranged from 71.5cm to 79cm at the time of their deployment, and the distance between mesocosms was no greater than 3m. The mesocosms were hammered 30cm into the sediments (as far as they would go) thus having about 1.3m of the mesocosms emerging from the water column.

For the Lake 632 deployment, 5 mesocosms were first washed with water from the lake and then deployed in a line along the southern edge of the lake in early June 2003. Water depth within the mesocosms ranged from 69cm to 77cm at the time of their deployment, and the distance between mesocosms was no greater than 5m. The mesocosms were deposited gently so as to have 60cm of the mesocosms pushed into the sediments with approximately 1m of the mesocosms emerging from the water column. Once deployed, both sites were left untouched for three-weeks to allow the disturbed sediments to return to an assumed equilibrium.

3.2.2 Preparation of the ^{202}Hg(II) Stock Solution

10 mg of ^{202}Hg-enriched elementary Hg (99.9% purity; 98.6±0.1% enriched in ^{202}Hg) was purchased from Trace Sciences International (Richmond Hill, Ontario). The remaining six Hg isotopes make up the remaining 1.4% in the following abundance:

^{196}Hg 0.0029%, ^{198}Hg 0.1989%, ^{199}Hg 0.3367%, ^{200}Hg 0.4610%, ^{201}Hg 0.2631%, ^{204}Hg 0.0979%. 5.00 mL Ultra-pure (Optima, SeaStar) concentrated HNO_3 was added into the original bottle containing the enriched ^{202}Hg. Once completely dissolved, it was transferred into a 125-mL pre-cleaned Teflon bottle and added with 95.00 mL of deionized water (Milli-Q Element). The final 100 mg/L $^{202}Hg(NO_3)_2$ stock solution was then doubly bagged and kept in a fridge.

3.2.3 Preparation of the ^{202}Hg Spray Bottles

On the day before the Hg addition experiments, 8 acid-cleaned 500-mL polypropylene bottles were labeled and added with various amounts of the ^{202}Hg stock solution (see Table A-1 in the Appendix). Bottles were then filled with deionized water, sealed and double bagged in zip-lock bags and kept in a fridge.

3.2.4 ^{202}Hg Field Addition

After waiting a three-week period to allow the sediments to regain equilibrium, ^{202}Hg was sprayed into the mesocosms by hand in the late evening in the form of $^{202}Hg(NO_3)_2$. A detailed description regarding the dose of ^{202}Hg added to each mesocosm is given in Table A-2 in the Appendix. Deionized water was brought to the site to rinse the bottles three times so that all the ^{202}Hg had been added to the mesocosms. Immediately following the addition, large black polyethylene sheets were used to cover the mesocosms for two days to minimise the losses of ^{202}Hg due to photoreduction of Hg(II)

to Hg^0. It is estimated that up to 75% of newly added Hg to enclosures can be photoreduced and lost to the atmosphere shortly after addition (P. Blanchfield, pers. comm.). By covering our mesocosms for two days, we allowed more time for the newly added ^{202}Hg to distribute throughout the overlying water and top layer of sediments within the enclosures to maintain as much ^{202}Hg within the experimental enclosures as possible. After 2 days, the plastic sheets were removed and the mesocosms were left for 3 weeks before the first sampling to allow the added ^{202}Hg to distribute throughout the enclosed system. Sampling of the MML of the three wetlands was done according to the dates found in Table 2.

Table 2. Dates of ^{202}Hg addition and sampling of the MML of the three wetlands.

Site	Date of ^{202}Hg addition	Profiling of the MML	Month(s) after ^{202}Hg addition
Delta Marsh	June 20, 2003	Jul-03	1
		Aug-03	3
		May-04	11
Lake 632	July 6, 2003	Aug-03	1
		Nov-03	3
		Jun-04	11
Baie St. Francois	No addition	Jun-03	N/A
		Sep-03	N/A
		Sep-04	N/A

3.3 Profiling the MML

Dialysis samplers (peepers) of the type described by Carignan et al. (1985) were used to collect sediment interstitial waters at a vertical resolution of 1cm. Each peeper consisted of a 30 × 15× 1cm Plexiglas plate in which 0.6 × 7.0 × 0.6cm compartments spaced 1cm center to center were machined. A 0.2-μm hydrophilic polysulfone membrane (HT-200,

Gelman) to cover the bottom plate was laid down, and a 0.2-cm thick Plexiglas cover sheet with windows matching the cells on the bottom plate was screwed in place to hold the peeper together. The Plexiglas components of the samplers were acid-washed in a solution of 5% HNO_3 for >24 hours, and kept under a N_2 atmosphere for >15 days 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 profile of redox-sensitive species such as sulfide and thiols (Carignan *et al.* 1994). The assembled peepers were replaced under a N_2 atmosphere again for 7 days before placement in the sediment.

Peepers were deployed three times in each mesocosm (Delta Marsh and Lake 632) about 3cm apart and three times in Baie St. Francois about 10cm apart between June 2003 and June 2004 (see Table 2). After 3 weeks of equilibration, the peepers were retrieved individually from the sediment and sampled immediately for different constituents. Samples (3ml) 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 5.5mmol L^{-1} $FeCl_3$ (20µl of each) for samples from Lake 632 and Baie St. Francois and 0.21mol L^{-1} DMPD and 0.22mol L^{-1} $FeCl_3$ (40 µl of each) for samples from Crescent Pond, due to the different concentrations of sulfide in these wetlands. Samples for thiols (2.5ml) and dissolved organic carbon (DOC) (2.5ml) were collected with polypropylene syringes and

injected through Teflon septa into N_2-purged amber glass vials. Samples (1ml) for pH measurements were taken using 1-ml syringes and measured for pH within 10-30 minutes in the field with an Orion pH meter and pH electrode (Model 290). Samples for Hg_T were obtained by piercing the peeper membrane with an Eppendorf pipette fitted with a plastic pipette tip and transferred into pre-cleaned Teflon or polypropylene bottles or Digi Tubes containing 80µl concentrated ultrapure HCl (Optima, SeaStar). Samples for $MeHg_T$ were collected in a similar way into amber high-density polyethylene bottles containing 80µl of concentrated ultrapure HCl. All Hg_T and $MeHg_T$ bottles as well as plastic pipette tips used for sampling were prepared in metal-free Class 10-1000 clean rooms and clean room enclosures at the Ultra-Clean Trace Elements Laboratory (UCTEL) of the University of Manitoba. The bottles were doubly bagged before and after sampling and the "clean hands, dirty hands" technique (St. Louis *et al.* 1994) was strictly followed while sampling for Hg. All samples and blanks were stored at 4°C in the dark and transported to the laboratory. Sulfide samples were analyzed within 48 hours, samples for Hg_T and $MeHg_T$ were frozen at –20°C, and samples for thiols were kept frozen at –79°C until analyzed.

3.4 Porewater Analysis

Thiols were analyzed using the optimized HPLC method after SBD-F derivatization (Zhang *et al.* 2004). Typical detection limits are in the range of 10-20nmol L^{-1}. $\Sigma[H_2S]$ was determined on a Cary 50-visible spectrophotometer at 670nm (Cline 1969). DOC was analyzed using the high-temperature combustion method (APHA method 5310B) on

a Shimadzu TOC-5000A carbon analyzer (detection limit of $1 mgL^{-1}$) in the laboratories of Freshwater Institute and Enviro-Test Laboratories. Hg_T was analyzed at UCTEL using cold vapor-atomic fluorescence spectroscopy (CVAFS) (U.S. EPA 2002) on a Tekran 2600 Hg analyzer. The method detection limit determined for Hg_T in the UCTEL was $0.2 ngL^{-1}$. Certified reference material used in the analysis (ORMS-2 from the National Research Council of Canada) was found to be within 95%-105% of the ORMS-2 certified value of $30.6 ngL^{-1}$. An operating standard (OPR) of 5 ngL^{-1} Hg was also used as the quality control sample and the recovery was always within 90%-110%. Isotopic data of Hg in the mesocosm studies was collected by coupling a Tekran 2600 Hg analyzer to an Elan DRC II Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). Operating conditions of the ICP-MS are shown in Table 3.

Table 3. ICP-MS equipment and operating conditions.

ICP-MS instrument	Perkin-Elmer DRC-II
Plasma conditions	
rf power	1100 W
Plasma gas flow	15 L min^{-1}
Nebulizer gas flow *	0.93 L min^{-1}
Cones	
Cones used	Platinum cones
Mass spectrometer settings	
Resolution	0.7
Dwell time	50 ms
Sweeps/reading	2
Readings/replicate	30
Isotopes monitored	^{196}Hg, ^{198}Hg, ^{199}Hg, ^{200}Hg, ^{201}Hg, ^{202}Hg, ^{204}Hg

* The overall nebulzier gas flow was 1 L min-1, with the remaining compensated by the Ar flow from the Tekran.

Hg_T samples collected from ELA and Delta Marsh (8ml sample volume) were diluted up to 16ml with Milli-Q Element water to facilitate the analysis. $MeHg_T$ was determined in the Laboratory of the University of Ottawa (Dr. David Lean) using capillary gas chromatography coupled with atomic fluorescence spectroscopy as described by Cai et al. (1996).

3.5 Isotopic calculations

The added ^{202}Hg was only 98.6% enriched in ^{202}Hg; therefore 1.4% of the Hg added was a combination of the other six stable isotopes of Hg present in their relative natural abundances. A step by step explanation of the calculation used to determine the ^{202}Hg added from the natural pool of ^{202}Hg present in the porewater samples collected can be found in the Appendix.

3.6 Speciation Modeling

Chemical speciation modeling was done by thermodynamic calculations using the Windermere Humic Aqueous Model (WHAM, Version 6.0; Natural Environmental Research Council, UK). This model is based on the Humic Ion Binding Model VI (Tipping, 1998). It is important to note that thermodynamic calculations are limited in the approach to determining species present in natural waters by, (i) equilibrium is immediately assumed whereas conditions may not be at equilibrium due to kinetics or biological activity at the site under investigation, (ii) the majority of thermodynamic

constants are determined for 25°C whereas natural waters are often at lower temperatures, (iii) thermodynamic constants found at low ionic strength (fresh water) may not be accurate when extrapolated for high ionic strength waters (marine or brackish water), (iv) speciation calculations can only be applied for those species with determined formation constants and (v) in terms of sulfide speciation, little is known regarding the crystalline forms of various metal-sulfides in natural waters along with their solubility products (Chapman *et al.* 1998). While there are limitations involved with the use of thermodynamic constants in chemical speciation, they are accepted with some caution as a predictive method in determining *in situ* chemical speciation in natural waters. The thermodynamic constants used in this experiment are listed in Table 4 and represent the "best available" constants for Hg complexes and are by no means complete.

Preliminary calculations revealed that WHAM 6.0 greatly over-estimated the importance of Hg-DOC complexes in the presence of sulfide. This is likely due to the incorrect thermodynamic constants used in WHAM 6.0 for Hg-DOC complexes. The formation constants used in WHAM 6.0 (Tipping, 1998) are 3 orders of magnitude higher than those used in an earlier version of WHAM (Tiping, 1994). Little explanation is given about the reasoning for these higher formation constants, but Tipping (1998) did mention that there was a problem when extracting the formation constants from a single literature study in the 1980s. An alternative approach was then used to estimate the upper limit of the relative importance of Hg-DOC complexes in the presence of sulfide. The approach involved the following assumptions: i) all the DOC was present as humic substances; ii) humic substances contain 50% C (mass ratio) (Buffle, 1988); iii) humic substances

contain up to 1 mol/kg S (Hesterberg et al., 2001); iv) up to 10% (molar ratio) of S in humic substances is present as –SH groups (Hesterberg et al., 2001), and v) At low [Hg], Hg binding to humic substances occurs exclusively at the -SH sites and the binding between Hg and DOC-SH is similar to that between Hg and simple, free thiols such as cysteine. The calculated results suggested that, even at this upper limit, Hg-DOC complexes were negligible in waters where sulfide was present at a concentration higher than 1 nM, which was always the case with the porewater samples collected in this work. Therefore, Hg-DOC complexes were excluded from the WHAM calculation of Hg speciation calculation throughout this thesis.

$HgS(s, cinnabar) + H^+ = Hg^{2+} + HS^-$	-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
$Hg^{2+} + Cl^- = HgCl^+$	7.3	NIST, 2003
$Hg^{2+} + 2Cl^- = HgCl_2$	14	NIST, 2003
$Hg^{2+} + 3Cl^- = HgCl^{3-}$	15	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
$H_2S = H^+ + HS^-$	-7.02	NIST, 2003
$Hg^{2+} + HS^- = HgHS^+$	22.29*	Benoit et al. 1999b
$Hg^{2+} + 2HS^- = Hg(HS)_2$	40.39*	Benoit et al. 1999b
$Hg^{2+} + HS^- = HgS^0 + H^+$	29.8*	Benoit et al. 1999b
$Hg^{2+} + 2HS^- = HgS(HS)^- + H^+$	34.6*	Benoit et al. 1999b
$Hg^{2+} + 2HS^- = HgS_2^{2-} + 2H^+$	25.51*	Benoit et al. 1999b

* Recalculated from I = 0.3 mol L^{-1} using the Davis equation.

Chapter 4. Results & Disscusion (I):

Presence of the MML in Wetlands

4.1 Presence of the MML at Baie St. Francois

Depth profiles of water chemistry and Hg speciation across the sediment/water interface from the years 2001 to 2004 from Baie St. Francois are shown in Figures 2 to 6. Data were gathered in June and September 2003 (Figures 4 and 5) and September 2004 (Figure 6). The earlier data from 2001 and 2002 (Figures 2 and 3) are courtesy of Richard Goulet from the Collaborative Mercury Research Network (COMERN) (Goulet et al., in preparation).

The horizontal dashed line within these figures represents the sediment/water interface with the negative values on the y-axis entering the overlying water and the positive values reaching down into the sediments of the wetland. Data points generally begin a few centimeters above the sediment/water interface and reach down up to 25cm into the sediments. This varies according to how the dialysis sampler was placed into the sediments at the time of sampling. The x-axis scales were kept constant among analytes for each site whenever possible. However, at times it was necessary to increase/decrease the scale to demonstrate the shape of the profile at the time sampled.

During July 2001 (Figure 2a), MeHg peaked 3.5cm below the sediment/water interface and again peaked in November 2001 (Figure 2b) 1.5cm below the sediment/water

interface. The July 2001 MeHg peak corresponds with an increase in dissolved sulfide while the November 2001 MeHg peak is above the peak in dissolved sulfide. In May 2002 (Figure 3a) MeHg peaks 2.5cm below the sediment/water interface with a corresponding increase in sulfide. July 2002 (Figure 3b) sulfide is present in the overlying water down into the sediments, however the presence of MeHg is at an extreme minimum lacking a peak at any depth. November 2002 shows a peak in MeHg half a centimeter above the sediment/water interface again corresponding to an increase of sulfide (Figure 3c).

The data provided by R. Goulet (Figures 2 and 3) demonstrate two important points. First, the corresponding peaks of sulfide and MeHg emphasis the relationship between the activity of sulfate-reducing bacteria and the production of MeHg. Second, MeHg is produced within a thin layer near or below the sediment/water interface of the wetland sediments. The presence of this layer of MeHg (the MML) is the basis of the remaining work of this thesis. It should be noted, however, that the study site at Baie St. Francois in this thesis is not the same as the site studied by R. Goulet and A. Tessier.

