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Mercury methylation in estuaries: Insights from using measuring rates using stable mercury isotopes

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Abstract

Rates of mercury (Hg) methylation and methylmercury (MeHg) demethylation in sediment of the Hudson River, Chesapeake Bay and Bay of Fundy were measured using stable isotopes of mercury (Hg) and methylmercury (MeHg). Methylation of the isotope correlated well with in situ MeHg concentration, and MeHg turnover times were on the order of days. It was concluded that methylation was more important than demethylation in controlling the differences in concentrations of MeHg among ecosystems. Also in situ MeHg concentration appeared to be a good indicator of methylation activity in sediment across ecosystems. Examination of a temporal data set, collected from the vicinity of Hart Miller Island, Chesapeake Bay between 1998 and 2002, and a spatial data set of a longitudinal transect of the main stem of the Chesapeake Bay, is used to further examine the controls on Hg methylation and MeHg concentration. Microbial activity and the formation of sulfide appear to be at least as important as Hg concentration in controlling MeHg concentration in estuarine sediment.

Keywords: Mercury; Methylmercury; Methylation; Demethylation; Estuary; Sediment

1. Introduction

The majority of the worlds' estuaries have been contaminated with heavy metals and organic contaminants, such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) (e.g. Birch and Taylor, 2000; Vanzoest and Vaneck, 1993). This is in large part because of the industrialization of their shores and tributaries. The accumulation of mercury (Hg) from industrial point sources (Tomiyasu et al., 2000; Hintelmann and Wilken, 1995) and atmospheric deposition (Mason et al., 1994; Mason et al., 1999) is of great concern as a portion of this Hg is methylated in the estuary, and it is methylmercury (MeHg) that bioaccumulates in the food web (Heyes et al., 2004; Kim et al., 2004, 2005; Sunderland et al., 2004; Kerhig et al., 2003; Hines et al., 2000; Bloom et al., 1999; Kannan et al., 1998). Concentrations of MeHg have reached levels of concern in estuarine and ocean fish (Baeyens et al., 2003; Myers et al., 2003), which has lead to specific fish consumption advisories for seafood issued by public health agencies such as the US FDA and EPA, Health Canada and the UK Food Standards Agency.

Most studies of Hg contamination in estuaries have focused primarily on point source contamination, such as the acetaldehyde factory in Minamata (Fujiki and Tajima, 1992) and the chlor-alkali associated industry

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in Lavaca Bay, Texas (Bloom et al., 1999). While these studies have been locally valuable and have provided insight into some of the controls on Hg methylation, they have been conducted where Hg concentrations are exceedingly high, perhaps biasing our understanding of Hg methylation and demethylation in less contaminated estuaries and the coastal margins (Schaefer et al., 2004, Choi and Bartha, 1994).

An examination of a subset of these studies yields interesting trends between Hg and MeHg concentration (Fig. 1). While sediment total Hg (T-Hg) concentrations varied by four orders of magnitude in these sample studies (Fig. 1a), MeHg concentrations changed by just two orders of magnitude (Fig. 1b). Overall, the percentage of T-Hg that was MeHg varied by an order of magnitude, and perhaps even decreased at higher MeHg concentrations (Fig. 1c). This lack of a direct dependence of MeHg concentration in sediment on T-Hg concentration is also apparent in a number of other ecosystems (Benoit et al., 2003). In a recent study of contaminated sites, Schaefer et al. (2004) suggest that the percent of Hg that occurs as MeHg decreases with increasing Hg concentrations in water due to enhanced demethylation resulting from the induction of specific Hg detoxifying pathways by the *mer* genes. *Mer* B cleaves Hg^{II} from the methyl group and *Mer* A reduces Hg^{II} to Hg^{0} (Barkay et al., 2003). It is also possible that at concentrations above 1 μmol g^{-1} (dry weight) in sediments that these *mer* B genes may be induced and demethylation of MeHg enhanced. What is apparent from the review by Benoit et al. (2003) is that, aside from some "control" sites and a few studies in sensitive areas, such as Florida Bay (Kannan et al., 1998), little research has been conducted on estuarine sites with low Hg deposition and relatively low sediment Hg concentrations. In fact, there is lack of ecosystem-scale understanding of the Hg methylation cycle in estuaries.

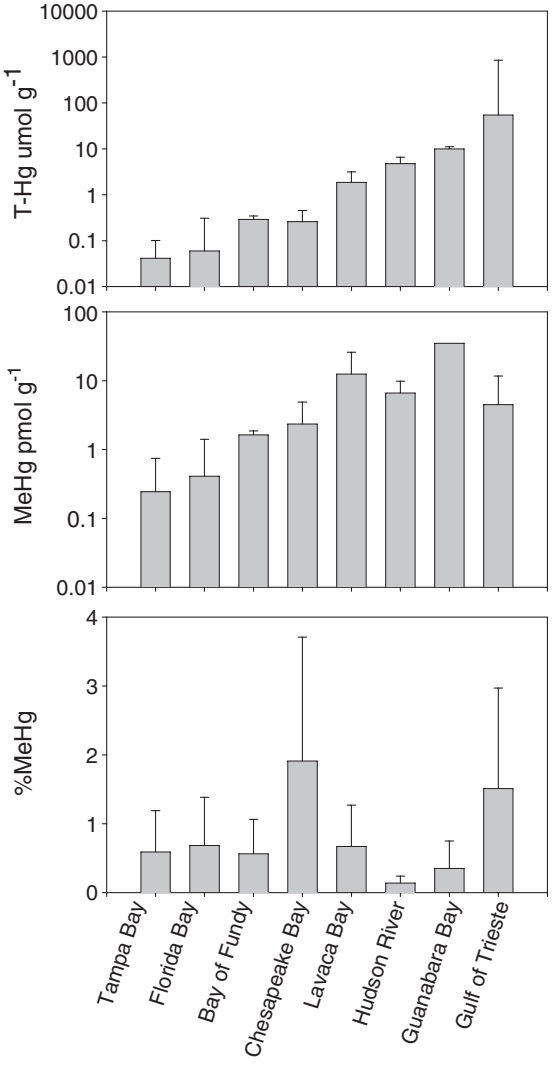

Fig. 1. Mercury (a), methylmercury (b) concentrations (Note log scale) and % of Hg that is MeHg (c) in sediments from Tampa Bay and Florida Bay (Kannan et al., 1998), Bay of Fundy, Canada (Sunderland et al., 2004), Chesapeake Bay (Kim, 2004), Lavacca Bay, Texas (Bloom et al., 1999), Hudson River, New York (Heyes et al., 2004), Guanabara Bay, Brazil (Kerhig et al., 2003) and Gulf of Trieste, Slovenia (Hines et al., 2000).

