SEDIMENT METHYLMERCURY CONCENTRATIONS AND PRODUCTION RATES IN COASTAL WETLANDS OF CHEQUAMEGON BAY (WI), LAKE SUPERIOR

A Manuscript Style Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Masters in Biology

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SEDIMENT METHYLMERCURY CONCENTRATIONS AND PRODUCTION RATES IN COASTAL WETLANDS OF CHEQUAMEGON BAY (WI), LAKE SUPERIOR

By Jacob Ogorek

We recommend acceptance of this thesis in partial fulfillment of the candidate's requirements for the degree of Masters in Biology, Aquatic Science. The candidate has completed the oral defense of the thesis.

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ABSTRACT

Ogorek, J.M. Sediment methylmercury concentrations and production rates in coastal wetlands of Chequamegon Bay (WI), Lake Superior. Master’s of Science in Biology/Aquatic Science, December 2009, 63 pp. (R. Haro)

Mercury in Lake Superior is understudied, and (1) geochemical factors in sediments that influence sedimentary methylmercury concentrations and (2) key habitats for methylmercury production are unclear. The primary methylmercury source in aquatic systems is typically inorganic mercury methylation by sulfate reducing bacteria (SRB). Mercury methylation is generally greatest in wetland sediments, and is mediated by inorganic mercury bioavailability and SRB activity, both of which are strongly influenced by sulfidic compounds and organic matter. We hypothesize that Lake Superior coastal wetlands are important sites for methylmercury production, and sediment methylmercury correlates with sedimentary geochemical factors such as inorganic mercury, sulfide/sulfate, organic matter, and mercury methylation potential. We measured sediment methyl and total mercury concentrations, mercury methylation potential (via isotopic mercury additions), organic matter, sulfide, and sulfate in three coastal wetlands (two of which are located at the mouth of a tributary) and one open-water site in Chequamegon Bay (Lake Superior). Sediment mercury levels were relatively low, with the highest concentrations measured in coastal wetlands, particularly when associated with a tributary. We found methylmercury levels were correlated with multiple geochemical parameters, and it appears that sediment organic matter is the primary driving force of SRB activities leading to methylmercury production.
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INTRODUCTION

Mercury is a highly toxic metal of great concern from local to global scales. Human activities have greatly increased observed environmental mercury levels (Schuster et al., 2002; Wiener et al., 2003; Lindberg et al., 2007), which are combined with an extremely complex set of global cycling pathways and processes, and have increased exposure of wildlife (Scheuhammer et al., 2007) and humans (Mergler et al., 2007) to mercury. Once in the environment, mercury interacts within multiple environmental compartments and exists in multiple oxidation states as well as both organic and inorganic forms. Of particular concern is methylmercury (MeHg), an extremely toxic and biologically available organic compound that is derived from microbial methylation of inorganic divalent mercury (Hg(II)).

Certain species of sulfate-reducing bacteria (SRB) are considered the primary mercury methylators in the environment (Benoit et al., 2002), although other groups of bacteria can produce MeHg as well (Warner et al., 2003; Fleming et al., 2006). Surficial sediments in aquatic ecosystems (e.g., wetlands, lakes, reservoirs and streams) are the primary sites of mercury methylation (Gilmour et al., 1998; St. Louis et al., 2004; Heyes et al., 2006; Hines et al., 2006). Methylmercury readily enters and biomagnifies in aquatic food webs, with the greatest magnification (about $10^5$ to $10^6$ fold) occurring between water and
primary producers (phytoplankton), and more limited increases (about 3 to 10 fold) between subsequent trophic levels, culminating in potentially harmful concentrations in top predators (Wiener et al., 2003; Scheuhammer et al., 2007).

Several geochemical factors influence sedimentary MeHg concentrations. Since Hg(II) is the dominant form of sedimentary mercury species and the primary constituent for methylation, it is hypothesized that the total mercury (HgT) levels control MeHg abundance in sediments. Across relatively uncontaminated sediments that span a wide range of environments there is a weak positive relation ($r^2=0.41$, $p < 0.01$) between HgT and MeHg (Benoit et al., 2002). Other environmental factors, including organic matter and sulfidic compounds, likely play an important role as well. Organic matter and sulfate, required for respiration of SRB, have been positively correlated with MeHg production in laboratory cultures (King et al., 2000) and surficial sediments (Gilmour et al., 1992; Lambertsson et al., 2006). However, organic matter and sulfide also limit the bioavailability of inorganic Hg(II) to SRB. Strong binding constants have been measured for reduced sulfur compounds (log $K^\prime$=37.5-42.5, Benoit et al., 1999) and dissolved organic matter (log $K^\prime$=10.6-11.8, Benoit et al., 2001). A negative relation between sediment mercury methylation and Hg(II) bioavailability due to complexation has also been observed (Gilmour et al., 1998; Hammerschmidt et al., 2004; Hammerschmidt et al., 2008).

Methylation of Hg(II) is a critical and necessary “first step” in MeHg bioaccumulation in aquatic systems and resident biota. Methylation rate
estimates are generally proportional to benthic MeHg concentrations (Gilmour et al., 1998; Hammerschmidt et al., 2006; Heyes et al., 2006; Kim et al., 2006; Drott et al., 2008) and have been correlated with MeHg levels in biota (Pak et al., 1998). Peak methylation usually occurs within a few centimeters of the sediment surface (Gilmour et al., 1998; Heyes et al., 2006; Hines et al., 2006) at the most biologically active and ecologically significant sediment horizon. Mercury-methylation values, determined by stable isotope additions, have been recently reported in marine and estuarine sediments (Lambersson et al., 2001; Hammerschmidt et al., 2004; Martin-Doimeadios et al., 2004; Lambertsson et al., 2006), freshwater sediments (Orihel et al., 2006), epilithion (Desrosiers et al., 2006), and the water column (Eckley et al., 2006). However, these rate measurements do not necessarily quantify absolute methylation rates because of difficulties in matching the true bioavailability of an administered tracer versus that of native Hg species (Hintelmann et al., 2000). Although some methodologies attempt equilibration of the Hg spike with native pore waters to compensate for in situ matrices, these tests are best described as estimated or potential rates and are most useful in a comparative context.

Overall, environmental conditions in Lake Superior seem relatively unfavorable for MeHg production. Pelagic Lake Superior is cold and unproductive (reduced microbial activity), contains low environmental mercury levels (Rolfhus et al., 2003), and given its relatively high surface area to shoreline and watershed ratios, is presumably relatively unaffected by littoral and watershed processes. However, elevated mercury levels are measured in
walleye and lake trout, prompting fish-consumption advisories (U.S. Environmental Protection Agency 2007, Ontario Ministry of the Environment 2007). Although coastal wetlands are a relatively small proportion of the overall area of Lake Superior, they may be a substantial source of MeHg to Lake Superior and its food web. Coastal wetlands are abundant on the south shore (Trebitz et al., 2002) and warm sooner than the rest of the lake. Wetlands are important sites for MeHg production and subsequent transport to aquatic systems downstream (Hurley et al., 1995; St. Louis et al., 1996). Wetlands at the mouth of a tributary may be particularly important because they receive additional watershed inputs of mercury and other important geochemical parameters from the associated tributary (Rolfhus et al., 2003) and from subsurface hydrologic flow (Stoor et al., 2006). The potential for MeHg uptake into the Lake Superior food web in coastal wetlands is also elevated. Isotopic analyses of food webs in Lake Superior coastal wetlands show that food-web foundations are benthic (Sierszen et al., 2006) and that the energetic pathway is largely detrital (Sierszen et al., 2004), linking the food web directly to the site of sedimentary MeHg production. Coastal wetlands also support a diverse biological community including many fish species (Jude et al., 1992) and are an important biogeochemical interface between the lower food webs of watershed, lotic, and lentic ecosystems.

