Geochemical factors influencing the production and transport of methylmercury in St. Louis River Estuary sediment

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A B S T R A C T

The production of methylmercury (MeHg), a bioaccumulative neurotoxin, in freshwater systems is primarily driven by naturally occurring sulfate reducing bacteria in anoxic sediment and waters. This research used laboratory microcosms to examine the influence of sulfate on MeHg production and partitioning in sulfate-impacted freshwater estuary sediment. A laboratory sulfate addition experiment exposed 20 cm diameter intact sediment cores with varying organic carbon content to sulfate concentrations in the overlying water ranging from 5 to 50 mg L−1. Results from the 6 month incubation suggest that net MeHg production in sediment from open-water areas of the St. Louis River Estuary was not directly related to overlying water sulfate. Mercury mobility, as indicated by porewater concentrations, appeared to be related to the quantity of organic carbon and sulfur in sediment. Laboratory flux estimates were consistent with porewater concentrations and provided a means to compare diffusion-driven MeHg loading from sediment to MeHg loading from upstream sources.

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1. Introduction

Methylmercury (MeHg) is a bioaccumulative organo-metallic neurotoxin that poses a risk to human and ecosystem health and has led to widespread fish consumption advisories around the world (National Research Council, 2000). The production of MeHg from inorganic mercury is known to be mediated by sulfate reducing bacteria (SRB) (Compeau and Bartha, 1985; Gilmour et al., 1992), though recent findings are rapidly advancing the understanding of which specific strains of SRB and other microorganisms possess the capacity to methylate mercury (Parks et al., 2013; Kerin et al., 2006; Yu et al., 2012; Gilmour et al., 2013). A vast body of empirical evidence suggests that SRB are the primary methylators of mercury in aquatic sediments. Sulfate reducing bacteria require sulfate (Gilmour et al., 1992; Jeremiason et al., 2006; Mitchell et al., 2008), organic carbon (King et al., 2000; Kim et al., 2011; Lambertsson and Nilsson, 2006; Mitchell et al., 2008), inorganic mercury (Benoit et al., 2003; Hammerschmidt et al., 2008; Hammerschmidt and Fitzgerald, 2004), and anoxic conditions (Compeau and Bartha, 1984; DeLaune et al., 2004) in order to produce MeHg. Rates of MeHg production (methylation) in anoxic sediments however, are often not directly related to either sulfate or organic carbon alone due to a host of interrelated biogeochemical processes (Fitzgerald et al., 2007; Benoit et al., 2003). A variety of bacteria are capable of mercury demethylation (Hintelmann et al., 2000) and the balance between MeHg production and degradation (net methylation) defines the quantity of MeHg found in aquatic sediments (Drott et al., 2008).

The addition of dissolved, labile organic carbon to aquatic sediment can yield high mercury methylation rates if sulfate is not limiting SRB metabolism (Mitchell et al., 2008). Additionally, the presence of abundant in situ organic carbon can provide favorable conditions for the production of MeHg (Lambertsson and Nilsson, 2006). However, solid-phase organic carbon is highly correlated with mercury apparent partitioning coefficients (Kd – the ratio of adsorbed to dissolved mercury), such that abundant solid-phase carbon can reduce the porewater inorganic mercury available to methylating microbial populations in some situations (Hammerschmidt et al., 2004; Turner et al., 2001; Bower et al., 2008).

Studies on many spatial scales have shown rapid increases in MeHg production in response to amended sulfate. Small scale lab studies on homogenized, well-mixed sediment and pure biological cultures have been utilized to identify the specific mechanisms regulating mercury methylation (Compeau and Bartha, 1985; Hintelmann et al., 2000; Parks et al., 2013), but there is a disparity
between these laboratory studies and in situ conditions due to competition among naturally occurring microbial populations and transport limitations of the natural setting (St. Louis et al., 1994; Balogh et al., 2006). Many field studies have focused on the production of MeHg in wetlands or peatlands (Harmon et al., 2004; Branfireun et al., 1999; Jeremiason et al., 2006; Mitchell et al., 2008) due to their role as net exporters of MeHg to freshwater and coastal systems. While performed under in situ field conditions, the sulfate additions in these studies were designed to mimic atmospheric sulfate loads from precipitation and were typically applied periodically to partially or seasonally inundated natural settings. This limits the utility of previous research in understanding the influence of sulfate loading on mercury methylation in fresh water sediments underlying rivers and lakes, which are continually inundated and can be subject to continually elevated sulfate concentrations in overlying water.

Though not as hydrologically dynamic as wetlands or peatlands, anoxic sediments underlying water with elevated sulfate have the potential to receive loadings of sulfate similar to or larger than that of direct atmospheric deposition due to passive molecular diffusion alone, a process which can be enhanced by bioturbation (Seliers et al., 2001; Hines et al., 2004). Additionally, aquatic sediments are continuously and directly connected to the water column where fish and other ecological receptors are exposed to MeHg. In slow-moving rivers, lakes, reservoirs, or estuaries, interactions with underlying sediment can be important in the context of upstream MeHg loads typically dominated by rain-event based inputs from upstream or adjacent wetlands (Balogh et al., 2006). In this research, sulfate loads were applied by carefully controlling sulfate concentrations in water above intact cores containing sediment from the open-water portions of a freshwater estuary (Berndt and Bavin, 2012a).

