Reservoir Water Level Fluctuation and Methylmercury Cycling

Final Project Report

Minnesota Power St. Louis River Hydroelectric Sediment Mercury Research Project

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1. Executive Summary of Findings

As part of the St. Louis River Hydroelectric Project Sediment Mercury Research Study supported by Minnesota Power and the Electric Power Research Institute, we investigated the role of water level fluctuation in the production and mobilization of MeHg in sediments from wetlands adjacent to a headwater reservoir (Boulder Lake), a peaking reservoir (Thomson Reservoir), and a nearby natural flowage lake (Alden Lake) used as a control. Significant findings from the research sections of the report include:

The Thomson Reservoir Shrub-Scrub wetland contains areas of buried sediment that have elevated Hg from an historic anthropogenic source. However, it is important to note that the amount of MeHg and the %MeHg are not proportional to the amount of THg in the soils. Most of the sites sampled with elevated Hg contaminated sediments in the Thomson wetland in fact have quite low absolute MeHg concentrations. (SECTIONS 6, 7)

The wetlands associated with the SLRP reservoirs tend to have higher MeHg concentrations in surface soils than the wetlands of natural lakes in the area, though a direct comparison is not possible since the wetland types are different. (SECTIONS 6, 7).

Localized zones of high MeHg concentrations found through more intensive surveys are related to favourable hydrological and/or biogeochemical conditions for methylation, not the abundance of THg (SECTION 7).

The Boulder wetland had a localized zone of elevated MeHg sediments in the surface peats as a consequence of enhanced methylation at the wetland-reservoir margin. The development of a steep redox gradient between the typically highly-reducing peat soils and the oxygenated and relatively nutrient rich reservoir water is an ideal Hg methylating environment. This zone is also subject to dewatering on an annual basis which promotes air entry, enhanced decomposition and sulfate reoxidation (SECTION 7).

Field data collected from the three target wetlands show that all of the wetlands exhibit hydrological and biogeochemical conditions conducive to Hg methylation. All exhibit periods of persistent saturation, promoting reducing conditions that are favorable for sulfate reduction - a prerequisite for Hg methylation to occur. Although there are apparent differences in MeHg concentrations and production among the three wetlands, it is not possible to determine the potential influence of the different water level fluctuation regimes from the dramatically different wetland types that existed at each study location (SECTION 8).

Laboratory experiments that used stable isotopic Hg methylation assays and microbial techniques to assess community diversity found that while some water level fluctuation treatments (monthly - Thomson and Alden) resulted in significantly higher ambient MeHg concentrations in wetland soils, others (static, daily) generally had an inhibitory effect on MeHg production and microbial community fitness (SECTION 8).

Although the overall biomass is different between the wetland sites and experimental treatments, the bacterial community was fairly similar at all sample locations (SECTION 8).
The sediments from the Thomson reservoir wetland had the highest quantity of sulfate reducing bacteria (SRB; known Hg methylators) biomarkers initially and showed statistically significant decreases for both the static and daily treatments. Neither the Boulder or Alden wetlands showed statistically significant change in SRB biomarkers for any treatments (SECTION 8).

The mobilization of THg and MeHg from associated reservoir wetlands to the actual reservoir through water level fluctuation may be a more important factor in the delivery of MeHg to surface waters than enhancement of MeHg production (SECTION 9).

The methylation potential of littoral sediments did not respond differently to either cooling or freezing treatments. The mixing of near-shore sediments appears to reduce overall Hg methylation potential in the near surface sediments (SECTION 10).

Recommendations include a better characterization of the distribution of MeHg and MeHg production in the most sensitive wetland classes (moss-lichen), and a focus on the role of variable hydraulic gradients driven by water level fluctuation as a key mechanism for the delivery of MeHg from surrounding wetlands to SLRP reservoirs. (SECTION 12).
2. Acknowledgments

The authors wish to acknowledge the contributions of many individuals, without whom this work would not have been possible. Minnesota Power - Allete, and the Electric Power Research Institute provided the funding for this research. We are grateful for their support, flexibility and patience as this project took turns in unexpected directions.

We offer our sincere thanks to the Fond du Lac Band of Lake Superior Chippewa. Their logistical support and knowledge of the waters of the St. Louis River, natural lakes and reservoirs was invaluable. We are honoured to have had the opportunity to work with Mr. Larry Schwarzkopf, who helped set the stage for the initial sampling program; this work is in his honor. Terry provided expert boat piloting on several occasions and his guidance and companionship during those times is remembered fondly.

Mr. Lowell Neudahl of Minnesota Power - Allete made commitments to this project that were far above the call. Whether it was the provision of data, long days in a boat out at the B__A__ Bog, or incredibly detailed and insightful edits on the project reports, we are indebted to him for his time and dedication.

Mr. Robert Goldstein of EPRI has likewise made important and insightful intellectual contributions that have resulted in the final products of this project being vastly superior than they would have been without his commentary. He has also exhibited patience and professionalism when technical difficulties, bureaucratic delays, and personal challenges sidetracked the project timeline.

At the University of Toronto, we thank our student assistants over the last few years Amanda Landre, Greg Bunker, and Jessica Iraci, all of whom have proceeded on to successful graduate research careers after starting on this path, in part, through this project. Ms. Susi Wanigaratne provided analytical support through much of this work. Carl Mitchell is now Dr. Carl Mitchell, a Professor at the University of Toronto - Scarborough, and his contributions to this work are too many and diverse to list. We are grateful to the graduate students and postdoctoral fellows associated with Dr. Fowle’s group who have been involved in this project previously at the University of Windsor - Great Lakes Institute for Environmental Research, and now at the University of Kansas. Our thanks also to the staff of the USGS Water Resources Division Mercury Lab in Middleton WI for their dedicated analytical support.

Finally, we thank all of the members of the advisory panel representing the Fond du Lac Reservation, Minnesota Power - Allete and the Minnesota Pollution Control Agency. The guidance received from this group has been welcome and significantly contributed to the success of this final report.
3. Project Scope and Objectives

3.1. Wetlands and Mercury Cycling

Wetlands, and in particular, peatlands (wetlands with an accumulation of organic soil > 0.4 m) have been identified as important locations in the landscape where inorganic mercury (Hg) species (notably Hg(II) complexes) are converted to methylmercury (MeHg) a potent neurotoxin with known bioaccumulative properties (Grigal, 2003; Munthe et al., 2007). A large number of studies have identified wetlands as key controls on watershed export of MeHg over a wide range of scales, from the individual wetland (e.g. Branfireun et al., 1996; Heyes et al., 2000), to the watershed (St. Louis et al., 1994; 1996), and to regional scale (Hurley et al., 1995).

Wetlands are frequently sites of elevated MeHg production relative to other terrestrial ecosystems because of the persistence of physical conditions that are conducive to biomethylation of Hg(II). It is now generally accepted that Sulfate Reducing Bacteria (SRB) are primarily responsible for this interconversion in freshwater ecosystems. SRB are obligate anaerobes, and given adequate labile substrate, a nutrient (sulfate) source, methylate bioavailable Hg(II) as a metabolic by-product (Compeau and Bartha, 1985). Gilmour and Henry (1992) first demonstrated the relationship between sulfate reduction and Hg methylation in natural lake and estuarine sediments, and the relationship has been demonstrated in a range of wetland environments either through the stimulation of MeHg production through the addition of sulfate (e.g. Branfireun et al., 1998; 2001; Jeremiason et al., 2006), or more directly through a coupling of sulfate reduction rates (SRR) and Hg methylation rates (King et al., 2001). The factors that control SRR (temperature, redox, pH, substrate and nutrient availability, SRB community structure, growth rates and biomass) will thus affect the rate at which Hg(II) is converted to MeHg. Problematically, much less is known about the processes that control the demethylation of MeHg in soils and sediments (Marvin-Dipasquale et al., 2000; King et al., 2001); photodemethylation in open waters are the only demethylation pathway which has been well quantified (see Sellers et al., 1996). Consequently, observed concentrations of MeHg in natural waters, sediments, and soils reflect the balance of the processes that control MeHg production and degradation. The use of stable isotopes of Hg as both Hg(II) and MeHg to assess rates of interconversion has proven to be a novel and valuable approach (Eckley and Hintelmann, 2006; Heyes et al., 2006), but remains limited in application given the specialized analytical infrastructure (dedicated ICP-MS) required for the work to be undertaken.

The ultimate impact of a wetland on the loading of a downstream ecosystem with MeHg is as much a function of its hydrological connectivity as MeHg production rates. A wetland that is hydrologically decoupled from the downstream system will have no impact of that system, whereas a system that is connected will have an impact that will vary as a function of the degree of within-wetland and downstream connectivity and the MeHg production potential of the wetland (Grigal, 2003; Munthe et al., 2007). In summary, one may consider a wetland with persistent saturated conditions, dominantly anaerobic soils, regions of deeply reducing conditions, and a consistent net lakeward hydraulic gradient to likely be a significant source of MeHg to receptor organisms.
3.2. Problem Statement

High fish mercury concentrations in the lakes, rivers and impoundments of the St. Louis River system in Northeastern Minnesota have been a focus of ongoing research since the early 1990s, with particular concern that reservoir operations may impact the availability of Hg to the foodchain. In 1992, an expert panel concluded that given the available data at that time, it was “not possible to evaluate the impact of reservoir operations on Hg in fish in [various] reservoirs. The issue is complicated by many factors including, but not limited to the covariance of environmental variables, potentially inadequate reference lakes, and a lack of process-oriented studies” (Panel Correspondence, 1992). Although, this expert panel noted that “fish Hg correlates positively with increasing water level fluctuation”, they could not confidently separate this effect from other environmental factors. Subsequent work has focussed on the documentation of the distribution of Hg throughout the St. Louis River system (Sorensen et al., 2004) and remediation strategies (same study), although taking a laboratory/mesocosm approach to study the impact of contaminated sediments on biological uptake proved challenging and somewhat inconclusive because of the high degree of heterogeneity in sediment properties and Hg concentrations (Sorensen et al., 2004). Further work on the question of water level fluctuation and increased exposure of the aquatic food chain to MeHg has been emerging in the literature. For example, Sorensen et al. (2005) have suggested that the covariance of Hg in fish and water level fluctuations in natural and regulated systems in Minnesota may be due to the production of sulfate in drying, aerated sediments, and the subsequent flushing and reduction of that sulfate upon rewetting, producing conditions conducive to high SRB activity and MeHg production. Certainly, the hydrologically-governed sulfide oxidation/sulfate reduction loop described in this work has been demonstrated in wetland systems elsewhere (see Devito et al., 1998; Eimers et al., 2007), supporting the theory of Sorensen et al. (2005). Sorensen et al. (2005) also argue clearly that although causation does not necessarily follow from correlation, water level fluctuation is not affected by the other co-varying chemical parameters, so the direction of influence is certain. Following from these conclusions, it is clear that sediments and soils that are subject to this wetting and drying are going to be found in the shoreline regions of water bodies, not in the deeper, permanently submerged basins.

Minnesota Power issued a request for proposals as part of the St. Louis River Hydroelectric Sediment Mercury Research Project to address the question “Do water level fluctuations caused by the SLRP hydropower operation increase the production or mobilization of methylmercury relative to natural water level fluctuations”? The RFP outlined the hypothesis that the frequency of reservoir water level fluctuations were affecting the production of MeHg in the surrounding wetlands, and/or the fluctuation was contributing to enhanced mobilization of MeHg from the wetland sediments. The RFP explicitly requested a laboratory based, experimental approach to the question. In the execution of the research project supported by these agencies, we investigated the role of water level fluctuation in the production and mobilization of MeHg in sediments from wetlands adjacent to a headwater reservoir, a peaking reservoir, and a nearby natural flowage lake used as a control.

3.3. Approach

Lacking preexisting data on the distribution of total mercury (THg) and MeHg in the wetlands of the St. Louis River system to guide the laboratory experiment, we were obliged to undertake two separate field studies in addition to the laboratory experiments. The first concerned the distribution of wetland soil THg and MeHg in a range of wetland types across a variety of natural lakes and impoundments in...
order to generate a contextualizing data set (Section 6). Using this data, and in collaboration with the
collaboration with the partner agencies, we selected three wetland sites for more intensive study. The distribution of THg and
MeHg in the soils of these three sites were investigated in more detail in order to assess the variability
in both THg and MeHg concentrations (Section 7). During this field study, we installed field monitoring
instruments to assess the range of water level fluctuation in the wetland soils and the reduction-
oxidation regime in order to compare the experimental conditions established in the laboratory to those
observed in the field. Upon completion of the tasks, a significant number of intact sediment cores were
retrieved from each of the study wetlands. These cores were used to experimentally assess the effect
of water level fluctuation on MeHg production (Section 8), MeHg mobilization (Section 9) and finally the
effect of winter draw down on both MeHg production and mobilization (Section 10). In the final section
of the report, we summarize the findings of all of this work, and draw specific conclusions about the role
of the SLRP hydropower operation on the production and mobilization of MeHg in surrounding wetlands
(Sections 11 and 12).
4. General Statement on Sample Collection, Laboratory Methods and Quality Assurance for the Determination of Total Mercury and Methylmercury Concentrations in Soils, Sediments and Waters

4.1. University of Toronto Mercury Research Lab

4.2. Overview of Laboratory Infrastructure

The University of Toronto Mercury Research Lab is fully equipped to undertake ultra-trace level determinations of total mercury (THg) and monomethylmercury (MeHg) using published, standard methods. Results produced by this laboratory have been published in numerous peer-reviewed scientific papers. The laboratory uses different instruments to determine Hg species in various media. A Tekran 2600 Automated Total Mercury System is used to determine ultra-trace THg concentrations primarily in water, but also in sediments and tissues with low THg concentrations (US EPA Method 1631). A Milestone DMA-80 direct mercury analyzer is used for the bulk of trace level Hg determinations for solid phase materials (tissues, soils, sediments)(USEPA Method 7473). A cold-vapour atomic fluorescence spectroscopy (CVAFS) system based around a Tekran 2500 detector is used for MeHg measurements (Horvat et al., Analytica Chimica Acta. 282: 153-168, 1993; Olson et al., Fresenius Journal Analytical Chemistry. 358: 392-396,1997). Our most sensitive instruments and samples are contained within a Class 100 Clean Room to minimize contamination of samples and equipment.

4.2.1. Summary of the Method for Determining Total Mercury in Peat, Soils and Sediments

a) Sample Acquisition: All sampling is undertaken using ultra-clean protocols. Technicians are gloved in the field with sterilized, clean-room grade trace-metal free gloves. Using what is commonly referred to as a “clean hands, dirty hands” method, one technician will handle sampling equipment and containers only, while the other will only come into contact with the peat samples. Surface peat samples are taken by directly plucking the surficial material and placing into a small leak-proof zip-closure bag, which is then rolled to exclude air, double-bagged, labeled and placed into a clean, dark cooler that either contains dry ice, or cooler packs to chill the samples until they can be returned to the facility and frozen. Deeper peat samples are handled similarly, but may be acquired either by cutting out a surface block of peat with a clean blade, measuring depth intervals and acquiring sample, or by using a suitable sampler for deep peat deposits (e.g. Russian peat corer). If a deep corer is used, then the sample for Hg analyses is taken from the inside of the core that was not in contact with the corer. Gloves are changed after every sample is taken. Samples are kept frozen at -15°C or lower until analyses can be performed.

b) Total Mercury Analysis: In the laboratory, individual samples are thawed, homogenized, and subsampled. A small mass is retained for oven-drying, and a minimum of two wet samples (<0.5 g wet weight each) are used for analyses. Remaining sample, if any, is kept frozen for replicate analyses if required. Samples are analyzed as wet weight. Final analytical concentrations are expressed as a standardized dry weight through the generation of a wet-to-dry weight conversion factor derived from the sub-sample used exclusively for oven-drying. This eliminates any matrix changes or Hg losses due to drying or heating.
The wet peat, soil or sediment sample is acid digested in a sealed high-pressure Teflon vessel (Savillex®). After digestion, an aliquot is diluted in 18.2 MOhm deionized water. Bromine Monochloride (BrCl) is added to the sample container to oxidize all forms of Hg to HgII oxidation state. After a minimum of 12 hours the BrCl is neutralized by addition of Hydroxylamine Hydrochloride (NH2OH•HCl). Following neutralization, the sample is introduced into the Tekran 2600 Automated Total Mercury Analyzer that is used for the determination of total mercury in diluted digestate. This system complies with EPA Method 1631. Stannous Chloride (SnCl2) is added to the sample in line to reduce the Hg from the HgII to the Hg0 oxidation state. The Hg0 is purged at a gas phase separator onto a gold-wire trap (sample). The mercury vapor is thermally desorbed to a second gold trap (analytical) and from that detected by cold vapor atomic fluorescence spectrometry (CVAFS). There is no difference in approach for riverine sediments, lacustrine sediments, mineral soils, or organic/peat soils.