Factors controlling Hg methylation that have been proposed can be grouped as those affecting the bioavailability of Hg for methylation and those affecting the activity of the methylating bacteria (Winfrey and Rudd, 1990; Choi et al., 1994, Benoit et al., 2003). The factors controlling the bioavailability of Hg depend on the dissolved and solid phase speciation of Hg (Benoit et al., 1999, 2001; Ravichandran, 2004). Sulfate reducing bacteria (SRB) have been identified as being the most important group of Hg methylating bacteria in estuaries, thus factors that affect the activity of SRB will effect Hg methylation (Gilmour et al., 1992; Choi et al., 1994). Since not all SRB methylate Hg, the activity of the entire population may not be a good indicator of Hg methylating activity (King et al., 1999).

Previously, rates of methylation in estuarine sediment have been measured using ^{203}Hg (Gilmour and Riedel, 1995; Stordal and Gill, 1995) and demethylation by ^{14}C-labeled MeHg (Marvin-DiPasquale et al., 2000, 2003). However, such approaches have several disadvantages, such as not being able to measure rates and concentrations in the same samples and the potential for incomplete determination of MeHg demethylation, because not all carbon from the CH_3Hg^{II} may be released as CO_2 and CH_4. We have rather used stable isotopes of Hg and

MeHg to measure these processes in the Hudson River and Bay of Fundy (Heyes et al., 2004; Sunderland et al., 2004) and in enclosure experiments (Kim et al., in press). We have also used stable isotopes in the Patuxent River (Mackall Cove), a tributary of the Chesapeake Bay. While the concentration of MeHg in sediments is more easily measured, the measure of Hg methylation rate reflects the short-term balance between MeHg production and MeHg demethylation. We have found sediment MeHg concentration and absolute MeHg production, measured from the addition of stable isotopes, to be well correlated in Hudson River sediment and in mesocosm studies with Baltimore Harbor sediment (Heyes et al., 2004; Kim et al., 2004, in press). The relationship between Hg methylation and MeHg demethylation potentials is discussed further here. In addition, the proposition that in situ MeHg concentration is a good indicator of current net methylation activity in estuaries is discussed in this paper.

Until recently, there has not been enough Hg, MeHg and ancillary data to begin the study of the physical and biogeochemical controls on Hg methylation in estuaries. Furthermore, there appears to be no spatial and/or temporal studies of Hg and MeHg in larger systems, such as estuaries, lakes or watersheds. In this paper we investigate the temporal variation in Hg and MeHg concentrations using results from an ongoing study (1998–2002) of Hg and MeHg concentrations near Hart-Miller Island (HMI), a dredge disposal facility, in the Chesapeake Bay. We will also investigate the spatial variation in Hg and MeHg concentrations from a longitudinal study of Chesapeake Bay sediment. As it has been demonstrated that in situ MeHg concentration is a good predictor of net Hg methylation activity in estuaries, we will use it as a surrogate in the examination of the controls on Hg methylation in estuaries.

2. Methods

2.1. Field sampling and incubations

Sediment was collected from the vicinity of Hart-Miller Island (39°14.5′N and 76°22′W) in the Chesapeake Bay using an Eckmann style grab sampler and from the main stem of Chesapeake Bay using a Cedar type box corer (Kim, 2004). The samples were collected from the vicinity of HMI in September of each year, and from the main stem of the Chesapeake Bay in May 1993. The upper 4 cm of sediment was scooped from the samplers into an acid washed polypropylene sediment cup. Sediment was collected by sub-coring sediment gathered using a modified Van Veen and a box corer from the Bay of Fundy and Hudson River, respectively (Heyes et al., 2004; Sunderland et al., 2004). Samples from the Hudson River were collected in February and June of 2001 and from the Bay of Fundy between May and August 2001. The sediment was collected from Mackall Cove, off the Patuxent River, by hand coring from a dingy in July 2000. Samples for analysis of Hg and MeHg concentration were placed on ice and frozen upon returning to the lab. The cores for incubation were transported to the laboratory under ambient temperature conditions and with overlying water to maintain redox conditions. Approximately 90% of the overlying water was replaced prior to incubating the cores after spike addition at the same temperature as the sampling site.

The method used to perform Hg methylation and demethylation rates is outlined in Heyes et al. (2004). ^{201}Hg isotope (Oak Ridge batch #176506) and ^{199}Hg (Oak Ridge batch #168490) of 98% and 92% purity were used in these experiments. The interferences from contaminant isotopes in these enriched mixtures were removed from all calculations by using matrix algebra. Me^{199}Hg isotope was synthesized by adding the ^{199}Hg to a solution of methylcobalamin, extracting the methylated ^{199}Hg formed into methylene chloride and then back extracting the methylated isotope into water. Sediment cores were injected with Hg or MeHg isotope that had been equilibrated with site porewater for 1 h prior to injection. The background solid phase concentration of Hg and MeHg were increased by approximately10% and 100%, respectively, by the spike addition, and were incubated for 4 h at the temperature of the site water. The cores were then sliced at 2-cm intervals and quick-frozen.

2.2. Laboratory

Total Hg (T-Hg) concentrations in sediment were determined by refluxing approximately 1 g of sediment in 5 ml of 60 °C 7:3 sulfuric/nitric acid for 4–6 h. The solution was diluted to approximately 50 ml and 1 ml of bromine monochloride was added. Prior to analysis, excess oxidant was reduced with hydroxylamine hydrochloride. Analysis of the digestate was based on EPA method 1631 and the method of Gill and Fitzgerald (1987). An aliquot of sample was placed in a bubbler with tin chloride ($SnCl_2$), and the reduced Hg purged from solution onto a gold trap. The gold trap was flash heated in a stream of argon and the Hg content measured by cold vapor atomic fluorescence spectroscopy (CVAF) (Tekran® 2500) or, in the case of isotope amended samples, detection was made using Induc-

tively Coupled Plasma Mass Spectrometry (ICP-MS) (HP 4500) (Hintelmann and Evans, 1997). For CVAF, the absolute detection limit was 15 fM, based on 3 standard deviation of the mean of the water blank. The actual detection limit for water was 45 fM and for sediments, 7.5 pmol g^{-1}, based on 3 times the standard deviation of the mean of the digestion blank. For ICP-MS, the absolute detection limit was similar at 50 fM and the operational detection limit was 2.5 pmol g^{-1}. The analytical error (defined as the standard error of duplicate samples) was 9% for T-Hg concentration and 10% for ^{199}Hg isotope concentration. Analysis of the estuarine SRMs IAEA-405 (3.84–4.23 nmol g^{-1}) and NIST 1646a (0.20 nmol g^{-1}) standard reference materials for Hg were performed. We obtained 3.69 ± 0.16 nmol g^{-1} for IAEA 405 and 0.21 ± 0.016 nmol g^{-1} for NIST 1646a.