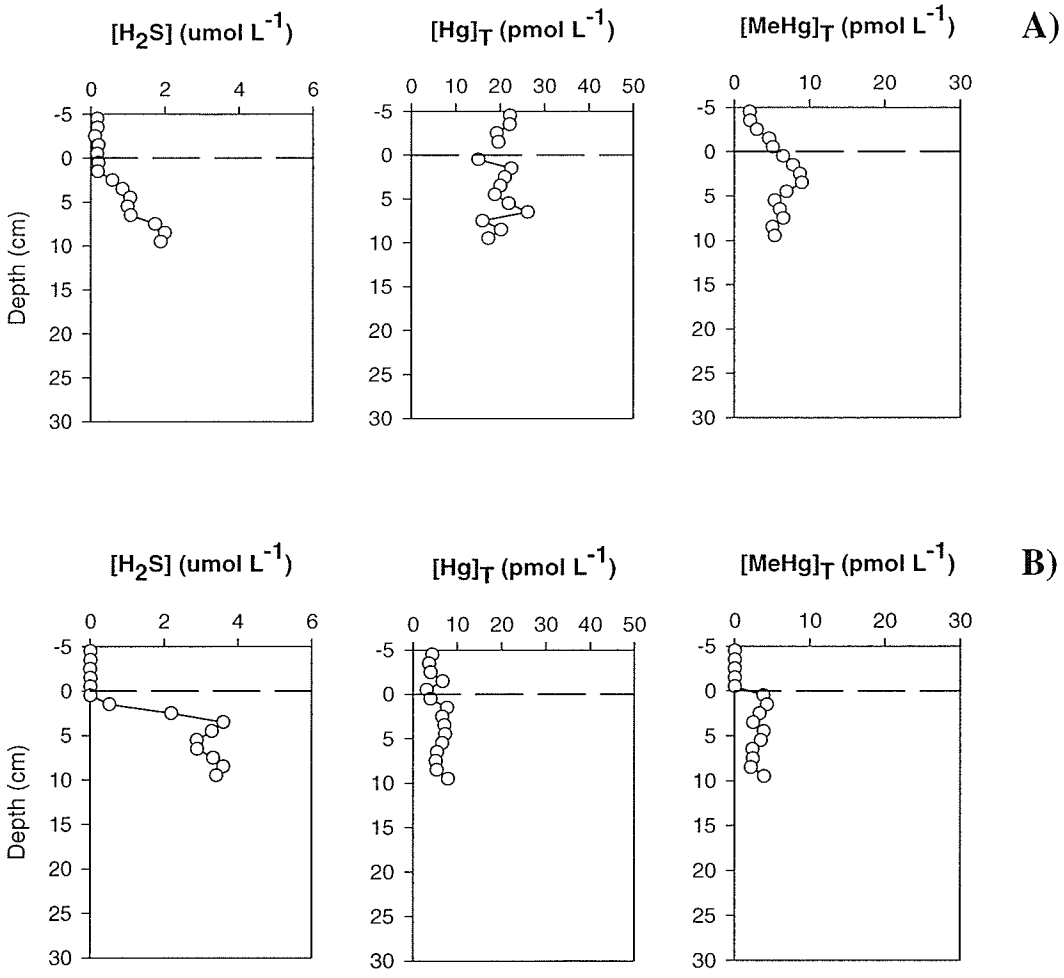

Figure 2. Porewater profiles of sulfide, Hg_T, and MeHg in A) July 2001 and B) November 2001 in Baie St. Francois. (Courtesty of R. Goulet).

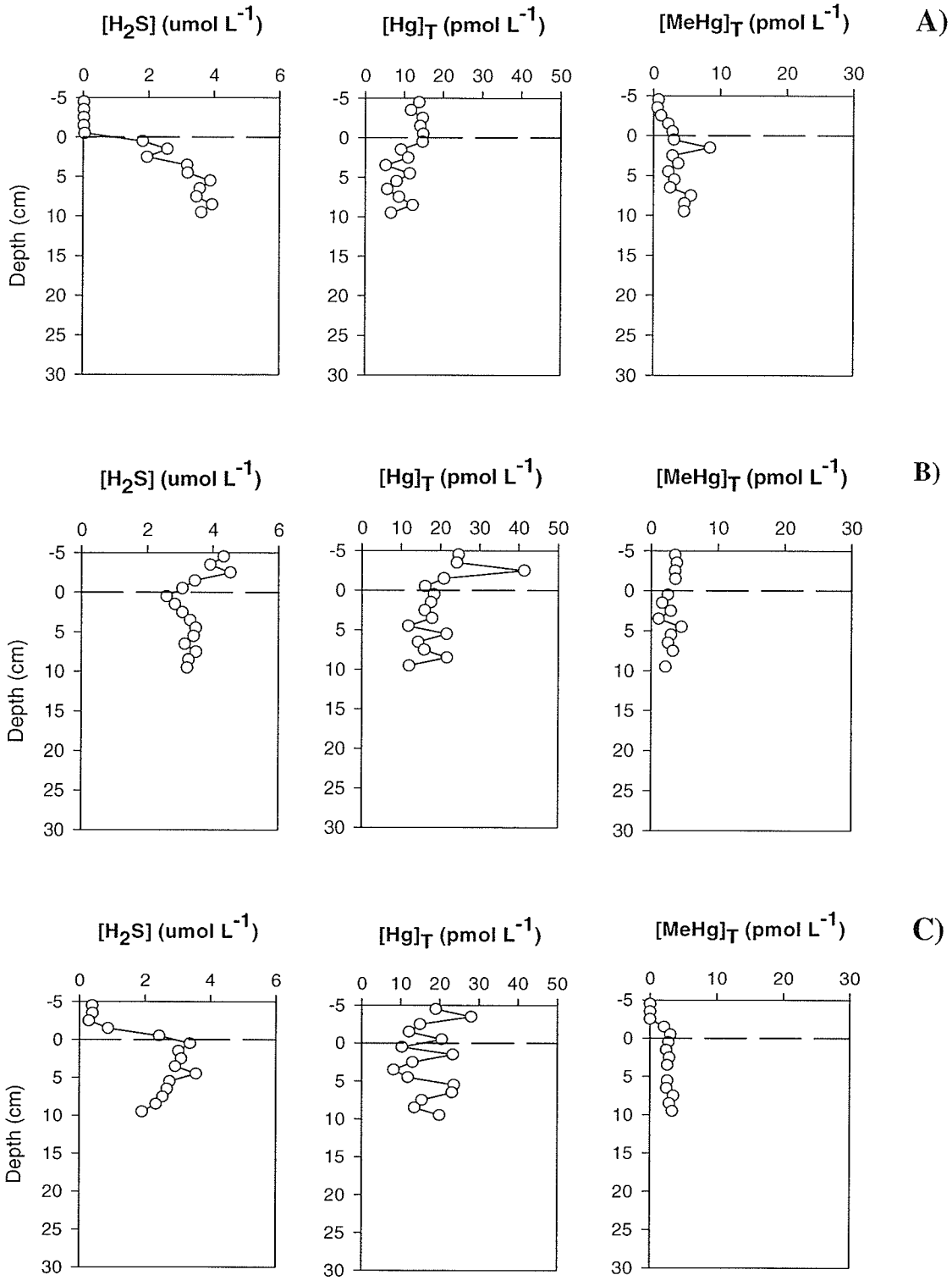

Figure 3. Porewater profile of sulfide, Hg_T and MeHg in A) May 2002, B) July 2002 and C) November 2002 in Baie St. Francois. (Courtesty of R. Goulet).

4.2 Porewater characteristics of Baie St. Francois

Figures 4 through 6 are porewater profiles of pH, sulfide, DOC, Hg_T and $MeHg_T$ in Baie St. Francois from June and September 2003 and September 2004. The major Hg species present in the system, as calculated by WHAM 6.0, are also shown. Hg-DOC complexes were excluded in the speciation calculation for the reasons described in Section 3.6.

pH profiles from 2003 and 2004 (Figures 4, 5 and 6) indicate a constant pH down through the sediments with a slight increase in pH immediately above the sediment/water interface. June and September 2003 show pH near 6.4 (Figures 4 and 5) while September 2004 shows pH closer to 7.0 (Figure 6). This variation of pH is likely a result of sampling a site 20m away from the 2003 site.

The sulfide concentration in sediment porewaters of Baie St. Francois was generally in the lower micromolar level. Sulfide peaked near 6cm depth in June 2003 and 8 cm depth in September 2003 (Figures 4 and 5) with September 2003 showing a very pronounced peak reaching 5μmol/L. The sulfide profile from September 2004 (Figure 6) demonstrates that the sulfide level was very low above the sediment/water interface and then fluctuated around 1μmol/L below the interface; the very high sulfide concentration of sulfide (~ 8μmol/L) at a depth of 25cm depth might be due to sampling/analytical errors, as the peak was defined only by 1 point. Peaks in sulfide represent the extent of sulfate reduction which produces sulfide and the sulfide fixation by iron and manganese which consumes sulfide. While only slightly pronounced in Figures 4 and 6, these peaks

demonstrate the presence of biotic sulfate reduction thus the presence of mercury methylating bacteria (i.e., sulfate reducing bacteria).

The slight increase in pH (Figures 5 and 6) corresponds with a sudden increase in sulfide in September 2003 and 2004 (pH data is lacking across the sediment/water interface due to irregular placement of the sampler at the time of deployment in June 2003, Figure 4). Maximum levels of pH are recorded at sites of major sulfate reduction, as sulfate reduction is an alkalinity generating process. For example, with sulfate reduction occurring in the presence of $Fe(OH)_3$ the reaction:

$$4Fe(OH)_3(s) + 4SO_4^{2-} + 9CH_2O = 4FeS(s) + CO_2 + 8HCO_3^- + 11H_2O$$

generates buffering capacity via the bicarbonate ions which in turn generate the alkalinity that raises the pH (Carignan and Nriagu, 1985; Strumm and Morgan, 1996). With the low sulfide levels in Baie St. Francois, only a slight increase in pH is observed.

The DOC concentration in sediment porewaters of Baie St. Francois ranged from 10-35 mg C/L. DOC is seen to peak at a depth of 5cm in June 2003 while in September 2003, the peak is seen directly across the sediment/water interface (Figures 4 and 5). These peaks show high levels of DOC both close to 35mg/L. Less structure is seen in September 2004 (Figure 10) with a much lower concentration of 10mg/L near sediment/water interface and stretching up to 20mg/L 25cm into the sediments. The

shape of the DOC profiles across the sediment-water interface results from a variety of complicated diagenetic processes that produce, transport, transform, and consume DOC.

Hg_T concentrations are seen to differ among the 3 profiles (Figures 4, 5 and 6). The highest concentrations are found in June 2003 reaching beyond 750pmol/L, where September 2003 Hg_T levels reach a maximum close to 500 pmol/L at 23cm depth into the sediments. The lowest concentrations were detected in September 2004 at 17cm depth close to 400pmol/L. These profiles lack any consistent structure.

The calculated major Hg species in Baie St. Francois are profiled in Figures 4, 5 and 6. The neutral complex HgS^0 is found to dominate Hg speciation. As a general rule, the neutral complex $Hg(HS)_2$ and the two charged complexes $HgS(HS)^-$ and HgS_2^{2-} were all found to exist in two, three and four orders of magnitude lower than the HgS^0 complex respectively. A slight increase in the presence of the $Hg(HS)_2$, $HgS(HS)^-$, and HgS_2^{2-} complexes is seen between 1cm and 8cm depth in June 2003 and September 2003, while this curve in the profiles is also demonstrated very sharply across the sediment/water interface in September 2004. The profile for the neutral HgS^0 remains vertical down into the sediments in June 2003 whereas it increases slightly at 20cm depth in September 2003 and at 6cm depth in September 2004. This Hg-speciation data will further be discussed in Chapter 6.

$MeHg_T$ data were obtained for June 2003 and September 2003 and plotted in Figures 4 and 5. Both profiles reveal distinctive peaks of 50pmol/L at 3cm depth in June 2003 and

5cm depth in September 2003. The June 2003 profile is noisy beyond the peak as it heads downwards into the sediments alternating above and below 30pmol/L. Whereas the September 2003 profile remains constant as it moves down into the sediments at a lower concentration of 20pmol/L. Both peaks of MeHg are found to overlap the peaks of sulfide at both times.

This finding of a MeHg peak is consistent with the data from 2001 and 2002 (Figures 2 and 3) again emphasizing the relationship between biotic sulfate reduction and MeHg production. More pressing, these strong peaks of MeHg coupled with the MeHg data from 2001 and 2002 emphasize the existence of the MML in Baie St. Francois. The peak of MeHg is found to exist from slightly above the sediment/water interface down to a depth of 4.5cm into the sediments between June and September 2003. The magnitude of the peak also varied from sub-picomolar levels in November 2002 (Figure 3c) up nearing 60pmol/L in June 2003. In comparing MeHg concentrations to Hg_T concentrations at the time of sampling, Hg_T concentrations seem to influence the concentration of the MeHg peak.