4.2.2. Summary of the Method for Determining Total Mercury in Water

a) Sample Acquisition: Following similar ultra-clean protocols as above, water samples for THg analyses are taken in either acid-cleaned Teflon bottles, or pre-sterilized polyethylene teraphthalate (PET) bottles. For surface water samples, the bottle is triple rinsed, then immersed for sampling. The bottle is double-bagged, labeled, and stored in a cool, dark container until it can be returned to the laboratory for processing. Samples that are to be analyzed as unfiltered are simply acidified with ultrapure concentrated HCl (0.5% by volume). Dissolved Hg samples are filtered using an acid-cleaned teflon filter apparatus (Savillex Inc) and pre-muffled (500°C) glass-fibre filters (Whatman GFF 0.7 μm) and then acidified as above. Acidified samples are stored doubled bagged in a cool, dark container. Refrigeration is not required.

b) Total Mercury Analysis: A Tekran 2600 Automated Total Mercury Analyzer is used for the determination of total mercury in water. This system complies with EPA Method 1631. From the addition of Bromine Monochloride (BrCl) the method is as above for diluted digestate.

4.2.3. Summary of the Method for Determining Methylmercury in Soils, Sediments and Water

a) Sample Acquisition: Following same ultra-clean protocols as for THg. In fact, sample splits can be used for the determination of both THg and MeHg in soils, sediments and water.

b) Methylmercury Analysis: Aqueous samples are first distilled to minimize matrix interferences. The samples are distilled at 135°C with the addition of potassium chloride (KCl), sulfuric acid (H2SO4), and copper sulfate (CuSO4). Solid samples are digested using the same apparatus with the solid sample introduced to the vessel with a volume of 18.2 MOhm deionized water. The pH of the distillate is adjusted to 4.9 using acetate buffer. The distillate is then ethylated using sodium tetracetethyl borate (NaTEB) and allowed to react for 15 minutes. Following reaction with NaTEB the distillate is purged with nitrogen gas (N2) for 20 minutes and the MeHg is collected on a Tenax® Trap. Mercury species are thermally desorbed from the Tenax® Trap, separated using a gas chromatography (GC) column, reduced using a pyrolytic column, and detected using a cold vapor atomic fluorescence spectrometry (CVAFS).

4.3. US Geological Survey Laboratory
4.3.1. Overview

The USGS laboratory in Middleton Wisconsin is under the directorship of Dr. D. Krabbenhoft. The Standard Operating Protocols and accepted EPA methods are all as described above for the University of Toronto Laboratory. In addition to the detection of ambient total mercury and ambient methylmercury, this laboratory is also equipped with an ICP-MS, which is used to make specific determinations of the abundance of particular isotopes of mercury. Details in this method are as described in Hintelmann et al., 1995 and 1997).

4.4. General Quality Assurance

a) Standardization: Standardization is performed at least at the beginning of a daily sample run. For all analyses, a standard curve is used to calculate sample concentrations measured from an instrument response. The curve is generated by measuring instrument responses for a series of standard solutions of the analyte. Sample concentrations are then calculated by interpolating between the standard points. A set of at least three standards that bracket the expected sample concentrations is used for standardization. Instrument responses used to generate the standard curve must be linear according to criteria established for the specific method or a second series of standard solutions are analyzed prior to analysis of any samples.

b) Precision – Duplicates: The precision of an analytical procedure is determined by performing replicate analysis of a sample and must meet the criteria established for the specific method. The indexes of precision used are relative percent difference (RPD) and relative standard deviation (RSD):

\[
\text{RPD} \, (\%) = \frac{(|X_1-X_2|)}{\text{mean}} \times 100
\]

\[
\text{RSD} \, (\%) = \left(\frac{\text{standard deviation}}{\text{mean}}\right) \times 100
\]

where \(X_1\) and \(X_2\) are the measured values for the first and second replicates, respectively. The Limit of Detection (LOD) is the concentration that is three standard deviations of multiple blank analysis (IUPAC definition for a 99% confidence level). Below this concentration, the analyte is considered to be undetectable. The region from three to five times the standard deviation of the blanks is the region of detection but not quantification. A concentration greater that five times the standard deviation of the blanks is the region of quantification. The RPD and RSD are applicable only in the region of quantification. If the RPD or RSD exceeds 10 percent for total mercury the sample must be reanalyzed.

c) Accuracy – Spikes: Sample accuracy is determined by adding a known amount of the analyte (spike) to the sample and measuring the change in concentration. The percent recovery is used as the index for measuring accuracy and is calculated as follows:

\[
\text{Percent Recovery} = \frac{(C_2-C_1)}{C_2} \times 100
\]

Where \(C_2\) is the spiked sample concentration and \(C_1\) is the sample concentration. Percent recoveries must meet criteria established for the specific method or a second spiked sample must be analyzed. If the second spike does not meet criteria then all sample data for that run are suspect and need to be reanalyzed or a flag is assigned to draw the project chiefs attention to that data.

d) Blanks: Method blanks will be analyzed to verify that the analytical system is free of contamination and sample carryover. The mean of the instrument responses from the blanks is used as the zero value in the calibration curve and in the calculation of the LOD. The LOD/volume of sample in liters, as calculated from the first three blanks, must be less than the expected sample concentration.
5. Site Description

The studies described in this report were undertaken in, and on wetland soils and lake sediments from, reservoirs and natural lakes in the St. Louis River Project Area in northeastern Minnesota (Figure 5.1). These sites include:

a) Two natural lakes that have not been affected by impoundment (Alden Lake; Linwood Lake)

b) Five headwater reservoirs with operations characterized by more seasonal water level drawdown over the winter season to maintain downstream flows (Island Lake, Whiteface Lake, Boulder Lake, Fish Lake and Wild Rice Lake)

c) One peaking reservoir with operation characterized by more frequent water level fluctuations (up to daily frequency) designed to match hydroelectric demand (Thomson Reservoir).

Figure 5.1. Location of the study lakes and reservoirs in northeastern Minnesota.
6. Methylmercury and Total Mercury in Surface Soils of Wetlands of the St. Louis River Project, Minnesota

6.1. Introduction

In the published literature, the presence of wetlands in a watershed has been shown to correlate with DOC-mediated THg flux (Mierle and Ingram, 1991), MeHg in surface water (St. Louis et al., 1994; 1996) and MeHg in fish and other organisms (Wiener et al., 2006; Evers et al., 2007). Although the role of wetlands as important sites of MeHg production is widely accepted and often generalized, it is much less frequently substantiated with empirical studies.

Certainly, there is ample evidence that both THg and in particular MeHg concentrations in wetland soils and pore waters vary widely, both within individual wetlands (Branfireun, 2004), and between wetland locations and types (e.g. Heyes et al., 2000). This variability in wetland MeHg distribution is driven by the same factors that govern methylation in any favourable environment; bioavailable mercury and nutrient loading, sediment type, organic matter character, microbial activity and hydrology (Munthe et al., 2007). Moreover, wetlands may be sites of MeHg production, but it is only with hydrologic connectivity to receiving waters that they become net sources of MeHg to receptor organisms (Grigal, 2003; Munthe et al., 2007).

The larger research project, of which this is a part, focused on the influence of reservoir water level fluctuation on Hg methylation in wetlands surrounding impoundments of the St. Louis River Reservoir Project (SLRP). The broader objective of the project was to determine if reservoir operations that influenced both the magnitude and frequency of water level fluctuation influenced the production of MeHg, and or the mobilization of MeHg from wetlands surrounding the impoundments. At the outset of this project, it was recognized that there was a considerable range of dominant wetland types in the reservoir and lake systems of the area, from open water with emergent aquatic vegetation, through densely-vegetated marshes, to open sphagnum moss bogs. This range of wetland type existed both within, and among reservoir systems. Although wetland maps existed for the systems, little first-hand information existed, and the classification was based only on the highest level categorization of the National Wetlands Inventory (Cowardin et al., 1979) and was incorrect in several locations, demanding further verification. Most importantly in the context of the broader study, no information existed on the THg or MeHg content of the soils of these various wetland types, making the selection of representative sites for the more intensive Hg study impossible without an initial scoping study.

The specific objectives of this work were to:

a) Assess the range of wetland types associated with five headwater reservoirs operated with seasonal drawdown, one downstream reservoir operated with a variable (peaking) water level according to electricity demand, and two nearby unimpounded, unregulated natural lakes.

b) Collect bulk surficial soil samples from the dominant wetlands in each of the above systems to analyze for THg and MeHg concentrations, assess spatial variability, and range of observed concentrations.
c) Use the above information to select representative field sites for more intensive study of the effect of water level fluctuation on wetland methylmercury production.

This purpose of this study was to survey a wide range of wetlands over eight different lakes and reservoirs, providing insight into the variability and range of THg and MeHg concentrations in wetland soils to guide future sampling programs. It was not intended to provide insight into the mechanisms behind the observed THg and MeHg concentrations, and as such no other ancillary data was collected. We report here the most extensive dataset on THg and MeHg concentrations from various wetlands in one geographic area, providing significant guidance not only to other aspects of the research project for which it was intended, but also for other research for which the spatial variability in THg and MeHg in wetlands may influence the loading of MeHg to surface waters, or land-use change/manipulations such as wetland drainage or impoundment might be of greater or lesser concern, depending on the amount of MeHg in the soils or sediments.

6.2. Methods

Sampling: A two-day sampling campaign was undertaken in July, 2004, prior to the beginning of the official contracting period, as the data derived from this effort was critical to the successful implementation of future work in the following field season. Over this two day period, the major wetlands of one peaking reservoir (Thomson Reservoir), five headwater reservoirs (Boulder Lake, Whiteface Lake, Fish Lake, Rice Lake and Island Lake), and two natural unregulated lakes (Linwood Lake and Alden Lake) were accessed by boat (See Figure 5.1). Thomson Reservoir is also a site of particular concern because it is known to be among the downstream reservoirs with elevated sediment THg concentrations as a result of past mercury-laden discharges from wood processing industries into the river at Cloquet, MN (Sorensen et al., 2004). Bulk sediment samples (0-10 cm depths relative to surface with living vegetation removed) were taken from all of the target lakes and reservoirs, and from multiple locations within each system. Where feasible, a short transect was sampled at each wetland location, with samples from the near-shore littoral sediments at the front of the wetland, within 5 meters of the interface between the wetland and open water, and from the interior of the wetland in a location beyond the direct influence of the open water system. Samples were acquired by cutting out surficial sediment with a clean stainless steel blade for more consolidated materials, or hand grabbing an integrated sample for less consolidated materials. Clean techniques used at all times (gloves; double bagged samples). A combination of reservoir/lake size, wetland extent, logistical challenges, site access and a focus on sites of concern resulted in varying numbers of samples being acquired from each site (Thomson n=13; Boulder n=8; Whiteface n=6; Fish n=2; Rice n=4; Island n=9; Linwood n=3; Alden n=3). The interpretation of mean and median values for sites with a small sample size is obviously to be done with caution.

Chemical Analysis: Samples were kept in coolers and frozen immediately upon the return to the field office. The samples were returned to the University of Toronto on dry ice, and stored frozen until analysis. THg and MeHg analyses were performed using standard protocols and are briefly described here. THg and MeHg analyses were performed in a Class 100 cleanroom at the University of Toronto by USEPA Methods 1630 and 1631. MeHg concentration was determined by aqueous phase ethylation and cold vapour atomic fluorescence spectroscopy (CVAFS) following distillation (Horvat et al., 1993; Olson et al., 1997). The MeHg in the soils was isolated from the sample matrix by atmospheric pressure water vapor distillation (Horvat et al., 1993). The sample distillate was ethylated with sodium tet-
raethylborate, buffered with sodium acetate, and purged with nitrogen onto glass traps filled with Tenax®. The Tenax® trap was then heated in a stream of argon, the mercury stream was speciated on a gas chromatography column, combusted to Hg0 using a pyrolytic column, and detected on a Tekran® 2500 by CVAFS.

THg concentration was determined using a Tekran® model 2600 CVAFS mercury detector with automated sampler. The day prior to analysis, 1 mL of BrCl was added to 40 mL of digestate. Analysis was by CVAFS with two-stage gold trap amalgamation and reduction by SnCl₂ as described in USEPA Method 1631.

QA/QC: Quality assurance and control measures varied from day to day. Among analytical runs, Limits of Detection for THg varied between ~0.16 and 0.8 ng/g (dry wt) and between 8 and 45 pg/g (dry wt) for MeHg based on three times the standard deviation of the analytical blanks. Analytical runs were rejected and samples re-analyzed if a) variation among replicates was greater than 10%, and/or b) analyses of THg standard reference material (IAEA-405) differed from certified values by + 10%. Aqueous standards are used for calibration in both the THg and MeHg determinations, and check standards did not vary within a run by >2%. Samples were analysed as wet sediment, oven-dried, and a wet to dry weight conversion applied to the determined concentration. The final data are reported as Hg concentration in ng/g dry weight.

Statistical Analysis: Since normality of the data distribution cannot be assumed, particularly when N<10 as is the case for some of the sites sampled, median concentrations will be presented and compared instead of means. A non-parametric test that is not sensitive to the nature of the population distribution (Kruskall-Wallis Test) was used to test for differences among sites and/or treatments. The Kruskall-Wallis test is used to compare independent samples, and test the null-hypothesis that several populations have the same continuous distribution, at least as far as their medians are concerned. The null-hypothesis is rejected if $H > \chi^2$ at the prescribed level of significance, or $p <=$ the prescribed level of significance. For this analysis all significant relationships are at the 95% confidence level, unless otherwise noted. All descriptive statistics and tests for significance were done using Aabel 2.4.2 for Macintosh (Gigawiz, Inc.).

6.3. Results

Wetland Types: Using the Canadian Wetland Classification System (2nd ed., National Wetlands Working Group, 1997) The dominant wetlands in the study reservoirs and lakes are Flat/Domed Bogs (with some small floating bogs eroded from the larger domed systems), Lacustrine Bay Marshes, Lacustrine Shore Marshes and small Open Water wetlands. The Bog systems are easily distinguished, and have their surface vegetation dominated by Sphagnum mosses, with low shrubs and occasional black spruce and tamarack. The Boulder, Rice and Whiteface headwater reservoirs were dominated by bog-type wetlands, with evidence of significant erosion at the reservoir-wetland interface. A narrow (<3 m) fringe of non-bog vegetation (mixed shrubs and tall rushes) was always present at the reservoir-wetland interface, indicating the direct influence of the more nutrient-rich reservoir water on plant community. The other reservoirs and lakes were dominated by marshes, both Lacustrine Bay Marshes (Island Lake, Thomson Reservoir) and Lacustrine Shore Marshes (Fish Lake, Alden Lake), differentiated only by the self-evident geomorphic position. These wetlands are characterized by a mixed vegetation community, but the Lacustrine Shore Marshes tended to have simple communities of low rushes,
whereas the Lacustrine Bay Marshes tended toward more complex assemblages of mixed graminoids and shrubs. All are influenced to greater and lesser degrees by water level fluctuations in adjacent water bodies. Only Linwood Lake was considered to have a predominantly open water wetland, with submerged aquatic vegetation only.