Methylmercury was extracted from sediment by distillation (Horvat et al., 1993). This was followed by aqueous phase ethylation, using sodium tetra-ethylborate, and by purging and trapping onto Tenax®. The Tenax® was flash heated in a stream of argon and the released Hg species thermally separated in a GC column (OV-3) (Bloom, 1989). All Hg complexes were converted to Hg° in a pyrolytic column at the GC outlet, and non-isotope amended samples were measured by CVAF. The ICP-MS was used as the detector for isotope amended samples. The absolute detection limit of the methods was 30 fM. The operational detection limit and analytical error (defined as the standard error of duplicate samples) for MeHg measured in sediment (~1 g) by CVAF was 0.05 pmol g^{-1} and 30%, respectively. For ICP-MS, the detection limit was 0.075 pmol g^{-1} and sample reproducibility was 10% for MeHg concentration and 23% for the Me^{199}Hg isotope concentration. Quality assurance was performed by analysis of IAEA-405 (24.4–29.9 pmol g^{-1}) estuarine sediment standard reference material for MeHg using both CVAF and ICP-MS detection and through spike additions. Recovery of spikes from averaged 87% and a concentration of 26.4 ± 2.5 pmol g^{-1} was obtained for IAEA-405. The ability of the ICP-MS to separate isotopes restricts the practical detection limit for any one Hg isotope to approximately 0.5% of the total ambient Hg concentration.

3. Results

3.1. Mercury methylation in estuarine sediments

The three estuaries studied have very different T-Hg and MeHg concentrations (Table 1). While T-Hg concentrations are very high in the Hudson River, the three sites have a similar range in % MeHg in the sediment. Vertical profiles and detailed discussions of the Hudson River and Bay of Fundy data have been published elsewhere (Heyes et al., 2004; Sunderland et al., 2004) and the data for the Patuxent River is shown in Fig. 2. Of these sites, only the Patuxent River had a "typical" profile for MeHg, with a maximum concentration of 1 pmol g^{-1} at the sediment–water interface. Methylmercury production potential, measured as pmol of isotope methylated g^{-1} h^{-1}, varied among the three estuaries (Fig. 3). The highest methylation potentials were observed in the Hudson River but the percentage of isotope methylated was highest in the Bay of Fundy. Concentrations of Hg are much higher in the Hudson sediments ($4 .1 \pm 0.7$ nmol g^{-1}) than both Patuxent River (0.13 ± 0.10 nmol g^{-1}) and the Bay of Fundy (0.15 ± 0.3 nmol g^{-1}) and to ensure detection of the isotope, we added 10 times more isotope to the Patuxent River and Hudson River sediments than the Bay of Fundy sediment.

Table 1
Mean mercury and methylmercury concentrations in sediment of the study sites

Site	Total Hg, nmol g^{-1}	MeHg, pmol g^{-1}	% MeHg
Hudson River	4.13 ± 0.76	7.98 ± 2.49	0.20 ± 0.07
Patuxent River	0.13 ± 0.10	0.49 ± 0.49	0.23 ± 0.49
Bay of Fundy	0.15 ± 0.3	1.1 ± 1.5	0.70 ± 0.26

Methylation in the Patuxent River sediments is highly skewed toward the surface sediments, as this site receives little tidal turbation. This is consistent with the observed MeHg distribution at the site. Both the Hudson River and Bay of Fundy sites are subjected to substantial sediment resuspension and tidal surge, and methylation rate is more uniform downcore. The seasonal results from the Hudson River suggest that temperature has an influence on Hg methylation with the Hg methylation potentials being higher in June than earlier in the year (Fig. 3).

When MeHg potentials (pmol g^{-1} h^{-1}) are plotted against the in situ MeHg concentrations, the slopes of the regression lines (which are significant) among the sites are very different (Fig. 4). In the case of the Patuxent River, the slope is relatively steep and the absolute amount of methylated isotope produced would equal the existing amount of MeHg in the sediment after just a few hours (Fig. 4a). Seasonal impacts on Hg methylation in the Hudson River are again clear in Fig. 4b. However, while in situ MeHg production quadruples in rate from 0.05 to 0.25 pmol h^{-1} from

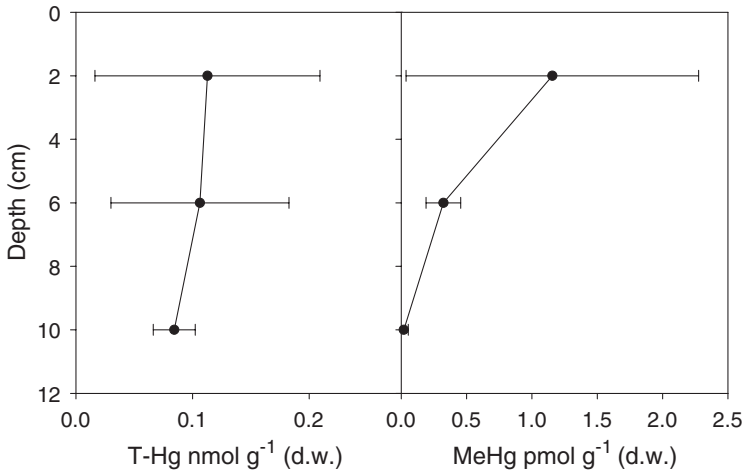

Fig. 2. Methylmercury (a) and total mercury (b) concentrations in sediment from Mackall Cove, a tributary off the Patuxent River, Chesapeake Bay, MD. Samples were collected July 2000.

February to June, the in situ MeHg concentration, which is a reflection of the relative rate of the two processes (methylation and demethylation), barely doubles. Obviously, demethylation also impacts the in situ, or steady state, MeHg concentration. In the Bay of Fundy, the slope of the regression line is the lowest, and methylation potential (pmol g^{-1} h^{-1}) correlates relatively poorly with MeHg concentration.