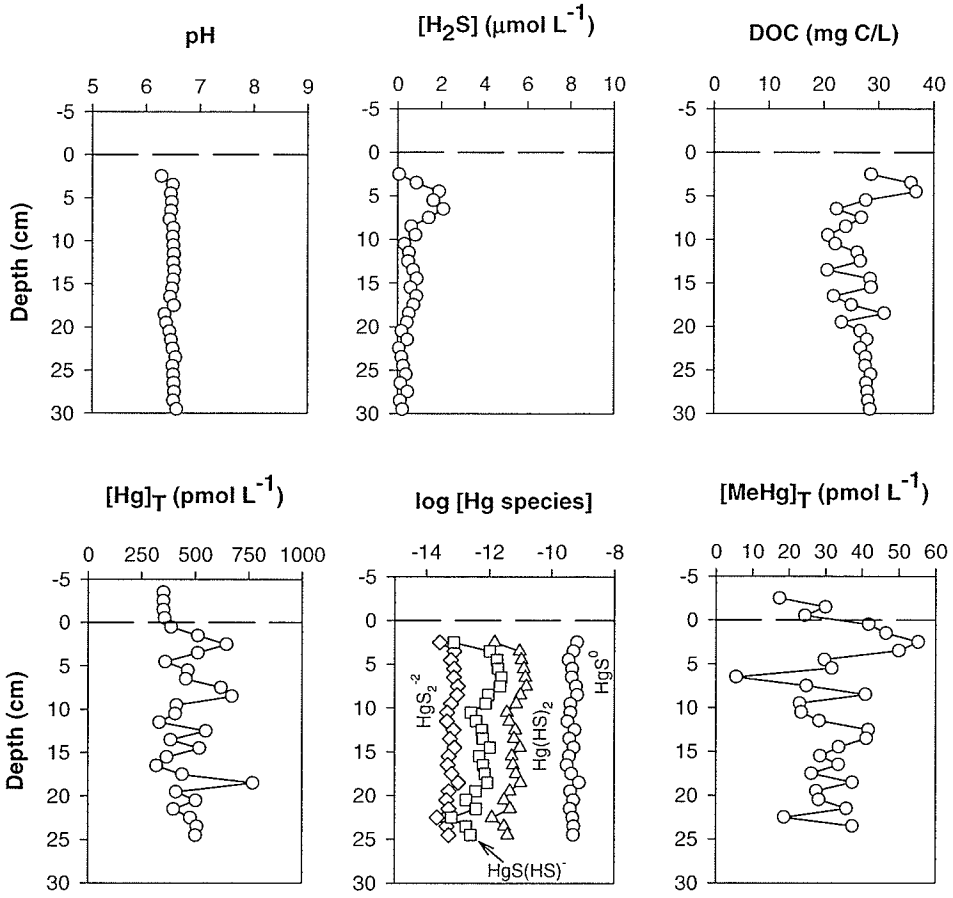

Figure 4. Porewater profiles of water chemistry and Hg speciation in Baie St. Francois

June 2003

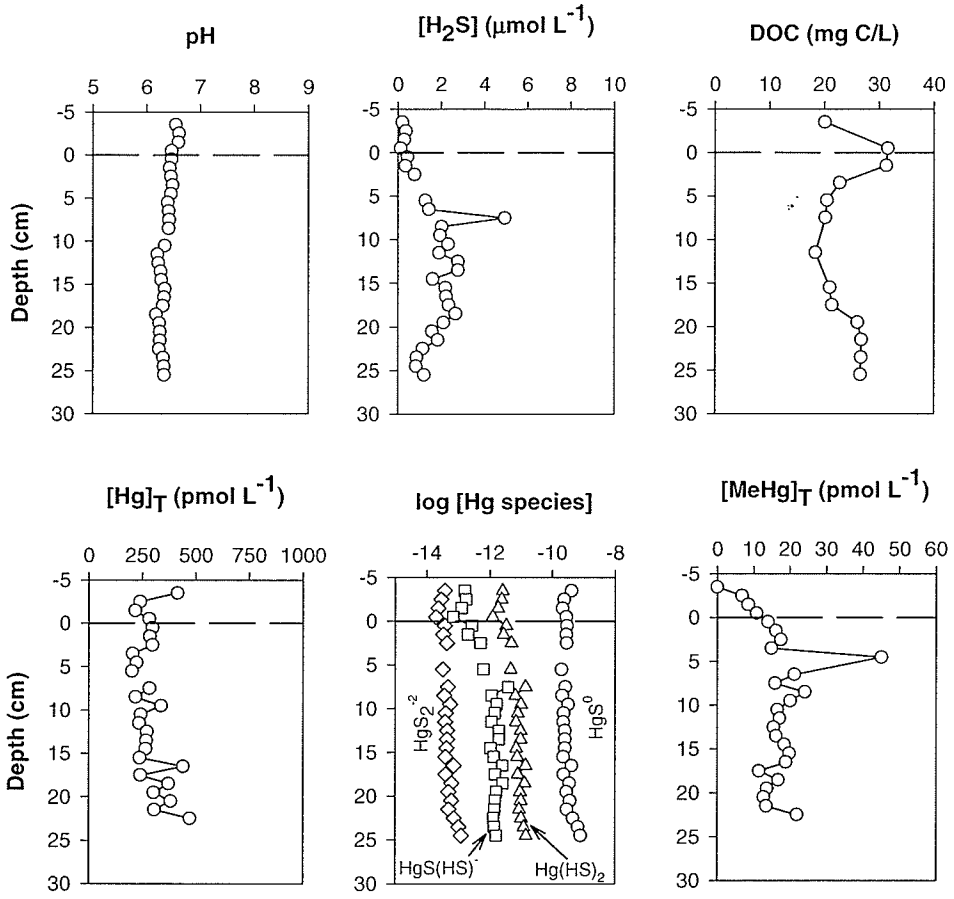

Figure 5. Porewater profiles of water chemistry and Hg speciation in Baie St. Francois September 2003.

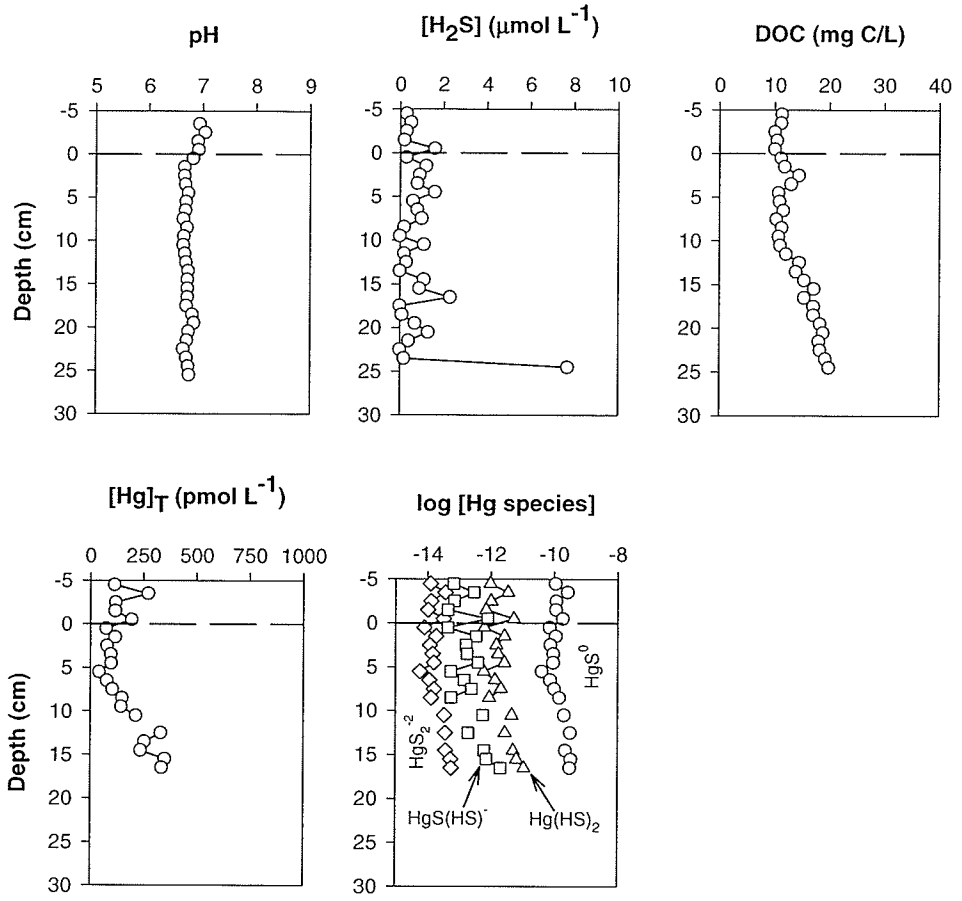

Figure 6. Porewater profiles of water chemistry and Hg speciation in Baie St. Francois September 2004.

4.3 Thiols across the MML

An investigation of the presence of five low molecular weight thiols (CYS: cysteine, TA: thiolglycolic acid, GSH: glutathione, NAC: N-acetyl-L-cysteine and MPA: 3-mercaptopropionic acid) was also undertaken in the Baie St. Francois wetland. The analytical method was developed mainly by Dr. Jingzhong Zhang, a postdoctoral fellow of our laboratory, whereas the author of this thesis collected all the porewater samples and was also involved in some of the sample analyses. The detailed technique is described elsewhere (Zhang et al., 2004), of which the present author was a co-author. A chromatogram of a mixed thiol standard and a chromatogram of a porewater samples from Baie St. Francois are shown in Figure 7. All five thiols analyzed were found to be present in the overlying water above the sediment/water interface and down across the MML into the sediment porewater. Sharp peaks are seen in Baie St. Francois slightly below the sediment/water interface for MPA, TA and GSH (Figure 8). The other two thiols (CYS and NAC) show sporadic profiles giving their profiles no defined structure. Concentrations of the thiols in the wetlands ranged from nanomolar to submicromolar levels which bracket those levels found in intertidal sediment porewaters (Shea and MacCrehan 1988) and in surface coastal waters (Al-Farawati and Van Den Berg 2001).

These peaks within the MML are caused by a variety of complicated chemical (e.g. redox reactions) and biological (e.g., bioturbation, and bioirrigation) processes. Comparing the thiol profiles from September 2003 (Figure 8) to that of inorganic sulfide of September 2003 (Figure 5) shows only a correlation between that of inorganic sulfide with GSH.

However, the other four thiols show major and minor peaks occurring at depths where inorganic sulfide does not peak. This finding may indicate that the abiotic formation of thiols from inorganic sulfide is of lesser importance than that of biotic formation (Zhang et al., 2004). Microbial degradation of sulfur-containing amino acids (Salsbury and Merricks, 1975) and microbial degradation of DMSP (Shea and MacCrehan, 1988) are biotic processes that may be occurring within the MML down into the deeper sediments of these wetlands. Thiols have recently been found to play an important role in MeHg speciation, however they are negligible when dealing with Hg speciation (Zhang *et al.* 2004).

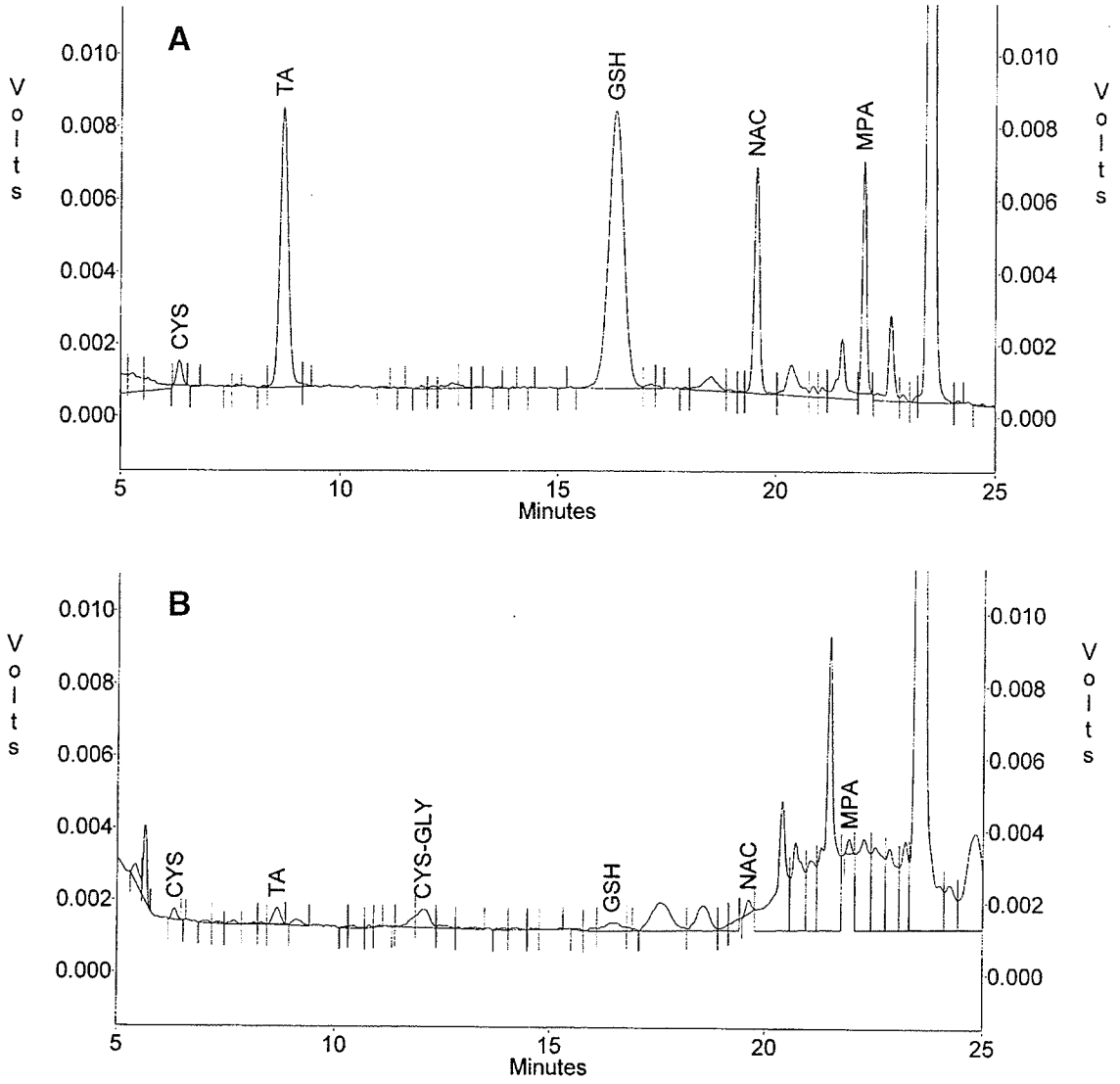

Figure 7. Typical chromatograms for (A) a 100 nmol L^{-1} mixed standard solution of thiols, and (B) a porewater sample from Baie St. Francois after 10-time preconcentration. CYS: cysteine; TA: thioglycolic acid; GSH: glutathione; NAC: N-acetyl-*L*-cysteine; MPA: 3-mercaptopropionic acid; Cys-Gly: N-cysteinylglycine (Cys-Gly).

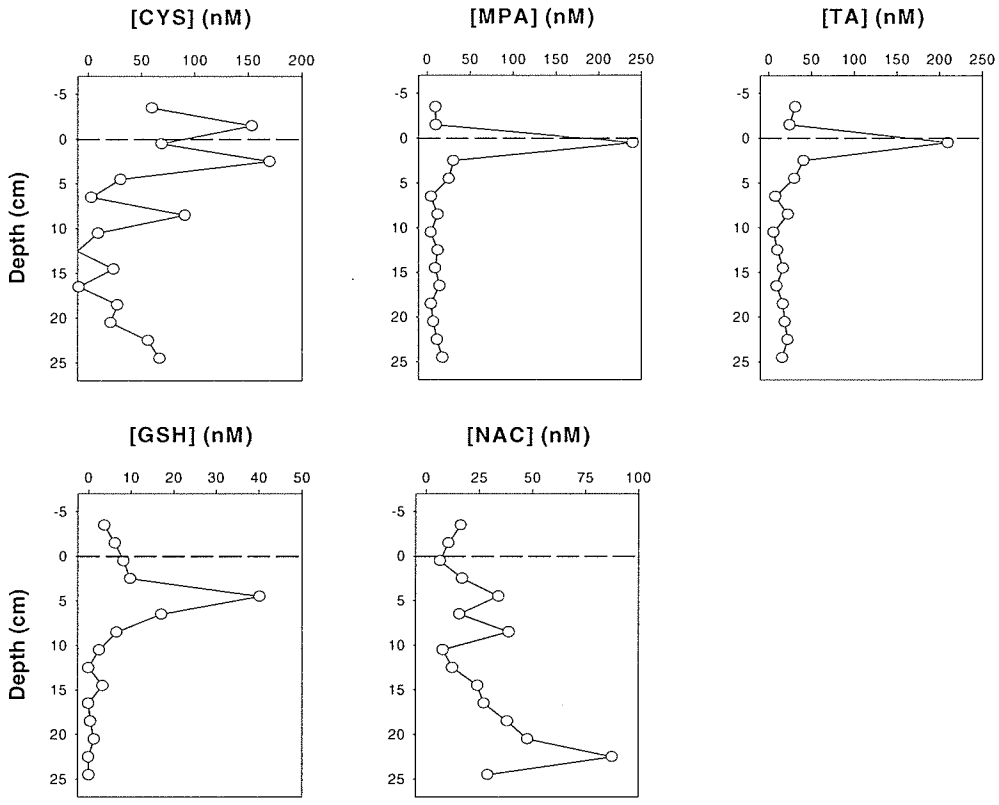

Figure 8. Porewater profiles of thiols in Baie St. Francois, September 2003.