Previous surveys of wetlands in this area conformed to the National Wetlands Inventory system of classification described by Cowardin et al. (1979). In some cases, these prior inventories were determined to be significantly in error during this survey; for example the extensive bogs (moss-lichen wetlands) of Rice Lake have been mislabeled emergent marshes in previous surveys. In the interest of conformity with this system, the NWI class equivalent of the Canadian system will be used in this report (Table 6.1).

<table>
<thead>
<tr>
<th>Canadian Wetland Classification</th>
<th>National Wetlands Inventory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flat/Domed Bog</td>
<td>Moss-Lichen Wetland</td>
</tr>
<tr>
<td>Floating Bog Mat</td>
<td>Moss-Lichen Wetland</td>
</tr>
<tr>
<td>Lacustrine Shore Marsh</td>
<td>Emergent Wetland</td>
</tr>
<tr>
<td>Lacustrine Bay Marsh</td>
<td>Shrub-Scrub Wetland</td>
</tr>
<tr>
<td>Open Water</td>
<td>Aquatic Bed</td>
</tr>
</tbody>
</table>

Table 6.1. Equivalent classifications between the Canadian Wetland Classification System and that used by the National Wetlands Inventory (this report).

Mercury and Methylmercury in Wetland Soils: Soil (0-10 cm integrated sample) THg and MeHg concentrations measured across all lake and reservoir systems (n=49) ranged from 7.6 to 320 ng/g d.w., and <MDL to 24.3 ng/g d.w. respectively (Table 6.2). The %MeHg ranged from 0 to 27% with no trend among systems other than a consistently low %MeHg for the two natural flowage lakes, Linwood and Alden (1% MeHg or less).
Table 6.2. Summary statistics for total mercury, methylmercury and percent methylmercury for the five study reservoirs and two study lakes.

<table>
<thead>
<tr>
<th>Variable:</th>
<th>Boulder</th>
<th>Fish</th>
<th>Island</th>
<th>Rice</th>
<th>Whiteface</th>
<th>Thomson</th>
<th>Linwood</th>
<th>Alden</th>
</tr>
</thead>
<tbody>
<tr>
<td>THg Min.:</td>
<td>33.0</td>
<td>144.4</td>
<td>7.6</td>
<td>13.3</td>
<td>33.2</td>
<td>31.8</td>
<td>23.5</td>
<td>32.5</td>
</tr>
<tr>
<td>ng/g Max.:</td>
<td>188.4</td>
<td>187.2</td>
<td>224.0</td>
<td>82.1</td>
<td>179.0</td>
<td>320.1</td>
<td>88.4</td>
<td>106.2</td>
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<tr>
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<td>165.8</td>
<td>85.3</td>
<td>57.0</td>
<td>80.2</td>
<td>158.2</td>
<td>61.9</td>
<td>59.3</td>
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<tr>
<td>Median:</td>
<td>63.5</td>
<td>165.8</td>
<td>73.0</td>
<td>66.2</td>
<td>47.5</td>
<td>114.9</td>
<td>73.8</td>
<td>39.1</td>
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<tr>
<td>MeHg Min.:</td>
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<td>2.6</td>
<td>&lt;MDL</td>
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<td>3.3</td>
<td>0.9</td>
<td>0.2</td>
<td>0.1</td>
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<td>11.3</td>
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<td>11.5</td>
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<td>1.3</td>
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<td>d.w. Mean:</td>
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<td>2.3</td>
<td>4.7</td>
<td>6.3</td>
<td>7.6</td>
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<td>0.5</td>
</tr>
<tr>
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<td>7.0</td>
<td>2.1</td>
<td>5.3</td>
<td>5.8</td>
<td>2.9</td>
<td>0.9</td>
<td>0.2</td>
</tr>
<tr>
<td>%MeHg Min.:</td>
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<td>2.0</td>
<td>0.0</td>
<td>2.0</td>
<td>2.0</td>
<td>1.0</td>
<td>1.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Max.:</td>
<td>23.0</td>
<td>6.0</td>
<td>6.0</td>
<td>12.0</td>
<td>17.0</td>
<td>27.0</td>
<td>0.7</td>
<td>0.7</td>
</tr>
<tr>
<td>Mean:</td>
<td>7.9</td>
<td>4.0</td>
<td>2.4</td>
<td>7.3</td>
<td>10.5</td>
<td>6.5</td>
<td>1.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Median:</td>
<td>6.5</td>
<td>4.0</td>
<td>2.0</td>
<td>7.5</td>
<td>12.0</td>
<td>2.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

N: 8 2 9 4 6 13 3 3

Total Mercury: Soil THg concentrations (0-10 cm) measured across all lake and reservoir systems does not statistically differ among the systems at the 95% confidence level (Figure 6.1).

Figure 6.1. Total mercury in wetland soils (0-10 cm) by reservoir/Lake. The horizontal line is the median of the data. Lower and upper whiskers are the 10th and 90th percentile respectively. All data for each category are displayed as points to illustrate sample size and range differences.

Fish Lake and Thomson Reservoir have higher median THg concentrations in their wetlands than the other sites, (165.8 and 114.9 ng/g d.w. respectively), but statistical significance is obfuscated by the small sample size for Fish Lake (N=2; boat access proved impossible due to dense aquatic vegetation), and the large range in THg concentration (nearly 300 ng/g d.w.) found in the Thomson Reservoir wetland. The two natural lakes shared a similar range of THg concentrations (23.5 - 88.4 ng/g d.w. for Linwood, and 32.5 to 106 ng/g d.w. for Alden). Although lower than many of the other waterbodies, there was not a statistically significant difference in the total Hg concentration between the two natural lakes and the SLRP reservoirs.

When the wetland soil THg concentrations are examined by lumping wetland type across the reservoirs/lakes, there are differences in both the median THg concentrations and the range among wetland types (Figure 6.2). The Shrub-Scrub class is significantly different from the Moss-Lichen (p=0.003) and the Emergent Wetland (p=0.040) class due to the elevated Hg in the Thomson Reservoir shrub-scrub wetland from historic Hg discharges to the river. Significance tests for the Aquatic Bed class are compromised by a small sample size (N=3). A larger sample size would likely reveal a significant difference between this class and the Shrub-Scrub wetland given the tendency observed in Figure 6.2.

![Figure 6.2](image)

Figure 6.2. Total mercury in wetland soils (0-10 cm) by wetland type. The horizontal line is the median of the data. Lower and upper whiskers are the 10th and 90th percentile respectively. All data for each category are displayed as points to illustrate sample size and range differences.

There are no statistically significant differences in THg concentrations by location relative to the reservoir/lake-wetland interface (Figure 6.3). This is an important finding as it indicates that the THg concentrations of the surficial littoral sediments in front of each wetland tend to reflect those of the wetland, and not the lacustrine sediments.

Figure 6.3. Total mercury in wetland soils (0-10 cm) by location relative to the reservoir/lake-Wetland interface. The horizontal line is the median of the data. Lower and upper whiskers are the 10th and 90th percentile respectively. All data for each category are displayed as points to illustrate sample size and range differences.

**Methylmercury:** MeHg concentrations measured across all sites (n=49) ranged from below the MDL to 24.3 ng/g d.w (Table 6.2; Figure 6.4).

Figure 6.4. Methylmercury in wetland soils (0-10 cm) by reservoir/lake. The horizontal line is the median of the data. Lower and upper whiskers are the 10th and 90th percentile respectively. All data for each category are displayed as points to illustrate sample size and range differences.

The Thomson Reservoir wetland was characterized by not only the highest individual MeHg concentration measured (24.3 ng/g d.w.) but also the greatest range (over 23 ng/g d.w.). Unlike for THg, the two natural lakes had statistically significantly lower MeHg concentrations than some of the other systems, although the same low sample size means that the significance of the difference should be inter-
interpreted with caution. MeHg concentrations in the Linwood Lake system were significantly lower than those of Boulder Reservoir \((p = 0.014)\), Whiteface Reservoir \((p = 0.020)\) and Thomson Reservoir \((p=0.013)\). MeHg concentrations in the Alden Lake system showed the same pattern of significant difference (Boulder Reservoir, \(p=0.025\); Whiteface Reservoir, \(p=0.020\); and Thomson Reservoir, \(p=0.013\)). The other statistically significant difference was between the Island Reservoir and Whiteface Reservoir systems, with significantly higher MeHg concentrations for Whiteface \((p = 0.005)\).

By wetland type, the Moss-Lichen class has a significantly higher MeHg concentration than the Emergent Wetland \((p=0.023)\) and Aquatic Bed classes \((p=0.021)\) (Figure 6.5). The Shrub-Scrubs Class is has significantly higher concentrations than the Emergent Wetlands \((p=0.040)\) and Aquatic Bed \((p=0.030)\) due to legacy Hg deposited from historic point discharges of mercury to the river.

![Box plot showing MeHg concentrations by wetland type](image)

*Figure 6.5. Methylmercury in wetland soils (0-10 cm) by wetland type. The horizontal line is the median of the data. Lower and upper whiskers are the 10th and 90th percentile respectively. All data for each category are displayed as points to illustrate sample size and range differences.*

The overall median MeHg concentration was significantly higher in the interior of the wetlands sampled than at either the littoral \((p=0.013)\) or margin locations \((p=0.020)\) (Figure 6.6).
There is no significant relationship between THg and MeHg concentrations in these wetland soils, either lumped or split by site, wetland type or sampling location.

**Percent Methylmercury:** Taken on its own, MeHg concentration is difficult to use to characterize the methylating environment. For example, higher MeHg concentrations may be associated with a very large THg pool, indicating a relatively inefficient conversion of inorganic Hg to MeHg, whereas lower concentrations but associated with a small THg pool may be indicative of an environment that has a high methylation potential. The calculated fraction of the THg that is MeHg is often considered to be a reasonable assessment of the ‘methylating efficiency’ of a particular media as it serves to normalize the differences in the size of the THg pool (e.g. Gilmour et al., 1998; Shanley et al., 2005).

The two natural systems, Linwood and Alden, had significantly lower %MeHg than many of the other systems (Boulder, p=0.032, 0.025; Rice, p=0.034, 0.034; Whiteface, p=0.020, 0.020; Thomson, p=0.026, 0.019). Boulder and Whiteface also had significantly higher %MeHg than Island (p=0.018 and 0.010, respectively) (Figure 6.7).
The highest individual values for %MeHg are found in the wetlands associated with Boulder Lake and the Thomson Reservoir (Figure 6.7). Although Fish Lake had some of the highest absolute MeHg concentrations (Figure 5.4), the %MeHg is relatively lower as compared to the other sites, demonstrating the utility of normalizing the THg pool among sites with %MeHg.

Figure 6.8 clearly indicates that the Moss-Lichen wetland class has a significantly higher median %MeHg than Emergent (p<0.001) and Aquatic Bed (p =0.024). No significant difference is found between the Moss-Lichen and Shrub-Scrub types. Although this trend is also evident in the absolute MeHg concentration (Figure 5.5), the %MeHg clearly differentiates this class from other wetland types with respect to MeHg production.
Although the absolute MeHg concentrations indicated differently, the interior wetland sampling locations were not statistically significantly different from the littoral and marginal locations with respect to %MeHg (Figure 6.9).

Figure 6.8. %MeHg in wetland soils (0-10 cm) by wetland type. The horizontal line is the median of the data. Lower and upper whiskers are the 10th and 90th percentile respectively. All data for each category are displayed as points to illustrate sample size and range differences.

Figure 6.9. %MeHg in wetland soils (0-10 cm) by sampling location. The horizontal line is the median of the data. Lower and upper whiskers are the 10th and 90th percentile respectively. All data for each category are displayed as points to illustrate sample size and range differences.
6.4. Discussion and Conclusions

The purpose of this study was to survey a wide range of wetlands over eight different lakes and reservoirs, providing insight into the variability and range of THg and MeHg concentrations in wetland soils to guide future sampling programs. Considerable differences among systems and wetland types indicate that the general class of ‘wetland’ cannot be applied as a uniform descriptor across the landscape with respect to the amount or speciation of mercury, with obvious implications for watershed modelling studies, and more intensive mechanistic research programs, such as those described here.

For these study systems in particular, important patterns for THg, MeHg and %MeHg emerged that are related to both the geographic and hydrologic position of the study lake and its wetlands, as well as the wetland type among systems. For this investigation, the range of THg in wetland soils (0-10 cm depth) measured is within the range of that reported for upland and wetland soils elsewhere (see Gri-gal, 2003), but with some observed concentrations at the top end of those observed at natural sites. Of particular note is the wetland in the Thomson Reservoir. Although minimum concentrations do not vary among systems, notably higher maximum THg concentrations (>300 ng/g d.w.) found here are likely the influence of the discharge of Hg from an historical upstream anthropogenic source. In contrast, the wetlands of the headwater reservoirs (Boulder, Fish, Island, Rice, Whiteface) have a similar range of THg concentrations in wetland soils (0-10 cm), despite the lumping of a number of wetland types in such a comparison. The two natural lakes (Linwood, Alden) have similar THg concentrations associated with their wetlands, and are not significantly different from the headwater wetlands. However, the Aquatic Bed wetland type that is only represented in these lakes are significantly lower in THg concentrations than other wetland types. The significantly higher THg concentrations associated with the Shrub-Scrub wetland type is influenced by the high concentrations in the Thomson system, which has a history of Hg contamination.

Methylmercury concentrations and %MeHg in the 0-10 cm layer of surface wetland soils are similar across wetlands in the reservoir systems, but are significantly higher than those of the natural lakes that were studied for this project, but were of a different wetland type. It is not possible to attribute this to impounded versus non-impounded status, as the relationship is driven by the dominance of wetland types with high MeHg concentrations and %MeHg in the impounded reservoir systems. Several of the reservoirs (Boulder, Whiteface and Rice) are dominated by the moss-lichen wetland type, which has on average significantly higher %MeHg than other wetland types. This distribution is a consequence of the preexisting wetland mosaic associated with each watershed.

6.5. Selection of Wetlands for Intensive Study

Thomson Reservoir was selected for more detailed study for several reasons: it is the only reservoir in the SLRP that is subjected to deliberate high frequency water level changes which may affect MeHg formation and transport; there is concern over high fish Hg concentrations associated with this system, and; the impacts of historical Hg releases upstream of this reservoir may have impacts on the amount of MeHg produced. Although neither site has extensive wetlands, Alden Lake was selected as the natural lake over Linwood because of the presence of emergent wetlands which could be easily accessed, sampled and monitored. Linwood Lake was dominated by very small areas of aquatic bed
type wetlands that are always fully wetted, not readily sampled, and not overly representative of the wetlands of the area. Boulder Lake was selected as the headwater wetland of interest because it was dominated by the moss-lichen wetland type that is abundantly associated with the headwater reservoirs, and the wetland was readily and quickly accessible from a boat launch at an interpretive centre on the lake.
7. Spatial Variability in Soil Total Mercury and Methylmercury Concentrations in Wetlands of the St. Louis River Project, Minnesota

7.1. Introduction and Rationale

Section 6 of this report describes a survey of the THg and MeHg concentrations in the surficial soils of a range of wetland surrounding candidate reservoirs and lakes of the SLRP. The data from this survey provided important guidance for the selection of the sites for intensive sampling for the laboratory experiment to study the effects of water level fluctuation on MeHg production. The wetlands for intensive study (Thomson East Marsh, Boulder Lake Bog, and Alden Lake Marsh) not only represented three water level regimes (peaking drawdown, seasonal drawdown, and natural fluctuation, respectively), but also three distinct wetland types (Shrub-Scrub wetland; Moss-Lichen wetland, and Emergent wetland).