The isotope addition methods suggest that demethylation potentials are very rapid, with large portions of the isotope demethylated within the period of the incubation (Fig. 5). Within the two sites there is little trend in demethylation potential, which suggests the factors

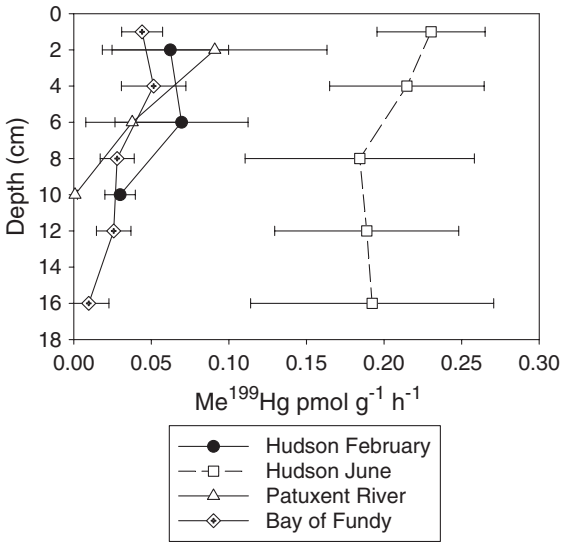

Fig. 3. Profiles of absolute mercury methylation of added Hg isotope from the Hudson River, Patuxent River and Bay of Fundy.

that affect demethylation are not entirely the same factors that affect Hg methylation. However, demethylation is greatest in the Bay of Fundy, which is consistent with the MeHg concentration data, and may explain the poor correlation observed in Fig. 4c. Unfortunately we did not measure demethylation in the Patuxent River sediment, where methylation rate was very rapid relative to the net MeHg concentration.

Methylation and demethylation activities between systems can be compared by examining the methylation and demethylation rate constants (Table 2). The calculation of rate constants is not trivial, incorporates various assumptions, and the impact of many factors that influence the respective rates are not well known. The mathematical steps in assessing the rate constants are discussed in detail by Martin-Doimeadios et al. (2004) and Hintelmann et al. (2000). Assuming a pseudo first order reversible reaction, the rate of methylation is:

$$\mathrm{d[MeHg]}/\mathrm{d}t = k_{\mathrm{m}}[\mathrm{Hg}] - k_{\mathrm{dm}}[\mathrm{MeHg}] \quad (1)$$

where k_{m} is the methylation rate and k_{dm} is the demethylation rate.

For methylation, the rate was determined by a simple product reactant expression, as formation of the reaction product is measured in this instance. The initial rate can be determined before the back reaction (demethylation of the methylated isotope (i.e. $k_{\mathrm{dm}}[^{201}\mathrm{MeHg}]$) becomes significant relative to the forward reaction (i.e. $k_{\mathrm{m}}[^{201}\mathrm{Hg}]$). Further, it was assumed that the added isotope is representative of the bioavailable pool of Hg, and that the spike was sufficiently large that substantial depletion did not occur during the assay

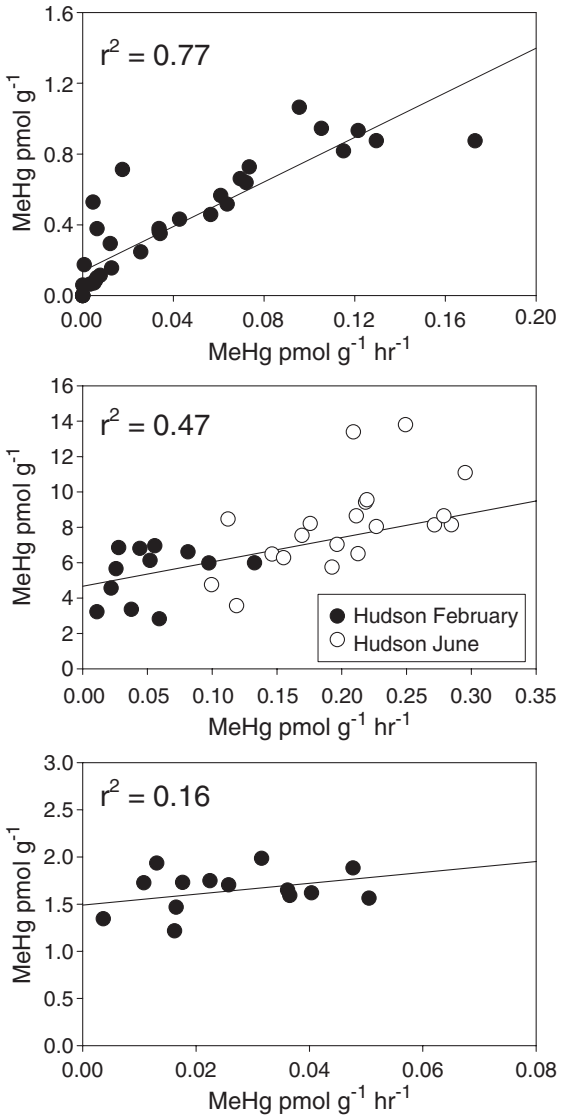

Fig. 4. Methylation of Hg isotope and in situ methylmercury (MeHg) concentration from (a) the Patuxent River, (b) Hudson River and (c) Bay of Fundy.

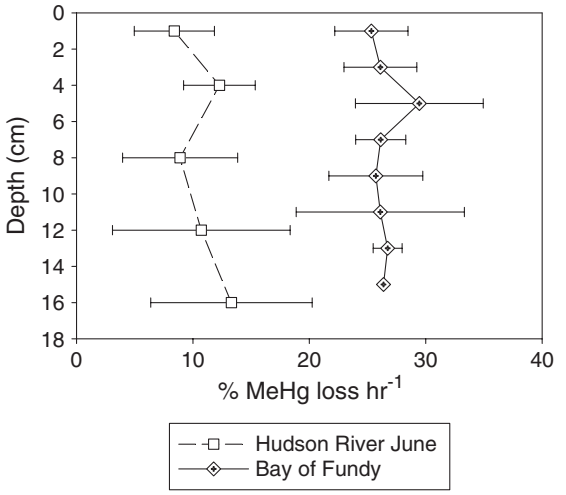

Fig. 5. Mercury demethylation profiles from the Hudson River and Bay of Fundy, expressed as % MeHg isotope loss per hour.

period. Given the low conversion rates, this assumption is valid. This resulted in Eq. (2):

$$k_m = \frac{[Me^{201}Hg]}{[^{201}Hg] \cdot t} \quad (2)$$

The amount of Hg isotope that is actually available for methylation is likely to be smaller than the amount of isotope added, but this is unimportant as long as the fraction that is bioavailable does not change over the course of the assay. This assumption is discussed further below. The isotope was equilibrated with porewater prior to injection in an effort to minimize changes in bioavailability during the assay.