Chapter 5. Results & Disscusion (II):

Movement of newly added Hg in contrasting wetlands

^{202}Hg addition experiments were further carried out to reveal the different behaviors of Hg in different wetland systems. The main findings of newly added mercury in two contrasting wetlands (Crescent Pond and Lake 632) over the course of one year (July 2003 to June 2004) are discussed below, starting from a detailed comparison of porewater chemistry (pH, sulfide and DOC) of the two wetland systems.

5.1 Porewater Chemistry of Crescent Pond

Crescent Pond is a eutrophic brackish wetland in Delta Marsh on the south shore of Lake Manitoba. A typical prairie wetland, the surface water of Crescent Pond is characterized with a high pH (up to 8.6) and conductivity (up to 1800 $\mu S\ cm^{-1}$), as detailed in Section 3.1 and Table 1. The discussion below is focused on the porewater chemistry of Crescent Pond.

5.1.1 pH

As indicated in Figures 9, 10 and 11 the pH of Crescent Pond ranged from 7.0 to 7.4 near the sediment/water interface over the year, but larger variations occurred in the pH of porewater from deeper sediment, as well as in the shape of the porewater pH profile. In July 2003 (Figure 9), a significant decrease of pH occurred at a depth between 6cm and

8cm depth and the pH from there downwards stabilized near pH 6.4. This gradient became much smaller in September 2003 (Figure 10) and almost disappeared in May 2004 (Figure 11).

The sharp gradient in pH in July 2003 coincides with the structure of the sulfide profile to be discussed below. The negative correlation between sulfide concentration and pH suggested that the pH was controlled by processes other than sulfate reduction, as sulfate reduction is an alkalinity-generating process. As described in Chapter 4, if the reduction of sulfate is the dominant process regulating the pH, the pH would have increased as the sulfate concentration decreases and sulfide increases. The sharp pH decrease at this depth thus must be caused by other acid-generating processes, possibly the respiration of algae (Stumm and Morgan, 1996).

5.1.2 Sulfide

As expected, the concentration of sulfide was near zero in the overlying water and increased sharply to sub-millimolar levels near or below the sediment/water interface (Figures 9, 10 and 11), suggesting it was produced by the reduction of sulfate. A profound peak was recorded in July 2003 (Figure 9), with a peak concentration near 500μM at a depth of 10cm. From there on the sulfide concentration decreased due to the precipitation of solids such as FeS(s). Concentrations of sulfide remained very high in porewaters at all the depths sampled in September 2003 (Figure 10) and May 2004 (Figure 11). The porewater sulfide concentration in Crescent Pond is unusually high

compared with most inland waters, due to the geological enrichment of gypsum and anhydrite in the region (DeVries and Wang, 2003).

5.1.3 DOC

DOC levels remained constant near the sediment/water interface at about 25mg C/L throughout the year (Figures 9, 10 and 11). Higher DOC concentrations were found in deeper porewaters in July 2003 and May 2004, with a peak reaching in excess of 100mg C/L at a depth of 20cm. However, in September 2003, the DOC profile remained constant at about 25mg C/L throughout the depth, except for a slight spike at 20cm.

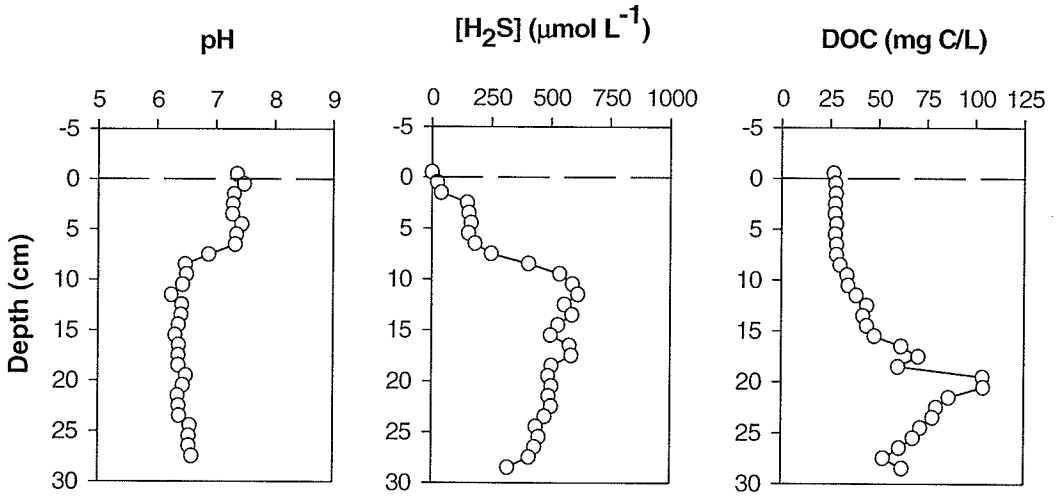

Figure 9. Porewater profiles of water chemistry in Delta Marsh July 2003.

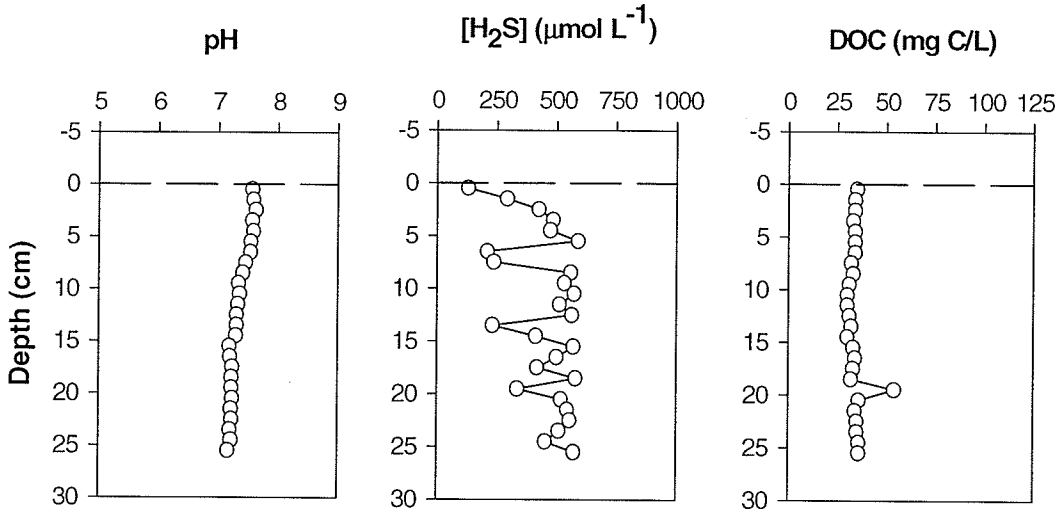

Figure 10. Porewater profiles of water chemistry in Delta Marsh September 2003.

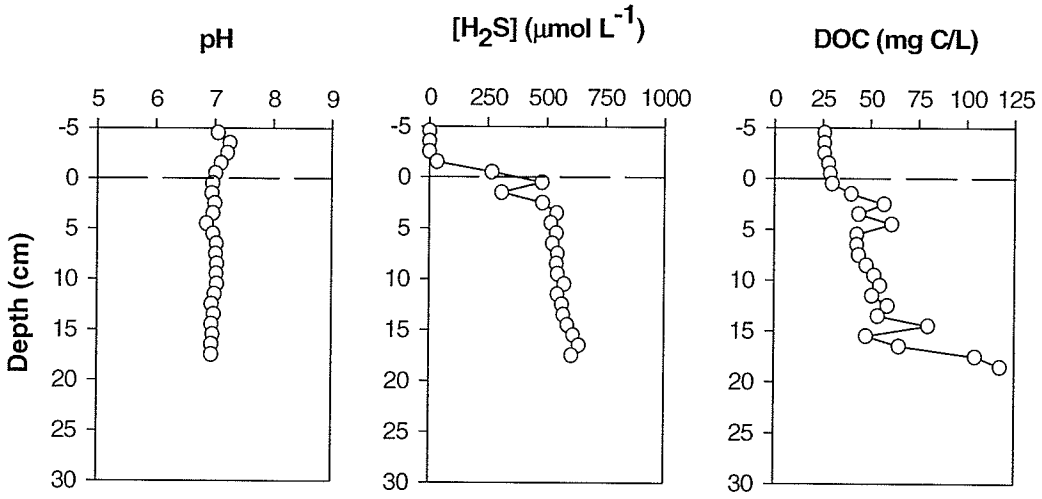

Figure 11. Porewater profiles of water chemistry in Delta Marsh May 2004.

5.2 Porewater Chemistry of Lake 632

Lake 632 is an oligotrophic wetland in the Experimental Lakes Area in northwestern Ontario. A typical Canadian Shield wetland, the surface water of Lake 632 is characterized with a slight acidic pH (~ 6.0) and very low conductivity (16 $\mu S\ cm^{-1}$), as detailed in Section 3.1 and Table 1. The discussion below is focused on the porewater chemistry of Lake 632.

5.2.1 pH

pH profiles demonstrated in Figures 12, 13 and 14 show pH remaining near 6 from the overlying water down across the sediment/water interface. August 2003 (Figure 12) shows no change in pH across the sediment/water interface while a slight increase in pH entering the overlying water is seen in November 2003 (Figure 13) and in June 2004 (Figure 14). Lake 632 is said to have a soft bottom indicating the sediments are extremely flocculent. The flocculent sediments may disturbed the sediment-water interface and destroyed any distinct structures in pH near the interface.

5.2.2 Sulfide

Sulfide profiles varied significantly over the year as seen in Figures 12, 13 and 14. August 2003 (Figure 12) saw sulfide present above the sediment/water interface and increase to a peak 5cm below the interface. Maximum values of sulfide are seen around

20cm depth at values near 20μmol/L. Sulfide values were lower in November 2003 (Figure 13) with a small peak of 7μmol/L slightly below the sediment/water interface with the second peak at 20cm below the interface reaching 10μmol/L. Sulfide concentrations were lowest in June 2004 (Figure 14) with only trace levels above the sediment/water interface and a peak of sulfide is seen at 5cm depth at a concentration of 5μmol/L.

The sulfide profiles at Lake 632 demonstrates a seasonal increase in sulfide peak in August 2003 (Figure 12), decreasing by November 2003 (Figure 13) and reaching a minimum the following spring in June 2004 (Figure14). Such a seasonal variation in sulfide has been reported elsewhere and is attributed to the seasonal variation in the activities of sulfate reducing bacteria (e.g., Ankley et al., 1996).

5.2.3 DOC

Figures 12, 13 and 14 all show a peak in DOC along or slightly above the sediment/water interface. DOC values are highest in August 2003 at 28mg C/L, less in November 2003 at 20mg C/L and are lowest in June 2004 reaching 14mg C/L deeper into the sediments. This clear trend of DOC peaking in the summer and decreasing over the next 10 months demonstrates the build up of settling organic matter from the summer months and decaying over the fall, winter and the following spring.

As demonstrated from Figures 9 to 14 and Table 1, these two systems differ significantly from one another. Not only in magnitude of pH and concentrations of dissolved sulfide and DOC, but the spatial and seasonal changes of these analytes indicate even greater differences among these two systems.

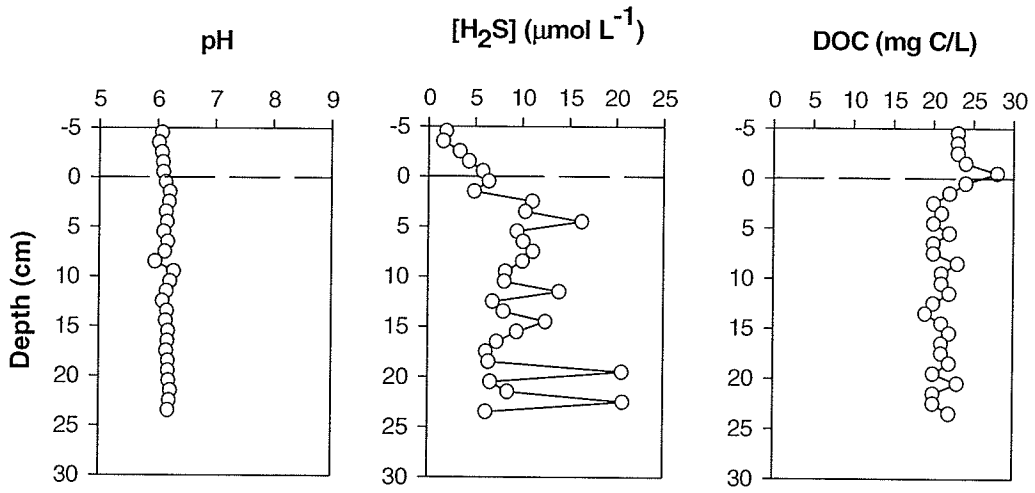

Figure 12. Porewater profiles of water chemistry in Lake 632 August 2003.

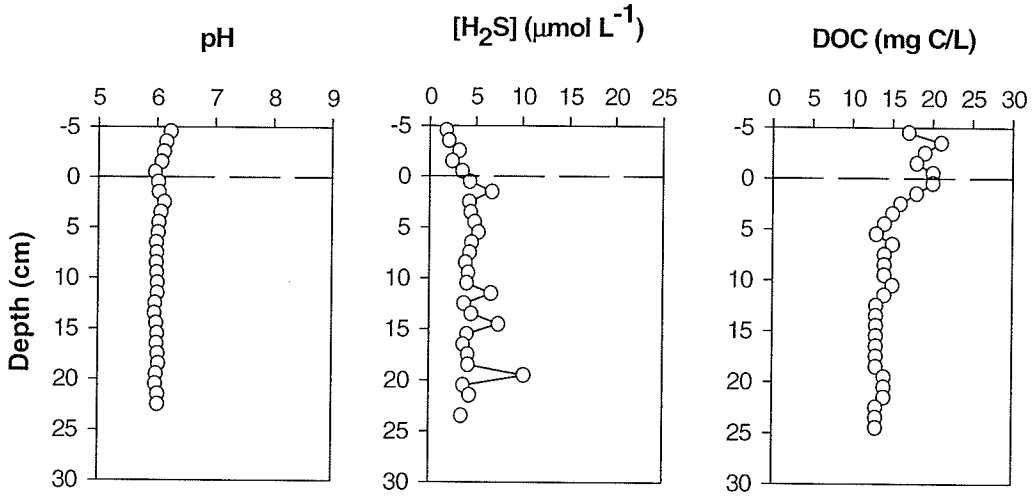

Figure 13. Porewater profiles of water chemistry in Lake 632 November 2003.