From the work presented in Section 6, it is apparent that the relatively small numbers of samples taken from each wetland were insufficient with respect to minimizing variability of measured Hg concentrations about a mean or median concentration. The more detailed assessment of this variability is necessary for at least two main reasons. Firstly, there have been no thorough evaluations of the wetland-scale spatial variability of THg or MeHg concentrations in soils reported anywhere in the literature to the best of our knowledge. An assessment of this variability is essential for effective inventory estimates to be undertaken, or for wetland Hg pools to be reasonably incorporated into models or mass balance estimates. Secondly, if experiments are to be undertaken with wetland soils, the assessment of in situ heterogeneity allows for at least a reasoned trade-off to be made between representativeness of the experimental manipulations and practical feasibility.

The larger research project of which this study is a part, focused on the influence of reservoir water level fluctuation on Hg methylation in wetlands surrounding impoundments of the St. Louis River Project (SLRP). The broader objective of the project was to determine if reservoir operations that influenced both the magnitude and frequency of water level fluctuation influenced the production of MeHg, and or the mobilization of MeHg from wetlands surrounding the impoundments. The central approach to evaluating this influence is to undertake a range of experimental manipulations of water level in representative intact soil cores from candidate wetlands and measuring methylmercury production over time. In order for a controlled laboratory experiment to be successfully implemented, within-wetland replicate sample variability needed to be minimized, particularly with respect to THg and MeHg concentrations, such that the effects of the experimental manipulations could be isolated. The limited sampling of each wetland in the preliminary reconnaissance provided some insight into the magnitude and range of concentrations that could be expected, however the sampling density was insufficient to identify trends in the spatial pattern of THg and MeHg. Moreover, the preliminary sampling effort only focused on soils in an integrated 0-10 cm sample relative to the surface. Given the potential for considerable variability in the depositional history of each wetland, particularly the Thomson East Marsh which historically received Hg-contaminated materials from an upstream anthropogenic source, information about the distribution of THg and MeHg deeper in the soil profile was essential in order to return to these three wetlands and retrieve between 20 and 30 intact soil cores of similar characteristics.
The specific objective of this work is to describe the spatial distribution of THg and MeHg in surface (0-5 cm) and subsurface (35-40 cm) soils of the Thomson, Boulder and Alden wetlands in order to identify relatively homogeneous candidate sites for the extraction of replicate intact soil cores. The data derived from each wetland will also be used to generate descriptive statistics to compare with future laboratory experiments in order to ensure that the experimental work is reasonably representative of the field conditions.

This purpose of this study was to describe in detail the spatial distribution of THg and MeHg in the soils of the three target wetlands, providing insight into the variability and range of THg and MeHg concentrations and guiding future sampling programs. It was not intended to provide insight into the mechanisms behind the observed THg and MeHg concentrations, and as such no other ancillary data was collected.

7.2. Methods

On June 1-3, 2005, the three target wetlands were intensively sampled to evaluate the variability in sediment THg and MeHg. These wetlands were selected based out the outcomes of the study reported in Section 5. The lakes selected, and the target wetlands, are presented in Figure 7.1.

One hundred and twenty three samples were retrieved from two depths in the peat layers (0-5 cm, and 35-40 cm), representing the top and bottom depths of the experimental cores to be taken from these sites for subsequent experiments. Samples were acquired by cutting out sediment for more consolidated materials, or hand grabbing an integrated sample for less consolidated materials. Clean techniques used at all times (gloves; double bagged samples). Samples were placed in coolers, frozen at the end of each field day, and transported on dry ice back to the University of Toronto where they were kept frozen until analysed for both THg and MeHg within two months.

Analysis: THg and MeHg analyses were performed using standard protocols and are briefly described here. THg and MeHg analyses were performed in a Class 100 cleanroom at the University of Toronto by USEPA Methods 1630 and 1631. MeHg concentration was determined by aqueous phase ethylation (Bloom, 1989) and cold vapour atomic fluorescence spectroscopy (CVAFS) following distillation (Horvat et al., 1993; Olson et al., 1997). The MeHg in the soils was isolated from the sample matrix by atmospheric pressure water vapor distillation (Horvat et al., 1993). The sample distillate was ethylated with sodium tetraethylborate, buffered with sodium acetate, and purged with nitrogen onto glass traps filled with Tenax®. The Tenax® trap was then heated in a stream of argon, the mercury stream was speciated on a gas chromatography column, combusted to Hg0 using a pyrolytic column, and detected on a Tekran® 2500 by CVAFS.

THg concentration was determined using a Tekran® model 2600 CVAFS mercury detector with automated sampler. The day prior to analysis, 1 mL of BrCl was added to 40 mL of digestate. Analysis was by CVAFS with two-stage gold trap amalgamation and reduction by SnCl2 as described in USEPA Method 1631.

QA/QC: Quality assurance and control measures varied from day to day. Among analytical runs, Limits of Detection for THg varied between ~0.16 and 0.8 ng/g (dry wt) and between 8 and 45 pg/g (dry wt) for MeHg based on three times the standard deviation of the analytical blanks. Analytical runs were
rejected and samples re-analyzed if a) variation among replicates was greater than 10%, and/or b) analyses of THg standard reference material (IAEA-405) differed from certified values by ± 10%. Aqueous standards are used for calibration in both the THg and MeHg determinations, and check standards did not vary within a run by >2%. Samples were analysed as wet sediment, oven-dried, and a wet to dry weight conversion applied to the determined concentration. The final data are reported as Hg concentration in ng/g dry weight.

Figure 7.1. Right panels: maps of the study reservoirs/lake. Shaded polygon in circle is the boundary of the sampled area in the study wetland areas. Left images are of the study wetlands for illustrative purposes.
7.3. Results

The descriptive statistics for THg and MeHg in each of the wetlands reveals marked variability in the median concentrations and range (Table 7.1).

Median THg concentrations are highest in Thomson followed by Boulder and Alden; the latter two having a similar range despite different means and medians. In Thomson and Boulder, THg concentrations are higher in the deeper samples, while the opposite is true for Alden. This more extensive sampling effort has revealed the Hg contaminated buried sediments from a historical Hg-emitting industry in the Thomson Marsh (THg > 2500 ng/g d.w. in one sample).

Median MeHg concentrations are similar between the Thomson and Boulder wetlands, and similar between depths. High MeHg concentrations are found in both Thomson (35-40 cm) and Boulder (0-5 cm) (nearly 30 and 20 ng/g d.w., respectively). The MeHg concentration in Alden Lake wetland soils are nearly 5 times higher than at 35-40 cm.

Calculation of %MeHg reveals greater differences among the three wetlands, with median values being highest in Boulder, followed by Thomson, then Alden. Values of greater than 2% of THg as MeHg in the solid phase are indicative of a consistently methylating environment. Maximum values in of >15% in the Boulder Moss-Lichen wetland, and >14% in the Thomson Shrub-Scrub wetland are the highest values for wetland soil solid-phase %MeHg in the reported literature to our knowledge. For example, Heyes et al. (2000) report %MeHg in the range of 0.5 to 3% for peats from a bog wetland in northwestern Ontario.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Thomson 0-5 cm</th>
<th>Thomson 35-40 cm</th>
<th>Boulder 0-5 cm</th>
<th>Boulder 35-40 cm</th>
<th>Alden 0-5 cm</th>
<th>Alden 35-40 cm</th>
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</thead>
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<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<tr>
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Table 7.1. Descriptive statistics for THg and MeHg data from the three target wetlands.
Spatial Distribution of Total Mercury and Methylmercury:

ALDEN: THg concentrations in the Alden wetland soils are relatively evenly distributed across the small wetland, with higher concentrations near the surface than at depth (Figure 7.2). MeHg concentrations are relatively low in the surface soils, except for one sample location with a concentration of 8.6 ng/g d.w. In contrast, MeHg concentrations at 35-40 cm are extremely low, with most sampling points between 0.1 and 0.4 ng/g d.w.

Figure 7.2. ALDEN WETLAND: Distribution of wetland soil THg (top), MeHg (middle) and %MeHg (bottom) at 0-5 cm (left) and 35-40 cm (right). The bounding polygon is for reference to Figure 6.1 and is the extent of the sampling area, not the wetland boundary. The southwest and northeast edges of the polygon are the wetland-lake margin, and terrestrial edges to the west and east. A weakly connected surface water channel is present.
through the middle of the sampling polygon, flowing from northeast to southwest. Circles are size proportional to relative concentration, with numerical legend for each variable at right.

As described in Section 6, a useful measure is the ratio of MeHg to THg (%MeHg) as an indication of methylating ‘efficiency’. The pattern of %MeHg in the Alden Marsh reveals a wetland with a generally low propensity for MeHg production, particularly in deeper soils. The lone sample in the southeast with elevated MeHg concentrations stands out in an otherwise uniformly low MeHg environment.

BOULDER: THg concentrations in the Boulder Moss-Lichen wetland (Figure 7.3) are similar to those observed in other mid-continental moss-lichen wetlands (see Grigal, 2003; Branfireun et al., 2005). The map of the distribution of THg shows no particular pattern over the sampling area, but higher concentrations at 35-40 cm depth than at the surface.

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**Figure 7.3.** BOULDER WETLAND: Distribution of wetland soil THg (top), MeHg (middle) and %MeHg (bottom) at 0-5 cm (left) and 35-40 cm (right). The bounding polygon is for reference to Figure 6.1 and is the extent of the sampling area, not the wetland boundary. The east edge of the polygon are the wetland-lake margin; the wetland

extends beyond the bounds of the sampling polygon in all other directions. Circles are size proportional to relative concentration, with numerical legend for each variable at right.

Although lower THg concentrations at the top of the peat profile in these dominantly precipitation-fed wetlands could be interpreted as a reflection of the generally observed decrease in THg in precipitation in North America over the last 20 years, some uncertainties in using peat as an archive of atmospheric Hg deposition have been expressed in the literature (see Biester et al., 2007).

MeHg shows considerably more spatial organization that was observed in the Alden wetland. At 0-5 cm, there is a tendency for the highest MeHg concentrations to be found within a few meters of the wetland-lake margin. This pattern is not evident at 35-40 cm, with a somewhat more even distribution over the sampling polygon. The pattern of %MeHg reflects the differences in MeHg concentrations, particularly in the surface soils, where high ratios point to a zone of strong Hg methylation in surface peats at the wetland-lake margin.

THOMSON: THg concentrations in the surface peats of the Thomson wetland are generally <300 ng/g (d.w.) which are at the high end of the range of concentrations seen in wetlands not subject to point-source contamination (Figure 7.4). The highly elevated THg concentrations noted above are found in the deeper peats at the front of the wetland, and are clearly the result of the accretion of Hg contaminated sediments during the flooding of the reservoir and subsequent wetland expansion.

The Thomson wetland shows a pattern of MeHg higher concentrations toward the easternmost edge of the wetland-lake margin adjacent to the upland (Figure 7.4). This pattern is evident both in the surface and deeper peats. One localized region of highly elevated MeHg concentration appears in the surface, and in particular deeper peats in the eastern part of the marsh. Although difficult to interpret, strong thermal gradients in surface waters in this wetland suggest that localized zones of groundwater discharge exist, which have been associated with zones of enhanced Hg methylation (Branfireun et al., 1996).

In the Thomson wetland, the %MeHg largely mimics the spatial pattern of MeHg concentrations, with the marginal surface peats showing the highest ratios (Figure 7.4). It is important to note that the amount of MeHg and the %MeHg are not proportional to the amount of THg in the soils. Most of the sites sampled with highly Hg contaminated sediments in the Thomson wetland in fact have quite low MeHg concentrations.
Figure 7.4. THOMSON MARSH: Distribution of wetland soil THg (top), MeHg (middle) and %MeHg (bottom) at 0-5 cm (left) and 35-40 cm (right). The bounding polygon is for reference to Figure 6.1 and is the extent of the sampling area, not the wetland boundary. The southern edge of the polygon is the wetland-lake margin. Circles are size proportional to relative concentration, with numerical legend for each variable at right.

7.4. Sampling Guidance

The objective of this field reconnaissance was to determine the degree of spatial variability in wetland soil THg and MeHg in order to identify likely locations where numerous replicate cores could be taken for the laboratory experiment (Section 8). Based on the results of this sampling, several candidate locations were identified. In the Alden wetland, replicate core samples were to be taken in the more uniform interior away from the terrestrial-wetland interface at the west and east sides of the wetland, avoiding areas of elevated THg and sporadically higher MeHg concentrations (Figure 7.2). In the Boulder wetland, replicate cores were to be sampled at the mid-point of the wetland, and no less than 100 meters from the shoreline to avoid the region of higher MeHg concentrations (Figure 7.3).
Thomson Reservoir wetland, very elevated THg concentrations in the shallower wetland soils approaching the shoreline to the west, and elevated MeHg concentrations to the east were to be avoided by acquiring core samples in the central-west portion of the wetland, not less than 50 meters from the shoreline (Figure 7.4).

7.5. Scientific Discussion and Conclusions

In the three wetlands sampled, a spatial pattern of MeHg and THg emerges that is either related to the depositional history of the wetland, or biogeochemical controls. The anthropogenic Hg signal is evident in both the Thomson and Boulder wetlands, but with the Hg originating from different sources. In the Thomson wetland, highly elevated THg concentrations in deeper wetland soils closer to the wetland-lake margin are clear evidence of historical contamination from upstream anthropogenic discharges. The burial of this sediment, and the presence of large woody debris and human-derived materials in the peat profile well back from the wetland-lake margin indicates that these sediments are accreting and the wetland margin moving lakeward, at least for some period in the recent history of this wetland.

The vertical THg profile in the Boulder wetland is suggestive of decrease in the atmospheric loading of Hg from the atmosphere in the wetlands recent history, although lack of a peat accumulation rate and a more detailed profile precludes further elaboration. This pattern would only be expected to be observed in the large open peatlands, where the hydrology of the wetland decouples the surface peat from the influence of surface flows, groundwater and lake water, resulting in precipitation being the only source of water, and hence Hg.

Zones of high MeHg concentrations, on the other hand, are not a reflection of external inputs, but of internal conditions that are conducive to sulfate reduction and Hg methylation. By absolute measure, the Thomson and Boulder wetlands have the greatest, and similar, total burdens of MeHg assuming relatively constant soil properties among the wetlands, because of the preservation of relatively high MeHg concentrations at depth. The burden of MeHg in the Thomson wetland is dominated by a relatively localized zone of high MeHg sediments, likely governed by local hydrological conditions such as localized groundwater discharge (see Branfireun et al., 1996). The pattern of MeHg concentrations in the Boulder wetland suggest a zone of high MeHg sediments in the surface peats at the wetland-lake margin. The development of a steep redox gradient between the typically highly-reducing sphagnum peat soils and the oxygenated and relatively nutrient rich lake water is an ideal Hg methylating environment. This zone is also subjected to the most dramatic dewatering on an annual basis, promoting air entry, enhanced decomposition, and sulfate reoxidation. Upon rewetting, the availability of carbon and sulfate in a soil environment that will rapidly move toward a reestablishment of deeply anoxic conditions because of low nutrient availability would lead to enhanced Hg methylation. This interface effect has been observed elsewhere (e.g. Krabbenhoft et al., 2007; METAALICUS watershed study; Krabbenhoft, Branfireun, Heyes, unpublished data).

The wetland at Alden Lake is, with the exception of one point, very low in MeHg despite THg concentrations in the range of the other wetlands. Methylation is moderated in this system by a difference in inorganic Hg bioavailability, nutrient limitation or an unsatisfactory biogeochemical environment for sulfate reduction (i.e. other nutrients are sufficiently abundant as to maintain relatively high redox values that preclude sulfate reduction or methanogenesis).
8. The Effect of Water Level Fluctuation on Methylmercury Production in Wetland Soils of the St. Louis River Project, Minnesota

8.1. Introduction and Rationale

In the reservoirs of the St. Louis River system, ongoing monitoring studies and previous research has found that fish Hg burden varies among the reservoirs and natural lakes, but that fish tissue Hg did not vary consistently with other physical factors. It has been hypothesized that the reservoir water level fluctuations were affecting the actual production of methylmercury (MeHg) in the surrounding wetlands, and/or the fluctuation was contributing to enhanced mobilization of MeHg from the wetland sediments. Based on previous work (see Sections 6 and 7 of this Report), wetlands located adjacent to a peaking reservoir with high-frequency water level fluctuation (Thomson Reservoir), a representative headwater reservoir (Boulder Lake), and, a natural unregulated flowage lake (Alden Lake) were selected for intensive study to examine these processes.