2. Study setting

The landscape of the uppermost regions of the St. Louis River watershed (9283 km²) has been heavily influenced for nearly 100 years by iron mining (Fig. 1) which has elevated sulfate concentrations in downstream surface waters. Sulfate loads from mining-impacted watersheds comprise a majority of sulfate in the river during some times of the year (Berndt and Bavin, 2012a) and concentrations in the main stem of the St. Louis River immediately downstream from the mining district routinely exceed 50 mg L⁻¹ during low flow conditions (Berndt and Bavin, 2009). Regional geology containing substantial carbonate (Zanko et al., 2008) has maintained near-neutral or alkaline pH in mining-impacted waters; however, recent proposals to initiate copper and nickel mining in the region have raised the collective awareness of sulfur-related issues in the minds of state resource managers. Since both sulfate in surface waters (Berndt and Bavin, 2012a) and MeHg in fish tissue (Wisconsin DNR, 2012) are elevated in the St. Louis River, understanding whether sulfate concentration in the overlying water is important in driving MeHg production and transport from river and estuary sediments is a necessary step for defining effective management solutions aimed at reducing MeHg in fish tissue.

The St. Louis River Estuary (SLRE) is a large (50 km²) freshwater estuary that is used extensively for recreation and serves as an important nursery habitat for western Lake Superior (Hoffman et al., 2010). The estuary contains diverse habitat, ecology, and geomorphology, and the lower portions of the SLRE remain one of the busiest ports on the Laurentian Great Lakes. Habitat Zones – areas delineated by local resource managers as having similar ecological significance (St. Louis River Alliance, 2002) – were used in this study to select sites that encompass the range of solid phase organic carbon quantity present in the open-water estuary sediment (2–6%, Online Supplemental Fig. S1). The Sheltered Bay (SB) site is characteristic of many sparsely vegetated backwater bays isolated from main-channel hydraulics in which primary production provides significant carbon inputs. The Upper Estuary Flats (UEF) site is outside the main historically dredged channel upstream near the point where the St. Louis River widens to become an estuary. The Lower Estuary Flats (LEF) site is outside the actively dredged channel near Lake Superior in an area

Fig. 1. St. Louis River Estuary with habitat zones and field sample locations; (inset) St. Louis River watershed, Cloquet River, and iron mining features at the western tip of Lake Superior in Northeastern Minnesota, USA.
routinely influenced by Lake Superior water and the effect of its seiche (Hoffman et al., 2010; Sorensen et al., 2004). Due to large upstream sulfate loads, water column sulfate concentrations are relatively constant throughout the estuary.

3. Methods

Intact sediment cores were collected from locations in three characteristically different habitat zones and analyzed for MeHg and related geochemistry. The sediment cores were then used to create laboratory microcosms, which were incubated in lab conditions with varying sulfate concentrations in the overlying water for six months.

3.1. Field methods and experimental design

Four large diameter (20 cm, polycarbonate) intact sediment cores were collected from field sites (Fig. 1) in August 2011. The cores were carefully transported to the lab and stored at 20 °C for one week in a climate control chamber with recirculating oxygenated salt water containing 15 mg L\(^{-1}\) sulfate. Triplicate subcores were extracted from each large intact core to obtain vertical profiles of solid phase THg, MeHg, and related chemistry for the three habitat zones (detailed methods in following section).

To initiate experimental sulfate treatments, lab microcosms were created by drilling two holes into the polycarbonate cores 10 cm above the sediment-water interface and connecting tubing. All overlying water experimental sulfate treatments were made using water from a tributary to the St. Louis River (Cloquet River, Fig. 1) containing dissolved organic carbon (DOC) and mercury concentrations similar to the SLRE (18–40 mg L\(^{-1}\) DOC, 1–6 ng L\(^{-1}\) Hg) but lower sulfate concentrations (2.5–5 mg L\(^{-1}\)) than those found in the SLRE (10–25 mg L\(^{-1}\)) (Berndt and Bavin, 2009). Three different sulfate amendment levels (sodium sulfate added to 50 L of Cloquet River water) were oxygenated and recirculated in the overlying water of microcosms from each habitat zone for a period of six months. Sulfate concentrations in the overlying waters were maintained at 50 mg L\(^{-1}\) (high treatment), 15 mg L\(^{-1}\) (medium treatment), and 5 mg L\(^{-1}\) (low treatment), respectively, which represent environmentally relevant concentrations in the SLRE. Fresh Cloquet River water was obtained and amended every two months during the laboratory study and monitored for sulfate concentration every two weeks (Online Supplemental Fig. S2). Following laboratory flux measurements, subcores were extracted from the experimental microcosms to characterize MeHg and related geochemical conditions at the end of the study.