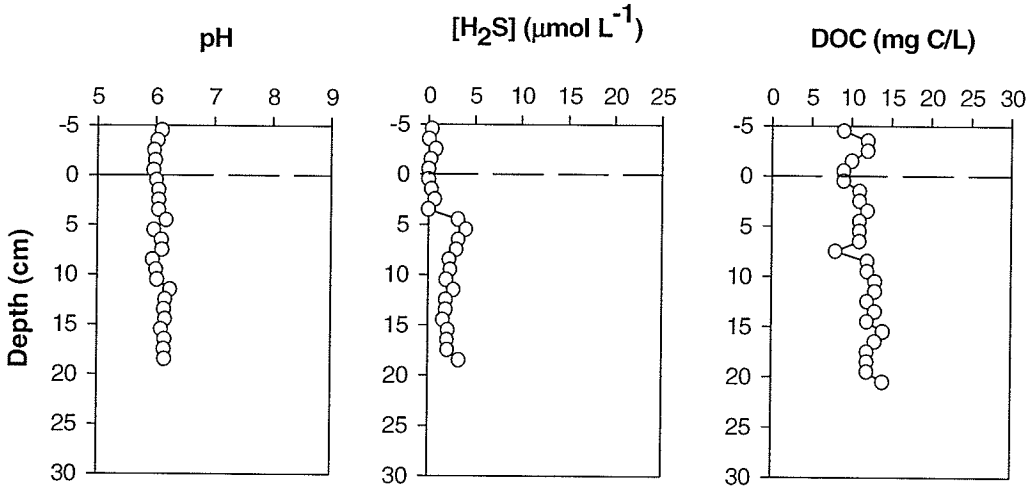

Figure 14. Porewater profiles of water chemistry in Lake 632 June 2004.

5.3 Vertical Migration of Newly Added ^{202}Hg in Two Contrasting Wetlands

To study the vertical movement of newly added Hg within wetland interstitial porewater, Hg in the form of ^{202}Hg was added in two dosages (with duplicates) to enclosures in the above two contrasting wetlands (see Table 3). ICP-MS was used to differentiate ^{202}Hg from the other six natural isotopes of Hg, and calculations using isotopic ratios were used to differentiate the newly added ^{202}Hg from the natural pool of ^{202}Hg (see appendix). The ICP-MS spectra in Figure 15 show a typical output of a natural sample containing no added ^{202}Hg. Alternatively, Figure 16 shows the ICP-MS spectra of a sample that contains enriched ^{202}Hg. The data presented here are time sequence profiles of the dissolved phase of the newly added ^{202}Hg at month 1, month 3 and month 11 after the ^{202}Hg addition.

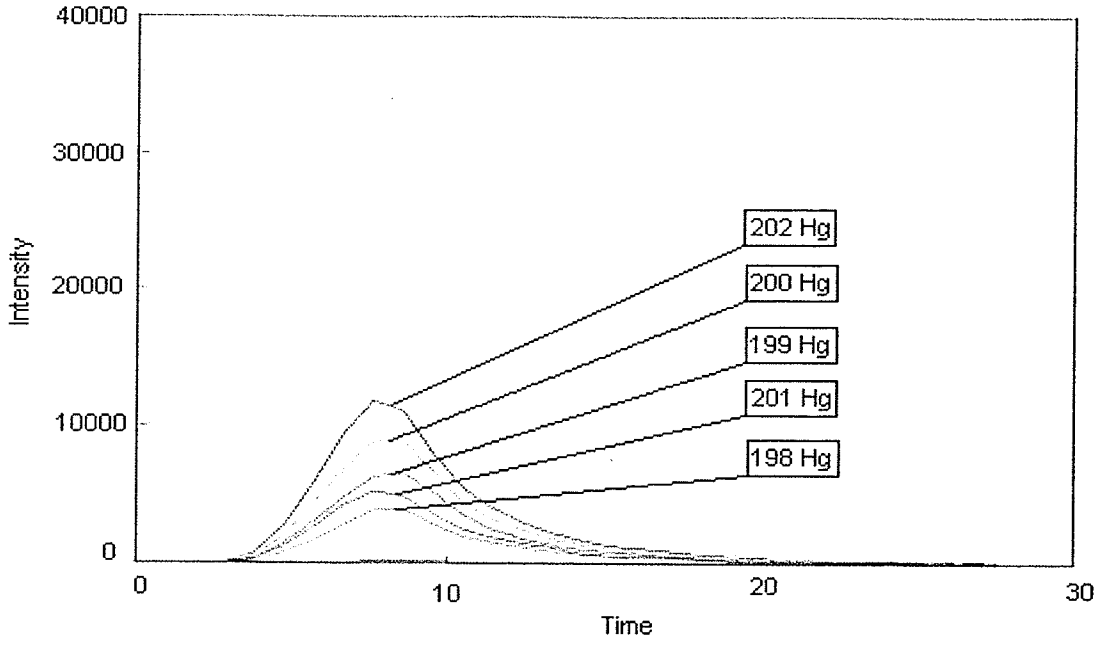

Figure 15. ICP-MS results of a water sample analyzed for five Hg isotopes collected from a peeper cell 2.5cm above the water-sediment interface from the ELA-control mesocosm in August 2003. The concentration of the water sample contained 12.4ng/L of natural Hg and no added ^{202}Hg.

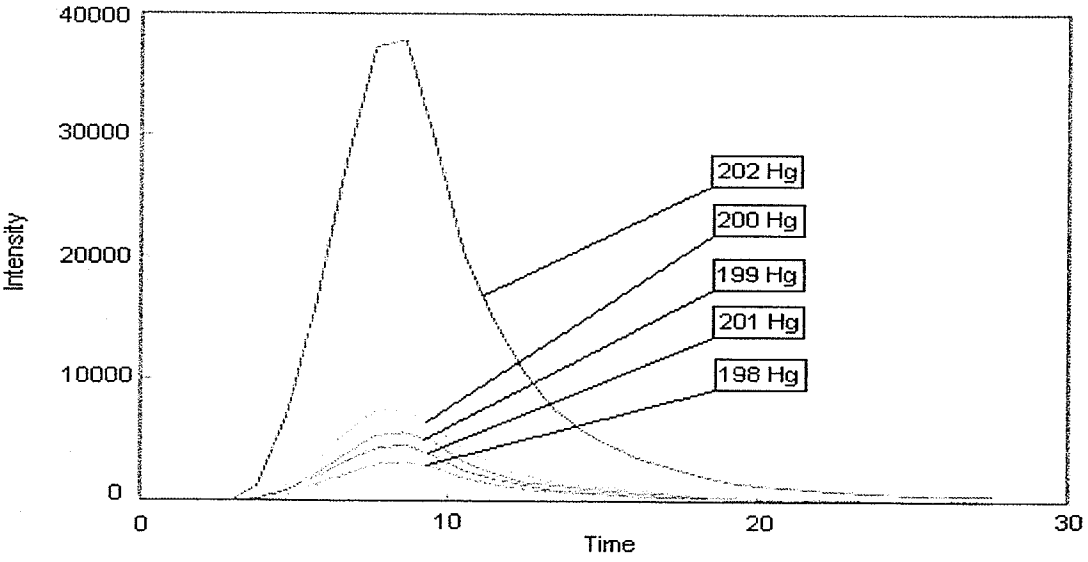

Figure 16. ICP-MS results of a water sample analyzed for five Hg isotopes collected from a peeper cell 2.5cm above the water-sediment interface from the ELA-5x1 mesocosm in August 2003. The concentration of the water sample contained 10.9ng/L of natural Hg and 10.4ng/L of added ^{202}Hg.

5.3.1 Delta 5-times Treatments

The vertical profiles of the added ^{202}Hg time sequence for the Delta 5x1 treatment are shown in Figure 17. At month 1 after addition, added ^{202}Hg is seen above the sediment/water interface at a concentration of 6ng/L. Immediately below the interface the ^{202}Hg increases rapidly to a concentration above 25ng/L at the depth of 6cm. This peak decreases down to 6ng/L over the next 10cm, then rapidly increases once again to 25ng/L at the last data point 20cm into the sediments. 3 months after addition, however, the ^{202}Hg concentration at all depth decreased dramatically from the results at 1 month after addition. Concentrations ranged from 1 to 4ng/L above the sediment/water interface and decreased as the profile descends into the sediments, with a slight peak of 3ng/L at a depth of 24cm. The profile conducted on month 11 demonstrates that nearly all the added ^{202}Hg was no longer in the dissolved phase.

Similar trends are seen in the 5-times replicate treatment (Delta 5x2) shown in Figure 18. A higher concentration of ^{202}Hg is seen one month after addition above the sediment/water interface with a maximum concentration close to 16ng/L, however the concentration and trend in the ^{202}Hg profile down into the sediments are very similar to those in the Delta 5x1 treatment. Higher overlying water ^{202}Hg concentrations were also observed in the Month 3 sampling. Month 11 shows a slight vertical migration downwards of the ^{202}Hg from the overlying water to form a peak of ^{202}Hg directly along the sediment/water interface. The base of this peak ends 3cm into the sediments where

the profile increases, then decreases, and increases again following the profile shape of month one though much lower in concentration.

Month 1 within the two Delta 5-times treatments showed remarkable similarities in magnitude and shape of the ^{202}Hg profiles. Large decreases in the dissolved concentration of ^{202}Hg were also seen among the two replicates, with Delta 5x2 having significantly more ^{202}Hg in the dissolved phase in the overlying water and top layers of the sediment porewaters at months 3 and 11.

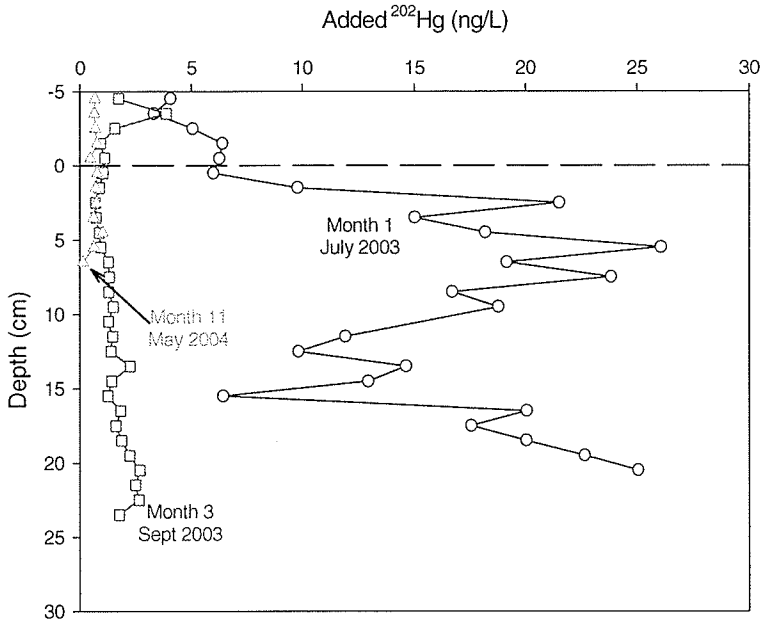

Figure 17. Added ^{202}Hg time sequence of the Delta 5x1 treatment.

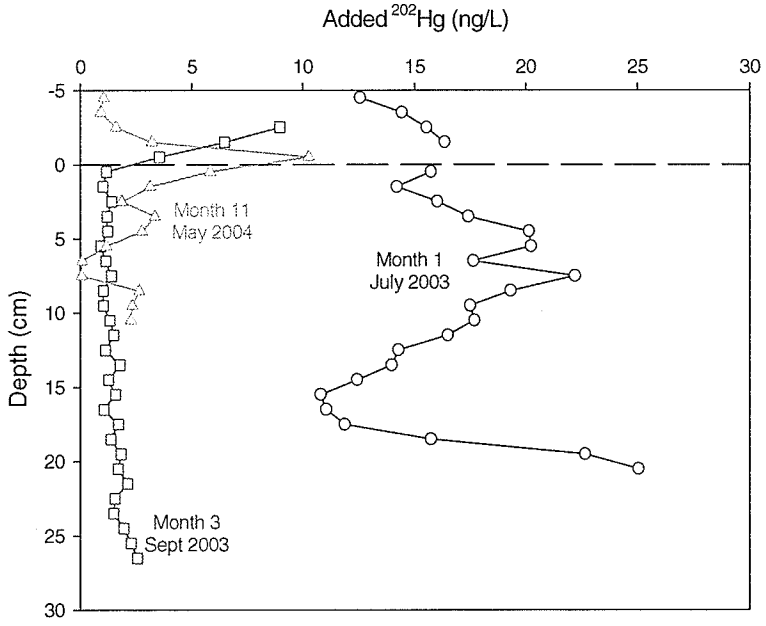

Figure 18. Added ^{202}Hg time sequence of the Delta 5x2 treatment.

5.3.2 Delta 10-times Treatments

Figure 19 shows the vertical time sequence profiles of added ^{202}Hg to the Delta 10x1 treatment. ^{202}Hg at month 1 is seen to decrease down to 2.5ng/L from above the sediment/water interface and remains constant from the interface down into the sediments, increasing slightly to 5ng/L below 20cm depth. Month 3 shows similarities to month 1 from the sediment/water interface down to 20cm depth (no data was collected above the sediment/water interface), with dissolved ^{202}Hg decreasing beyond 20cm depth. At month 11, about 2ng/L of added ^{202}Hg remained above the sediment/water interface. A small peak of ^{202}Hg is seen at this time along the sediment/water interface indicating the deposition of ^{202}Hg from above the interface. This peak slowly decreases down to 10cm depth with ^{202}Hg found only in trace levels.

The replicate treatment, Delta 10x2 (Figure 20), however demonstrates dissimilarities when compared to the Delta 10x1 treatment. Month 1 shows little ^{202}Hg present above the sediment/water interface while the concentration of ^{202}Hg increases as the profile descends into the sediments reaching a concentration of 13ng/L at 10cm depth. At month 3, the profile indicates an increase in ^{202}Hg above the sediment/water interface with this concentration of 2.5ng/L remaining constant across the interface down to 10cm depth where the dissolved ^{202}Hg rapidly disappears. At month 11 the ^{202}Hg profile took the form of a large broad peak across the sediment/water interface with a maximum concentration nearing 8ng/L. This peak rapidly diminished 10cm into the sediments.

The ^{202}Hg time sequence profiles of the two 10-times replicate treatments do not demonstrate reproducible results as the 5-times replicate treatments did. Among the treatments, it was found that the concentrations in ^{202}Hg were consistently higher in the 5-times treatments when compared to the 10-times treatments. This is contradictory to what was predicted and at this time we could not determine what caused such irreproducible and contradictory results.

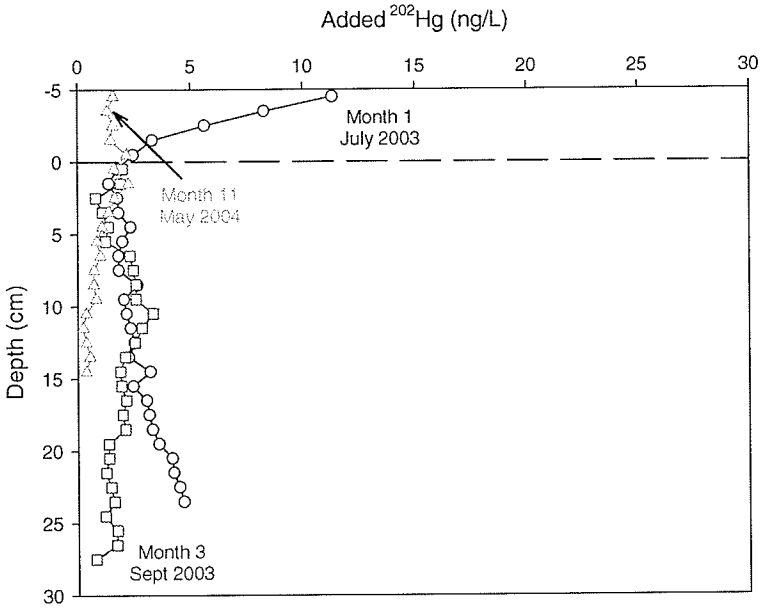

Figure 19. Added ^{202}Hg time sequence of the Delta 10x1 treatment.