There are considerable challenges in attempting to isolate the biophysical controls on Hg speciation in natural soils, sediments and waters. The interconversion of bioavailable inorganic Hg to MeHg is recognized as a dominantly microbial process, but the processes of mercury methylation and demethylation are still not well characterized, either genetically, or metabolically. The amount of MeHg present in a system is of course a consequence of the net outcome of methylation and demethylation, but little can be inferred about the rates or controls on these processes by the absolute burden of MeHg in the environmental compartment. Certainly, the solid phase pool to which MeHg has been partitioned can be considered a more stable reflection of the dominant biogeochemical milieu with respect to Hg speciation, in contrast with the aqueous phase, which may be expected to be more dynamic.

One approach to interpreting the methylating ‘efficiency’ of a particular sediment, soil or water is to calculate the simple ratio of MeHg to THg, with higher ratios indicating that a greater proportion of the total amount of Hg in the media has been converted to MeHg, hence a more favourable environment for methylation. More recently, advances in stable isotope techniques permit the direct measurement of the interconversion of specific, enriched isotopes of Hg added to an environmental sample over time. This measure, often denoted ‘mercury methylation potential’ is an accepted approach, but with limited application because of the limited availability of stable mercury isotopes, and the required dedicated instrumentation to measure them. Measuring changes in mercury methylation potential using stable Hg isotope tools in an experimental context offers a powerful method to directly evaluate changes in the methylating environment as a consequence of experimental manipulation.

Given that mercury methylation is a dominantly biotic process, coupling changes in the structure and function of soil microbial communities to changes in mercury methylation can be a conclusive means by which to explain the effect of water level fluctuation. Determining the community structure and metabolic status of a microbial assemblage in environmental samples or microcosm studies remains a difficult challenge. Unlike many eukaryotic organisms, bacterial morphology provides little to no information concerning the ecological role of the organisms. Therefore direct observation via microscopy is of little utility when attempting to study biogeochemical processes. Likewise, the use of culturing and enrichment cultures, while useful in some situations, is hampered by evidence that nearly 95%
of viable microorganisms are unculturable in the laboratory. Modern molecular approaches that are now being applied to environmental samples can produce detailed descriptions of the species and sometimes strains present within a microbial community. Genetic tools present exciting new opportunities. 16s rRNA gene cloning and quantitative RT-PCR for analysis of the microbial community can theoretically be used to couple specific community analysis to biogeochemical processes, however unless specific knowledge is in hand regarding the genetic fingerprint of important microbial communities and specific strains, the problem can quickly become intractable.

Phospholipid fatty acid (PLFA) analysis is another approach which also quantifies microbial community structure without a reliance on culturing techniques. Specific advantages of this technique include: 1) beyond community structure the technique provides total microbial biomass from the same sample; 2) results integrate the entire sample without bias from PCR and/or primers and colony picking; 3) techniques can directly be applied without bias to soils, and sediments; 4) relative to the visual and molecular techniques this biochemical method is time and cost effective; 5) results are precise and reflect only the viable populations in the samples.

Returning to the original problem of whether or not water level fluctuation exerts a direct effect on MeHg production, we propose the following conceptual model:

Methylating bacterial communities require a supply of a) bioavailable inorganic Hg; b) nutrients (e.g. sulfate), and; c) labile substrate. A positive relationship has been identified between water level fluctuations and methylmercury concentrations in the environment (e.g. Sorensen et al., 2005), and the highest levels of MeHg in wetland soils and porewaters tend to be in the zone of water table fluctuation (see Branfireun et al., 1996; Branfireun and Roulet, 2002). The periodic oxidation of wetland soils will provide for the enhanced release of inorganic Hg, sulfate and DOC into pore waters, promoting MeHg production upon rewetting. Given that microbial communities can respond very quickly to changes in redox conditions (see Mitchell and Branfireun, 2007), we hypothesize that MeHg production is positively related to the frequency of water level fluctuation. This is a hypothesis that is to be tested; it is not a statement of fact or opinion.

We suggest that the combination of stable Hg isotope methods and microbiological community analysis methods in an experimental framework provides the strongest combination of approaches to isolate the impact of the differing physical conditions on MeHg production, the biogeochemical process of interest.

8.2. Methods

Given that hydrology, vegetation and soil properties varied widely among the different wetland types found in association with the lakes and reservoirs of the area, isolating the specific effect of water level fluctuation on MeHg production and mobilization would not be possible in a field context. Therefore, the objective of this study was to experimentally determine if water level fluctuation alone (as driven by reservoir operation or natural hydrologic variability) affects the production of MeHg in the soils of wetlands surrounding SLRP reservoirs.

FIELD METHODS: For this experiment, approximately 50 intact wetland soil cores (5 cm dia. X 40 cm long) were acquired from Thomson Reservoir Shrub-Scrub wetland, Boulder Lake Moss-Lichen...
wetland, and Alden Lake Emergent wetland (see Sections 5 and 6). A relatively homogeneous area of each wetland was selected based on the information derived from the work described in Section 6. Cores were extracted with a custom piston corer that inserted a polycarbonate liner into the sediment, allowing the sample to be fully sealed in the tube that would ultimately form the experimental apparatus in the laboratory. Maintaining the core in this tube from the time of sampling until the end of the experiment was important, as it was critical to not contaminate the sediment core with bacteria that would affect the microbial analyses. Sediment compression in the cores was <10% by volume. Core samples were fully saturated with pore water, capped tightly at both ends and placed upright in a dark cooler. Once acquired, cores were kept chilled, and transported promptly back to the University of Toronto. Cores were stored in the dark in an environmental chamber at 4°C until the beginning of the experiment (approximately 3 months after sampling). At the same time that the cores were sampled, approximately 120 L of Boulder Lake Reservoir water was filtered in the field, and transported back to the University of Toronto. This water would be used as the standard water to be circulated through all of the experimental cores, and started with uniform THg and MeHg concentrations of 2.52 and 0.08 ng/L respectively.

LABORATORY EXPERIMENTAL DESIGN: An experiment was designed to subject cores from each of the three wetlands to three different water level fluctuation regimes. Four replicate cores from each of the three sites (12 cores total) was subjected to three treatments: a static water level with a slow flushing of reservoir water for three months; a monthly treatment with a slow flushing of reservoir water for one month, a complete draining for one month, then a return to flushing for one month; and a daily cycle of complete wetting and draining, run continuously for three months. Water delivery for treatment was governed by a digital 12-channel precision peristaltic pump (Carter®) controlled by a Campbell Scientific® CR-10x datalogger. Water was supplied by a separate 25L carboy of filtered Boulder Lake water (see above), pumped to the bottom of each core tube, with overflow collected and returned to the carboy. Water overflow from the cores had to be returned to the carboy, which were periodically replenished with stored lake water, because of practical limitations of transporting 100s of L of lake water to the laboratory. The water supply carboys were re-filtered (0.45um) weekly. It was decided that, for the experimental cores which were to be maintained in a fully-wetted state (Static water level treatment, and Monthly treatment during the wetted segments of the experiment), water would be pumped through the cores in order to maintain biological activity through nutrient supply. A wetted core with no water exchange would quickly become fully depleted in nutrients and go permanently deeply anoxic until the carbon substrate was exhausted; a condition not likely observed in the field. Since the experiment was concerned with water level, not degree of stagnation, the pumps were set to deliver the lowest flow rate possible, which corresponds to 130 ml per day, very close to the mean drainable pore water volume for the soil cores (determined as part of the experimental work in Section 8). The entire experiment was conducted in an environmental chamber at 20°C. One core from each wetland in each treatment was continuously monitored for redox using Eh microelectrode arrays in order to compare core conditions to measured field conditions.

CORE SUBSAMPLING METHODS: A full set of cores (4 x 3 wetlands) were destructively sampled at the outset of the experiment by extruding the soil from the core under nitrogen, slicing the core into thirds by length, homogenizing and subsampling for THg and MeHg analyses, Hg isotope addition for methylation potential, and microbial community analyses. All samples except for the Hg isotope addition samples were frozen immediately and remained so until analyses were performed (described be-
low). The same procedure was followed for all of the experimental cores at the end of the three month experiment.

**ISOTOPE ADDITIONS:** In order to quantify the conversion of inorganic 200Hg to 200MeHg as a measure of methylation potential, 1.0 mL of high purity (99%) inorganic 200Hg isotope was added to a 5.0 g subsample of soil from each slice of each core. The amount of isotope added was a function of the concentration of ambient THg in the initial sample. With mean ambient soil THg concentrations for Thomson, Boulder and Alden of 0.049, 0.010 and 0.024 ug/g, respectively, a stock solution of 0.13 ug/mL 200Hg was diluted to three different concentrations for each of the three wetland soil, ultimately adding 0.060, 0.012 and 0.028 ug of 200Hg in 1.0 mL of water to the 5 g of homogenized sediment. These spiked samples were incubated for 12 hours, and frozen immediately until analyzed for 200THg and 200MeHg by the USGS Mercury Laboratory in Middleton Wisconsin (Krabbenhoft).

**MERCURY ANALYTICAL METHODS:** Samples were analysed for both the ambient Hg species and the added isotopic 200Hg. Peat soil and vegetation samples were digested at 80 °C using a 7:3 (vol/vol) mixture of concentrated HNO3 and H2SO4. After BrCl oxidation, THg content in the digests was determined by SnCl2 reduction, gold trap preconcentration, and detection using flow injection cold vapor ICP-MS (Perkin Elmer Elan 6100). For THg analyses of filtered pore waters, samples were subjected to a BrCl oxidation, stannous chloride reduction and purging with Hg-free nitrogen onto gold traps, which were processed as described elsewhere (Hintelmann et al., 1995, 1997; Olson and DeWild, 1999). MeHg in water, soils, and vegetation was isolated from the sample matrix by atmospheric pressure water vapor distillation (Horvat et al., 1993). MeHg in the distillates was derivatized using sodium tetraethylborate and preconcentrated onto Carbo® traps. Quantification was achieved after thermodesorption, GC separation, and detection by ICP/MS (Hintelmann et al., 1995, 1997). Typical limits of detection (LOD) for THg were 0.04 ng/L in water and 0.2-1 ng/g in vegetation and soils, and for MeHg they were 1 pg/L in water and 1-10 pg/g in soils. LOD for the 200Hg isotope in the various samples are dependent on the concentration of native mercury in the respective sample. To precisely quantify the applied isotope concentration, it must be ≥0.5-1% in excess of the native mercury concentration. The exact LOD varied with the precision of the isotope ratio measurement that was achieved during each run.

**MICROBIAL COMMUNITY ANALYSIS:** Initially we proposed and indeed used a combination of 16s rRNA gene cloning and quantitative RT-PCR to examine the mercury resistance (mer) operon, which encodes bacterial resistance to MeHg and involves the sequential action of two enzymes, organomercurial lyase (MerB) and mercuric reductase (MerA; see Schaefer et al., 2004) and/or sulfate-reducing genes for analysis of the microbial community and the mercury methylation activity. These methods were abandoned when initial community analysis reveal significant diversity of the SRB population which presented issues (time and significant costs beyond the scope of this project) with the development of specific primers for gene expression assays.

Phospholipid fatty acids are an important component of all cellular membranes to maintain fluidity and to enable transport of nutrients into the cell, and to eliminate metabolic byproducts. Analysis of these lipids are based the extraction and separation of the different lipid classes from the sample, followed by quantitative analysis via gas chromatography and/or mass spectrometry (GC or GC-MS). Each class of lipids can be utilized as a signature lipid biomarker (SLB) with phospholipids (PLFA) pro-
viding a mechanism to quantify viable microbial biomass, community composition, and nutritional status (White et al., 1998).

Lipids were analyzed at the Microbial Geochemistry Laboratory at the University of Kansas (Fowle) using the modified Bligh and Dyer method (Smith et al., 1986). Extractions were performed using a one-phase chloroform-methanol buffer extractant. Lipids were recovered, dissolved in chloroform and fractioned on disposable silicic acid columns into neutral-, glyco-, and polar lipid fractions. The polar lipid fraction was transesterified in with mild alkali to recover the PLFA as methyl ester in hexane. PLFA were analyzed by gas chromatography with peak conformation performed on GC/MS.

PLFA QA/QC: Samples for PLFA were prepared and analyzed according to the standardized operation protocol utilized by the USEPA. Negative controls were used to monitor for laboratory contamination. No QC issues were detected during the course of the analysis. All laboratory equipment and instruments utilized were calibrated and operating within acceptable ranges. Solvents were validated for purity.

FIELD INSTRUMENTATION: In order to assess if the physical conditions in the experimental cores captured the hydrological and biogeochemical variability observed in the field, measurements of water table fluctuation, and soil temperature and redox potential (at -10cm and -40cm relative to the ground surface) were made every 30 minutes over the ice free season of 2005. Logged by a Campbell Scientific CR-10x datalogger, water levels were measured using a Keller® pressure transducer in a 2” ID fully penetrating well. Soil temperature and redox measurements were made using custom fabricated electrodes with embedded high precision thermistors and a pure platinum wire electrode coupled to an Orion® Ag/AgCl Reference electrode.

STATISTICAL METHODS: To test whether or not an experimental treatment resulted in a change in a chemical or biological variable, all experimental treatments were compared to the initial measurements made at the beginning of the experiment. A simple one-way Analysis of Variance was conducted, using the Tukey-Kramer multiple comparison of means with a significance ascribed at 95%. All descriptive statistics and tests for significance were done using Aabel 2.4.2 for Macintosh (Gigawiz, Inc.).

8.3. Results

COMPARISON OF EXPERIMENTAL CONDITIONS WITH FIELD DATA

Field data from the instrumented sites in the Thomson, Boulder and Alden wetlands show anticipated different patterns of water table fluctuation, redox potential, and temperature (Table 8.1; Figures 8.1-3). Average soil temperatures over the entire study period were nearly identical among the wetlands at both depths. An instrument failure about one third of the way into the season at the Alden site prevents the calculation of a seasonal average, but the early season temperatures track those of the other two sites. On average, redox potential indicates a generally aerated environment in the surface soils of the Thomson wetland, but in the range of iron-reducing conditions at -40 cm (Table 8.1).
Table 8.1. Mean soil temperature and redox potential at two depths in the three study wetlands.

The Boulder wetland shows on average more reducing conditions at the surface than at depth, but with both values again in the range of iron-reducing conditions (100 to -100 mV). Only the deeper redox probe in the Alden wetland recorded an average condition that was clearly in the range of sulfate reduction (-100 to -200 mV).

These mean values do not reveal the significant variability in these values over time. The Thomson Shrub-Scrub wetland exhibited a water level fluctuation of close to 30 cm on a regular basis in the earlier part of the season, while in the late summer and fall showed a consistent downward trend, likely coupled to a drier period and water level drawdown in the reservoir (Figure 8.1). In general, these water level fluctuations did not affect soil redox potentials, even in the near surface soils, suggesting that the water level fluctuations were not sufficiently frequent, nor prolonged, to significantly disrupt the biogeochemical conditions in the soils. Only during the period of drawdown later in the season did the water table fall below the elevation of the -10 cm electrode for a sufficient period to permit air entry and the establishment of fully oxic conditions (> +300 mV) (Figure 8.1)
The Boulder Moss-Lichen wetland exhibits quite a different pattern, driven by the different operation of the headwater reservoir (Figure 8.2). The water table position shows a seasonal rise and fall driven by changes in storage in the reservoir. The water table data are misleading since at no time was the...
Boulder wetland flooded to a depth of 40 cm. With the water table well anchored several meters into the deep peat, we physically observed that the buoyant surface peats had risen in elevation roughly this amount in response to the increasing water levels in the adjacent reservoir, effectively keeping the water table at the ground surface. Subsequently, the peat surface subsided again as the water levels fell later in the season.