The objectives of this study were three-fold: (1) to characterize MeHg and HgT concentrations in sediments from three contrasting coastal wetlands, varying in tributary influence, and one non-wetland near-shore environment; (2)
to examine the relation of sedimentary MeHg concentrations to known controlling factors, such as HgT levels, ambient and isotopically estimated mercury methylation rates, pore water sulfide/sulfate concentrations, and organic matter content; and (3) to evaluate mercury levels in wetlands and near-shore environments in the context of MeHg bioaccumulation in Lake Superior biota. We hypothesize that: (1) among sampling sites the greatest HgT and MeHg concentrations are in wetland-influenced sediments; (2) that relations exist between sedimentary MeHg and HgT concentration, pore water sulfide/sulfate, and organic matter; and, (3) that MeHg concentrations will positively correlate with isotopic estimates of sedimentary mercury methylation.
STUDY AREA

Chequamegon Bay is a large embayment (surface area 146 km²) on the Wisconsin shoreline of southwestern Lake Superior. Most of the bay is separated from Lake Superior by a narrow spit of land, but is connected to Lake Superior by a relatively deep channel along its northwestern side. Relative to pelagic Lake Superior (the largest freshwater lake in the world by surface area), Chequamegon Bay is shallow with a mean depth of 8.6 m and a maximum depth of 23 m (Ragotzkie et al., 1969). Water depths in the southwestern and eastern portions are typically less than 5 m. Chequamegon Bay is covered with ice about 105-120 days/yr (Assel, 2003), and receives an average of 84.3 cm/yr of precipitation (National Climatic Data Center, 2005). The communities of Ashland and Washburn, with a combined human population of about 11,000, are located on Chequamegon Bay (U.S. Census Bureau, 2000).

Chequamegon Bay sediments are described by Edsall et al. (2004) to be in good condition and only lightly contaminated with toxic substances. Compared to the St. Louis River Estuary, a heavily used freshwater shipping port at the western end of Lake Superior, Chequamegon Bay sediments are relatively free of recalcitrant anthropogenic debris (mine tailings, taconite pellets, coal, and combusted coal waste). Sediments in the deeper open water areas of the bay are largely inorganic, and are composed of clay or mixed clay and fine sand in
the southwest, fine and coarse sand in the east, and coarse sand in the north (Edsall et al., 2004). Highly organic sediments are found in shallow near-shore zones of the bay that are protected from wave action. These areas of organic matter entrainment are generally associated with coastal wetlands, being located in depositional areas adjacent to or within the wetlands.

Water movement and thermal stratification are complex in Chequamegon Bay and have been extensively described by Ragotzkie et al. (1969). Water circulation is generally counterclockwise but varies spatially and temporally, and is associated with wind-driven thermocline movement and seiche activity. Chequamegon Bay thermally stratifies from June to September, but stratification is often disrupted by strong winds and changing wind direction, resulting in the flushing of the bay with water from Lake Superior every 5 to 10 days. Chequamegon Bay has a main seiche period of about 8 hours (although substantial oscillations also occur at shorter intervals), with seiche amplitude most frequently ranging from 10 to 15 cm.

The Chequamegon Bay watershed covers 501 km² and includes three distinct drainages (Northwestern Shore, Fish Creek, and Kakagon River). The watershed on the northwestern shore of the bay is primarily forested and high relief where many small streams (generally less than third-order streams) arise and drain directly into the bay. The Fish Creek watershed (241 km²) lies west of the bay and is primarily forest (58.3%) and agricultural land (26.8% pasture or hay and 3.8% cultivated), with a small amount of wetland area (4.5%). The
western portion of the Fish Creek drainage is a highland lakes district dominated by groundwater seepage lakes that have no surface water connection to Fish Creek. Fish Creek is a fifth-order stream at its confluence with Chequamegon Bay and is a major contributor of sedimentary material due to highly erodible clay bluffs within the watershed (Fitzpatrick et al., 1999). The Kakagon River watershed (89 km²) lies along the southeastern corner of the bay and is primarily forest (63.8%), with a relatively high proportion of wetlands (15.7%) and small amount of agricultural land (9.0% pasture or hay and 6.0% cultivated). Three major streams drain the Kakagon River watershed (Kakagon River, Bear Trap Creek, and Wood Creek) converging into a fourth-order stream complex of interconnected sloughs near the bay.

Chequamegon Bay is an ecologically important embayment Lake Superior system. Relative to the whole system, Chequamegon Bay is shallow and stratifies weeks before Lake Superior (Ragotzkie et al., 1969), warming more rapidly than the rest of the lake in the spring. Chequamegon Bay supports many benthic macroinvertebrate species relative to pelagic Lake Superior (Back et al., 2003), including Hexagenia mayflies (Edsall et al., 2004) and Unionid bivalves (author, unpublished data from 2005), both of which are considered indicators of good ecosystem health (Elder et al., 1991; Edsall 2001). Chequamegon Bay also supports a diverse community of fish (Hoff et al., 1999), including both cool- and cold-water species, and supports an intensively managed sport fishery (Devine et al., 2005).
Three expansive coastal wetland systems are located along the shores of Chequamegon Bay. The Fish Creek Wetland is located at the southwestern end of the bay near the mouth of West Fish Creek. The Kakagon River Wetland is located at the eastern end of Chequamegon Bay at the mouth of the extensive Kakagon River stream complex of interconnected sloughs. Sand Cut Slough Wetland is located in the eastern end of the bay (near the Kakagon River Wetland) in an embayment lacking a tributary stream. These three coastal wetlands, as well as one mid-bay open-water site, were selected for detailed evaluation by this study. These sites were chosen anticipating differences in sediment mercury concentrations, sediment MeHg production rates, and ancillary variables. Locations of the wetlands and the open water site are shown in Figure 1.
Figure 1. Map showing proximity of study area to Lake Superior and locations of Fish Creek (FC), Kakagon River Wetland (KRW), Sand Cut Slough (SCS), and the open water (OW) sampling sites within Chequamegon Bay.
METHODS

Sample Collection

The methodology for sediment collection varied somewhat among years. In 2003, preliminary surficial sediment samples (0-3 cm) for ambient mercury analyses were collected with an Eckman or Ponar dredge. In 2004 a single intact sediment core for isotopic mercury methylation estimates was collected at each site, while additional surficial sediment samples (0-3 cm) for ambient mercury analyses were collected from the shallow portion of sediment cores. These homogenized surficial sediments were stored in sealed polycarbonate jars and frozen (-20°C) within 8 hours. In 2005, ten or more intact sediment cores (5 cm in diameter and at least 5 cm long) were collected at each sampling site. Five cores from each site were analyzed for ambient mercury concentrations and were frozen in the core liner within 8 hr. The remaining 5-6 cores from each site were used to estimate mercury-methylation rates. Sediment cores were collected in acrylic core liners with a gravity corer at Kakagon River and Sand Cut Slough. Core liners were fitted to a custom-fabricated fiberglass pole to obtain cores from Fish Creek Mouth, while at the open-water reference site cores were taken manually by a SCUBA diver. In the field, we retained only cores that had (1) at least 5 cm of overlying water, (2) a continuous sediment core within
the core liner, and (3) an undisturbed sediment-water interface. After collection, cores were sealed with caps and tape to prevent water leakage.

Before sediment sampling, overlying water (2004 and 2005) and pore water (2005) were collected from each site. Pore water was collected from the 1-3 cm sediment horizon as described by Krabbenhoft et al. (1998). A series of thin slots were cut through to the inner chamber of a Teflon piezometer over a 2-cm interval. The piezometer was attached through an acrylic plate, which maintained desired sampling depth and slowed the penetration of overlying water into sediments during pore water sampling. The piezometer, which was connected to a peristaltic pump by a Teflon line, was inserted into the sediment until the plate rested on the sediment surface. Vacuum was gently applied to maintain a slow pore water flow from the sediments through the piezometer. Pore water for sulfide analysis was preserved in an equal volume of sulfide anti-oxidizing buffer, and then stored in 15-ml polypropylene vials without headspace, and analyzed within 1 week of collection. Overlying water was collected below the water surface directly into a clean 500-mL Teflon bottle. Overlying water and pore water samples for mercury and sulfate determinations were acidified to 1% (v/v) with concentrated HCl until analysis.

**Stable Mercury Isotope Spikes**

Mercury methylation was measured through the application of a mercury isotope tracer, or “spike”, which is then tracked over time via inductively coupled plasma mass spectrometry (ICP-MS). The method used to spike sediment cores
with the mercury isotopes was first described by Gilmour et al. (1998). Mercury isotopes were added to sediment cores within 8 hr of sampling. Five holes (1-mm diameter) were drilled through the core liner at 1-cm increments in the top 5 cm of the sediment core. A glass syringe equipped with a stainless steel needle was used to add 903 ng of inorganic mercury (as $^{202}$HgCl) and 0.50 ng of organic mercury (as Me$^{199}$HgCl) isotope to each 1-cm section of the upper core. Each isotope was injected across the diameter of the sediment core, and the injection holes were then sealed with duct tape. Each core was then incubated for 24 hours at room temperature (~20°C), and then frozen (~20°C).