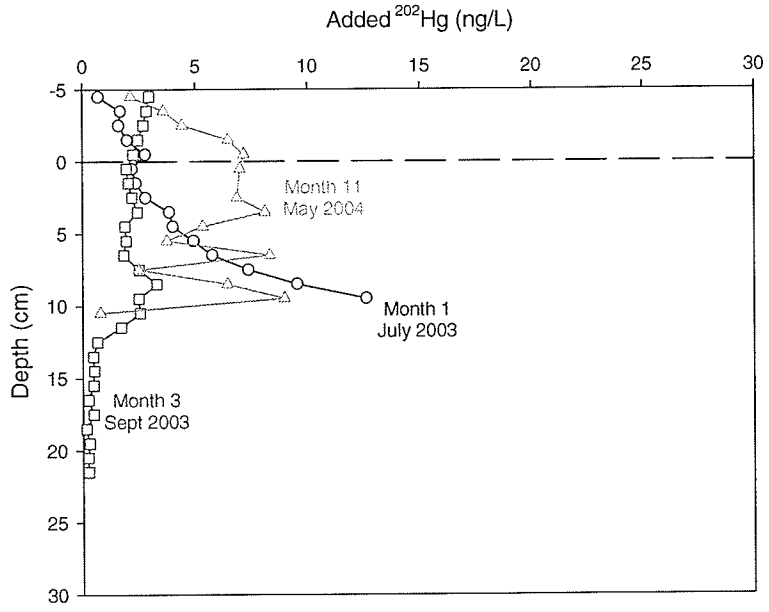

Figure 20. Added ^{202}Hg time sequence of the Delta 10x2 treatment.

5.3.3 ELA 5-times Treatments

^{202}Hg time sequence profiles of the ELA 5x1 treatment are shown in Figure 21. One month after addition, ^{202}Hg seems to peak 1 cm above the sediment/water interface where it decreased in concentration yet penetrating 5cm into the sediments. Trace levels of ^{202}Hg are seen further into the sediments. Month 3 sees a decrease in ^{202}Hg above the sediment/water interface with a downward vertical movement of the ^{202}Hg peak now present half a centimeter below the interface. At month 11, the ^{202}Hg peak is still present immediately below sediment/water interface, although peaking at a much lower concentration.

Differing from the 5x1 treatment, ^{202}Hg within the 5x2 treatment (Figure 22) was far more prevalent above the sediment/water interface at month 1 reaching a concentration near 20ng/L, more than double that of the 5x1 treatment. However, mainly similar trends are found in the 5-time dosage replicate. Slight peaks are replicated near the sediment/water interface at month 3 and again in a lower concentration at month 11.

Comparing the replicates of the two 5-time dosage treatments indicate reproducible results were obtained with these two treatments. The dissolved phase of the ^{202}Hg decreased in concentrations over the year as well the downward vertical migration of the ^{202}Hg was demonstrated within the two treatments over the time that sampling occurred.

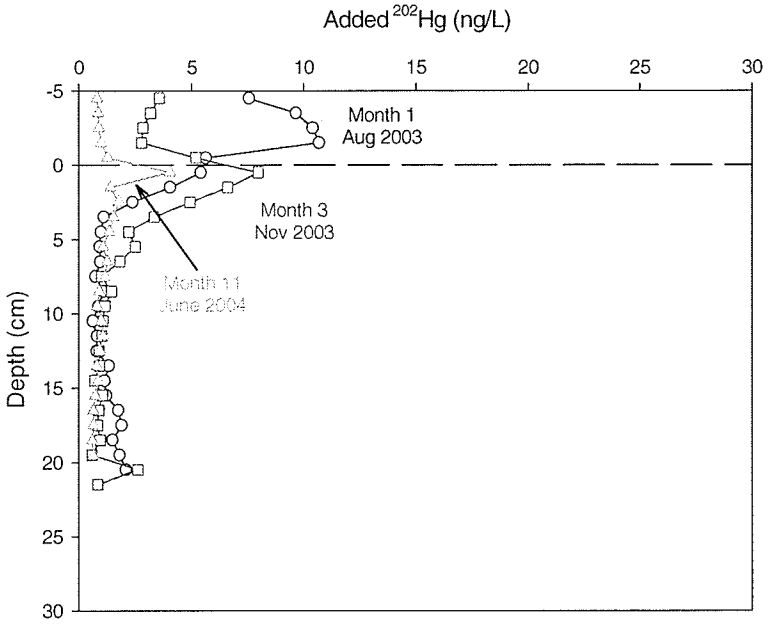

Figure 21. Added ^{202}Hg time sequence of the ELA 5x1 treatment.

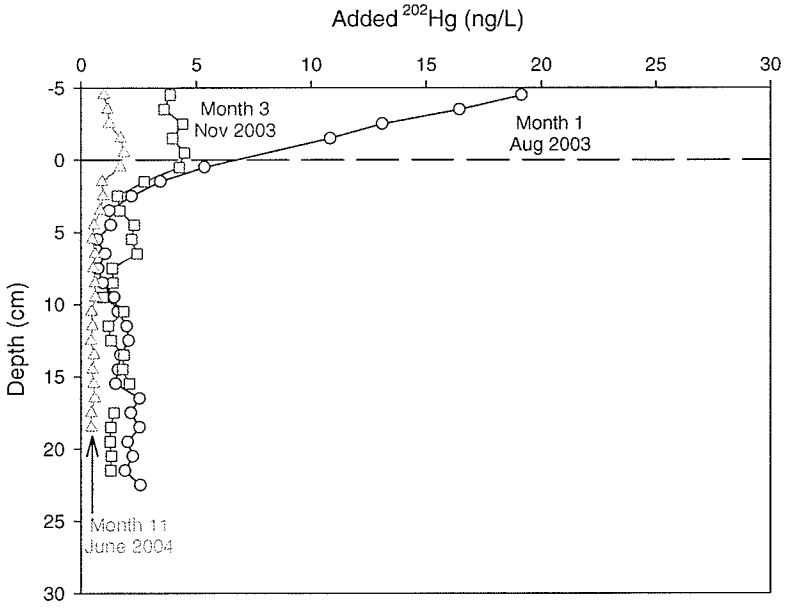

Figure 22. Added ^{202}Hg time sequence of the ELA 5x2 treatment.

5.3.4 ELA 10-times Treatments

^{202}Hg time sequence profiles of the ELA 10x1 treatment are shown in Figure 23. One month after addition, ^{202}Hg was present at nearly 30ng/L above the sediment/water interface. The ^{202}Hg then decreased sharply across the interface where it penetrated 25cm into the sediments maintaining a concentration near 4ng/L from 5cm depth to 20cm depth. The ^{202}Hg then increased slightly in concentration over the next 5cm. At month 3, a large decrease in ^{202}Hg was seen above the sediment/water interface causing a peak of ^{202}Hg to occur 2cm below the sediment/water interface. Below 5cm into the sediments, the profile demonstrated another smaller peak 9cm below the interface where the ^{202}Hg then was shown to decrease in concentration down to the depth of 25cm. Month 11 demonstrates the downwards vertical movement of the ^{202}Hg peak where it is now located 4cm below the sediment/water interface at a reduced concentration. The remaining profile at month 11 resembles a slightly noisy vertical line reaching into the sediments.

The replicate 10-time dosage treatment (10x2) is shown in Figure 24. As in the 10x1 treatment, month 1 reveals a peak of ^{202}Hg immediately above the sediment/water interface with a maximum concentration nearing 23ng/L. The concentration rapidly decreases downwards across the interface into the sediments reaching a minimum at 5cm depth where upon the concentration again begins to increase. A small sharp peak results 9cm into the sediments at a peak concentration of 5ng/L, which then slightly decreases further down into the sediments. Month 3 shows a large decrease in ^{202}Hg above the

sediment/water interface causing a very broad peak of ^{202}Hg that begins on the interface and ends 10cm into the sediments. The peak is diminished greatly by month 11 but is however still present from the sediment/water interface down to 5cm depth.

Comparing the data from the two 10-times treatments demonstrates comparable results were obtained. Month 1 and month 11 results were very similar in shape and magnitude. However, month 3 differed slightly with the appearance of a second smaller peak in the 10x1 treatment as opposed to only one large peak that occurred in the 10x2 treatment.

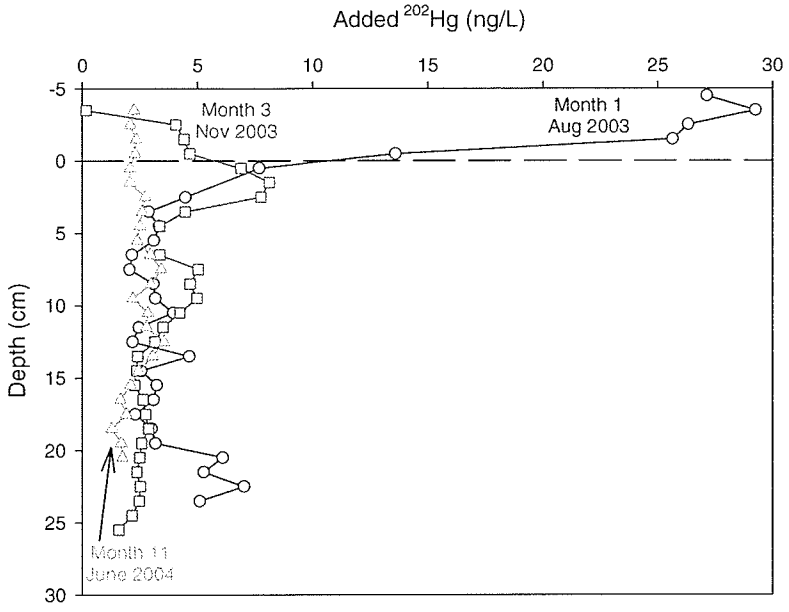

Figure 23. Added ^{202}Hg time sequence of the ELA 10x1 treatment.

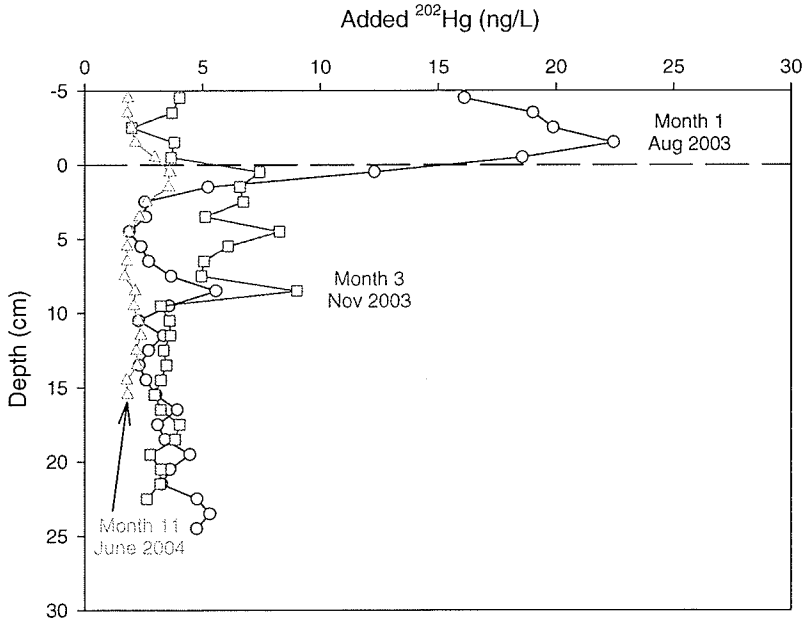

Figure 24. Added ^{202}Hg time sequence of the ELA 10x2 treatment.

5.4 Comparison of the Vertical Distribution of Newly Added ^{202}Hg to Lake 632 and Crescent Pond.

Unprecedented differences have been observed in the ^{202}Hg profiles among the two wetlands. The ELA treatments demonstrate a logical and reproducible downward movement of the ^{202}Hg, with ^{202}Hg slowly descending across the sediment/water interface over the year. While the Delta Marsh treatments show an immediate presence of the ^{202}Hg deep within the sediment porewaters (>10cm) within one month after addition in three of the four treatments. This begs the question as to how the newly added ^{202}Hg reached such depths within the sediment porewater so fast in Delta Marsh, relative to ELA.

The experimental setup of the study may help explain the rapid movement within the Delta Marsh enclosures. Delta Marsh as mentioned previously is a highly productive wetland and thus provides habitat to a high number of aquatic biota, whereas Lake 632 is oligotrophic and thus less productive. Upon the deployment of the mesocosms at both sites, no time was spent in attempting to remove any aquatic organisms from inside of the enclosures. Although no attempts were made in identifying or recording the presence and abundance of benthic invertebrates in the mesocosms, at least no visible aquatic organisms could be seen within the ELA enclosures; it was however evident that there were some visible aquatic biota present within the enclosures setup in Delta Marsh, mainly tadpoles. Within four weeks after the addition of the ^{202}Hg, the tadpoles had matured to frogs. It is possible the physical activity of the frogs could have caused the

^{202}Hg to be introduced to the deeper sediments seen in Figures 17, 18 and 19. However, this increase in ^{202}Hg is not seen in the Delta 10x1 treatment (Figure 19). Minnow traps could have been placed within the mesocosms for several days immediately following the deployment of the mesocosms at each site to remove large biota from the enclosures (Mailman, 2004). However, this act would cause the data to be less representative of this overly productive system.

While sampling the Delta 5x2 treatment in July 2003, a duck was discovered floating dead in the mesocosm. The struggle that resulted from the presence of a large waterfowl within the enclosure surely disturbed the sediments. Looking at the ^{202}Hg profile sampled after this event (Month 1, Figure 18), the occurrence of the waterfowl may be the cause for such a profile. However, the exact same profile shape of ^{202}Hg is also found in the Delta 5x1 treatment (Month 1, Figure 17) where no dead duck was found. No covers were used on the mesocosms throughout this experiment. Mesh covering are used to keep waterfowl out of enclosures routinely at the ELA (P. Blanchfield, pers. comm.). This strategy should have been adopted in this experiment.

The most likely explanation for the complicated ^{202}Hg dynamics in Crescent Pond is the high productivity of the marsh. Chironomids exist in high densities in this wetland, thus creating a dynamic benthic community that exists across the sediment/water interface. With so many chironomids traveling up and down across the sediment/water interface, this would allow the ^{202}Hg to be distributed within the sediments fairly rapidly. Chironomids and other tube-dwelling invertebrates are mainly found within the first five

to seven centimeters of the sediments (D. Warbluski, pers. comm.), however they are also found to be present deeper within the sediments. The bioturbation and bioirrigation of benthic invertebrates within this productive system may be the cause for the rapid vertical movement of added ^{202}Hg. In addition, Crescent Pond also has high density of aquatic algae and plants, the roots of which will also create heterogeneous microenvironments in sediments. DeVries and Wang (2003) recently reported that the porewater sulfide concentration in Delta Marsh is extremely heterogeneous.