Diffusion calculations suggested that six months was a sufficient time for overlying water to diffuse approximately 10 cm into sediment. To track diffusional transport from the overlying water into the sediment, chloride was added as a conservative ion tracer to all overlying water treatments (sodium chloride, 200 mg L\(^{-1}\) as chloride). The initial porewater chloride concentrations were consistent at all depths among all sediment microcosms (10 ± 2 mg L\(^{-1}\)). At the end of the 6 month experiment, chloride from the overlying water had diffused to greater than 20 cm depth in each sediment microcosm (Online Supplemental Fig. S3) indicating that diffusion of amended solutes (chloride and sulfate) in the overlying water treatments occurred over the timescale of the experiments in all sediment microcosms. Though the potential for sodium chloride to affect results was not directly assessed, chloride is a relatively weak ligand for mercury in anoxic waters relative to dissolved organic carbon and sulfide (Benoit et al., 1999). Additionally, at only 1–2% of sea water strength, these monovalent ions are not likely to have ionic-strength effects (Stumm and Morgan, 1996) or be an important factor in defining bacterial communities in light of other experimental manipulations.

3.2. Sediment coring and porewater collection

At the beginning and end of the experimental sulfate treatment, sub-cores (2.54 cm diameter, polycarbonate) were extracted in triplicate from the 20 cm diameter sediment microcosms. Subcores were sectioned into 0–4 cm, 4–10 cm, and 10–20 cm intervals and placed directly into trace-metal clean glass jars that were immediately filled with nitrogen gas to minimize the alteration of redox conditions. Within 15 min of sub-coring, sample jars were transferred to an oxygen-free glove box (97.5% nitrogen, 2.5% hydrogen) where samples were homogenized and subsampled for mercury, SRB abundance, AVS, and porewater constituents. Subsamples for MeHg, Thg, and SRB abundance were allocated to clean sample jars in the glove box and stored at −20 °C until analysis. Subsamples for AVS and porewater were processed immediately to minimize any error introduced by changes in redox conditions. Porewater samples were obtained by sealing a sediment aliquot in 50 mL polypropylene centrifuge tubes under an oxygen-free atmosphere and centrifuging (10,000 rpm for 20 min). Supernatant was then filtered through 0.45 μm polyethersulfone filters in an oxygen-free atmosphere directly into clean sample vials for short-term storage prior to quantification.

To determine within-microcosm variability, triplicate sub-cores in one microcosm from each habitat zone were analyzed separately during pre-treatment characterization. In microcosms that did not have individual sub-cores analyzed, sub-cores (3) were composited at each respective depth prior to analysis. The concentration quantified in homogenized, composited samples from triplicate subcores is directly comparable to the average of the three concentrations quantified for individual sub-cores. To preserve the vertical structure of the microcosm sediment during pre-treatment characterization, a thin-wall butyl tube was inserted into the sediment microcosms prior to sub-coring to prevent the sediment from falling into the void created by the sub-core. Then sub-cores from a sacrificial microcosm (20 cm diameter) collected at each site were used to fill each hole from which an analytical sub-core was removed. No significant difference was observed in the bulk or porewater concentrations of sacrificial microcosm as compared to experimental microcosms (Online Supplemental Table S1).

At the conclusion of the experiments, Thg flux out of the sediment was estimated in each microcosm. Fresh Cloquet River water was obtained in 50 L acid-cleaned carboys and five volumes of unamended water (3.14 L = one volume) was allowed to flow over each microcosm to ensure fresh Cloquet River water was sitting on top of each microcosm. Then flow ceased, a sample was collected, and water sat on top of the sediment for 36–48 h with a gentle, small stream of air bubbling through PTFE tubing to mix overlying water and maintain oxic conditions. After 36–48 h, the overlying water was sampled again, filtered (0.45 μm polyethersulfone) directly into trace-metal-clean PETG bottles, and acidified with hydrochloric acid to a final concentration of 0.5%. After flux measurements, apparent partition coefficients (Kd) for MeHg and Thg were determined in sediment of experimental microcosms. The top 10 cm of sediment from a sub-core (6.35 cm diameter, polycarbonate) was extruded into a 3.8 L Ziploc sample bag and transferred into an oxygen-free atmosphere. Following thorough homogenization, sediment was transferred into 50 mL polypropylene centrifuge tubes and centrifuged (10,000 rpm for 20 min). The supernatant was filtered and preserved similar to overlying water mercury samples. A subsample of homogenized sediment was removed before centrifuging and placed in a trace-metal clean scintillation vial for solid phase Thg and MeHg analysis.
3.3. Analytical techniques

Solid phase samples were stored frozen and freeze dried prior to THg and MeHg analysis by ICP-MS at the University of Toronto using methods outlined by Mitchell and Gilmour (2008). Total mercury in sediment samples were extracted with heated H$_2$SO$_4$/HNO$_3$ digestions followed by dilution of the extract in deionized water. The sediment extract was analyzed using an ICP-MS coupled with a Perkin Elmer flow injection autosampler. Total mercury concentrations in water samples were measured on a Tekran 2600 automated Hg system coupled with a cold vapor atomic fluorescence spectroscopy (CVAFS) detector, as described in US Environmental Protection Agency Method 1631 Revision E (USEPA, 2002). MeHg concentrations in water and sediment samples were measured on a GC–ICP–MS using isotope dilution, as described in Hintelmann and Evans (1997).