**Isotopic and Ambient Mercury Analyses in Sediments**

Sediment cores were sectioned into 1-cm intervals for the top 5 cm and into 5-cm intervals in deeper sections. These sediment sections, as well as homogenized surficial sediments from 2003 and 2004, were lyophilized to a constant weight, and homogenized with a mortar and pestle. Processed sediments were stored in clean plastic bags in a freezer until analysis. All sediments were analyzed following lyophilization and are reported on a dry weight (dw) basis.

Methylmercury in sediments, overlying water, and pore water, was extracted by distillation as described by DeWild et al. (2002) before analysis to dissolve solid phase MeHg, facilitate chloride anion exchange, and to remove potential matrix interferences. Dried sediments (0.1 – 2.0 g) or aqueous samples (up to 90 mL) were weighed into Teflon distillation vessels and brought up to 90 mL total volume with purified water, to which 1 mL each of 25% (w/v) CuSO$_4$ and
50% (w/v) H₂SO₄, and 0.5 mL of 20% (w/v) KCl was added. The vessels were then heated in an aluminum block (130 °C) and purged with N₂ gas, with the resulting vapor carried to and condensed in chilled Teflon receiving vessels. Distillation was stopped when approximately 25% of the water remained in the distillation vessels.

Distillates were analyzed by adding an aliquot to a 125-mL sparging flask, followed by 0.1 mL of 1% (w/v) sodium tetraethylborate and 0.2 mL of acetate buffer solution (27.2 g of sodium acetate dissolved into 100 mL of an 11.8% acetic acid solution). Ethylation of the aqueous MeHg resulted in volatile ethylmethylmercury, which was purged from solution with N₂ gas and sequestered onto carbon traps. Ethylmethylmercury was thermally desorbed from the traps, separated from other ethylated mercury species with a gas chromatography column (OV-3 stationary phase), and thermally decomposed to Hg⁰ (at 800 °C) before detection by cold vapor atomic fluorescence spectroscopy (CVAFS).

Before analysis for HgT, aqueous samples were prepared following EPA method 1631 (USEPA, 2002). Sediments were digested and oxidized to reduce matrix interferences and to convert all mercury species to Hg(II). Dried sediment (0.1–2.0 g) was weighed into a 50-mL disposable digestion cup, to which 10 mL of concentrated sulfuric/nitric acid in a 3:7 ratio (v:v) was added. Each digestion cup was equipped with a disposable reflux cap and digested at 90 °C in a hot block for 3 hr. Following acid digestion, 30 mL of 0.02 N bromine chloride was
added to the digestate, which was then heated to 40 °C for 12 hr to oxidize all mercury to Hg(II). Immediately before detection, the BrCl-oxidized digestate and aqueous samples were pre-reduced with hydroxylamine hydrochloride to remove free halogen interference.

Ambient MeHg and HgT were detected by CVAFS (Tekran Model 2500) at the University of Wisconsin-La Crosse. Stable isotopes of mercury were quantified by ICP-MS (Blum et al., 2007) at the US Geological Survey Wisconsin District Mercury Laboratory in Middleton, WI.

**Analysis of Ancillary Variables**

Ancillary variables, including organic matter in sediment, and sulfide and sulfate in pore water, were measured in samples taken in 2005. Organic matter in sediment was estimated from the loss of mass following the heating of 2-3 g of freeze-dried and ground sediments at 550°C for 2 hr (Dean 1974), and are reported as loss on ignition (LOI). Sulfate and sulfide concentrations in pore water were determined by ion chromatography with a Dionex ICS-90 IC System and ion specific electrode, respectively (in Standard Methods for the Examination of Water and Wastewater 1995, methods 4110 and 4500-G, respectively).

**Quality Assurance and Control**

Appropriate clean techniques were applied throughout sampling, processing, and analysis to minimize the risk of contamination and undesirable mercury transformations. The clean hands/dirty hands technique was used
during all stages of sample collection, processing, and analysis. Only mercury-free reagent water (nominal resistivity of 18.0 MΩ) and ACS grade trace-metal-clean chemicals were used for sample preparation and analysis. To prevent mercury contamination before use, equipment was acid washed in 50% concentrated HCl (Teflon and glass) or 10% concentrated HNO₃ (other plastics) for 24 hr. Following acid washing, equipment was rinsed with copious amounts of reagent water, dried under a HEPA-filtered laminar flow hood in a mercury free laboratory, and double bagged in plastic bags.

Quality assurance protocols included the use of analytical and procedural blanks, evaluation of matrix interferences with standard additions, precision measurements (as the percent relative standard deviation (RSD)) through triplicate analysis of 10% of the samples, and the analysis of standard reference materials. For ambient MeHg analysis, the recovery of MeHg from a mussel tissue standard reference material (Muss 2976) was 108.8 ± 3.3% (n = 17). The RSD of MeHg concentrations for triplicate analyses of sediments averaged 15.6%, with an average recovery of MeHg spike in those sediments of 139%. Recovery of MeHg quantified via ICP-MS from Muss 2976 was 97.3 ± 3.6% (n = 6) with an average RSD of 10.9% for triplicate analyses of sediments. For ambient HgT analysis, the recovery of HgT from marine sediment (Mess 3) was 91.0 ± 1.4% (n = 15). The average RSD of HgT concentrations for triplicate analyses of sediments was 18.3%, with an average recovery of mercury spike in those sediments of 95.5%. Recovery of HgT via ICP-MS from Mess 3 was 99.8
± 2.3%, and averaged a RSD of 12.7% in triplicate samples and a 98.9% recovery of HgT from spiked triplicates.
RESULTS

Sediment Composition

The texture and composition of the sediment cores varied substantially among the study sites selected for this project. Cores collected at the Kakagon River sites were composed of fine grain sediment with abundant organic matter. The uppermost strata in the Kakagon River cores were dominated by the presence of coarse organic matter, presumably from the abundant emergent wetland vegetation adjacent to the sampling site. Sediment cores from the Fish Creek wetland were primarily comprised of compact clay with some coarse organic material. Sediments at the mouth of the Fish Creek wetland were similar to those observed within the wetland but also included fine sand. Cores from the Sand Cut Slough differed from those at the other study sites, containing shallow (depth ~ 5 cm) coarse sandy strata overlying coarse organic matter. Visual examinations of bulk sediments from this site revealed that benthic macroinvertebrates were abundant in the sandy zones; hence, sediment samples collected in 2005 were targeted from these areas. Sediment cores from the open-water study site were a mixture of medium and fine sand with relatively little organic material.
Ambient Mercury Concentrations in Sediments and Pore Water

Sampling conducted in 2005 was the most comprehensive and significant to the interpretations of this research, however, data (when available) from preliminary samples collected in previous years are also presented. HgT was measured only in samples collected during the 2005 field season, whereas MeHg was measured in sediment samples from all years. To maintain consistency among years, sediment mercury concentrations are reported for surficial sediments, defined here as the 0-3 cm horizon, and are concentrations from homogenized surficial samples (years 2003 and 2004), or the average of the top three sections of sediment cores (2005).

Mean mercury concentrations in surficial sediments and pore waters from Chequamegon Bay are presented in Table 1. Surficial sediment MeHg and HgT concentrations varied several fold among the three coastal wetland sites and the open water site. The greatest HgT concentrations were observed for the cores taken at the Kakagon River wetland and mouth (42.6 and 32.7 ng/g dw, respectively), and at the mouth of the Fish Creek wetland (19.0 ng/g). HgT concentrations were lowest in samples from the Sand Cut Slough wetland (3.6 ng/g) and at the open water site (5.6 ng/g). Similarly, sediment MeHg concentrations in 2005 sediments followed the same trend as HgT among sampling sites. Sedimentary MeHg concentrations were greatest in the Kakagon River wetland and mouth (0.43 and 0.66 ng/g, respectively), as well as the mouth...
Table 1. Observed HgT and MeHg mean, standard error, and range for sediment and pore water samples.