The occurrence of the Month 1 profiles in Delta Marsh either demonstrates an error in experimental setup, or emphasizes the difference in behaviour exhibited by newly added Hg between these two wetlands. Two other field experiments are being conducted at the present time that can help interpret the finding of this project. The Aquatic Cycling of Mercury in the Everglades (ACME) is a large-scale field project conducted in the Florida Everglades to determine the cause of widespread Hg contamination in the Florida Everglades. One component of this project is the addition of stable isotopes of Hg to mesocosms. While this project is also taking place in a highly productive system, data have yet to be published. Another large-scale field experiment is the Mercury Experiment to Assess Atmospheric Loading in Canada and the United States (METAALICUS). This ecosystem level experiment presently being conducted at the ELA is responsible for adding different Hg isotopes to the upland, lake, and wetland of a Canadian Shield watershed over the past three years. It is understood that sediment cores have been sampled during the past three years of this study, however no data have been published for us to compare results. While not a productive system as Delta Marsh, the

comparison of the data from specifically the wetland area of the METAALICUS project would help us to solidify conclusions regarding the Month 1 profiles in Delta Marsh.

Chapter 6. Results & Discussion (III):

Speciation of Newly Added ^{202}Hg in Two Contrasting Wetlands

Vertical profiling of the added ^{202}Hg across the MML down further into the sediments indicates the position and movement of the newly added Hg, but does not reveal the complexes that have been formed within these contrasting systems. By collecting pH and sulfide data along with the ^{202}Hg data within each of the treatments, the speciation of the newly added ^{202}Hg was calculated using WHAM (V6.0), as detailed in Section 3.6. Results from one of each duplicated treatment are presented.

6.1 Added ^{202}Hg Speciation Profiles in Delta Marsh

The two neutral species HgS^0 and $Hg(HS)_2$, and two charged species $HgS(HS)^-$ and HgS_2^{2-} were the four major species to be formed in both Delta 5x1 (Figure 25) and Delta 10x1 (Figure 26) treatments. The three species HgS^0, $Hg(HS)_2$ and $HgS(HS)^-$ were the major species formed and exist at the same order of magnitude. However, it cannot be said that one complex dominates above any of the other two. The species HgS^0 is more prevalent immediately above the sediment/water interface (Figures 25 and 26) at all times of the year in both treatments. Month 1 in both treatments demonstrates the greatest difference above the sediment/water interface, with month 3 showing this difference to a lesser extent. At all times of the year however, this species becomes equally abundant with the other species as soon as the profiles reach below the sediment/water interface.

The species HgS_2^{2-} is present in both treatments, however its concentration is four orders of magnitude lower than the other species throughout the year.

Although there was found to be less ^{202}Hg in the 10-times treatment than the 5-times treatment, no change in speciation was observed among treatments. No distinctive peaks of any of the Hg species have formed across the MML, whereas it is seen that once in the sediments, the species formed seem to remain constant down through the sediments. There is a leftwards shift across the x-axis of all the species in the figures representing the decrease of dissolved ^{202}Hg over time.

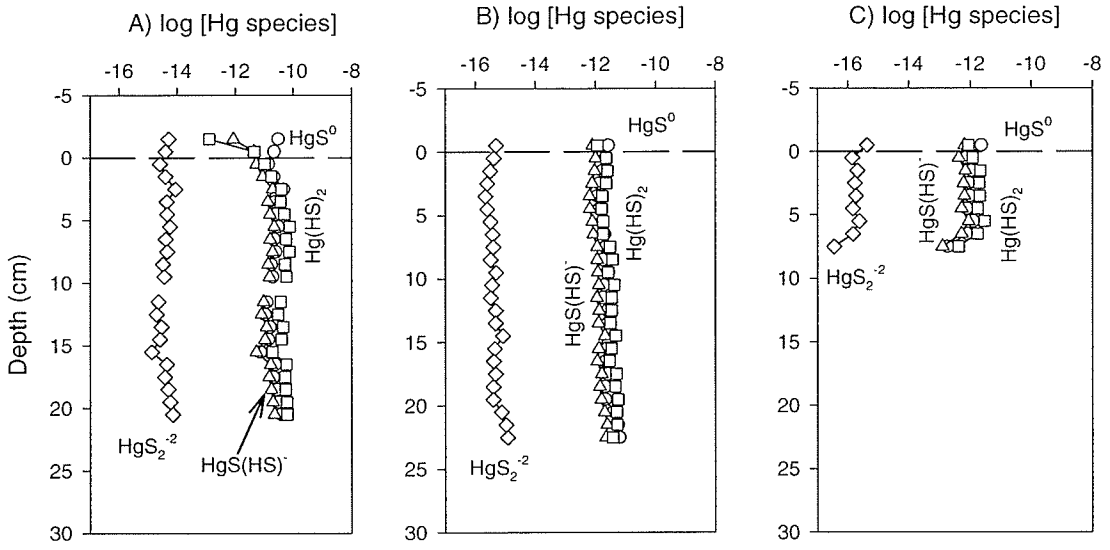

Figure 25. Added ^{202}Hg speciation profiles from Delta 5x1 enclosure at time (A) 1 month, (B) 3 months, and (C) 11 months after the ^{202}Hg addition.

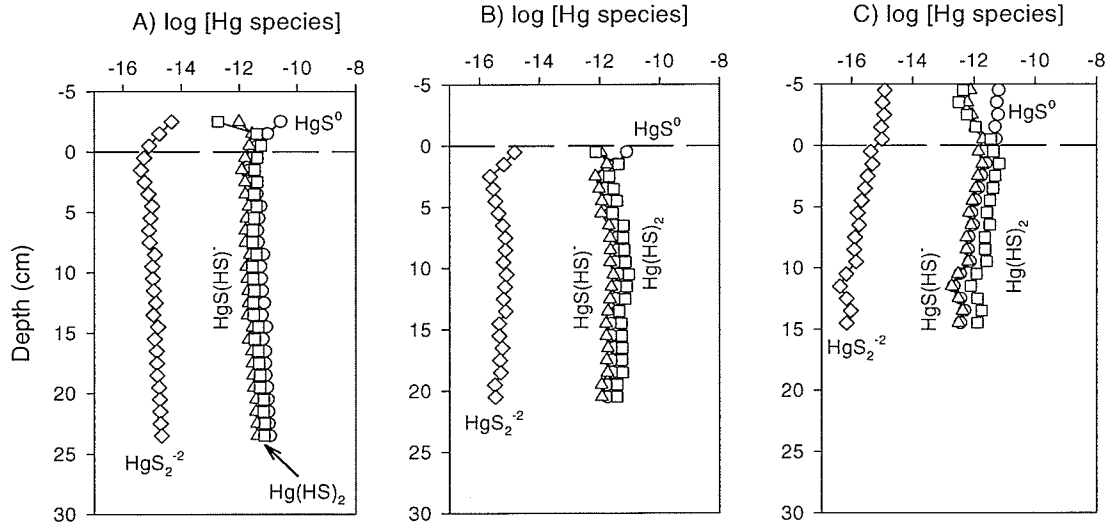

Figure 26. Added ^{202}Hg speciation profiles from Delta 10x1 enclosure at time (A) 1 month, (B) 3 months, and (C) 11 months after the ^{202}Hg addition.

6.2 Added ^{202}Hg Speciation Profiles in Lake 632

Similar to Delta Marsh, the two neutral species HgS^0, and $Hg(HS)_2$, and the two charged species $HgS(HS)^-$, and HgS_2^{2-}, again were the four major species to be formed in both ELA 5x1 (Figure 27) and ELA 10x1 (Figure 28) treatments. It is clear that the neutral HgS^0 species dominates the Hg speciation in these two treatments throughout the year. The charged species $HgS(HS)^-$ and the neutral species $Hg(HS)_2$ are the next abundant species with $HgS(HS)^-$ occurring one order of magnitude lower than HgS^0, but always in greater concentration than $Hg(HS)_2$. Similar to the Delta Marsh treatments, the charged species HgS_2^{2-} occurs routinely four orders of magnitude lower than the dominant HgS^0 species in both treatments throughout the year.

More structure is evident in the ELA ^{202}Hg speciation profiles than in the Delta ^{202}Hg speciation profiles. Figure 27 shows in month 1 and month 3 within the ELA 5x1 treatment a slight decrease in concentration of all the species as the profiles move down from the sediment/water interface. While Figure 28 demonstrates an inverse peak in all the species (with the exception of HgS^0) below the sediment/water interface occurring in month 1 and month 3. The profiles seem to remain constant in month 3.

The higher ^{202}Hg concentration above the sediment/water interface in the ELA 10x1 treatment compared to the ELA 5x1 treatments in month 1 had little impact on altering the Hg speciation between the two treatments. The neutral $Hg(HS)_2$ species decreased in the 10x1 enclosure, however this change was minimal.

It is clear in Figures 17 to 24 that the ^{202}Hg is present in a significant concentration across the sediment/water interface down into the top layer of the sediments where the MML is expected to exist in these two wetlands. The differences in ^{202}Hg concentrations 2cm above the sediment/water interface between the ELA 5x1 and ELA 10x1 treatments showed no significant differences in Hg speciation among these treatments. This difference in Hg concentration that was originally predicted to have an impact on Hg speciation proved to be unable to alter the dominance of the Hg-species. It is clear that the only factor that was able to alter Hg speciation was the concentration of sulfide in the system.

Over the year there is a decrease in concentration of the ^{202}Hg species due to the decrease of ^{202}Hg in the dissolved phase. As Hg undergoes precipitation/adsorption reactions within the sediments, the Hg becomes unavailable to methylation organisms decreasing its bioavailabilty.

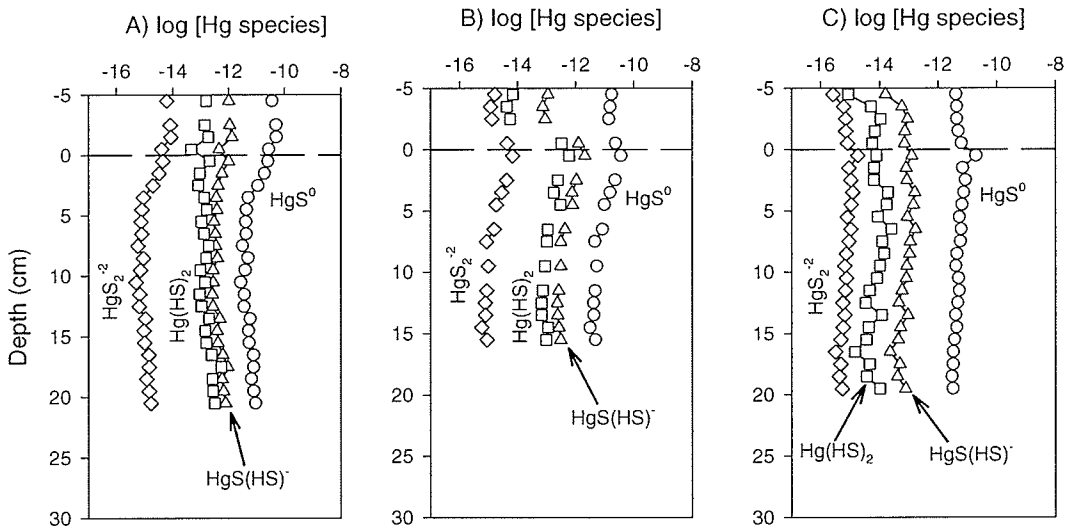

Figure 27. Added ^{202}Hg speciation profiles from ELA 5x1 enclosure at time (A) 1 month, (B) 3 months, and (C) 11 months after the ^{202}Hg addition.

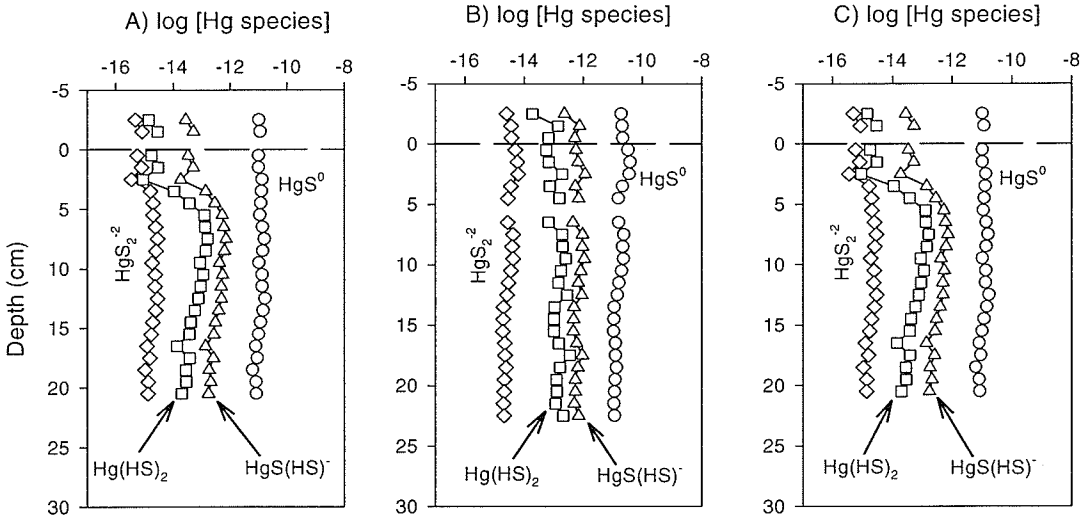

Figure 28. Added ^{202}Hg speciation profiles from ELA 10x1 enclosure at time (A) 1 month, (B) 3 months, and (C) 11 months after the ^{202}Hg addition.

6.3 Comparison of Hg speciation Across all Three Wetlands

The neutral species HgS^0 is clearly dominant in Lake 632, while in Delta Marsh it is one of the three major species present in the same order of magnitude. This shift in dominance in Hg speciation in Delta Marsh from Lake 632 is due to the high sulfide concentrations in Delta Marsh. Lake 632 exhibits sulfide concentrations below $1x10^{-5}M$, while Delta Marsh has sulfide concentrations above $1x10^{-4}M$. This overall increase in concentration of charged Hg species in the highly sulfidic environment of Delta Marsh coincides with the findings presented by Benoit *et al.* (1999a) that demonstrates this shift in Hg speciation dominance with increasing sulfide levels. With the shift away in Hg speciation dominance from the neutral HgS^0 species, a decrease in MeHg production is also predicted in the Delta Marsh system, however that is beyond the scope of this thesis.

The neutral HgS^0 species does become the dominant Hg species across sediment/water interface in Delta Marsh (Figures 9 to 11). The appearance of the increase of this species is related to the decrease in sulfide across the sediment/water interface. With Delta Marsh being a highly productive wetland, oxygen is replenished to the overlying waters each day through photosynthesis. This constant source of oxygen is primarily responsible for the redox transformation of sulfide to sulfate across the sediment/water interface. This redox reaction prevents any sulfide from being detected above sediment/water interface as is shown in the sulfide profiles in Figures 9 to 11. Thus in this micro-environment across the sediment/water interface the dominance of the neutral HgS^0 species is evident. With the hypothesis of HgS^0 as the most bioavailable species

available for methylation (Benoit et al. 1999a, 2001b), this micro-environment found within highly sulfidic environments could prove to be important in the production of MeHg within this prairie wetland.