This dynamic ground surface elevation in turn maintained a nearly constant water table position, allowing for a continuous decrease in redox potential over the season as nutrients were depleted (Figure 8.2). It is unclear why the -40 cm Eh is higher than the -10 cm, but significant heterogeneity in sphagnum peat structures would reduce further interpretation of this data to pure conjecture.

The Alden Emergent wetland water table exhibits a damped seasonality, marked by a modest summer drawdown, punctuated by episodic increases in water table elevation driven by rainfall events (Figure 8.3). The redox potential in contrast, shows the strongest variability of all of the wetlands, ranging from deeply reducing (<-200 mV) to fully oxic (~300mV). The relatively wet early season maintains a stable and low redox potential at both soil depths. Drawdown of the water table around day 240 allows air entry first at 10 cm, followed by -40 cm as the water table continued to depress. The restoration of the water table to closer to the ground surface around Day 260 resulted in the rapid restoration of deeply reducing conditions at -40 cm, with a slower recovery of the soil at -10 cm, due to the more prolonged period of aeration.

In the laboratory, temperature in the environmental chamber was set at a constant 20˚C for the entire experiment. This temperature is effectively identical to the mean seasonal surface soil temperature at the field sites, but about 25% warmer than the deeper soil temperatures. Redox potential measured at 2 cm intervals in one of each of the wetland soil types in each treatment (9 cores total) were highly variable, reflecting the inherent micro-heterogeneity of the intact sediments. However, in the middle and lower sections of all of the cores that were wetted, reducing conditions were established that were very much in line with those observed in the field. For example, data from a core of Thomson wetland soil subjected to a daily water level fluctuation shows stable and highly reducing conditions at depth (Figure 8.4; compare to Eh at -40 cm in Figure 8.1). The automated water flows in the core set up a very strongly oscillating redox potential in the middle of the core, varying between fully aerated and strongly reducing over a two day drying and rewetting cycle. This strong pattern was not observed in the field data because, as it turns out, the water level fluctuations in the Thomson reservoir did not occur to this degree with this frequency. The surface redox potentials in the cores clearly responded to the drying and rewetting, but were fully oxic for the duration of the experiment (e.g. Figure 8.4). The Eh data for the monthly treatments reflected the 30 day wetted/30 day drained/30 day wetted sequence, with fully oxidized conditions occurring under air entry conditions under drained conditions in the intact cores from all of the wetlands. During the wetted periods, the monthly treatments were similar to the static treatments, with a general tendency toward more stable reducing conditions throughout the cores at all depths ranging between ~50 mV and -225 mV. During the 30 day drained period in the monthly treatment, the surface soils were the fastest to establish fully oxidized conditions, while the deeper soils gradually became oxic over the 30 day period. The monthly treatment soils were relatively slow to respond upon rewetting with a gradual decrease in Eh to minima of only between +175 and +50 mV, never returning to the more deeply reducing pre-drained levels.
Figure 8.2. BOULDER MOSS-LICHEN WETLAND: Water table position relative to the ground surface (top panel), and in situ redox potential at two soil depths over the ice-free study period, 2005.
Figure 8.3. ALDEN EMERGENT WETLAND: Water table position relative to the ground surface (top panel), and in situ redox potential at two soil depths over the ice-free study period, 2005.

WATER LEVEL FLUCTUATION EXPERIMENT RESULTS

It was decided before further analyses were undertaken to see if there were statistically significant differences among the three core slice depths (top, middle, and bottom) in the key variables being considered here. As there were none, the value from each slice is treated as an additional data point for each core. Therefore, for each site and for each treatment, there are 4 replicate cores and 3 samples from each core, for a total of 12 values. This lumping of slices for each site and treatment improves statistical robustness by increasing sample size.

TOTAL MERCURY: We would expect that there would be marked differences in the total mercury concentrations in the sediments of the different field locations and at different depths, but that THg would not be different for cores from the same location across treatments, given that the cores were randomly sampled in the field and randomly assigned to an experimental treatment. We also would not expect the experiment to alter the bulk THg concentration of the sediments in the cores. The similarity of THg across treatments is, in fact, essential to the acceptability of the experimental outcomes. The following figures demonstrate clearly that there is variability for each location, but that replicates within and across treatments do not differ with any statistical significance in THg concentration (Figure 8.5). The high ambient THg concentrations in the Thomson wetland cores are a consequence of legacy Hg
contamination from an upstream source of Hg contamination. The concentrations in the Boulder and Alden wetlands are similar to those observed in other peat dominated systems and in previous measurements made as part of this project.

Figure 8.5. AMBIENT TOTAL MERCURY: Bars are the mean for each treatment. Error bars are one Standard Error about the mean. An asterisk (*) above the bar indicates that the mean of the experimental treatment is statistically significantly different from the Initial mean value at the 95% confidence interval.

METHYLMERCURY: Like THg, MeHg concentrations in the core soils are different among the wetland sites (Figure 8.6). This observation, and the range of concentrations, are consistent with those reported earlier in this document.

From the experimental perspective, we importantly observe that unlike THg there are significant differences in ambient MeHg concentration in some of the core soils for some of the different treatments (Figure 8.6). The Thomson wetland soils subjected to the monthly water level fluctuation had statistically significantly higher ambient MeHg concentrations than they did initially (p = 0.015). For the Boulder wetland, the statistically significant difference is for the soils subjected to the static water level treatment (p = 0.031) (Figure 8.6).
PERCENT METHYLMERCUARY: As an expression of the ‘efficiency’ of each system to convert ambient inorganic Hg to MeHg, we can also examine the fraction of THg that is MeHg (MeHg/THg) to consider effects of the different water level treatments (Figure 8.7). These data show the same statistically significant differences as ambient MeHg, with the addition of the monthly treatment for the Alden wetland soil core. (Thomson Monthly p = 0.001; Boulder Static p = 0.020; Alden Monthly p = 0.015). In soils from two of the three wetlands, the extended wetting/drying/rewetting treatment has resulted in a significant increase in the fraction of THg that is MeHg.

As with other work both as part of this project and others, there is no relationship between Ambient THg and MeHg in these soil cores (data not shown).
Figure 8.7. PROPORTION OF TOTAL MERCURY AS METHYLMERCURY: Bars are the mean for each treatment. Error bars are one Standard Error about the mean. An asterisk (*) above the bar indicates that the mean of the experimental treatment is statistically significantly different from the Initial mean value at the 95% confidence interval.

ISOTOPIC MERCURY AND METHYLATION POTENTIAL ASSAYS: High purity inorganic 200Hg was used at the beginning and at the end of the experiment to assess the potential conversion rate of inorganic Hg to MeHg by measuring the amount of 200MeHg produced over a 12 hour incubation. The data are presented as a % conversion of inorganic Hg to MeHg per day (Figure 8.8).

Firstly, it is clear that, using this method of assessing potential MeHg production rates, the soils from the Alden wetland has the greatest initial potential to convert inorganic Hg to MeHg. This is quite contrary to the ambient MeHg data, however our interpretation of this result is that the Alden Marsh supports the requisite biological and physical conditions for methylation, but that the sediments may be inorganic mercury limited. When the additional small amount of highly available spike mercury was added, it was readily converted to MeHg relative to the other sites. With a similar contrast, the Boulder wetland that has the highest ambient %MeHg initially, has the lowest 200MeHg production rates measured initially.

In all experimental treatments for all sites, the MeHg production rate was statistically significantly lower at the end of the experiment than it was initially (all treatments p <0.001).
We may conclude that the instantaneous measure of MeHg production is a reflection of only what that state in the core is at the time of measurement, while the bulk sediment ambient MeHg concentrations are a better reflection of the longer term MeHg production in the sediment. The lower MeHg production rates at the end of the experiment after 3 months suggest that the experimental cores became either limited in the important nutrients or substrates required for microbial metabolism that is responsible for methylation (e.g. sulfate, labile carbon), or the microbial communities responsible for methylation were either reduced in size or were otherwise compromised. From the redox data presented in previous reports, it would appear as though the general biogeochemical conditions in the cores mimic those of the field.

MICROBIAL COMMUNITY ANALYSES: PLFA nomenclature follows the pattern of A:BωC. The “A” position identifies the total number of carbon atoms in the Fatty Acid. Position B is the number of double bonds from the aliphatic (ω) end of the molecule. Position C designates the carbon atom from the aliphatic end before the double bond. This is followed by a “c” for cis or a “t” for trans configuration. The prefixes “i” and “a” stand for iso and anteiso branching. Midchain branching is noted by “me” and cyclopropyl fatty acids are designated as “cy”. Example 18:1ω7c is 18 carbons long with one double bond occurring at the 7th carbon atom from the ω end.
BIOMASS: Phospholipid fatty acids (PLFA) are found within the membranes of all living cells but decompose quickly upon cell death. Thus, measuring PLFA content provides a quantitative measure of microbial biomass (White et al., 1979; Balkwill et al., 1988). There is an overall trend evident, that with increasing frequency of water level fluctuation, the total live cell biomass decreased (Figure 8.9). Significant differences are only found for the Thomson and Boulder daily treatments ($p = 0.002$ and $0.046$ respectively).

This PLFA biomass data represents a change from a mean of approximately $1 \times 10^7$ cells/g dry weight in the initial treatments to as low as $2 \times 10^6$ cells/g dry weight in the daily treatments. Importantly, although total biomass was lower, the experimental cores maintained viable, live bacterial communities after 3 months of experimental treatment.

COMMUNITY STRUCTURE: In order to relate the complex mixture of PLFA to the bacterial community, structural group interpretation is employed. In certain instances, this association is so strong that fatty acid biomarkers have been identified for individual organisms (sulfate reducing bacteria are a good example of this). Table 8.2 describes the six major structural groups employed.

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**Figure 8.9. TOTAL MICROBIAL BIOMASS:** Bars are the mean for each treatment. Error bars are one Standard Error about the mean. An asterisk (*) above the bar indicates that the mean of the experimental treatment is statistically significantly different from the Initial mean value at the 95% confidence interval.
Table 8.2. PLFA Structure Groups

<table>
<thead>
<tr>
<th>PLFA Structure Group</th>
<th>General Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monoenoics (Monos)</td>
<td>Found in Gram negative bacteria, fast growing, use many carbon substrates, adaptable</td>
</tr>
<tr>
<td>Terminally Branched Saturated (TerBrSats)</td>
<td>Gram positive bacteria, some SRB cell membranes may have these lipids</td>
</tr>
<tr>
<td>Branched Monoenoic (BrMonos)</td>
<td>Generally obligate anaerobes such as SRB</td>
</tr>
<tr>
<td>Mid-chain Branched Saturated (MidBrSats)</td>
<td>Actinomycete spp., SRB, and some Gram positives</td>
</tr>
<tr>
<td>Normal Saturated (Nsats)</td>
<td>Both prokaryotic and eukaryotic kingdoms</td>
</tr>
</tbody>
</table>

Community structure of the various treatments is shown in Figure 8.10. This comparison plots indicate that although the overall biomass is different between sites and treatments the community structure is fairly similar. The concentrations of Nsats or General Fatty Acids are in proportion with other biomarkers which is indicative of a fairly diverse community and therefore strong evidence that the soil cores are a good analogue for their natural counterparts. In all cases daily treatments appears to decrease the relative proportions of dissimilatory iron reducing bacteria, and also SRB for the daily treatments of Boulder and Thomson.

![Figure 8.10. MICROBIAL COMMUNITY STRUCTURE.](image-url)
SULFATE-REDUCING BACTERIA: Figure 8.11. depicts the amount of specific biomarkers for SRB in umol/g. The biomarkers used were for the genus Desulfovibrio (i17:1ω7c) and Desulfobacter (10me16:0). The marker for Desulfbulbus (17:1ω6c) could not positively be identified. It would appear that sediments from the Thomson Scrub-Shrub wetland had the highest quantity of SRB biomarkers initially, and showed statistically significant decreases for both the static (p=0.020) and daily treatments (p<0.001). Neither the Boulder Moss-Lichen wetland, nor the Alden Emergent wetland showed statistically significant change in SRB biomarkers for any treatments. Generally, SRB biomarkers follow the overall trends of the total biomass.

![Figure 8.11. TOTAL SULFATE REDUCING BACTERIAL BIOMARKERS: Bars are the mean for each treatment. Error bars are one Standard Error about the mean. An asterisk (*) above the bar indicates that the mean of the experimental treatment is statistically significantly different from the Initial mean value at the 95% confidence interval.](image)

IRON-REDUCING BACTERIA: Figure 8.12 shows the concentrations of iron reducing biomarkers as a function of treatment (13me15:0, 10me17:0, 18:2ω6c, 18:1ω9c & 18:3ω3c). There are more specific biomarkers for iron reducing organisms than SRB and thus we will not compare overall of abundances of dissimilatory iron reducing bacteria (DIRB) to SRB. Although the overall pattern of changes in the DIRB populations over the experimental treatments are similar to the SRBs (Figure 8.11), much higher variability about the means result in no statistically significant differences between initial conditions and experimental treatments despite the appearance of the data in Figure 8.12. Visually, it appears that there is a substantial decrease in total DIRB in the Daily Treatment for Thomson, and a substantial decrease in all treatments for Boulder. DIRB abundance is low initially, and for all treatments in Alden.
**Figure 8.12. TOTAL DISSIMILATORY IRON REDUCING BACTERIAL BIOMARKERS:** Bars are the mean for each treatment. Error bars are one Standard Error about the mean. An asterisk (*) above the bar indicates that the mean of the experimental treatment is statistically significantly different from the Initial mean value at the 95% confidence interval.

**Metabolic Activity:** The data presented in Figure 8.13 was generated by taking the ratio of cyclopropyl fatty acids to monoenoic fatty acids. In gram negative bacteria the monoenoics (16:1ω7c & 18:1ω7c) are converted to cyclopropyls (cy17:0 & cy19:0) as microbes move from log to stationary phase of growth (e.g. slowing of growth). These ratios can vary, depending upon environment and organism, but usually the range is 0.1 (log) to 5.0 (stationary). A lower ratio infers a higher turnover rate. (ratios cy17:0/16:1ω7c and cy19:0/18:1ω7c)

This graph suggests that not only did the total biomass decrease in the daily treatments, but so did the growth rate (Figure 8.13). The metabolic ratios for the daily treatments are statistically significantly higher for all of the wetland locations (Thomson p = 0.018; Boulder p = <0.001; Alden p <0.001). If carbon turnover rates correlate directly with mercury methylation rates then the daily treatments clearly would have the lowest MeHg generation per day based on this metabolic indicator. This conclusion is bolstered by the specific carbon requirements of SRB. If the fermenting bacterial community is actively growing (e.g. high carbon turnover) and producing short-chained organic molecules for consumption by the SRB community then we would expect significant sulfate reduction and in turn mercury methylation. Plotting metabolic activity against 200MeHg production supports this contention, with elevated rates of 200MeHg production only occurring during the growth phase (low metabolic ratio) (Figure 8.14).
Figure 8.13. METABOLIC RATIO: Bars are the mean for each treatment. Error bars are one Standard Error about the mean. An asterisk (*) above the bar indicates that the mean of the experimental treatment is statistically significantly different from the Initial mean value at the 95% confidence interval.

Figure 8.14. POTENTIAL METHYLMERCURY PRODUCTION RATES VS METABOLIC RATIO.