The abundance of SRB was characterized using quantitative real time polymerase chain reaction (qPCR). A MoBio PowerSoil extraction kit was used to extract total DNA from sediment samples. Quantitative PCR was used to estimate the abundance of SRB by quantifying copies of the dsrA gene as outlined in Schippers et al. (2006) and modified by Oster (2012). Sediment samples analyzed for total carbon (TC) were dried for 48 h in a 60 °C oven to remove moisture and analyzed on a Thermo Scientific Flash 1112 Combustion CHNS Analyzer (detection limit less than 100 μg/g). Since inorganic carbon comprised less than 10% of total carbon at the sites investigated in this study, TC is used as a proxy for organic carbon. Acid volatile sulfide (AVS) was measured using the Brouwer Diffusion Method (Brouwer and Murphy, 1994) and extract concentrations quantified with a sulfide ion-selective electrode. The detection limit for AVS was less than 0.5 μmol/g based on the mass of sediment and detection limit of the electrode. All solid phase measurements are expressed in terms of dry weight mass.

Sulfate and chloride in porewater samples were measured using a Dionex ICS-1100 Integrated IC system (AS-DV Autosampler). Sulfate and chloride standards (0.3–30 mg/L) were made using sodium sulfate (Na$_2$SO$_4$) and sodium chloride (NaCl) in Millipore water, and were checked against a Thermo Scientific anion standard. Samples for dissolved organic carbon (DOC) were sub-sampled from the 0.45 μm filtered water aliquot and acidified to pH 2 to remove any dissolved inorganic carbon (DIC). Samples were analyzed in a Shimadzu TOC-VCSH high temperature carbon analyzer with a sparging time of 3.5 min. Dissolved sulfide, manganese, and iron in sediment porewaters were measured after methods from Brendel and Luther (1995) (Online Supplemental Information contains method details).

4. Results and discussion

4.1. Geochemical trends and sediment mercury

Sediment from the Sheltered Bay (SB) habitat zone possessed the highest solid-phase carbon relative to the Upper Estuary Flats (UEF) and Lower Estuary Flats (LEF) habitat zones (Fig. 2a). In addition to overall carbon abundance, down core trends differed among the habitat zones. In the UEF and SB habitat zones %TC decreased with depth in the sediment profile, which is characteristic of the degradation of organic carbon over time with increasing depth in a depositional system (Meyers and Ishiwatari, 1993). In contrast, there was no discernible %TC trend with depth in the LEF habitat zone, likely due to boat traffic and seiche currents producing more energetic hydraulics in the outer harbor which could mix surficial sediments.

The SB habitat zone sediment also had the greatest abundance of solid phase acid volatile sulfides (AVS) (Fig. 2b), particularly in surficial sediments. Solid phase AVS concentrations in the SB sediment decreased with depth (from 42 μmol g$^{-1}$ dw) while the UEF and LEF sediment had very low AVS concentrations near the sediment water interface that increased to 10 to 15 μmol g$^{-1}$ with depth. Although dissolved sulfide was not detected in sediment porewaters (0.5 μmol L$^{-1}$ detection limit, Online Supplemental Figs. S4 and S5), AVS quantified prior to experimental sulfate additions suggest that sulfate was being reduced rapidly in surficial sediment (0–4 cm) of the SB habitat zone compared to the UEF and LEF habitat zones. Higher abundance of organic carbon in SB sediment and the potential for oxygen depletion in bottom waters (backwater, slow moving area) could be driving this more rapid sulfide accumulation.

Solid phase total mercury (THg) was consistently higher in the SB habitat zone relative to the UEF and LEF habitat zones (Fig. 2c), possibly due to a higher abundance of organic carbon (Hammerschmidt and Fitzgerald, 2004). Total mercury concentrations were the most variable in the LEF habitat zone, ranging from 60 to 250 ng g$^{-1}$ in the top 10 cm of sediment. Since solid phase THg concentrations varied among habitat zones and with depth, MeHg concentrations (Online Supplemental Fig. S6) were normalized to THg in order to compare the net capacity of sediment to
produce MeHg on a relative basis. Similar to observations in other saltwater and freshwater surficial sediments (Sunderland et al., 2006; Bloom et al., 1999; Goulet et al., 2007; Hines et al., 2004), a narrow range of %MeHg in the solid phase was observed with depth and among the habitat zones (Fig. 2d, 0.6–0.96%, excluding 10–20 cm LEF sediment). Despite evidence that the SB habitat zone experienced substantially greater sulfate reduction in surficial sediment (Fig. 2b), %MeHg was consistently higher in sediment from the UEF habitat zone (Fig. 2d).

Unlike observations in marine and other depositional systems (Hammerschmidt et al., 2008; Sunderland et al., 2006; Hammerschmidt and Fitzgerald, 2004), sediment THg was not closely related to organic carbon in the overall dataset (Fig. 3a). Carbon and THg were most variable in the LEF habitat zone which is the area of the estuary most subject to anthropogenic disturbance. Solid-phase MeHg concentration was closely related to solid-phase total mercury concentration across the dataset regardless of the quantity of carbon present (Fig. 3b) suggesting that all sites contain a relatively similar capacity to produce MeHg from the inorganic mercury pool present.