<table>
<thead>
<tr>
<th>Site</th>
<th>Collection Date</th>
<th>Sample Size(^a)</th>
<th>Sed. HgT mean, range (ng/g dw)</th>
<th>Sed. MeHg mean, range (ng/g dw)</th>
<th>MeHg/HgT (%)</th>
<th>Porewater MeHg Mean and range (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRW (wetland)</td>
<td>Aug. 2003</td>
<td>7</td>
<td></td>
<td>0.43 ± 0.11, 0.08 - 0.88</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KRW (wetland)</td>
<td>Sept. 2004</td>
<td>2</td>
<td></td>
<td>0.14 ± 0.01, 0.13 – 0.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>KRW (wetland)</td>
<td>July 2005</td>
<td>5, 4</td>
<td>42.6 ± 2.4, 33.7 – 46.4</td>
<td>0.43 ± 0.20, 0.20 – 1.22</td>
<td>1.1</td>
<td>1.8 ± 0.4, 0.8 – 2.9</td>
</tr>
<tr>
<td>KRW (mouth)</td>
<td>July 2005</td>
<td>5, 4</td>
<td>32.7 ± 1.9,27.3 – 37.3</td>
<td>0.66 ± 0.19, 0.22 – 1.09</td>
<td>1.9</td>
<td>5.1 ± 1.0, 3.0 – 7.9</td>
</tr>
<tr>
<td>FC (wetland)</td>
<td>Aug. 2003</td>
<td>7</td>
<td></td>
<td>0.46 ± 0.09, 0.06 – 0.71</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FC (wetland)</td>
<td>July 2004</td>
<td>6</td>
<td></td>
<td>0.71 ± 0.21, 0.12 – 1.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FC (mouth)</td>
<td>Aug. 2003</td>
<td>7</td>
<td></td>
<td>0.20 ± 0.04, 0.05 – 0.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FC (mouth)</td>
<td>July 2004</td>
<td>6</td>
<td></td>
<td>0.14 ± 0.04, 0.03 – 0.31</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FC (mouth)</td>
<td>July 2005</td>
<td>5, 2</td>
<td>19.0 ± 1.4, 14.7 – 22.8</td>
<td>0.33 ± 0.01, 0.30 – 0.37</td>
<td>1.8</td>
<td>9.1, 8.4 – 9.8</td>
</tr>
<tr>
<td>SCS</td>
<td>Aug. 2004</td>
<td>7</td>
<td></td>
<td>0.42 ± 0.08, 0.17 – 0.74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCS</td>
<td>July 2005</td>
<td>5, 4</td>
<td>3.6 ± 0.5, 2.1 – 4.8</td>
<td>0.06 ± 0.02, 0.01 – 0.12</td>
<td>1.4</td>
<td>1.4 ± 0.5, 0.4 – 2.9</td>
</tr>
<tr>
<td>OW</td>
<td>Aug. 2003</td>
<td>6</td>
<td></td>
<td>0.01 ± 0.004, 0.004 – 0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OW</td>
<td>July 2004</td>
<td>20</td>
<td></td>
<td>0.05 ± 0.01, 0.01 – 0.13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>OW</td>
<td>July 2005</td>
<td>5, 4</td>
<td>5.6 ± 0.4, 4.9 – 6.9</td>
<td>0.02 ± 0.01, 0.01 – 0.04</td>
<td>0.4</td>
<td>1.5 ± 0.7, 0.1 – 3.3</td>
</tr>
</tbody>
</table>

\(^{a}\) Sample number for sediments and followed by pore waters if sampled.
of the Fish Creek wetland (0.33 ng/g). Sampling was not done in the Fish Creek wetland in 2005; however, MeHg in samples taken from this site in 2003 and 2004 were relatively high (0.46 and 0.71 ng/g, respectively). The lowest sediment MeHg concentrations were observed in samples from the Sand Cut Slough wetland (0.06 ng/g) and at the open water site (0.02 ng/g).

Methylmercury concentrations in pore waters varied from 1.4 to 9.1 ng/L but did not reflect the spatial trends observed for the sediment MeHg concentrations. Pore water MeHg concentrations were highest at the mouths of the Fish Creek (9.1 ng/L) and Kakagon River (5.1 ng/L) wetlands, and were relatively low at the other sites (between 1-2 ng/L).

Vertical profiles of mercury concentrations are presented for five sediment cores collected from each site in 2005 in Figure 2. Sediment cores were at least 5 cm deep, with deeper core sections analyzed if successfully retrieved. HgT concentrations in shallow sediments (< 5 cm) were relatively consistent with depth at each site; however the stratigraphy of HgT in the deeper sediments at several sites varied substantially. HgT in deeper strata (below 15 cm) in the Kakagon River wetland decreased by roughly half of those measured in the surficial sediments. In contrast, HgT concentrations in the deeper sediments from the mouth of Fish Creek wetland and from the Sand Cut Slough were approximately 2 and 7 times greater (respectively) than those in the shallow sediments. Vertical profiles of MeHg in sediment cores display a rapidly decreasing trend in concentration with depth at the Kakagon River sites and at the mouth of the Fish Creek wetland, a commonly observed pattern (Hines et al.,
Figure 2. Concentration profiles for HgT and MeHg (ng/g, dw) in 2005 sediment cores. Error bars represent the standard error in concentrations per sediment depth observed in field replicates (see Table 1).
2004; Hammerschmidt et al., 2006; Heyes et al., 2006). MeHg concentrations in the shallow (< 5 cm) sandy sediments at Sand Cut Slough and the open water site were comparably low and show little change with depth. However, similar to the trend observed with the HgT concentration profile, a marked increase in MeHg concentrations occurred in deeper sediments at Sand Cut Slough, corresponding with the transition of sand to coarse “sawdust-like” organic matter.

**Fraction of HgT as MeHg in Sediments**

The fraction of HgT as MeHg was calculated from ambient mercury concentrations in 2005 sediment cores. Overall, the mean MeHg fraction in surficial sediments (top 3 cm) were low (< 2%) and percent MeHg at all wetland associated sites exceeded the open water site by factor of 3-5 (Table 1). The highest mean % MeHg was found at the mouths of the Kakagon River (1.9) and Fish Creek wetland (1.8). Vertical profiles for the fraction of HgT as MeHg are shown in Figure 3. At the Kakagon River, Fish Creek, and Sand Cut Slough

![Fraction of HgT as MeHg in Sediments](image)

**Figure 3.** Depth profile of ambient MeHg/HgT ratio in 2005 sediment cores. Error bars represent the standard error in the ambient MeHg/HgT ratio observed in field replicates (see Table 1).
sites, the MeHg fraction in the surficial sediments are relatively high but decrease rapidly by a factor of 2 – 8 by the 5-10 cm depth interval. Similar stratigraphic patterns have been observed elsewhere (Gilmour et al., 1998; Roulet et al., 2001; Hines et al., 2004). The MeHg fraction at Sand Cut Slough returns to relatively high levels in the highly organic deep sediments.

**Isotopically Determined Mercury Methylation in Sediments**

Mercury methylation rates were estimated by injecting an enriched isotope source of inorganic mercury $^{202}\text{HgCl}$ into intact surficial sediments (0-3 cm depth) of 5 – 6 replicate cores collected from each site in 2005. Preliminary single core estimates collected from each site the previous year (2004) are also included; however, sampling in 2005 was the most comprehensive and significant to the interpretations of this research. The mean observed conversion rate of inorganic $^{202}\text{Hg}$ to $\text{Me}^{202}\text{Hg}$ (also known as the methylation rate constant, expressed as fraction of added inorganic Hg methylated per day) from all sites is presented in Table 2. In 2005, observed methylation rate constants varied substantially between sampling sites, spanning about a 5-fold difference. In these cores, methylation rate constants were highest for the Kakagon River wetland and mouth (0.12 and 0.17 $\text{Me}^{202}\text{Hg}/\text{202HgT day}^{-1}$, respectively), intermediate in Sand Cut Slough and open water (0.067 and 0.075 $\text{Me}^{202}\text{Hg}/\text{202HgT day}^{-1}$), and lowest in the mouth of the Fish Creek wetland (0.030 $\text{Me}^{202}\text{Hg}/\text{202HgT day}^{-1}$). Although not sampled in 2005, three cores from the Fish Creek wetland in 2004 showed notable variability with a 27-fold range in methylation, from 0.03 to 0.81 ($\text{Me}^{202}\text{Hg}/\text{202HgT day}^{-1}$). Estimates of MeHg
Table 2. Estimated mercury methylation rate constant (mean, standard error, and range) in surficial (top 3 cm) sediments from the study sites. Results are calculated based on the 24 hour incubation time following injections of inorganic $^{202}\text{Hg}$ into intact sediment cores.