8.4. DISCUSSION AND CONCLUSIONS

From the basic field data collected at the three target wetlands, it is apparent that all of the wetlands exhibit hydrological and biogeochemical conditions conducive to Hg methylation. That is, they all exhibit periods of persistent saturation, allowing redox potentials to descend into reducing conditions that are in the range of sulfate reduction - a prerequisite for Hg methylation in freshwater systems. Although the results of Sections 5 and 6 reveal apparent differences in MeHg concentrations and production (through the calculation of %MeHg) among the three wetlands, there was no way to mechanistically decouple the potential influence of the different water level fluctuation regimes from the dramatically different wetland types that existed in the three watersheds on their propensity to convert inorganic Hg to MeHg.

In this section, we presented the results of an ambitious experiment that involved returning over four dozen 5 x 40 cm intact soil cores from the field to the laboratory, and subjecting them to a range of water level fluctuations to attempt to isolate the effect of this physical variable on MeHg production. Three main measures emerge as key points upon which to focus when evaluating the overall influence of the different water level fluctuation treatments and interpreting the outcomes of the experiment: changes in the abundance of ambient MeHg in the wetland soils; changes in the proportion of ambient THg that is MeHg; and the relationship between the microbial metabolic ratio as a measure of substrate limitation, and the fraction of 200-Hg converted to MeHg per day.

The monthly water level fluctuation treatment resulted in significantly higher ambient MeHg concentrations in the Thomson wetland soils, and when normalized as the ratio of MeHg to THg, the same treatment also resulted in a significantly higher ratio for both the Thomson and Alden wetlands. The Boulder Reservoir showed the highest ambient MeHg concentrations and %MeHg under static water level conditions. The monthly and static treatments did not have statistically different microbial biomass measures, or metabolic ratios from the initial condition (Figures 8.9 and 8.13 respectively), indicating the preservation of an abundant, viable and growing microbial community with a relatively higher turnover rate. Community structure analyses also show that the monthly and static treatments maintain a consortia that reflects the initial condition (Figure 8.10).

In contrast, high frequency (e.g. daily) water level fluctuation results in statistically significantly lower bacterial biomass (Figure 8.9) and lower metabolic activity (Figure 8.13). Mean ambient MeHg concentrations and mean %MeHg for the daily treatments were the same or higher than initial values, however they were not statistically significantly different. Although only statistically significant for the Thomson Reservoir wetland, there is clearly the greatest decrease in the abundance of the most well-known group of methylators, the SRB (Figures 8.10, 8.11) under the higher-frequency daily fluctuation. Notably, there is also the greatest (but non-statistically significant) decrease in the abundance of DIRB (Figures 8.11, 8.12), a group of bacteria that are recognized as capable of methylating Hg (see Kerin et al., 2006).

The use of stable isotopes of Hg to assess methylmercury production potential has produced mixed results using this experimental design. At the outset of the experiment, the stable Hg isotope incubation reveals that the Alden Lake wetland, which has among the lowest MeHg concentrations measured over the entire study, shows the greatest methylation potential. This finding clearly suggests that the low MeHg concentrations at this site are not because of the inability of the microbial community to
methylate Hg, but because the biogeochemical conditions in situ are not conducive for sulfate-reduction (insufficient sulfate or labile carbon substrate), and/or the inorganic Hg at this site is not particularly bioavailable. These data are also an excellent illustration of why single measures of either ambient MeHg, or experimental assays, may not fully reveal the Hg dynamics of a particular environment. At the end of the experiment, it is clear that the ability of the soils from all three wetlands to convert inorganic Hg to MeHg is much lower than under initial conditions under all experimental treatments. As these measurements were only made at the beginning and end of the experiment, it is impossible to know what the nature of this shift was.

In light of these results, we must reject our opening conceptual model and hypothesis and propose an modified conceptual model:

Methylating bacterial communities require a supply of a) bioavailable inorganic Hg; b) nutrients (e.g. sulfate), and; c) labile substrate. The periodic oxidation of wetland soils will provide for the enhanced release of inorganic Hg, sulfate and DOC into pore waters, promoting MeHg production upon rewetting. The period of air-entry must be sufficiently long to allow for the oxidation of organic matter and reduced-S. Equally importantly, in order for Hg methylation to be significantly promoted the period of rewetting must be sufficient to allow for the maintenance of a viable microbial community that can utilize the metabolize the available nutrients and substrate.

**EXPERIMENTAL CHALLENGES:** Experimentally, it would appear as though some of the biogeochemical properties of the soils were preserved through the experiment, particularly those illustrated by the microbial community analyses. This information gives us some confidence that the experimental treatments were not fundamentally flawed and changed or eliminated the native bacteria consortia. The measurements of redox potential in the soil cores across all of the experimental treatments also lent confidence to the experimental design in that even in soils subjected to a high frequency (i.e. daily) water level change, reducing conditions were readily established, and rapidly restablished after air entry. If the microbial consortia had been dramatically negatively impacted by the experimental design, a rapid reestablishment of reducing conditions would not have been observed.

In contrast, the isotopic Hg methylation potential assays undertaken at the end of the 3 month experiment, and the measures of metabolic ratio and biomass suggests that substrate limitation led to an attrition of the native microbial consortia or simply a significant slowing of growth. The experimental design could be improved through a more reliable maintenance of the microbial environment, or through more repeated destructive sampling over the course of the experiment, rather than simply an initial and final sampling.

Logistical issues associated with designing, establishing and troubleshooting the experimental apparatus resulted in a holding time of approximately three months between the sampling of the cores in the field and the initiation of the experiment. Initial samples were taken at the beginning of the experiment, not at the time of sampling, so they represent a true starting point for the experiment. However, it is possible that continued microbial metabolism at 4°C impacted the nutrient and substrate pool in the core soils. However, given that the cores were rewetted with oxic reservoir water, and permitted to equilibrate for one week under steady flow conditions prior to the sampling of the initial cores and the beginning of the experiment, we have confidence that the biogeochemical function of the cores were not profoundly compromised. The one week equilibration time is based on other work done in our labo-
ratory where peat samples frozen at -20°C have been thawed and incubated along with fresh peats. Microbial respiration as measured by carbon dioxide and methane production increased in the thawed peats to the same level as fresh soils within seven days in all treatments (C. Mitchell, unpublished data, 1997).

Given the challenges associated with attempting to maintain in situ field conditions in a laboratory setting, it is strongly recommended that these kinds of environmental controls on Hg methylation and wetland soil biogeochemistry be explored using a mesocosm approach in the field. Using a factorial design and field manipulations of water table position, one would be faced with technical challenges, but benefit from representative physical environmental conditions, the ability to repeatedly sample from a much larger and integrated pool of soils and pore waters, and a far lower likelihood of experimental artifacts such as substrate or nutrient limitation. Mitchell et al. (2008) provides an excellent example of the kind of explanatory power that may be gained from a mesocosm experiment with factorial treatments.
9. Mobilization of Total Mercury and Methylmercury from Wetland Soils of the St. Louis River Project, Minnesota.

9.1. Introduction and Rationale

Factors that exert various controls over the production of methylmercury in soils, sediments and water have been widely considered in the literature, including microbial activity, nutrient and substrate controls, mercury bioavailability and other biogeochemical factors such as reduction-oxidation potential. The processes responsible for physically conveying MeHg that is produced in the environment to receptor organisms is, in contrast, frequently overlooked. Work has been done to assess diffusional processes from lacustrine, estuarine and marine sediments, but very little work has considered the mobilization of MeHg from soils and sediments by hydrologic processes. These processes are particularly important in environments known to produce MeHg such as wetlands that hydrologically coupled to lacustrine or riverine systems with variable water levels. These dynamics drive wetting and draining episodes that alternately promote the production of MeHg under anoxic conditions, and then transport it to the adjacent aquatic system. This is particularly salient to the study of Hg dynamics in the SLRP system, where water level fluctuation influences the production of MeHg in the soils of adjacent wetlands (Section 8), but may also facilitate the transport of that MeHg to the reservoirs. Indeed, it is possible that the transport aspect of the Hg cycle in these reservoirs is of equal or greater importance than MeHg production by facilitating the exposure of aquatic organisms to wetland-derived MeHg. Here we report the first experimental study to assess the role of soil wetting and draining on the mobilization of THg and MeHg in wetland soils.

9.2. Methods

FIELD SAMPLING: Six soil cores (5 cm dia. X 40 cm long) for this experiment were acquired from each of the Thomson Shrub-Scrub wetland and Boulder Moss-Lichen wetland at the same time that the cores were taken for the experiment described in Section 7. Field methods and core handling are as described in Section 8. The cores were stored at 4˚C for approximately 6 months before this experiment was undertaken.

EXPERIMENTAL DESIGN: Two carboys each with 15L of filtered Boulder Lake Reservoir water (Initial THg = 2.52 ng/L; MeHg = 0.08 ng/L) were used to supply water to rewet the cores between each draining cycle. The storage of these cores prior to the experiment led to some concern that the soils and porewaters would be somewhat depleted of nutrients and labile substrate. Since this experiment was not to assess ambient rates of MeHg production or compare production potential, but to assess Hg mobility, we elected to amend the supply water with sodium sulfate and glucose monohydrate in molar ratios to raise the concentration of sulfate to 5 mg/L in order to stimulate the activity of sulfate reducing bacteria, thus generating a new pool of pore water MeHg (see Mitchell et al., 2008).

In an environmental room temperature regulated to 20˚C, the 12 experimental cores were connected to the digital low-flow peristaltic pumps used in the experiment described in Section 8. Each carboy supplied three cores from each wetland. This water was circulated through all of the cores for
one week at a rate of 130 mL/day to refresh the sediment pore waters after a period of storage, with
water returning to the carboys, as described in Section 7.

At the beginning of the experiment, the pump circulation was stopped. 0.68 mg of lithium bromide
was injected in 1 ml of the reservoir water solution into each of the cores through side ports spaced at 1
cm increments. The bromide is known to be a relatively conservative hydrologic tracer that allows for
the comparison of the physical dewatering mechanisms of the different sediments, independent of the
potentially different sorptive capacities of the sediments for Hg. One of the 15L carboys was amended
with 73 mg of molybdate to inhibit sulfate reduction and Hg methylation in one set of the experimental
cores to isolate the effect of the wetting and draining on MeHg mobility versus production. The pumps
were reversed, and all of the drainage water recovered and analysed for THg and MeHg using methods
previously described. Br was measured on a Dionex DX-500 Ion Chromatograph in our lab at the Uni-
versity of Toronto. The rewetting and draining procedure was repeated 5 times over 24 hour cycles.

9.3. Results

HYDROLOGIC TRACER: The Br tracer showed a predictable and consistent decrease in mass flux
with each drainage cycle as the mass of initial tracer injected into each core was depleted (Figure
9.1A). All of the experimental cores released Br similarly, suggesting that, even though the two wetland
soils were of a different character and derived from completely different plant communities, the active
porosity of each was similar.

When presented as a percentage of the original mass of Br tracer injected, there is a steady de-
crease of from between 15 and 23% at Drain_1 to between 2.1 and 4.4% by Drain_5 (Figure 9.1A). The
total mass of Br recovered ranged from between 34.6 and 50.7%, with no significant difference be-
tween wetland types. This data indicates that, over the 5 daily draining cycles, between approximately
35 and 50% of the original pore waters at the beginning of the experiment were mobilized out of the
cores. Given the heterogeneity of natural soils, the consistency of these data are surprising.

DISSOLVED TOTAL MERCURY: The mass of THg mobilized in the dissolved phase exhibited a pat-
tern of release from pore waters that resembles that of the conservative tracer, except that THg shows
a decline between Drain_1 and Drain_2, and then a more consistent release from then on (Figure
9.1B). Curiously, the non-molybdate amended Boulder wetland cores did not show the initially higher
mass flux. This can only be explained as between-core variability and is, in fact largely due to a lower
mass flux of drainage water from this core set due to differences in porosity. Although the reservoir
water used to wet the cores had a THg concentration of 2.52 ng/L, the drainage water concentrations
ranged from an average of 24.6 ng/L for the first drain to between 11.0 and 11.5 ng/L for subsequent
draining events. This additional THg must be being derived from the solid phase through desorption or
dissolution, and moved into pore waters. The kinetics of this release are able to maintain a stable mass
flux of dissolved THg over subsequent wetting and draining cycles. The Boulder wetland cores re-
leased a total of 11.6 and 4.2 ng THg over the 5 draining cycles, the later due to the lower water yield;
the Thomson wetland cores released a relatively consistent total of 11.6 to 15.3 ng THg. If the THg in
the drainage water were conservatively derived from the supply water then the total flux of THg would
only be between 3.8 and 6.3 ng. Therefore the cores are a net source of dissolved THg.
Figure 9.1. Pore water draining experiment summary of results. Each column of panes corresponds to the site from which the soil cores were obtained. (I) indicates the cores that were subjected to the molybdate inhibitor. A) is the percent recovery of the total mass of Bromide tracer added before the first drainage cycle. B) is the mass of THg removed in the dissolved phase. C) is the mass of MeHg removed in the dissolved phase. D) is the fraction of the THg drained from the core as MeHg. Points are means of three replicates and error bars are plus and minus one standard error.
METHYLMERCURY: The mass flux of MeHg from all of the experimental core sets reflected neither the pattern of the Br tracer nor dissolved THg (Figure 9.1C). Although the Boulder wetland core set show a more damped mass flux because of lower volumes of drainage water recovered, all of the core sets show an initial decrease in dissolved MeHg release, then an increase up to Drain_4, then a subsequent decrease during the final drainage. The mean total mass of MeHg mobilized for the Boulder wetland core sets was 0.73 and 1.47 ng and 1.54 and 1.72 ng for the Thomson core sets. If the MeHg in the drainage water were conservatively derived from the supply water then the total flux of MeHg would only be between 0.12 and 0.2 ng. Therefore the cores are a net source of dissolved MeHg.

The molybdate addition had no inhibitory effect on the release of MeHg from the inhibited cores. There are several potential explanations. The molybdate may not have been introduced to the sites of methylation because only a fraction of the pore waters were exchanged during each draining cycle, or the molybdate became immediately bound to the organic matter in the soil core as it was introduced. The MeHg mobilized during the experiment may have been derived entirely from partitioning from the solid phase, or other microbial communities may be involved in the methylation process that are not inhibited by molybdate (i.e. non-SRB). In hindsight, the most likely explanation is that the molybdate was bound to the organic matter in the soil core, precluding it’s inhibitory effect on SRB in pore waters. Molybdate has been used effectively to suppress methylation by SRB in culture and solution, but not in higher bulk density intact sediments.

Examining the percent of THg as MeHg over the course of the experiment reveals a dynamic %MeHg with an increasing trend at least during the middle of the experiment (Figure 9.1D). This suggests that partitioning is unlikely to be the only mechanism of MeHg release, unless inorganic Hg and MeHg form distinctly different associations with the solid-phase.

9.4. Discussion and Conclusions

In contrast with other aspects of this project which are concerned with the biophysical factors governing MeHg production, this section focussed on the physical mechanisms of pore water mobilization and the movement of MeHg from the wetland soils into the adjacent surface water systems. This experiment, simply put, filled intact soil cores with water, held them for a day, and then drained the water and analysed that drainage water for its chemical constituents. A chemical tracer was introduced at the beginning of the experiment that was expected to not interact with, or be retained by the soil matrix. If other chemical species behaved like this tracer, then they might be reasonably considered relatively inert, with their release governed dominantly by the physical removal of pore waters that had a pre-existing chemistry that is not easily, or at least quickly changed (supply limited). If they behaved differently than the tracer, then we may infer that they are being governed by other processes in the soil matrix that continue to supply the chemical to pore waters (transport limited).