For demethylation, we again assumed a first-order reaction, and that the back reaction was initially unimportant (Eq. (3)):

$$[Me^{199}Hg] = [Me^{199}Hg]_0 e^{k_{dm}t} \quad (3)$$

A better approach could be to assess both constants based on time series data. Indeed, if such an approach is taken, it is possible to obtain both rate constants after the addition of only one isotope, although the use of two isotopes provides confirming evidence for the reversibility of the reaction. This approach is explained in Martin-Doimeadios et al. (2004). However, this approach would require time dependent methylation and demethylation rate data collected for each location, which was not obtained in the studies described here. At steady state, from Eq. (1), it can be concluded that

Table 2
Rate constants of mercury methylation (k_m), demethylation (k_{dm}), ratios of the rate constants and of methylmercury and mercury concentrations from the methylation demethylation cores of the study sites

Site	k_m	k_{dm}	k_m/k_{dm}	MeHg/T-Hg
Whole core				
Hudson	1.05×10^{-4}	0.66	1.6×10^{-4}	1.76×10^{-3}
Bay of Fundy	1.11×10^{-3}	0.24	4.7×10^{-3}	4.51×10^{-3}
Patuxent River	4.61×10^{-4}			
Most active				
Hudson	0.85×10^{-4}	0.64	1.38×10^{-4}	1.97×10^{-3}
Bay of Fundy	1.83×10^{-3}	0.15	1.24×10^{-2}	3.33×10^{-3}

Constants for the whole core and for the slices where methylmercury MeHg production was greatest are given under "most active".

the ratio of the rate constants is equivalent to the ratio of the bioavailable fractions of [Hg] and [MeHg]:

$$k_m/k_{dm} = [MeHg]_{bio}/[Hg]_{bio} \quad (4)$$

Overall, the Hudson River k_m is an order of magnitude lower than the Bay of Fundy k_m regardless if the calculation is for the whole core or the zone of greatest methylation potential (Table 2). The k_{dm}'s for the Bay of Fundy and the Hudson River were within a factor of 3, thus the difference in demethylation rate has less impact on the relative MeHg concentration between sites. The ratio of the rate constants is similar to the ratio of MeHg/Hg concentration for the Bay of Fundy, as expected from Eq. (4), if the relative bioavailability of each is similar. This is less so for the Hudson River (Table 2) but overall, the similarity between these two ratios implies that the cycling of the isotope and the in situ Hg and MeHg are similar, overall, and that our assumptions about the proportionality of the bioavailability of the added Hg isotope and in situ Hg have some merit.

The relationship between in situ MeHg concentration and MeHg production (Fig. 4; slope=[MeHg]/($[Me^{201}Hg]\cdot t^{-1}$) can be used to independently estimate the MeHg demethylation rate, which then can be compared to the value estimated from the isotope additions. Assuming that the in situ Hg and MeHg are at steady state, then the [MeHg] concentration can be expressed in terms of [Hg] and the rate constants, as derived from Eq. (4), and $[Me^{201}Hg]$ can also be similarly expressed, based on Eq. (2), so that the slope of the relationship is given by the following expression (Eq. (5)):

$$\text{Slope} = \frac{1}{k_{dm}} \frac{[Hg]}{[^{201}Hg]} \quad (5)$$

From Fig. 4, the slope of the relationship for the Hudson River is 11.4 h. As the isotope spike of inorganic ^{201}Hg was a tenth of the in situ Hg concentration, $k_{dm}=0.87\ h^{-1}$ from Eq. (5). Similarly, for the Bay of Fundy, the slope from Fig. 4 is 6.25 h; therefore, $k_{dm}=1.6\ h^{-1}$. These rates are faster than what were estimated based on the change in MeHg isotope (Table 2). Therefore, the MeHg isotope addition short-term incubation method is likely underestimating Hg demethylation rate, perhaps because some portion of the isotope becomes less available over time, or, more likely, because the high rates of conversion invalidate the assumption that the back reaction can be neglected. In sediments, the site of maximum demethylation may be in close proximity to the site of Hg methylation, and may even involve the same organisms (Pak and Bartha, 1998). Thus, the newly produced MeHg may perhaps be more available for demethylation, and this may lead to differences in the two estimations, because of the different approaches taken.

3.2. Temporal and spatial variation in MeHg concentrations in estuarine sediment

The total Hg and MeHg concentration has been determined in sediments at 35 sites from 1998 to 2003 around Hart Miller Island (HMI), a man-made dredge disposal facility in the Chesapeake Bay, as part of a project with the Maryland Department of the Environment (MDE, 2003). This data and future data on these sites will be available through the EPA system "http://epa.gov/storet". The variability of T-Hg and MeHg concentrations among the stations was substantial (Fig. 6). Over this period, T-Hg concentrations from the 36 stations ranged from 0.04 to 8.6 nmol g^{-1}; with a mean of 0.99, a median of 0.87 and a standard deviation of 0.93 nmol g^{-1} (variability or RSD=100* stdev/mean=94%). Concentrations of MeHg among the stations also had a wide range, from 0.20 to 16.7 pmol g^{-1}; with a mean of 4.45, median of 3.83 and standard deviation of 3.06 pmol g^{-1} (RSD=69%). The percent of T-Hg that occurred as MeHg appeared more consistent, with the mean of 0.54%, median of 0.39%, and a standard deviation of 0.46%, although the RSD was also high (85%). Although substantial inter annual variability is present, as indicated by the error bars, some long term consistency exists for some stations such as MDE 2 and MDE 24, where T-Hg concentrations are always low, and MDE 37, where T-Hg concentrations are always high. This is also true for MeHg, and as the % MeHg indicates, locations with sediment low in T-Hg concentration are often low in MeHg concentration (Fig. 6c).

The variability that exists in T-Hg concentration among the sites can be largely attributed to the organic matter content (OM) (Fig. 7). The sediments high in T-Hg concentration that appear as outliers are from the more contaminated Back River. The percentage OM appears to be a good indictor of T-Hg concentration around HMI (Fig. 7a), especially for the lower OM sediments. At higher OM content and T-Hg concentration, the relationship breaks down. A similar lack of correlation at high OM was found for Baltimore Harbor (Mason and Lawrence, 1999). Like inorganic Hg, MeHg also has a high affinity for organic matter. However, the relationship is not as strong (Fig. 6b), nor is there a relationship between % MeHg and % OM (Fig. 6c).