<table>
<thead>
<tr>
<th>Sample Site</th>
<th>Sample Year (n)</th>
<th>Methylation Rate $(\text{Me}^{202}\text{Hg}/\text{Me}^{202}\text{HgT day}^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRW (wetland)</td>
<td>2004 (1)</td>
<td>0.14</td>
</tr>
<tr>
<td>KRW (wetland)</td>
<td>2005 (6)</td>
<td>$0.12 \pm 0.02, 0.04 - 0.21$</td>
</tr>
<tr>
<td>KRW (mouth)</td>
<td>2005 (6)</td>
<td>$0.17 \pm 0.03, 0.08 - 0.27$</td>
</tr>
<tr>
<td>FC (wetland)</td>
<td>2004 (2)</td>
<td>0.06, .03 - .09</td>
</tr>
<tr>
<td>FC (wetland)</td>
<td>2004 (1)</td>
<td>0.81</td>
</tr>
<tr>
<td>FC (mouth)</td>
<td>2004 (1)</td>
<td>0.10</td>
</tr>
<tr>
<td>FC (mouth)</td>
<td>2005 (5)</td>
<td>$0.03 \pm 0.01, 0.01 - 0.06$</td>
</tr>
<tr>
<td>SCS</td>
<td>2004 (1)</td>
<td>0.11</td>
</tr>
<tr>
<td>SCS</td>
<td>2005 (6)</td>
<td>$0.07 \pm 0.02, 0.02 - 0.12$</td>
</tr>
<tr>
<td>OW</td>
<td>2004 (1)</td>
<td>0.10</td>
</tr>
<tr>
<td>OW</td>
<td>2005 (5)</td>
<td>$0.08 \pm 0.02, 0.04 - 0.16$</td>
</tr>
</tbody>
</table>

demethylation (data not presented) were also attempted via injections of an enriched isotope source of organic mercury $(\text{Me}^{199}\text{HgCl})$ into the sediments. Demethylation (>75%) of the Me$^{199}\text{Hg}$ isotope was almost complete; however, the necessary mass of Me$^{199}\text{Hg}$ injected into the sediments for detection by ICP-MS was underestimated and analyses for the $^{199}\text{Hg}$ isotope (as Me$^{199}\text{Hg}$ as well as the demethylation product $^{199}\text{Hg(II)}$) was typically at or below our analytical capabilities.
Mercury Concentrations in Surface Waters

Total mercury concentrations for surface water samples are shown in Table 3. Overall, the observed concentrations were quite low and varied little among sites. The HgT values observed here are similar to those reported

Table 3. Observed HgT and MeHg results for filtered and unfiltered surface water samples.

<table>
<thead>
<tr>
<th>Site</th>
<th>Sample Date</th>
<th>MeHg (ng/L)a</th>
<th>Part. MeHg (ng/L)</th>
<th>HgT (ng/L)a</th>
<th>Part. HgT (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRW (wetland)</td>
<td>Sept. 2004</td>
<td>0.18, (0.08)</td>
<td>0.10</td>
<td>1.0, (0.49)</td>
<td>0.52</td>
</tr>
<tr>
<td>KRW (wetland)</td>
<td>July 2005</td>
<td>0.29</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KRW (mouth)</td>
<td>Sept. 2004</td>
<td>0.15, (0.05)</td>
<td>0.10</td>
<td>1.1, (0.31)</td>
<td>0.82</td>
</tr>
<tr>
<td>KRW (mouth)</td>
<td>July 2005</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FC (wetland)</td>
<td>Aug. 2004</td>
<td>0.18, (0.17)</td>
<td>0.01</td>
<td>2.1, (0.29)</td>
<td>1.8</td>
</tr>
<tr>
<td>FC (mouth)</td>
<td>Aug. 2004</td>
<td>0.05, (0.05)</td>
<td>-0.001</td>
<td>0.41, (0.42)</td>
<td>-0.01</td>
</tr>
<tr>
<td>FC (mouth)</td>
<td>July 2005</td>
<td>-0.03</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCS</td>
<td>Aug. 2004</td>
<td>NA, (0.22)</td>
<td>NA</td>
<td>0.44, (0.34)</td>
<td>0.10</td>
</tr>
<tr>
<td>SCS</td>
<td>July 2005</td>
<td>0.09</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OW</td>
<td>Aug. 2004</td>
<td>0.27, (0.05)</td>
<td>0.22</td>
<td>0.32</td>
<td>-0.03 b</td>
</tr>
<tr>
<td>OW</td>
<td>July 2005</td>
<td>0.07</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a MeHg and HgT concentration (respectively) in filtered sample follows in parenthesis if measured

b Dissolved HgT estimated from other sampling sites
previously for this region (Rolfhus et al., 2003; Stoor et al., 2006). In unfiltered water, HgT concentrations were greatest in the Fish Creek wetland (2.1 ng/L) and in the Kakagon River wetland (1.0 ng/L) and mouth (1.1 ng/L). Unfiltered surface waters from the other sites were low (range 0.32 – 0.44 ng/L) and similar to HgT concentrations in filtered surface waters (range 0.29 – 0.49 ng/L). The highest MeHg concentration in unfiltered waters was observed at the open water site in 2004 (0.27 ng/L); however, at the same site in the following year MeHg was near the detection limit of 0.04 ng/L. For the other sites included in this study, the MeHg results for unfiltered waters showed more consistency across years. The largest average 2-year MeHg concentrations were measured in the Kakagon River wetland (0.24 ng/L) and mouth (0.14 ng/L). Although not sampled in 2005, unfiltered water from the Fish Creek wetland in 2004 was also relatively high (0.18 ng/L). At the mouth of the Fish Creek wetland and at the Sand Cut Slough, MeHg concentrations in unfiltered waters were at or near the detection limit (0.04 and 0.09 ng/L, respectively). For filtered surface water samples, MeHg was low (< 0.1 ng/L) with the exception of the Fish Creek wetland (0.17 ng/L) and in the Sand Cut Slough (0.22 ng/L).

**Sulfate/Sulfide in Pore Waters and Sediment Carbon**

Measurements of pore water sulfate/sulfide concentrations and sediment organic content made in 2005 are presented in Table 4. Sulfate concentrations varied little among the sampling sites (mean 40.6 μM, range 34.2 – 47.3 μM). In contrast, sulfide and sediment carbon content varied considerably among sites.
Table 4. Results for pore water sulfate and sulfide concentrations (µM) and organic matter content estimated using the loss on ignition (LOI) proxy in sediments.

<table>
<thead>
<tr>
<th>Sample Site</th>
<th>Sulfate</th>
<th>Sulfide</th>
<th>Sediment Carbon (% LOI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRW (wetland)</td>
<td>36.3 ± 2.9</td>
<td>40.3 ± 8.0</td>
<td>14.9 ± 0.98</td>
</tr>
<tr>
<td>KRW (mouth)</td>
<td>40.2 ± 2.0</td>
<td>36.5 ± 7.7</td>
<td>13.0 ± 1.0</td>
</tr>
<tr>
<td>FC (mouth)</td>
<td>45.1 ± 0.9</td>
<td>10.4 ± 2.2</td>
<td>2.5 ± 0.14</td>
</tr>
<tr>
<td>SCS</td>
<td>34.2 ± 1.6</td>
<td>6.7 ± 1.3</td>
<td>2.5 ± 0.95</td>
</tr>
<tr>
<td>OW</td>
<td>47.3 ± 2.6</td>
<td>5.7 ± 1.2</td>
<td>0.9 ± 0.05</td>
</tr>
</tbody>
</table>

Sulfide concentrations in pore water ranged approximately 7-fold, with the greatest concentrations in the Kakagon River wetland and mouth sites (40.3 and 36.5 µM, respectively). The mouth of the Fish Creek wetland had intermediate sulfide concentrations (10.4 µM), and the Sand Cut Slough and open water site had the lowest of all the sites (6.7 and 5.7 µM, respectively). Organic matter content in sediments, measured as percent loss on ignition, in the Kakagon River wetland and mouth were relatively high (14.9% and 13.0% LOI, respectively), while the sediments from the other sites contained comparably little organic matter (0.9% – 2.6% LOI).
DISCUSSION