Percent MeHg, indicative of an environment’s net capacity to transform inorganic Hg to MeHg, was not strongly related to TC in the overall dataset, though some weak trends did exist within sites (Fig. 3c). While not specifically investigated in this study, differences in the nature of the organic carbon present in the sediment of the hydrologically and ecologically diverse SLRE could account for the lack of a consistent relation between MeHg and OC. For instance, the isolated nature of the SB setting likely results in a larger proportion of autochthonous carbon inputs than the river-dominated UEF and lake influenced LEF settings. Previous work in the SLRE using stable carbon isotopes has suggested a difference in dominant organic carbon sources to sediment along a transect of the river and lake-influenced portions of the estuary (Hoffman et al., 2010).

4.2. Effect of overlying water sulfate on sediment methylmercury

Sulfate concentrations in sediment microcosm porewater showed a rapid decrease from the sediment – water interface to 10 cm depth into sediment (Fig. 4). This pattern was present at the beginning and end of lab incubations and is consistent with biological reduction of sulfate in surficial sediment of all habitat zones. Over the 6-month experimental period, different sulfate treatments clearly induced different porewater sulfate gradients, thereby delivering variable amounts of sulfate to surficial sediment in each sulfate-amended microcosm (Fig. 4d).

The diffusional sulfate fluxes to surficial sediment driven by the three different sulfate amendments in the overlying water were estimated based on observed concentration gradients and rates of molecular diffusion at the end of experimental treatments. The estimated fluxes provide a basis to compare among treatments and place the applied loads in the context of other mercury-related freshwater sulfate addition experiments that have shown a positive response in MeHg production with increasing sulfate load (Jeremiason et al., 2006; Coleman Wasik et al., 2012; Mitchell et al., 2008). Sulfate fluxes to surficial sediment were estimated at the end of the 6 month experiment using Eq. (1) where $F$ is the flux ($\text{mg m}^{-2} \text{d}^{-1}$), $\delta C$ is the concentration difference ($\text{mg m}^{-3}$), and $\delta x$ is the distance (m) over which a concentration difference was observed. The diffusion coefficient of sulfate in water, $D_w$ (9.48 \times 10^{-9} \text{ cm}^2 \text{s}^{-1} \text{ at } 20^\circ \text{C}$) was adjusted using estimates for porosity ($\phi$), (Boudreau, 1996) made from water content measurements for each habitat zone. For the purposes of defining flux into surficial sediment, $\delta x$ was defined as the distance from the sediment–water interface to 7 cm depth, the center of the second sediment porewater measurement.

$$F = \frac{\phi D \delta C}{\delta x}$$

(1)

$$\phi^2 = \frac{1}{\phi^2}$$

(2)

Since sulfate was depleted to near detection limits by 7 cm in all experimental microcosms, sulfate flux into the sediment estimated at the close of experimental treatments was proportional to overlying water amendment level (Fig. 4d). Sulfate concentration gradients in the lab microcosms suggest diffusive sulfate loadings ranging from 5 to 13 kg ha$^{-1}$ yr$^{-1}$ in the low treatments, 15-40 kg ha$^{-1}$ yr$^{-1}$ in the medium treatments, and 50-120 kg ha$^{-1}$ yr$^{-1}$ in the high treatments. These diffusive sulfate loadings represent a
conservatively low estimate since, in some cases, sulfate was depleted at depths less than 7 cm in experimental microcosms. However, the applied loadings span a range that encompasses experimental sulfate additions to wetlands in the region which were estimated at 4× annual sulfate deposition (32 kg ha\(^{-1}\) yr\(^{-1}\), Jeremiason et al., 2006).

Abundance of SRB was measured in sediment at the end of experiments to determine if sulfate concentrations in the overlying water influenced SRB activity during the experiment. Consistent with observations at the outset of experiments, all microcosms showed a maximum near the sediment-water interface and decreasing SRB abundance with increasing depth (Online Supplementary Fig. S7). Observed sediment SRB abundance in the 0–4 cm depth interval at the end of experimental treatments was related to sulfate amendment level in microcosms from all three habitat zones (Table 1). In the SB microcosms, SRB abundance in the high and medium sulfate treatments were significantly larger than SRB abundance in the low sulfate treatment (p < 0.05). In UEF microcosms, SRB abundance in the high and medium sulfate amended treatments were larger than SRB abundance in low sulfate treatment, but differences were less significant (p = 0.17 and 0.07, respectively). In LEF microcosms, SRB abundance in the high treatment was significantly higher than SRB abundance in the low treatment (p < 0.01) and SRB abundance in the medium treatment was intermediate. These observations suggest that the combination of experimental conditions and addition of sulfate to the overlying water stimulated the activity of SRB in the surficial sediment of the high and medium sulfate treatments relative to the low sulfate treatment. The lack of a consistent difference in SRB abundance between medium and high sulfate amended microcosms is consistent with half-saturation constants of 15–65 mg/L reported for sulfate reduction in surficial freshwater aquatic sediments (Pallud and Van Cappellen, 2006).