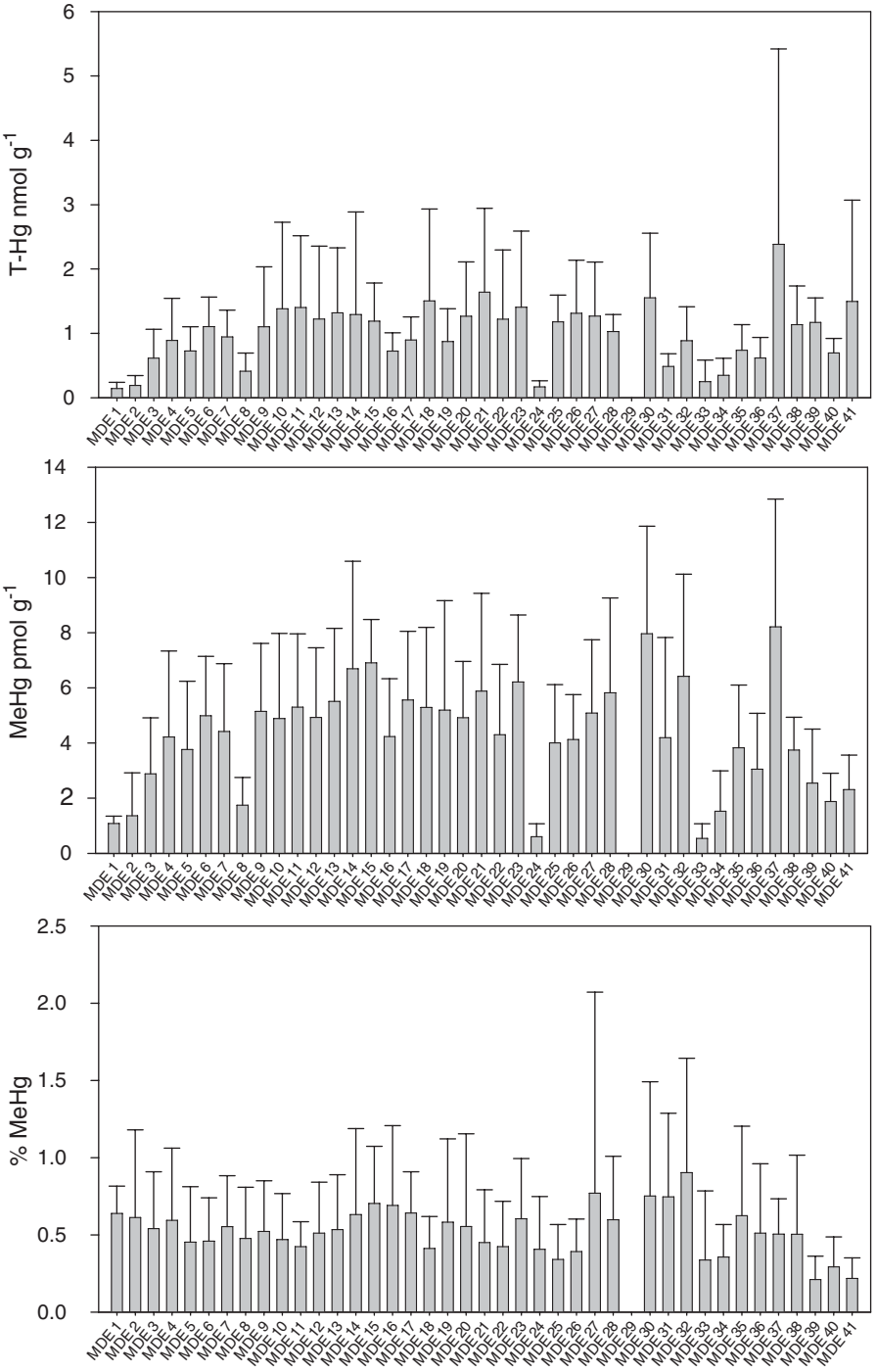

Fig. 6. Variability of Hg and MeHg concentrations around Hart Miller Island Disposal Facility, Chesapeake Bay 1998–2003.

The 1996 transect down the Chesapeake Bay revealed that the highest T-Hg concentrations were in the northern third of the Bay (Fig. 8). Concentrations of T-Hg in sediment quickly decreased below 0.2 nmol g^{-1} by the mid-Bay and remained below this concentration until the end of the transect (Kim, 2004). Concentrations of MeHg were also highest in the upper Bay but were lowest in the mid-Bay (Fig. 7). As a result, the percent of T-Hg that was MeHg remained constant at approximately 1% of T-Hg for the upper two thirds of the Estuary. However, in the lower Bay, MeHg concentration increased to approximately 4% of the T-Hg

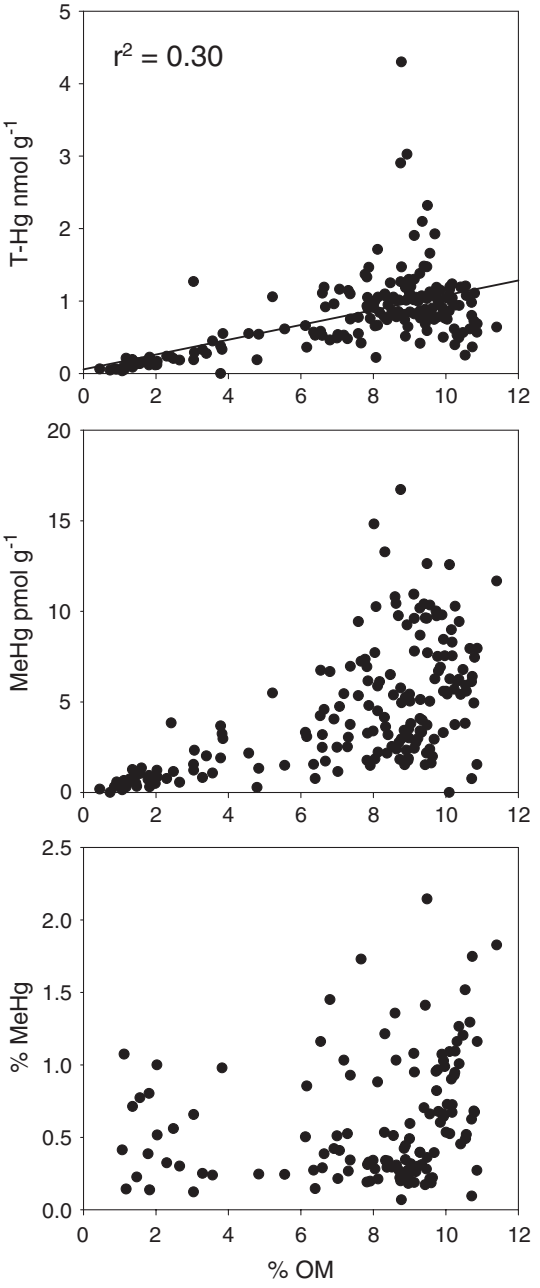

Fig. 7. The relationship between percentage organic matter (% OM) and (a) mercury (T-Hg), (b) methylmercury and (c) percentage of mercury that is methylmercury in sites around the Hart Miller Island Disposal Facility, Chesapeake Bay.

concentration in sediment on average, although there was high variability. The mid-Bay has the highest level of sulfide present in the porewater (Marvin-DiPasquale et al., 2003), which may inhibit methylation (Benoit et al., 1999), while sulfide was undetectable at the other locations in previous studies (Roden and Tuttle, 1993). Overall, T-Hg is correlated with OM down Bay (r^2=0.60; n=36) and with MeHg (r^2=0.61; n=36), which contrasts what was found around HMI for MeHg (Kim et al., 2005; Fig. 6b). The MeHg pool will be more variable as it is continually being formed via methylation, and destroyed by demethylation, and this could account for the weaker correlation around HMI, and overall compared to Hg and % OM.