Spatial Distributions of Mercury Concentrations

Our first objective was to examine the sedimentary-mercury concentrations in three coastal wetlands and one non-wetland near-shore environment in Chequamegon Bay. Despite the biological importance of Lake Superior coastal wetlands, reports of sedimentary HgT and MeHg in such habitats are few. We selected sampling sites anticipating differences in tributary influence, benthic conditions, and sedimentary mercury levels, with the intent of reflecting conditions of relatively pristine coastal wetlands found elsewhere on the south shore of Lake Superior. We found contrasting conditions among our sampling sites, with little evidence of localized human contamination, in contrast to the impacts reported for the St. Louis River estuary near Duluth, MN (Edsall et al., 2004). Our sites included various size fractions of sand and organic matter, clay, and silt typical of Lake Superior. Isotopic analysis of carbon from our sites (presented by Wiener et al., 2008) show that δ^{13}C values in the wetland areas (mean -26.5 ‰, range -20.5 – -31.7 ‰) are consistent with δ^{13}C values in terrestrial vegetation (Deines, 1980) suggesting that sedimentary carbon at these sites are largely of allochthonous origin. Conversely, δ^{13}C values in sediments from the open water sites (range -16.0 – -19.3 ‰) are closer to the isotopic signature in atmospheric CO₂ (-8 ‰), and may reflect more recent and direct
autochthonous fixation of atmospheric CO$_2$. Sediment in the Sand Cut Slough had the lowest $\delta^{13}$C value (-31.7 \text{‰}), suggesting the highest amount of terrestrial influence. This is surprising considering that it lacks riverine inflow and accordingly has less direct connection with the watershed than the other two wetland sites. We suspect that much of the organic matter in Sand Cut Slough was remnant woodchips from the extensive wood milling that occurred in Ashland from approximately the 1870’s to the early 1900’s. Substantial quantities of wood waste from milling operations were disposed of in the bay, with remnants up to 2 m thick still covering the historic lakebed near Ashland (USEPA, report ID# WISFN0507952). Submerged saw-logs are still present in the slough and are evidence of debris from historic wood milling legacy in Chequamegon Bay. Benthic macroinvertebrates (isopods, amphipods, hexegenia mayflies, fingernail clams, and unionid bivalves) and fish (Johnny Darter and yellow perch) were collected at all sites (Wiener et al., 2008), indicating that all of our sites were important habitat for Lake Superior biota.

Overall, our data show that Chequamegon Bay sediments are lightly contaminated with HgT, containing concentrations that are less than those in many sediments (Figure 4) from other lightly contaminated aquatic ecosystems (Benoit et al., 2002). Similarly, HgT concentrations in Chequamegon Bay sediments tend to be less than those that were collected from pelagic Lake Superior. Measurements of sediment HgT levels from the main lake ranged 83 – 88 ng/g dw (Rolfhus et al., 2003; Marvin et al., 2004), and were substantially higher than measurements at the open water site (5.6 ng/g) as well as in the
wetland areas (3.6 – 42.6 ng/g) in Chequamegon Bay. There is some history of point-source mercury pollution in Lake Superior, primarily from mining and smelting near Thunder Bay and the Keeweenaw Peninsula (Kerfoot et al., 1999), which may have resulted in elevated sediment HgT concentrations in the main lake basin. There is no history of such activities near Chequamegon Bay, which evidently was not impacted by historic point sources of mercury.

Sedimentary MeHg concentrations were also relatively low in Chequamegon Bay (Figure 4) compared to other uncontaminated systems around the world (Benoit et al., 2002). While comparisons with data from ecosystems in other geographic areas are useful, they must be made carefully. Unlike HgT, sediment MeHg concentrations are extremely dynamic, with ambient MeHg concentrations varying in response to methylation and demethylation reactions that depend on a
complex suite of variables that influence the bioavailability of Hg(II) substrate and metabolic activity of SRB's. Although we would like to compare our sediment MeHg measurements to other Lake Superior coastal wetlands, reports of this nature are lacking. However, compared to other regional ecosystems the sedimentary MeHg concentrations in Chequamegon Bay (mean 0.29, range 0.01 – 0.71 ng/g dw, respectively) are similar to levels in lakes at Voyageurs National Park in Minnesota (0.04 – 0.25 ng/g dw, Rolfhus, unpublished data) and in the Lake Superior basin (0.21 ng/g dw, Rolfhus et al., 2003). Within the scope of global and the limited regional measurements, it appears that sediments from Chequamegon Bay are relatively uncontaminated with MeHg.

Sediment mercury concentrations were high relative to the open water site in all of the coastal wetlands in Chequamegon Bay (Figure 5). These results were

![Graph](image)

Figure 5. Mean MeHg and HgT concentrations in sediments at each site. Error bars represent the standard error in concentration from replicate samples (see Table 1).
expected because the highly organic ecological state in wetlands favors solid phase HgT binding and MeHg formation in sediments relative to the sandy inorganic conditions in the open water site. However, HgT and MeHg concentrations were further elevated in the wetlands that had tributary influence. It is reasonable to assume that the transport of mercury associated with particulates and organic matter from upstream sources, including the watershed, would increase mercury levels in the receiving wetlands. The riverine wetlands were relatively enriched with terrestrial carbon (Wiener et al., 2008) which directly influences sediment mercury levels and suggests a watershed Hg source. In the Tahquamenon River, another Lake Superior tributary, Stoor et al. (2006) observed elevated mercury concentrations in unfiltered waters (HgT 2 – 10 ng/L, MeHg 0.1 – 0.35 ng/L) relative to levels in Lake Superior (HgT <1 ng/L, MeHg <0.015 ng/L in Rolfhus et al., 2003), indicating downstream transport, with the highest surface water mercury concentrations corresponding with spring snowmelt and flooding. We found particulate mercury concentrations were only slightly elevated relative the open water for HgT (and not convincingly so for MeHg) during our July and August sampling (Table 3). At our riverine wetland sites enrichment with mercury from upstream is a likely significant contributor to sediment mercury concentrations, however transport is temporally variable and was not observed during our sampling period.

Watershed size was not as important as watershed type and processes in determining wetland mercury levels within Chequamegon Bay. Mercury levels in the Kakagon River wetland were greater than those in Fish Creek, even though
the Fish Creek watershed was 2.7 times larger than the Kakagon River basin, and was a substantial contributor of erosional material to the bay (Fitzpatrick et al., 2004). The Kakagon River watershed is within 16 km and generally downwind of a coal-fired power plant, and has a larger proportion of wetland cover (15%) relative to Fish Creek (4.5%). The higher HgT and MeHg concentrations in the Kakagon River wetland may be the result of increased local deposition of inorganic mercury in the watershed, coupled with a relatively large wetland presence.

Sediment MeHg concentrations were relatively consistent at each site across years, however there were a couple of notable exceptions. The MeHg concentration in Kakagon River wetland sediment (0.14 ± 0.01 ng/g) in 2004 was substantially less than the range of measurements in samples taken from this site during other years (0.43 – 0.66 ng/g). This might reflect the heterogeneous nature of sediments, but is more likely the result of seasonal changes in net methylation that cause temporal variability. Methylmercury is the product of in situ production, and the turnover time of sedimentary MeHg pools is relatively short (Gilmour et al., 1992). There was approximately a 50- and 80-day difference from the 2003 and 2005 (respectively) sampling dates, with the 2004 sediments being sampled late in September. Although surface water temperatures were not recorded, it is likely that they were much lower in 2004 than for the other years, and could have led to a reduced rate of MeHg formation. The other notable exception was for the 7-fold difference of MeHg concentration at the Sand Cut Slough, which we attribute to heterogeneity of the sediments.
across years. Samples from 2004 were relatively organic (mean LOI = 20.3%) and were collected from the “wood chip” substrate that is favorable to MeHg formation, while the surficial sediments from 2005 contained little organic matter (mean LOI = 0.9%) and were almost entirely coarse sand.

Vertical profiles of sediment HgT concentration varied substantially with depth in sediment cores, however, clear trends across all sites were not observed (Figure 2). Attenuation of HgT values to a constant “baseline” value, as reported in other studies (Hines et al., 2004; Lindberg et al., 2007), did not occur in our sediment cores. Several factors may have caused this. There was considerable difference in sediment composition across sampling sites, which may influence sediment-HgT complexation, particularly in the inorganic sandy sediments from Sand Cut Slough and the open water site. Concentrations at our sites were also low, with the most recent surficial sediment layer already within the range of background concentrations (16 – 48 ng/g dw, Rossmann 1999) measured in the lake basin. It is unlikely that the cores were deep enough to sample preindustrial sediments. Kerfoot (1999) measured background HgT levels at sediment depths greater than 15 cm in pelagic Lake Superior were sedimentation rates are probably much lower than those in Chequamegon Bay.

Vertical profiles of sediment MeHg concentration varied substantially with depth in sediment cores (Figure 2), with concentrations decreasing rapidly within the first 10 cm at the Kakagon River and Fish Creek sites. This is a typical sediment core MeHg profile and is thought to reflect the changing redox potential in sediments, which is most favorable to SRB activity and MeHg production in the
top 2-5 cm of sediments. Interestingly, MeHg substantially increased in the deeper sediments of Sand Cut Slough at the transition of coarse sand to organic matter. The shallow inorganic sediments (0 – 5 cm) at this site are not favorable to mercury retention, contain little organic matter supportive of MeHg formation, and would be easily penetrated by oxygenated overlying water causing the oxic-anoxic interface favorable to SRB to occur deeper in the sediments.