The results of this experiment show that pore water drainage mobilizes significant masses of THg that are being derived from the solid phase. Although the mass of THg is greatest at the beginning of the experiment, this mass flux is sustained over multiple wetting and draining cycles. The establishment of a constant export mass over time suggests that there is a partitioning equilibrium that ultimately limits the amount of inorganic Hg that will be released. Using the average solid phase concentrations
of THg in the Thomson and Boulder wetland soils (388 and 132 ng g⁻¹ respectively) and the data from this experiment (mean pore water concentrations of 13.20 and 9.27 ng L⁻¹, respectively not including the first flush), we can calculate a distribution coefficient (log Kᵩ = log ([Hg]particle/[Hg]aq); L kg⁻¹) for THg of 4.46 and 4.15 for the Thomson and Boulder wetland soils. The log Kᵩ for Thomson is slightly higher than that reported elsewhere for peats, but the Boulder log Kᵩ is precisely the same as that determined for other sphagnum peats in northwestern Ontario (Branfireun et al., 2005).

MeHg on the other hand does not present any sort of equilibrium export behaviour, yet the cores are a net source of MeHg to the drainage water. Pore water drainage is therefore mobilizing MeHg that is derived from either the solid phase, in situ methylation, or a pre-existing pool in pore waters. Given that there is little concordance between MeHg release and THg or the Br tracer, it is difficult to draw clear conclusions as to the source of the MeHg in the drainage waters. The variability in the drainage water MeHg concentrations and mass fluxes could be explained by the variable interaction of filling and draining water with regions of the core where MeHg production (methylation) is taking place, or MeHg desorption from the solid phase.

RECOMMENDATIONS: A pattern of predictable partitioning of THg from wetland soils to pore waters is emerging in the literature. Further work to more specifically examine this partitioning behaviour over time is warranted, particularly to determine the limits of, and controls on, the release of THg from the solid-phase. The wetland soils in the experimental cores were a net source of MeHg, but the pattern of release was not predictable, as it was with THg. A more systematic approach to isolating biomethylation versus solid-liquid phase partitioning would reveal the controls on this variability. A longer time course for a similar experiment would again further illustrate the limits to this production and/or release from the solid phase.
10. Effect of Winter Drawdown on Methylmercury Production in Littoral Wetland Sediments of the St. Louis River Project, Minnesota.

10.1. Introduction and Rationale

In small reservoirs in temperate climates, water levels in managed reservoirs are typically at their lowest over the winter season. This drawdown frequently leads to near-shore sediments being either fully dewatered and frozen solid over the winter season, or partially to fully dewatered, but retains a cover of ice and snow, preventing the sediment from fully freezing. In the spring, these dewatered sediments are rewetted by rising water levels in the impoundment, thaw/warm, and are often disturbed by ice floating and rafting. This winter dewatering is effectively an extended drying and wetting cycle coinciding with a thermal cycle and sediment disturbance. All of these factors are known to influence MeHg production.

The objective of this study is to experimentally mimic the conditions described above, and using measures of mercury methylation, determine if:

a) reservoir near-shore dewatering during the winter season promotes enhanced MeHg production in the near shore sediments of reservoirs, and

b) if there is any difference in MeHg production potential among dewatered frozen, unfrozen and disturbed sediments.

10.2. Methods

FIELD SAMPLING: Twelve 30 cm sediment cores were acquired from the near shore zones in front of the intensively studied wetlands of the Thomson and Boulder Reservoirs. Unfortunately practical field limitations precluded the acquisition of more replicate cores. These sediment cores were handled and processed in the same manner as those described in Section 8. Upon return to the University of Toronto, all of the sediment cores were stored at 4°C, fully saturated with water. The cores were held in this fashion for approximately 3 months.

EXPERIMENTAL METHODS: At the outset of the experiments, all of the cores were fully gravity drained. Two cores from each of the reservoirs (4 total) were returned to 4°C storage, and two cores from each of the reservoirs (4 total) were placed in a freezer and stored at -20°C. One additional core from each of the reservoirs was drained and kept under the cold and frozen conditions to be used for the sediment disturbance experiment. Cores were stored under these conditions for 3 months. After 3 months, the frozen cores were moved to the 4°C environmental chamber and allowed to thaw for one week. After this period, all of the cores were rewetted with the standard filtered Boulder Lake reservoir water used for other experiments described here amended with 5 mg/L sulfate and the molar ratio of glucose monohydrate to stimulate the microbial consortia (see Section 9). The wetted cores were moved to the 20°C environmental chamber and held for one week. At the end of this holding period, the 4 cold-treatment and 4 frozen-treatment cores were extruded and sliced into 0-2 and 2-10 cm sections. This scheme was decided upon because the exchange of THg and MeHg between near-shore submerged sediments and the water column is dominated by diffusion, bioturbation and surficial erosion; Hg processes occurring deeper in the profile do not typically influence the overlying water chemis-
try. Sections were physically mixed, spiked with inorganic 200Hg, incubated at ambient temperature for 12 hours and then flash frozen (see Section 7 methods for details on the methylation potential assay method). These samples were then subsequently analysed for both 200THg and 200MeHg to assess Hg methylation potential (see Section 8). The single core from each reservoir subjected to the cold and frozen treatments underwent the same processing, except that the top half of the core was fully mixed when the cores were rewetted, disturbing the existing sediment profile.

MeHg production rates measured as part of this experiment cannot be directly related to other work because of the nutrient amendment. Only the within-experiment treatments are comparable as they all received the same additions.

10.3.Results

Lack of sufficient replication means that the results of this experiment cannot be statistically evaluated.

COLD VERSUS FROZEN TREATMENT: Ambient THg concentration was uniform amongst the duplicate cores within each wetland type and across experimental treatments (Figure 10.1). As discussed in Section 8, this is to be expected, but nonetheless is an important simplifying observation when discussing further speciation. Thomson Reservoir littoral sediments were on average about 3x higher THg concentration than those of the Boulder Lake Reservoir.

![Figure 9.1](image)

*Figure 9.1. Ambient THg in experimental cores of littoral sediments from a) Thomson Reservoir (left; dark grey), and b) Boulder Lake (right, light grey).*

Ambient MeHg does not share the uniformity of distribution of THg either among duplicate cores, or across experimental treatments. For the Thomson reservoir littoral sediments, the highest mean ambient MeHg concentrations were from the Chilled treatment at 0-2 cm, with the 2-10 core slice only moderately lower. The Freezing treatment exhibited much more variability about the mean concentration, but were all lower in MeHg concentration than the Chilled treatment.

The pattern of MeHg concentrations in the Boulder lake littoral sediment treatments was a mirror image of those from Thomson. The two Chilled treatments and the 0-2 cm Freezing treatment had uniformly low MeHg concentrations, but a high MeHg concentration in one of the duplicate cores (38.1 ng/g d.w.) sets the 2-10 cm slice of the Boulder Lake core in the -20°C treatment apart from the other data (Figure 10.2).

![Figure 10.2. Ambient MeHg in experimental cores of littoral sediments from a) Thomson Reservoir (left; dark grey), and b) Boulder Lake (right, light grey).](image)

This anomaly is reflected in the values for 200MeHg production, where the same Boulder lake sediment core slice shows an unprecedented 74% conversion of inorganic 200Hg spike to 200MeHg (Figure 10.3). Although all laboratory QA/QC supports this value, the lack of replication makes interpretation difficult.
Even if this value is set aside, an important pattern is evident. Methylation potential is not differentially affected by Chill or Freeze treatments in the Thomson Reservoir sediments, and is generally higher than that of the Boulder lake sediments. However, in the Boulder Lake sediments, the Freezing treatment cores show a higher methylation potential for the over the chill treatment. Without additional information, interpretation of this result is difficult, but it could be hypothesized that during the 3 month holding period at 4°C, the Boulder Lake sediments supported a more active low-temperature microbial community that became limited, reducing total biomass. The frozen treatment would have stopped metabolic activity until the sediments were thawed, preserving total biomass to a greater degree.
UNMIXED VERSUS MIXED TREATMENT: Both Ambient THg and MeHg concentrations were as described above, with no significant difference amongst the duplicate cores within each wetland type or slices with the exception of the Boulder lake sediment 2-10 cm Freeze treatment as discussed.

The mixed Cold and Freeze treated cores did not differ, so they were combined to provide a duplicate value in keeping with the other treatments. For both the Thomson and Boulder reservoir sediments, it would appear that sediment mixing does not homogenize methylation potential, but significantly reduces it at both depth slices in the near surface sediment (Figure 10.4).

![Figure 10.4. Freezing/Mixing treatment 200MeHg production rate in experimental cores of littoral sediments from a) Thomson Reservoir (left; dark grey), and b) Boulder Lake (right, light grey).](image)
10.4. Discussion and Conclusions

Although the lack of experimental replication constrains our ability to draw unequivocal conclusions about the effect of overwinter littoral dewatering, cooling and/or freezing, and mixing, some interesting observations can be made from the limited data.

In general, it would appear as though the littoral sediments from the two systems behave differently with respect to the experimental treatments. This finding suggests that it may be challenging to generalize these results either among different wetland/lake systems, or perhaps beyond the specific locations from which the samples were taken.

The results of this experiment indicate that Thomson reservoir littoral sediments did not respond differently to either cooling or freezing treatments (i.e. the methylation rates as measured by the 200-Hg methylation assay were the same across treatments), although the ambient MeHg concentrations suggested a slightly lower concentrations for the freezing treatments.

Freezing of Boulder Lake sediments showed a similar pattern among most of the sediment cores, but one resulted in markedly higher methylation potentials when frozen than when the sediments were only cooled. Given that this one core also had dramatically higher ambient MeHg concentrations, it would appear as though significant spatial heterogeneity in methylation potential could hamper investigations of this process in littoral sediments. More consistent results from the wetland soil cores either suggests good fortune, or that the wetland soils tend to be more homogenous in their biophysical properties.

Finally, the mixing of near-shore sediments appears to reduce overall Hg methylation potential in the near surface sediments of the Thomson reservoir, with the results from the Boulder reservoir being more inconclusive because of the very high MeHg production rates measured in one core.
11. Overall Summary

The objective of this project was to attempt to answer the question “Do water level fluctuations caused by the SLRP hydropower operation increase the production or mobilization of methylmercury relative to natural water level [fluctuations]?” A lack of information about the distribution of THg and MeHg in the systems of interest required a detailed scoping field study of the distribution of Hg species in the wetlands of the SLRP. In many respects, this foundational data has as high a utility as some of the more challenging experimental outcomes.

CONCERNING THE DISTRIBUTION OF TOTAL AND METHYLMERCURY IN THE SLRP WETLANDS: The range of THg in wetland soils measured is for the most part within the range of that reported for upland and wetland soils elsewhere. Thomson Reservoir has areas of highly elevated THg in buried sediments that are the result of legacy contamination. The wetlands of the headwater reservoirs have a similar range of THg concentrations in wetland soils, despite the lumping of a number of wetland types in such a comparison. Methylmercury concentrations and %MeHg are similar across wetlands in the reservoir systems, but are significantly higher than those of the natural lakes. It is not possible to attribute this to impounded versus non-impounded status, as the relationship is driven by the dominance of the wetland types chosen for this study with high MeHg concentrations and %MeHg in the impounded reservoir systems. The impounded systems study sites were moss-lichen wetland type, which has on average significantly higher %MeHg than other wetland types.

Zones of high MeHg concentrations are a reflection of internal conditions that are conducive to Hg methylation. The burden of MeHg in the Thomson wetland is dominated by a relatively localized zone of high MeHg sediments. The Boulder wetland also has a localized zone of high MeHg sediments in the surface peats as a consequence of enhanced methylation at the wetland-lake margin. Methylation is moderated in the Alden Lake Emergent wetland by either nutrient limitation or an unsatisfactory biogeochemical environment for sulfate reduction.

CONCERNING THE ROLE OF WATER LEVEL FLUCTUATION ON MEHG PRODUCTION: It is apparent that all of the wetlands exhibit hydrological and biogeochemical conditions conducive to Hg methylation. The experimental work to attempt to isolate the effect of water level fluctuation was not definitive. However, the monthly water level fluctuation treatment resulted in significantly higher ambient MeHg concentrations in the Thomson wetland soils, and when normalized as the ratio of MeHg to THg, the same treatment resulted in a significantly higher ratio for the Thomson and Alden wetlands. Similarly an increase in MeHg concentrations in the static treatments for soils from the Boulder wetland is significant, as it suggests that periods of persistent saturation are important in maintaining an environment conducive to MeHg production. It should be noted that the static treatments had a static water level, but were subjected to a slow flow of new water; an aspect of the experimental design that means that static water level is not synonymous with stagnant conditions. The duration of the apparent enhanced methylation effect for these treatments are unknown. More frequent water level fluctuation appears to have an inhibitory effect on MeHg production and microbial community fitness, although the duration of this effect is unknown.

CONCERNING THE ROLE OF WATER LEVEL FLUCTUATION ON MEHG MOBILIZATION: Pore water drainage results in a significant release of THg that is being derived from the solid phase based on a comparison of distribution coefficients with other wetland studies. Pore water drainage also re-
sults in the net export of MeHg that is derived from either the solid phase, *in situ* methylation, or both but the pattern of release is not systematic, as with THg.

Concerning the role of dewatering, cooling, freezing and sediment mixing: The littoral sediments examined did not respond differently to either cooling or freezing treatments (i.e. the methylation rates as measured by the 200-Hg methylation assay) were the same across treatments), but apparent heterogeneity in the methylation potential of the sediments obfuscates a clear interpretation. The mixing of near-shore sediments appears to reduce overall Hg methylation potential in the near surface sediments.
12. Recommendations Based on Project Outcomes

1. Based on the broad similarities among the different headwater reservoirs in the upper watershed, a more intensive study of a Moss-Lichen wetland (Bog) is recommended, given the dominance of this wetland class by total area (20-48\% of total wetland area in the headwater reservoir systems; Minnesota Power and Light Co., 1991). Improved wetland delineation and classification of the headwater reservoirs is warranted. Rice Lake has no documented Moss-Lichen (Bog) wetland, yet first-hand reconnaissance revealed that most of the wetlands in this system were misclassified. The sphagnum peat soils of these wetlands have a propensity for efficient interconversion of inorganic Hg to MeHg as illustrated by high \%MeHg. The soils of this wetland type (peats) should not be subject to significant physical or hydrological perturbation when connected to surface waters. The high \%MeHg in these systems would likely result in a significant transfer of MeHg to aquatic receptors.

2. Related to 1, the zone of high MeHg concentrations and \%MeHg at the wetland-lake interface at the Boulder Moss-Lichen wetland present a potential area of concern given that methylation may be enhanced in this region because of water level variability and nutrient delivery, and it is directly hydrologically connected to surface waters. Certainly, reservoir systems that are dominated by Moss-Lichen wetlands may have a higher total loading of MeHg than those without. More focussed investigations on a) the production of MeHg at these wetland-reservoir interface zones and b) the mass transport of water, THg and MeHg from the wetlands to the reservoirs would be justifiable.

3. Capturing the spatial heterogeneity in the distribution of THg and MeHg is not trivial, and requires a more extensive sampling scheme than executed here. Further work should be undertaken to improve burden estimates for the purposes of ecosystem modeling. Ancillary soil chemistry and physical properties should be measured concomitantly.

4. Water level fluctuation alone is not a first-order control on MeHg production. The experimental results suggest an optimal hydroperiod for MeHg production that is characterized by more extended periods of both wetted, and drying conditions. Despite the different operating cycles of the reservoirs, it would in fact appear as though the wetland hydroperiods all tend to follow a more seasonal pattern of wetting and drawdown, not dissimilar from that of the natural wetland systems. That is, the wetland water tables do not directly track changes in the reservoir levels. Despite this, the natural wetland had low MeHg concentrations overall, indicating that other biogeochemical factors are at least as important in regulating MeHg production as water level fluctuation.

5. Water level fluctuations may, however, set up hydraulic gradients that can a) drive the export of Hg-laden wetland pore waters to surface waters, and b) return more nutrient and substrate rich surface waters that may promote further methylation. Further work to develop a better understanding of the hydrologic exchanges between the reservoirs and their surrounding wetlands is warranted.


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