4. Discussion

4.1. Mercury methylation and demethylation potential

There are many factors that can hinder the measurement of true Hg methylation and MeHg demethylation rates. To simulate in situ rates, the incubations performed here were: (1) run for short durations (2–4 h), so that the initial rate estimation approach was valid; (2) the isotope was equilibrated with porewater prior to injection to minimize speciation changes during the assay; and (3) spike concentrations were kept low—10–20% of the in situ concentration to minimize the potential increase in Hg bioavailability that may have occurred with excess Hg addition. Clearly, the method is a compromise between the ability to detect changes in concentration and the maintenance of realistic microbial and geochemical conditions. It is therefore recognized that there are caveats associated with these measurements and the subsequent rate calculations.

The magnitude of the Hg methylation and demethylation rate constants suggest that the turnover time ($(k_m+k_{dm})^{-1}$) for MeHg in estuarine sediment is on the order of days. Therefore the ambient concentration of MeHg in the sediment reflects recent events. The relationship between methylation production rate (pmol g^{-1} h^{-1}) and concentration is stronger for the Hudson River and the Patuxent River, than for the Bay of Fundy. This may largely be the result of a small range in MeHg concentration found in the sediments of the Bay of Fundy cores sampled for the study, which does not represent the range of the Bay of Fundy as a whole (Table 1), rather than any intrinsic difference between the sites. As stated above, at steady state, for first order reversible reactions, the relative concentration of MeHg is directly related to the ratio of the rate constants, and inversely related to the magnitude of the demethylation rate.

The methylation rate constant (k_m) increased as: Bay of Fundy>Patuxent River>Hudson River (Table 2). The first order rate constant for methylation, as formulated in Eq. (2), should be independent of the amount of Hg isotope added, which was much larger in the case of the Hudson and Patuxent Rivers. Given the small con-

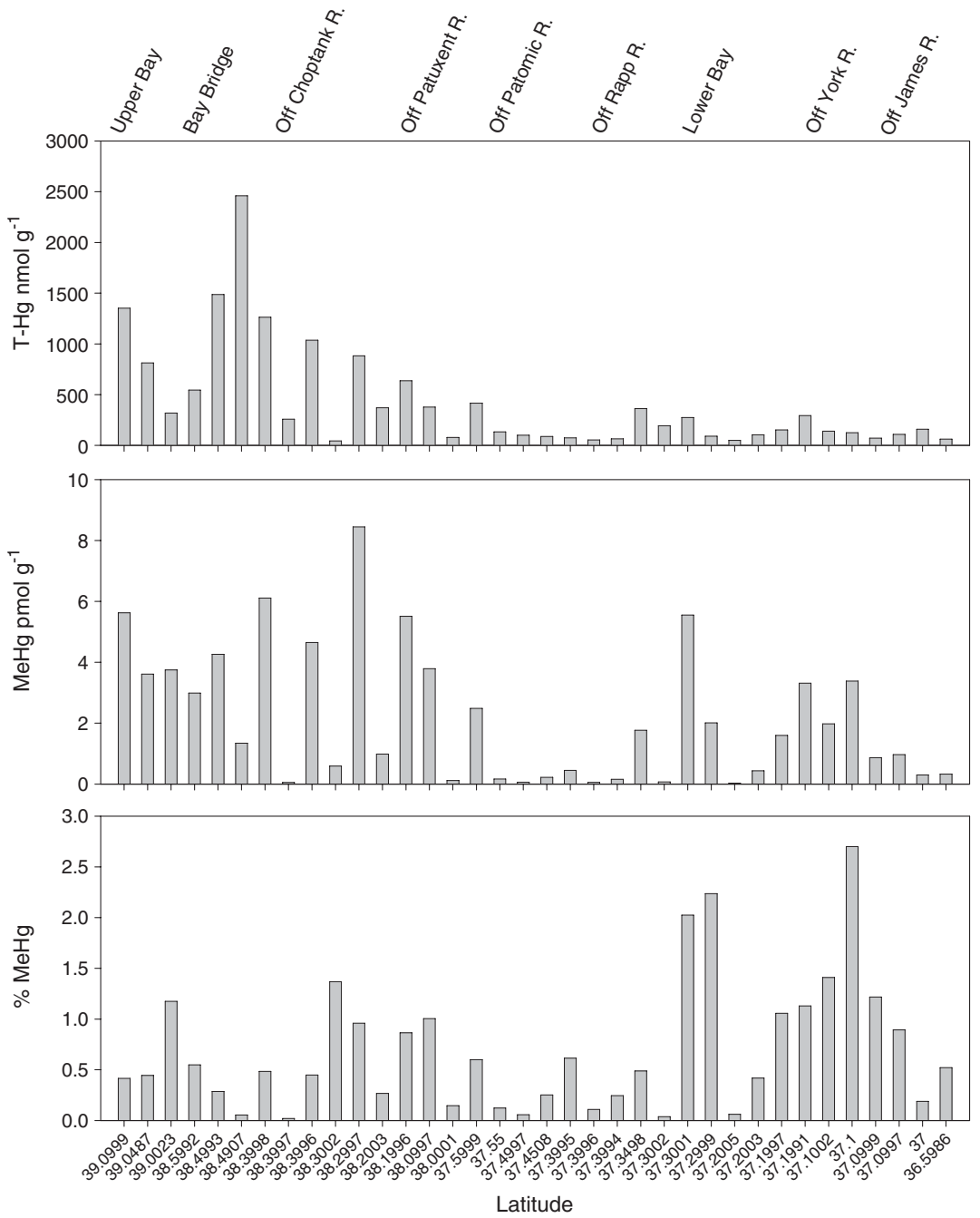

Fig. 8. Concentrations of (a) mercury (T-Hg), (b) methylmercury (MeHg) and (c) percentage of mercury that is methylmercury (% MeHg), along the main stem of the Chesapeake Bay.