**Relation of Sediment MeHg to Hypothesized Controlling Factors**

The second objective of this study was to assess factors that potentially influence sediment MeHg levels in Chequamegon Bay. The formation of sediment MeHg, which leads to uptake into the food web, is regulated by environmental factors that control (1) activity of methylating bacteria and (2) the bioavailability of the inorganic Hg(II) in sediments for methylation (Marvin-DiPasquale *et al.*, 2009). We measured environmental variables--including sulfate, sulfide, HgT, and organic carbon content--that potentially control MeHg production. These environmental variables affect both mercury bioavailability and bacterial activity and can have competing effects on MeHg production (Figure 6). Bacterial activities (i.e., sulfate-reduction rates) were not measured directly; however, the methylation activities of these bacteria were approximated with measurements of the methylation of added inorganic mercury isotope tracers.
Across sampling sites there was a positive relation ($t = 2.9$, $p = 0.063$) between sediment MeHg and HgT concentrations (Figure 7). This relation supports the hypothesis that MeHg concentrations are directly influenced by HgT content; however, caution should be used in extrapolating this relation beyond our sampling sites, especially outside of the Lake Superior basin. The relation between sediment HgT and MeHg ($r^2 = 0.74$), with about 1.5% of HgT as MeHg (Figure 6), fits well within the data set (Figure 4) from a wide range of
ecosystems examined by Benoit et al. (2002). It is important to note that our measurements of HgT concentration do not necessarily reflect the bioavailability of Hg\textsuperscript{II} to SRB for methylation, which can strongly influence MeHg concentrations (Orihel et al., 2008; Marvin-DiPasquale et al., 2009).

Figure 7. Observed relation between mean MeHg and HgT concentrations in individual sediment samples. Error bars represent the standard error of the mean for replicate samples.

The fraction of HgT measured as MeHg (MeHg/HgT) is a reasonable indicator of methylation rate (Marvin-DiPasquale et al., 2009), reflecting recent production of MeHg in sediments while simultaneously accounting for demethylation reactions. In general, we found a higher proportion of MeHg in wetland sediments (1.1 – 1.9%) relative to the open water site (0.4%), indicating that methylation rates are higher in the wetland areas, particularly at the mouths of the Kakagon River (1.9%) and Fish Creek (1.8%). However, MeHg/HgT measurements as a proxy of mercury methylation do not account for diffusional
losses of mercury to overlying water, which may be significant, particularly in relatively inorganic sediments. Vertical profiles of MeHg/HgT were elevated in the 0 – 5 cm horizon and decreased rapidly with depth in wetland sediments (Figure 3.). This likely reflects the expected trend of elevated methylation rates that decrease with depth at the Kakagon River and Fish Creek wetlands, probably due to the greater microbial activity of methylating bacteria in shallow sediments. However, the observed MeHg/HgT ratio does not likely reflect methylation rates in the relatively mercury-free sandy inorganic 0-5 cm sediment horizon of Sand Cut Slough. Although MeHg/HgT was within the range of other wetland sites (and followed the typical decreasing trend), elevated MeHg/HgT ratios in sediments at Sand Cut Slough were potentially biased high due to analytical variability inherent in the analyses of sediments with extremely low mercury concentrations. We suspect that mercury methylation is primarily occurring in the relatively organic deep sediments (> 5 cm) at Sand Cut Slough, which is evident in the relatively high MeHg/HgT (1.0 – 2.6%).

Mercury methylation rates were also directly estimated by the addition of inorganic mercury isotopes to sediment cores from each site and are presented in Table 2. In 2005, the highest relative mean methylation rates (expressed as the fraction of added Hg(II) methylated per day) from multiple cores at each site were observed in the Kakagon River wetland, whereas estimates at the Sand Cut Slough and Fish Creek mouth were similar and less than the open-water site. We expected estimations at the open-water site would be low, and less than the wetland sites. The relatively elevated methylation rates at the open-water site
may be explained by low levels of sulfide and organic matter, and has been attributed to increased methylation potential due to increased bioavailability of Hg(II) in marine sediments (Marvin-DiPasquale et al., 2009). Sulfide and organic matter were similar in the surficial sediments (0-3 cm) of Sand Cut Slough and the open-water sites (Table 4), potentially leading to the observed similarity in methylation rate estimates. However, this explanation fails to account for elevated rates at the sulfide- and organic-rich Kakagon River sites. Although Fish Creek was not sampled in 2005, several cores were collected in 2004. The estimated relative methylation rates for two of these cores (0.03 and 0.09) were within the range observed in 2005 and likely reflect the typical range for much of the wetland. However, methylation of the mercury spike was highly variable and nearly 100% in some sections of one core from the Fish Creek wetland. Although methylation rate estimates by all methods, including the stable isotope approach used here, are often fraught with problems that complicate interpretation, the very high rates observed at the Fish Creek wetland indicate that conditions are conducive to MeHg production by methylating bacteria.

Isotopic methylation rates estimated in this study were highly variable at each site and were high relative to estimates in other published papers (Table 5). This could be the result of several factors. First, we did not “precondition” the inorganic added mercury with native pore water to equilibrate and complex the raw spike with naturally occurring sulfide and dissolved organic matter; thus the added Hg(II) may have been more bioavailable in our study. However, Hammerschmidt et al. (2004) shows that complexation of added inorganic Hg(II)
Table 5. Mean and/or range of methylation rate estimates from other studies.

<table>
<thead>
<tr>
<th>Environment</th>
<th>Methylation Rate (% d⁻¹)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Canadian lake sediments</td>
<td>1.2 – 1.6</td>
<td>Hintelmann et al., 2000</td>
</tr>
<tr>
<td>Estuarine sediments</td>
<td>3.3</td>
<td>Martin-Domeadios et al., 2004</td>
</tr>
<tr>
<td>Canadian lake water</td>
<td>No detection – 14.8</td>
<td>Eckley et al., 2006</td>
</tr>
<tr>
<td>Estuarine sediments</td>
<td>0.2 – 4.4</td>
<td>Heyes et al., 2006</td>
</tr>
<tr>
<td>Freshwater, brackish, and marine sediments</td>
<td>&lt;0.1 – 8.0</td>
<td>Hines et al., 2006</td>
</tr>
<tr>
<td>Estuarine sediments</td>
<td>&lt; 1</td>
<td>Kim et al., 2006</td>
</tr>
<tr>
<td>Freshwater and brackish sediments</td>
<td>0.1 - 5</td>
<td>Drott et al., 2007</td>
</tr>
<tr>
<td>Marine sediments</td>
<td>4.6 (1.2 – 11.5)</td>
<td>Hammerschmidt et al., 2008</td>
</tr>
<tr>
<td>Chequamegon Bay</td>
<td>9.4ᵇ (2.8 – 13.6, 81.1ᵃ)</td>
<td>This study, 2004</td>
</tr>
<tr>
<td>Chequamegon Bay</td>
<td>9.1 (3.0 – 16.7)</td>
<td>This study, 2005</td>
</tr>
</tbody>
</table>

ᵃ Methylation rate of single core in Fish Creek wetland, not included in reported mean or range
ᵇ Average of methylation rates in six cores

was very rapid in pore waters following injections, and the lack of a preconditioning step in our isotopic additions may not have been a significant factor. We increased the relative abundance of ²⁰²Hg from 5 to 25 fold with our mercury spike, potentially saturating native microbial communities with inorganic mercury. Sediment HgT concentrations (native and isotopic) were less than 250 ng/g, which is within the range of uncontaminated sediments, and were not correlated with methylation rates. Alternatively, our study area may be highly sensitive to new mercury inputs and the existing sedimentary Hg in this
ecosystem may be largely unavailable for methylation. Ambient measures of methylation show that only 1% to 2% of mercury in sediments is methylated (Table 1) and is much less than isotopic estimates, and no relation is evident between the isotopic and ambient methylation estimates (Figure 8). These

![Figure 8](image-url)

Figure 8. Observed relation between the calculated methylation rate constant and the observed ambient MeHg/HgT ratio in surficial (top 3 cm) sediments. Error bars represent the standard error of the mean for replicate samples.

isotopic mercury methylation estimates could reflect the natural mercury methylation rates; however, they may also be a result of a enhanced methylation of newly added mercury. Orihel *et al.* (2008), who used stable mercury isotopes as tracers to simulate mercury deposition to a Canadian lake, found that recently deposited mercury is more bioavailable and rapidly methylated in the sediments and incorporated into the food web at a higher rate. The highly bioavailable “raw” mercury spikes that we used may be analogous to newly deposited atmospheric
Hg(II) that has not been equilibrated with native organic and sulfide-containing complexing agents. Our study similarly suggests that relatively uncomplexed mercury added to Chequamegon Bay sediments will be rapidly methylated.