Concentrations of AVS at the end of experimental exposures showed a large decrease (80%) in surface (0–4 cm) sediment of the SB microcosms (Supplemental Online Fig. S8). This was likely indicative of the oxidation of reduced sulfur species in surface sediment due to experimental manipulation. Initial AVS was low in the surface sediment of UEF and LEF microcosms and did not change significantly in response to experimental manipulation. The oxidation of sulfide in the 0–4 cm interval of the SB microcosms likely increased the sulfate loading to all treatments for SB sediment. However, the SRB abundance in all three sediment types at the close of experiments suggests that sulfate concentrations in the overlying water of experimental microcosms did influence the microbial population known for MeHg production on the timescale of the experiments (Table 1).

Based on observed changes to the sediment AVS, particularly in the SB microcosms, it was clear that laboratory conditions altered the redox state from in situ conditions regardless of sulfate treatment. Therefore, the interpretation presented here regarding the effect of overlying water sulfate concentration on %MeHg relies on comparisons among sulfate treatments within a habitat zone at the end of the six month incubation, rather than a comparison of %MeHg at the end of the lab incubation to %MeHg at the beginning of the incubation. For this reason, the microcosm sulfate

![Fig. 4. Porewater sulfate concentrations for (a) SB, (b) UEF, and (c) LEF habitat zones including overlying water concentrations for high (solid lines), low (dashed lines), medium (dotted lines), and initial profiles (gray double line and empty symbols). Depth into the sediment is given relative to the sediment-water interface. Horizontal bars represent the standard deviation of the three initial sediment microcosms from each habitat zone. (d) Estimated diffusive fluxes of sulfate into sediment at the end of 6 month lab incubations. Dashed horizontal line represents load associated with wetland sulfate addition studies (Jeremiason et al., 2006, Mitchell et al., 2008).](image-url)
amendment experiments must be considered indicative of the potential for sulfate’s influence on MeHg in sediments rather than a definitive representation of an in-situ response.

Total mercury at the end of the experiments was consistent with those measured initially (Online Supplemental Table S2). Overall, observations of %MeHg after the 6 month lab incubations at different depth intervals were not clearly related to sulfate concentrations in the overlying water for sediment from the SB and UEF microcosms (Fig. 5a and b).

The 0–4 cm depth interval in the UEF microcosm amended with low sulfate displayed a significant increase in %MeHg relative to the other UEF microcosms (Fig. 5b). While the cause of this apparent increase is unknown, geochemical trend analysis (Fig. 3c) suggests that this data point is an outlier when compared to the entire data set and it was excluded from the interpretation. In LEF microcosms, %MeHg was similar in the low, medium, and high sulfate treatment microcosms for the 0–4 cm depth interval (Fig. 5c). Between 4 and 10 cm, %MeHg displayed a decreasing trend from the high sulfate to low sulfate treatment; however, the differences in %MeHg between 4 and 10 cm among LEF microcosms were not statistically significant and porewater sulfate observations suggest little sulfate reached this depth interval during the study (Fig. 4).

The absence of a clear, consistent response in solid phase sediment %MeHg following significant changes in sulfate loads to intact sediment from the SLRE was unexpected given previous results in field, mesocosm, and laboratory sulfate addition studies. Dissolved sulfide concentrations quantified in this study (<1 µM, Online Supplemental Figs. S4 and S5) were not high enough to inhibit methylation by altering the bioavailability of inorganic mercury (Gilmour et al., 1998; Benoit et al., 1999; Hsu-Kim et al., 2004; Drott et al., 2007). Some previous field and mesocosm sulfate addition studies have used porewater MeHg soon after sulfate addition as a proxy for methylation activity (Mitchell et al., 2008; Jeremiason et al., 2006) and the results presented here rely on measurements in the solid phase after six months of continuous sulfate loads. The quantity of MeHg in the solid phase is considered a good approximation for the long-term, net MeHg production efficiency of an environment and is a relevant means of comparison given the continuous sulfate loads in this experiment. While we cannot rule out the possibility that a six-month timeframe was insufficient to significantly affect the pool of solid-phase MeHg, observations of seasonal differences in field measurements of both porewater and solid-phase MeHg (Bloom et al., 1999; Gill et al., 1999; Hammerschmidt et al., 2004) and sediment incubation responses in solid-phase sediment MeHg (Johnson et al., 2010; Hintelmann et al., 2000) suggest that the timescale of the experiment was sufficient to affect the pool of MeHg present on the solid phase. Our interpretation is that sulfate reducing microbes in these sulfate amendment experiments with intact freshwater estuary sediment were supplied with varying amounts of sulfate from the overlying water (Fig. 4 and Table 1), but that other geochemical factors such as the quantity of carbon or solid-phase partitioning may have been important in defining the net production of MeHg.