The pH, itself a master variable in determining the speciation of elements within natural waters, differs one and a half orders of magnitude between Delta Marsh and both Lake 632 and Baie St. Francois. This difference is not responsible for the difference in speciation that is shown within the data presented.

In comparing the Hg species formed in Baie St. Francois (Figures 4, 5 and 6) to those formed in Delta Marsh and Lake 632, it is clear that the Hg speciation in Baie St. Francois is similar to that of Lake 632 showing a clear domination of the neutral HgS^0 species. While sulfide concentrations were lower in Baie St. Francois than Lake 632, they were present in the same order of magnitude in both sites. This similarity in magnitude of sulfide in the Baie St. Francois and Lake 632 systems is the cause for the similar Hg species produced across these two sites.

It is however important to be reminded again that the above speciation results were not analytically measured; instead, they were estimated by thermodynamic calculations. As mentioned in Section 3.6, cautions are needed when interpreting these calculations. In particular, one should note that the thermodynamic constant for the formation of the neutral HgS^0 species has never been experimentally determined. The value used in this thesis and elsewhere (e.g., Benoit et al., 1999a) was simply extrapolated from formation

constants for CdS^0 and ZnS^0 (Dyrssen, 1989; Dyrssen and Wedborg, 1991); at least the formation constant for CdS^0 was recently found to be incorrect (Wang and Tessier, 1999). There is clearly a pressing need for experimentally determining the formation constant for HgS^0 and many other Hg-sulfide complexes.

Chapter 7. Conclusions

Understanding Hg dynamics and speciation results within interstitial porewaters is expected to provide critical information in future attempts to predict the production of MeHg in natural waters. The majority of MeHg found in fish originates as bioavailable Hg species in porewaters where MeHg is produced. The research conducted in Baie St. Francois demonstrated that MeHg is produced within a thin layer that we have termed the Mercury Methylation Layer. This layer along with other water chemistry (pH, DOC, sulfide, thiols, Hg_T) varies on a seasonal and yearly basis.

The ^{202}Hg addition experiment at the ELA and Delta Marsh demonstrate drastic differences in the mobility of newly added Hg within these two systems. Depth profiles across the MML of these manipulated systems clearly show the increased penetration of newly added ^{202}Hg across the MML within one month after addition in Delta Marsh when compared to Lake 632 at the ELA. Although Delta Marsh is a highly productive system when compared to Lake 632, comparisons to the ACME (a eutrophic wetland) and METALLICUS (a oligotrophic dimictic lake) projects would be beneficial to cross-examine the findings. Within 11 months after addition, the majority of added ^{202}Hg was no longer in the dissolved phase in both wetlands.

This research project demonstrated the differences in Hg speciation dominance across the MML in three contrasting wetlands that differed in pH, sulfide and DOC. Speciation calculations using the limited thermodynamic database revealed that in the wetlands Baie

St. Francois and Lake 632 with low sulfide ($<1x10^{-5}M$), the neutral complex HgS^0 was clearly the dominant Hg species. Whereas in Delta Marsh with high sulfide ($>1x10^{4}M$), the neutral species HgS^0, $Hg(HS)_2$ and the charged species $HgS(HS)^-$ were all found to exist in the same order of magnitude. DOC and pH were found to play less important roles in Hg speciation within these three contrasting systems.

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Appendices

Appendix 1. Tables from Chapter 3

Table A-1. ^{202}Hg Spray bottles preparation procedure.

	Bottle ID	100 mg/L ^{202}Hg added (mL)	Optima HNO3 added (mL)	^{202}Hg in the bottle (µg)
Delta	Delta-5x1	1.236	10	123.6
	Delta-5x2	1.236	10	123.6
	Delta-10x1	2.473	10	247.3
	Delta-10x2	2.473	10	247.3
ELA	ELA-5x1	1.236	10	123.6
	ELA-5x2	1.236	10	123.6
	ELA-10x1	2.473	10	247.3
	ELA-10x2	2.473	10	247.3

Table A-2. Dose of ^{202}Hg added to each mesocosm.

Treatment*	Mesocosm ID	Surface area (m^2)**	Bottle to be used	^{202}Hg added	
				(µg)	(µg/ m^2)
Crescent Pond					
Control	Delta Con	1.77	-	0	0
5X	Delta 5x1	1.77	Delta-5x1	123.6	69.98
5X	Delta 5x2	1.77	Delta-5x2	123.6	69.98
10X	Delta 10x1	1.77	Delta-10x1	247.3	140.01
10X	Delta 10x2	1.77	Delta-10x2	247.3	140.01
Lake 632					
Control	ELA Con	1.77	-	0	0
5X	ELA 5x1	1.77	ELA-5x1	123.6	69.98
5X	ELA 5x2	1.77	ELA-5x2	123.6	69.98
10X	ELA 10x1	1.77	ELA-10x1	247.3	140.01
10X	ELA 10x2	1.77	ELA-10x2	247.3	140.01

* The natural deposition rate of mercury is assumed to be 14 µg m^2/yr (based on the data for ELA)

** Based on a diameter of the mesocosm of 1.5 m.

Appendix 2. Calculations Used in the Determination of Added ^{202}Hg from Background ^{202}Hg

The total Hg concentration in a porewater sample is denoted as $[Hg]_s$ (subscript "s" denotes sample). The isotopic ratios of Hg are determined by ICP-MS. The fraction to be calculated (x), is the ^{202}Hg that was resulted from the Hg we spiked in the mesocosms. It is important as a reminder that the added ^{202}Hg was only 98.6% enriched in ^{202}Hg therefore 1.4% of the Hg added was a combination of the other six stable isotopes of Hg present in their relative natural abundances.

We begin by writing out the mass balance equations for two isotopes in the sample, ^{202}Hg (1) and another isotope. Here we have chosen ^{200}Hg (2). For the mass balance equations, the fraction of the spiked Hg in the $[Hg]_s$ is x, and the fraction from the natural background is 1-x. The mass balance equations are:

$$[^{202}Hg]_s = [Hg]_T(1-x)a_{202,1} + [Hg]_T x a_{202,2} \quad (1)$$

$$[^{200}Hg]_s = [Hg]_T(1-x)a_{200,1} + [Hg]_T x a_{200,2} \quad (2)$$

where $a_{202,1}$ and $a_{202,2}$ in Equation (1) and $a_{200,1}$ and $a_{200,2}$ in Equation (2) denote the abundance of ^{202}Hg and ^{200}Hg in the background Hg (natural abundance) and the spiked Hg (which is known from the supplier), respectively. If we divide Equation (1) by Equation (2), we obtain:

$$\frac{[^{202}Hg]_s}{[^{200}Hg]_s} = \frac{(1-x)a_{202,1} + xa_{202,2}}{(1-x)a_{200,1} + xa_{200,2}} \quad (3)$$

If we denote

$$R_s = \frac{[^{202}Hg]_s}{[^{200}Hg]_s}$$

as the ratio of ^{202}Hg to ^{200}Hg in the sample, Equation (3) can be solved as:

$$x = \frac{a_{200,1}R_s - a_{202,1}}{(a_{200,1} - a_{200,2})R_s + a_{202,2} - a_{202,1}} \quad (4)$$

Since R_s is known from the ICP-MS analysis, $a_{200,1}$ and $a_{202,1}$ are known from natural abundances, and $a_{200,2}$ and $a_{202,2}$ are known from the supplier, Equation (4) can be solved for x. This value (x) is then multiplied by the value obtained for Hg_T from the analysis to obtain the concentration of ^{202}Hg resulted from the spiked Hg in the mesocosm.

Appendix 3. A Discussion on Potentially Contaminated Data from the Lake 632 and Delta Marsh Control Mesocosms.

Hg_T concentrations from the control mesocosms in the ^{202}Hg addition experiment were highly variable over the course of the year reaching such extreme levels that contamination from some unknown source(s) is the only explanation for such data. Due to such high values, these data were removed from this thesis. However, we thought it valid to include these data in the Appendix to attempt an explanation, to demonstrate the diligence we undertook to avoid such problems and to inform others of the experiences we encountered during the time of this experiment. Hg_T values presented in Figures 29 and 30 differ greatly among and within these enclosures deployed in these two wetlands. While both sites show an increase in Hg_T concentrations throughout the year, the reasoning for these values may differ for each wetland.

Delta Marsh saw Hg_T concentrations increase from 50pmol/L to 100pmol/L up to 1000pmol/L across the sediment/water interface in the exact same location throughout the year (Figures 29). As 100pmol/L is the extreme upper limit on natural Hg concentrations (Morel et al. 1998), and Delta Marsh is not contaminated with Hg as is demonstrated in the June 2003 Hg_T profile, contamination is likely the cause for such increasingly higher values (Figures 29A and 29B). While the July 2003 (Figure 29A) and September 2003 (Figure 29B) data may in fact represent true *in situ* Hg_T concentrations, the large Hg peak of 2000pM in May 2004 (Figure 29C) is significantly

higher than the data of the previous year. As well, that concentration is unrealistic of uncontaminated natural systems such as that of Delta Marsh.

With such a strong structured peak in the May 2004 profile (Figure 29C), contamination of the peeper itself is unlikely as a contaminated peeper would show an even or random distribution of Hg across all of the peepers cells. A possible cause of the contaminated Hg could be dead biota found at the time of sampling in May 2004. At that time, 10 dead song birds were found floating in the mesocosm. They were removed and counted, however it was impossible to see if any other birds were at the bottom near the sediments as duck weed covered the surface.

Hg_T concentrations also varied tremendously in the Lake 632 enclosure throughout the year. Concentrations below the sediment/water interface ranged from 20pmol/L to 100pmol/L to 1000pmol/L (Figure 30). Similar to the Delta Marsh control enclosure, the two profiles taken in August and November of 2003 (Figure 30A and 30B) may in fact represent the *in situ* concentration of Hg_T at the time sampled. However, the profile from June 2004 (Figure 30C) peaks near 1000pMol/L. This concentration of Hg_T is again excessively high to be said it is the ambient level.

It is important at this time to mention the occurrence of some type of "oil" substance that surfaced from the ELA 5x1 and ELA 5x2 treatments while removing the peepers in August 2003. The ELA control mesocosm was within inches of the ELA 5x1 mesocosm. To our knowledge, this was an uncontaminated site. Over the past decade, no motorized

boats and no pretreated wood have been allowed within the watershead of Lake 632. While sampling, without question there was found to be some type of "oil" present with the sediments of this wetland that was released with the removal of the peepers. This finding was unexpected and may have contributed to the higher levels of Hg_T present in November 2003 (Figure 30B) and the large peak in June 2004 (Figure 30C).

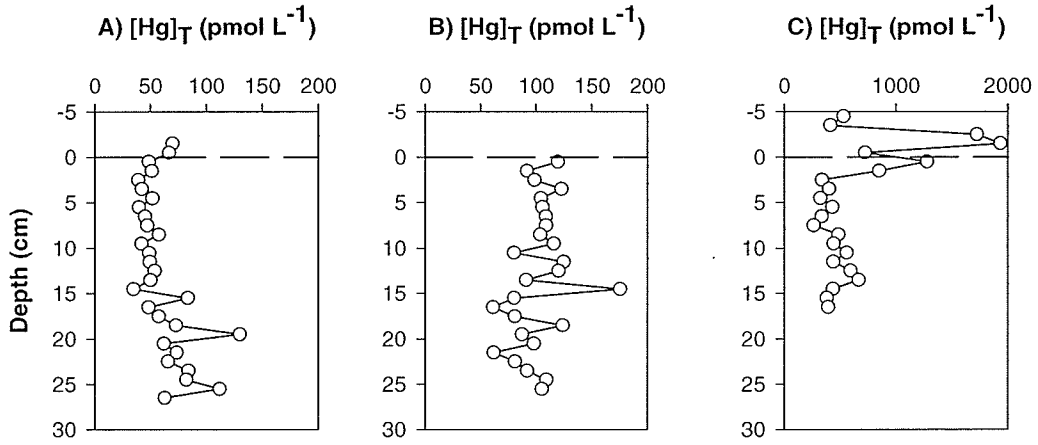

Figure 29. Hg_T profiles from the Delta-control mesocosm from (A) July 2003, (B) September 2003 and (C) May 2004.

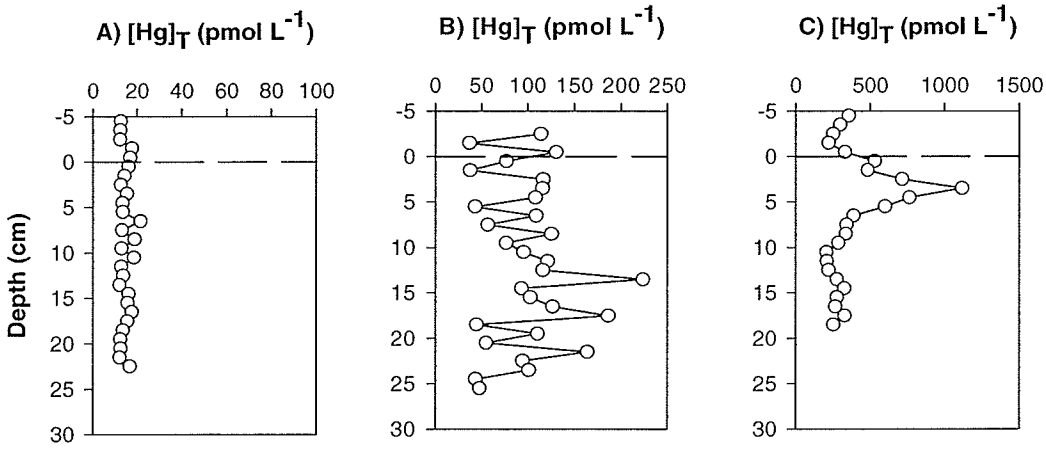

Figure 30. Hg_T profiles from the ELA-control mesocosm from (A) August 2003, (B) November 2003, and (C) June 2004.

To determine if the contamination arose from the peepers, a sample of 4ml was taken from a peeper cell with an acid cleaned pipette tip using the "clean hands dirty hands" technique after the peeper had been acid washed, purged for 14 days, assembled with Milli-Q grade water and purged for 7 days. This "blank" sample was found to have a Hg_T concentration of 3ng/L. This is well above the 0.2ng/L concentration that this water has before contact with the Plexiglas peeper, but well below the values that we have found to exist *in situ* across the MML of these wetlands. If the peeper cells contain an initial concentration of 3ng/L at the time of deployment, an initial diffusion of Hg out of the peeper will occur until the peeper cells lowers to the concentration outside of the peeper where equilibrium will be established (if the *in situ* Hg concentration is lower than 3ng/L). With a three-week deployment, this is enough time for all the Hg within the peeper cell to diffuse out and to give a representable value for the natural Hg concentration. Peepers used in this experiment were prepared in the exact same manner with the exceptions of the September 2004 peepers used in Baie St. Francois that were assembled in the UCTEL clean lab and not a typical working lab. This does not explain the difference in Hg concentration seen between and within Delta Marsh and Lake 632 control mesocosms.