One of the most important relationships that we examine in this study was that between estimated methylation rate and sediment MeHg concentrations. In sediments sampled in 2005 we found a modest positive relation \((r^2 = 0.50)\) between mercury methylation estimates and sediment MeHg concentrations (Figure 9). This statistical relation improved substantially \((r^2 = 0.97)\) when the data point for the Fish Creek mouth was excluded. During the 2005 sampling effort, flooding in Fish Creek was eroding a substantial amount of sediment from the wetland to the wetland mouth. The relatively MeHg rich sediments

![Graph showing observed relation between mean MeHg concentrations and the methylation rate constant in surficial (top 3 cm) sediments. Error bars represent the standard error of the mean for replicate samples.](image)

Figure 9. Observed relation between mean MeHg concentrations and the methylation rate constant in surficial (top 3 cm) sediments. Error bars represent the standard error of the mean for replicate samples.
transported from the wetland could have temporarily elevated sediment MeHg levels at the mouth; the 2-year average of sediment MeHg concentrations from the previous years was half that in 2005. It appears that in Chequamegon Bay coastal wetland sediments, mercury methylation rates are related to (and perhaps controlling) ambient MeHg concentrations. Isotopically derived mercury methylation rates are an approximation of actual methylation rates and have been correlated with in situ sediment MeHg concentrations in other ecosystems (Gilmour et al., 1998; Hammerschmidt et al., 2006; Heyes et al., 2006; Kim et al., 2006; Drott et al., 2008; Marvin-DiPasquale et al., 2009).

![Graph showing the observed relation between pore water sulfide concentration and sediment carbon content in surficial (top 3 cm) sediments. Error bars represent the standard error of the mean for replicate samples.](image)

\[
y = 2.56x + 2.53 \\
{r^2} = 0.993
\]

Figure 10. Observed relation between pore water sulfide concentration and sediment carbon content in surficial (top 3 cm) sediments. Error bars represent the standard error of the mean for replicate samples.

We also examined sediment variables that potentially influence MeHg concentrations through inorganic mercury bioavailability or stimulation of SRB. Sulfate levels were relatively uniform across sampling sites with no clear trends
observed, and no apparent relation to MeHg levels. This was unexpected given the considerable differences in location and sediment types among our sample sites. However, we found considerable variability in pore water sulfide and organic matter content among sampling sites. There appears to be a relatively strong relation ($r^2 = 0.99$) between sediment carbon content and sulfide levels (Figure 10), suggesting that sediment carbon controls sulfide levels via bacterial sulfate reduction, which may in turn influence sediment MeHg concentrations. There is relatively good positive relations ($r^2 = 0.69$ and $r^2 = 0.74$, respectively) between sediment MeHg concentration and organic matter (Figure 11) and pore water sulfide (Figure 12). Additionally, the Kakagon River wetland and mouth had the greatest organic matter and sulfide levels, and subsequently the highest mercury methylation rate estimates. At Sand Cut Slough, which had the greatest range of values, organic matter and MeHg concentrations were strongly correlated ($r^2 = 0.75$). We suggest that among our sample sites and potentially in other Lake Superior coastal areas, organic matter is driving sulfate reduction, for which the end results of sulfide and MeHg are being coproduced.

The Implications of This Research on Lake Superior and its Biota

The primary impetus for this research was to examine mercury in near-shore areas of Chequamegon Bay, focusing primarily on south shore coastal wetlands, in the context of bioaccumulation in Lake Superior food webs supporting fish production. Although Lake Superior and its watershed are lightly contaminated
with mercury, predatory game fish contain high levels of MeHg. In spite of this, there are only a limited number of reports since the development of sensitive analytical techniques of mercury concentrations and MeHg formation rate.
estimates in Lake Superior waters or sediments. Coastal wetlands are abundant on the southern shore, are critical habitat for Lake Superior biota, and are habitats most likely for MeHg formation. We focused on methylation in sediments, which are the primary site of MeHg formation and presumably important sources of MeHg entering the base of the food web in Lake Superior coastal wetlands.

We found sediment HgT levels are extremely low in our study area compared to generally observed levels (Kerfoot et al., 1999; Rolfhus et al., 2003). Total mercury concentrations in sediments were not high, indicating that the elevated biotic concentrations in game fish are not the result of intensive mercury pollution. To our knowledge, these are the first in-depth reports of sediment HgT and MeHg concentrations in Lake Superior coastal wetlands. However, since direct atmospheric deposition is the primary source of mercury to the lake (Rolfhus et al., 2003) we suspect that many of the other pristine Lake Superior coastal wetlands are also lightly contaminated. Sedimentary MeHg concentrations were correspondingly low but we are cautious about extrapolation to other Lake Superior wetland areas. Although this study shows a good relation of MeHg with HgT levels (which we predict to be low), MeHg concentrations were also related to other variables (sulfide and organic matter). In general, we would expect sediment MeHg concentrations in uncontaminated coastal wetlands to be low and to vary in relation to site specific conditions in the wetland.

Compared to the open water Lake Superior site, coastal wetland sediments are relatively high in MeHg concentrations. These wetland areas are important
habitat for Lake Superior biota. Although absolute concentrations are low, it is likely that much of the biological MeHg is produced in situ in coastal wetland sediments and transferred to the food web within these areas. Mercury concentrations in benthic invertebrates and small yellow perch (a prey fish that feeds on zooplankton and small zoobenthos) were higher in the Kakagon River wetland relative to the open water site (Table 6, Wiener et al., 2008). In the same study, mercury concentrations in a benthivorous prey fish (Johnny Darter) were also elevated at the Fish Creek mouth and Sand Cut Slough. This suggests that MeHg levels in the lower trophic levels reflect production and

<table>
<thead>
<tr>
<th>Site</th>
<th>BI MeHg (ng/g dw)</th>
<th>YP HgT (ng/g dw)</th>
<th>JD HgT (ng/g dw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KRW (wetland)</td>
<td>55</td>
<td>153 (60)</td>
<td></td>
</tr>
<tr>
<td>FC (wetland)</td>
<td></td>
<td>64 (35)</td>
<td></td>
</tr>
<tr>
<td>FC (mouth)</td>
<td></td>
<td>89 (25)</td>
<td>109 (40)</td>
</tr>
<tr>
<td>SCS</td>
<td>21</td>
<td>68 (78)</td>
<td>94 (32)</td>
</tr>
<tr>
<td>OW</td>
<td>15</td>
<td>61^b (15)</td>
<td>62^b (34)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>40^c (66)</td>
</tr>
</tbody>
</table>

^a Sample size in parenthesis following value
^b Collected near Chequamegon Point
^c Collected at the open water site

Table 6. Methylmercury concentrations (from Wiener et al., 2008) in pooled benthic invertebrates (BI), and HgT concentrations in Yellow Perch (YP, total length <90 mm) and Johnny Darter (JD, total length 35 – 73 mm) collected in Chequamegon Bay.
abundance of MeHg in the local sediments, and may be an important intermediate transfer agent to the upper trophic level prey species.

We also examined suspected controls of sediment MeHg to gain insight on the processes driving sediment concentrations and subsequent exposure to Lake Superior biota. There was a positive and relatively strong relation between HgT and MeHg levels in sediments of Chequamegon Bay, and measured methylation rates were correspondingly high at this site. These observations yield the conclusion that Chequamegon Bay, and probably other near-shore areas of Lake Superior, are sensitive to mercury inputs. Changes in atmospheric emissions and deposition of mercury could have a substantial influence on MeHg in Lake Superior and its biota. Wetland MeHg concentrations were also positively related to the presence of a tributary as well as organic matter content and sulfide levels in sediments. Changes within the relatively undeveloped Lake Superior watershed that resulted in increases in the mobilization and transport of particulate bound mercury, allochthonous organic matter in the receiving wetland, and eutrophication of near-shore habitats could simultaneous increase sediment MeHg levels downstream.
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