### 4.3. Porewater mercury partitioning

Total- and methyl-mercury were measured in porewater extracted from bulk sediment from the top 10 cm in each of the microcosms at the close of the experiments (Table 2). No discernible trend was observed for porewater THg or MeHg among high, medium, and low sulfate treatments, and values from each habitat zone were averaged for partitioning analysis. Dissolved organic carbon in sediment porewater was relatively uniform among habitat zones and sulfide concentrations remained low in all estuary sediment (Table 2). Porewater total mercury concentrations varied among sites (4.5–26.3 ng L⁻¹), while porewater MeHg was consistent, with all sites averaging between 0.32 and 0.46 ng L⁻¹ (Table 2). Sediment from the LEF habitat zone had the most

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**Table 1**

<table>
<thead>
<tr>
<th>High sulfate treatment 10⁵ dsrA copies g⁻¹</th>
<th>Med sulfate treatment 10⁵ dsrA copies g⁻¹</th>
<th>Low sulfate treatment 10⁵ dsrA copies g⁻¹</th>
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<tbody>
<tr>
<td>SB</td>
<td>2.77 ± 0.26</td>
<td>4.05 ± 0.62</td>
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<tr>
<td>UEF</td>
<td>1.32 ± 0.42</td>
<td>1.68 ± 0.50</td>
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<tr>
<td>LEF</td>
<td>1.10 ± 0.09</td>
<td>0.84 ± 0.30</td>
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**Fig. 5.** Solid phase percent methylmercury (%MeHg) in the experimental microcosms after sulfate treatment in (a) SB, (b) UEF, and (c) LEF habitat zones. Horizontal bars represent the standard deviation of the three initial sediment microcosms from each habitat zone. Vertical bars represent the length of section that each data point represents. Discrete data points have a slight vertical offset to minimize overlap of horizontal error bars.
variable total- and methyl-mercury concentrations on the solid-phase (Online Supplemental Table S2) and dissolved-phase (Table 2).

Apparent partitioning coefficients ($K_d$) were calculated as the ratio of solid concentration to porewater concentration and, for total mercury, appeared to be related to the solid phase concentrations of total carbon and AVS (Fig. 6). The SB habitat zone sediment had higher concentrations of both carbon and AVS that may have increased the solid phase partitioning of mercury relative to the UEF and LEF sediment (Hammerschmidt and Fitzgerald, 2004; Bower et al., 2008; Liu et al., 2008). The SB microcosms appeared to have the greatest potential for sulfate reduction, but the pool of dissolved inorganic mercury was much lower than that in the other habitat zones (Table 2) and could be responsible for the absence of a clear response in net methylation (%MeHg) as a result of sulfate treatments in sediment from the SB habitat zone.

The relationship between STC and THg apparent partition coefficients observed in the SLRE sediments (Fig. 6) is similar to that observed in a saltwater estuary (Hammerschmidt et al., 2004), and suggests that one or both of AVS and solid phase carbon influence mercury partitioning. In the results presented here, AVS and TC concentrations were covariate in their relationship with THg partitioning making it difficult to interpret the factor that had the stronger influence on dissolved THg concentrations. However, the UEF and LEF habitat zones had similar %TC (3.2% and 3.1%, respectively) and $K_d$ in surficial sediment, but markedly different AVS concentrations (8.7 μmol g$^{-1}$ and 2.7 μmol g$^{-1}$, respectively, Fig. 6) suggesting that carbon may play a more important role than AVS in solid phase partitioning of inorganic mercury.

Organic carbon and solid phase iron sulfides also bind MeHg strongly (Miller, 2006) and may control the partitioning of MeHg to the solid phase; however, MeHg partitioning coefficients did not appear related to AVS or %TC (Table 2). In addition, MeHg apparent partition coefficients measured in sediments from the SLRE (3.2–3.65 L kg$^{-1}$) were higher than many reported in the literature for marine environments (Hammerschmidt et al., 2004; Fitzgerald et al., 2007).

### 4.4. Mercury flux from sediment

Flux measurements were made in laboratory microcosms at the close of the experiments in order to estimate the potential for diffusive MeHg and THg flux from sediment to overlying water in the SLRE. THg flux was determined by measuring the change in THg concentration in the overlying water of each microcosm over a 36–48 h time-period and normalizing concentration changes to

<table>
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<th>Table 2</th>
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<td>Porewater and solid phase MeHg and THg concentrations averaged in each habitat zone ($n = 3$) for the top 10 cm of sediment at the close of experiments. ±Standard deviation of three microcosms for each habitat zone.</td>
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* Calculated as the mean of $K_d$ for individual samples.

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<th>Table 3</th>
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<tr>
<td>Overlying water MeHg and THg concentrations for initial and final flux time points, measured THg flux, and estimated MeHg flux from lab experiments performed at the close of experiments. ±Standard deviation of three microcosms for each habitat zone.</td>
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* More than half of quantified values below detection limit, 0.08 ng L$^{-1}$ MeHg. 

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Fig. 6. Average THg apparent partition coefficients (bars) and volume weighted average TC (square symbols) and AVS (circle symbols) from the top 10 cm of each sediment microcosm. Error bars represent standard deviation of 3 microcosms for each habitat zone.

Fig. 7. Total mercury flux, porewater total mercury concentrations, and total mercury in the solid phase of laboratory microcosms.
the volume of overlying water and microcosm sediment surface area (Hammerschmidt and Fitzgerald, 2008).

Consistent with porewater measurements, no discernible trend was observed in THg flux measurements among high, medium, and low sulfate treatments, so results were averaged for each habitat zone. Measured THg flux in experimental microcosms was between 15 and 40 ng m⁻² d⁻¹ (Table 3). The SB habitat zone had the lowest porewater THg concentration as well as the lowest THg flux of the three habitat zones despite having highest THg on the solid phase (Fig. 7). The LEF microcosms had a THg flux two times higher than the UEF habitat zone, which was also consistent with higher porewater THg concentrations (Fig. 7). Measured THg fluxes from experimental microcosms were within the range reported in saltwater estuary systems (Gill et al., 1999; Hammerschmidt and Fitzgerald, 2004), but would be considered relatively low compared to heavily contaminated systems (Choe et al., 2004; Covelli et al., 1999; Mason et al., 2006).

Though porewater MeHg concentrations were consistently higher than dissolved MeHg measured in the overlying water, MeHg flux from sediment could not be directly calculated from observations possibly owing to demethylation of MeHg. In place of direct measurements, estimates were made using an effective mass transfer coefficient ($k_{mt}$) calculated from the THg flux experimental results. Using measurements of THg flux, overlying water THg concentration, and porewater THg concentration, an effective mass transfer (effective diffusion) coefficient, $k_{mt}$ (L m⁻² d⁻¹) for THg was calculated for each habitat zone (average, $n = 3$) using Eq. (3). Without any other information, the mass transfer coefficient for MeHg was assumed to be equal to the average mass transfer coefficient for THg in each habitat zone. Since the overlying water and porewater MeHg concentrations are known, MeHg flux can be estimated using Eq. (3).

\[
\text{Flux} = k_{mt} \times (\text{Concentration}_{\text{porewater}} - \text{Concentration}_{\text{OverlyingWater}})
\]

Flux = $k_{mt}$ (Concentration$_{\text{porewater}}$ – Concentration$_{\text{OverlyingWater}}$) (3)

Estimates of MeHg flux were between 0.8% and 11% of measured total mercury fluxes (Table 3), which is of similar magnitude to MeHg porewater concentrations between 1.7% and 6.6% of THg. The SB habitat zone had the largest estimated MeHg flux (1.75 ng m⁻² d⁻¹) as a result of having the largest $k_{mt}$. The UEF and LEF habitat zones had lower MeHg flux estimates of 0.15 and 0.72 ng m⁻² d⁻¹, respectively. Estimated MeHg fluxes in the experimental microcosms are on the low end of a range reported in other estuary systems (Choe et al., 2004; Covelli et al., 1999; Mason et al., 2006; Hammerschmidt and Fitzgerald, 2008; Benoit et al., 2003).

5. Implications for St. Louis River freshwater estuary

The finding of a strong correlation between total- and methylmercury (Fig. 3b) in this study is consistent with observations in other sediment systems not heavily contaminated with mercury (Benoit et al., 2003) and suggests a similar net MeHg production capacity in all of the sediments studied. The disparity in the response of SRB abundance and %MeHg in the solid phase during sulfate amendment microcosm experiments suggests that biogeochemical factors other than sulfate loading may play important roles in the net production of MeHg in the open-water, relatively low organic carbon sediment of the SLRE.

Though diffusive mercury fluxes measured under experimental conditions could differ from those realized under field conditions, sediment flux estimates from this study are in the range of those measured in other aquatic sediments in field settings. Lab-quantified diffusive fluxes were scaled to the total area of each of the three habitat zones studied to obtain sediment loading estimates (mg d⁻¹) to the SLRE. Diffusive sediment MeHg loads from the LEF, UEF, and SB habitat zones (45% of total Estuary area) were estimated at 7.6, 3.7, and 6.3 mg d⁻¹, respectively, while THg load estimates totaled 632 mg d⁻¹ (Table 4). Upstream mercury loading to the estuary was also calculated using dissolved THg and MeHg concentrations measured by Berndt and Bavin (2009, 2012b) and coincident discharge measurements from a USGS gauging station (USGS Station ID: 0402400). The upstream MeHg load to the estuary was calculated to be 173.3 mg d⁻¹ during low flow (14% flow exceedance) and surpassed 7700 mg d⁻¹ during high flow (97% flow exceedance) conditions. If summed, the total estimated diffusive MeHg load from the open-water sediment of the three habitat zones investigated in this study represents 0.2–10% of the total amount of MeHg delivered to the estuary from the upstream river, depending on the flow regime.

Some areas not included in this study contain sediments that are contaminated with respect to mercury (>1000 ng g⁻¹ THg) (Crane, 2006), which could result in small portions of the estuary contributing disproportionately large amounts of mercury to the overlying water column (Covelli et al., 1999). Additionally, this study targeted sediment from open water areas of the estuary and did not measure MeHg or geochemistry in higher organic carbon wetlands in the system. Relatively few wetlands remain in the lower portion of the estuary due to hardening of the shoreline for industrial infrastructure. However, given the importance of wetlands as net exporters of MeHg and the results of previous sulfate addition experiments in them, a rigorous study of MeHg production and export in wetlands of the SLRE must be conducted to discern the role of surface water sulfate in controlling MeHg dynamics and to obtain a more complete picture of MeHg cycling in the system.

Acknowledgements

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The text contains a list of references and supplementary material. It appears to be a scientific paper discussing the role of organic matter in the dissolved phase speciation and mercury methylation in aquatic environments. The references include various studies on mercury metabolism and its environmental implications. The supplementary material contains additional data that can be explored for further analysis.


WI DNR (Wisconsin Department of Natural Resources), 2012. Fish Consumption advice for the St. Louis River Area of Concern. WI DNR Memo.