version rates, the Hg isotope added was in excess of the short-term bioavailable pool for methylation, and was likely partitioned to the readily available sites on particles and colloids after spiking into the core. Over a timescale which is likely longer than the assay period, such Hg may become bound more strongly and therefore become unavailable for methylation. The kinetics of such adsorption reactions cannot be accounted for given the method of estimating the Hg methylation rate constant, but are assumed to be small given the timescale of the measurement. Similarly, the kinetics of adsorption–desorption reactions could impact the bioavailability of MeHg for demethylation, but given the high demethylation rates, most of the MeHg spike must

be bioavailable during the assay. If the kinetic effects are similar for both assays, then the ratio of these measured rate constants will provide a comparable measurement of the in situ processes; i.e. is comparable to the relative MeHg concentration (Table 2). The results of the on-going METAALICUS studies in Canada (http://www.biology.ualberta.ca/old_site/metaalicus//metaalicus.htm), where Hg isotope is being intentionally added to a lake, and its watershed, support the notion that both Hg and MeHg are transferred into a refractory component within the sediment over time such that they are preserved from further reaction, and not be readily available for methylation or demethylation.

From this study of methylation potential (and the measure of the relative rate constants), we conclude that MeHg concentration is a good predictor of net methylation activity, and this is likely due to the ubiquity of demethylation rates, and the similarity in rates measured in the estuaries, and the relative rapid nature of both processes. Thus, changes in MeHg concentration in sediments appear driven more by differences in the methylation rate than the demethylation rate. Given this conclusion, the spatial and temporal distribution of Hg and MeHg in the Chesapeake Bay sediments are examined, and discussed here in terms of the factors that influence Hg methylation.

As mentioned above, the factors that affect Hg methylation can be separated into those that affect the bioavailability of Hg to the methylating organisms and those that affect the activity of the Hg methylating bacteria. The relative importance of these categories with regard to controlling Hg methylation in estuaries is difficult to assess, as, for example, DOC concentration and composition could impact both Hg bioavailability and microbial activity. While the concentration of Hg in estuarine sediment has some impact on the absolute MeHg concentration (Fig. 1), the response is not linear. This suggests that the largest fraction of Hg in sediment is not immediately available for Hg methylation and factors other than total Hg concentration are at least as important. This is well known and the primary factors influencing methylation have been previously outlined (e.g. Benoit et al., 2003), and will be examined in the context of estuaries below.

Mercury concentration is well correlated with % OM in the Chesapeake Bay, and other locations (Conaway et al., 2003), but % OM is less strongly related to MeHg concentration. Neither Hg nor MeHg are strongly related to % OM at high % OM levels in the Chesapeake Bay. While carbon is required by Hg methylating organisms, the measurement of total OM content has little relevance in terms of the concentrations of organic substrate, such as pyruvate and acetate, which are required by the Hg methylating organisms. We conclude that the overall OM content of estuarine sediments is a better indicator of the Hg binding capacity (i.e. of Hg bioavailability) than of its impact on the microbial activity.

Benoit et al. (1999) proposed that neutral Hg–S complexes in water, principally HgS^0, control the transfer of Hg into sulfate reducing bacteria (SRB). The formation of this neutral complex is dependent on a number of factors, the most significant of which is the concentration of dissolved sulfide. It is generally assumed that the pool of Hg available for methylation is dissolved, but culture studies with sediment suggest that resupply of the dissolved pool by desorption from sediment must occur to maintain the methylation rates observed in culture experiments (Benoit et al., 2001).

The other processes that affect Hg methylation are those that affect microbial activity. Increases in temperature will increase both microbial methylation and demethylation rates (Bloom et al., 1999; Gilmour et al., 1998; Korthals and Winfrey, 1987; Regnell et al., 1997) and in our study of the Hudson River, Hg methylation rates increased by a factor of four between the winter (3 °C) and the summer (22 °C). Whether the two processes respond to a similar degree to changes in temperature is not clear, but a different response would be one way of explaining the higher MeHg sediment concentrations in the summer.

Environmental evidence and studies of cultured bacteria suggest that the ability to methylate Hg at environmental concentrations is largely restricted to a subset of sulfate reducing bacteria (SRB) (Choi et al., 1994; Gilmour et al., 1992; King et al., 1999; Ekstrom et al., 2003). The number of SRB that have been tested is far from exhaustive, and it still remains possible that other classes of bacteria, such as iron reducers (Gilmour et al., 1996) and methanogens, can methylate Hg, although Warner et al. (2003) found no evidence for methylation under Fe-reducing conditions in freshwater sediments. Demethylation appears to be a process that is more widely spread across genera (Warner et al., 2003; Marvin-DiPasquale et al., 2000).

For sulfate reducing bacteria (SRB), the availability of sulfate and carbon are the primary substrate-limiting factors. For example, it has been suggested that sulfate reduction in the Chesapeake Bay is often carbon limited (Marvin-DiPasquale et al., 2003; Marvin-DiPasquale and Capone, 1998). Gilmour et al. (1992) demonstrated that increasing sulfate concentration stimulated MeHg production potential in estuarine sediments, and surpris-

ingly, Marvin-DiPasquale et al. (2003) has shown that sulfate can limit the activity of SRB throughout the Chesapeake Bay. Higher sulfate reduction rates (SRR) have been found in the mid and lower Chesapeake Bay (Fig. 9; Marvin-DiPasquale et al. 2003), with porewater sulfate concentrations ranging widely from 8 mM in the upper and mid-Bay to 20 μM in the lower Bay. However, the weak sulfate gradient and higher SRR do not result in an increase in MeHg concentration down-Bay (Fig. 9). MeHg concentration tends to decrease down-Bay, with the lowest MeHg concentrations, relative to SRR, in the mid-Bay where the measured SRR was greatest, but sulfide was the highest (Fig. 9). Indeed, the highest relative MeHg concentration (to SRR) was in the upper Bay. While sulfate additions can stimulate methylation, the accumulation of sulfide can inhibit methylation, thus the interaction of factors influencing bioavailability and microbial activity become interdependent (Gilmour et al., 1998).

As discussed earlier, Benoit et al. (2003) have shown the influence of sulfide concentration on Hg speciation and bioavailability to SRB. Concentrations of 1–2 mM sulfide have been observed in the porewaters of the mid-Chesapeake Bay but sulfide was not detected elsewhere (Marvin-DiPasquale et al., 2003; Roden and Tuttle, 1993). Charged Hg–S complexes will dominate the speciation of Hg at concentrations as low as 3 μM, and thus bioavailability of Hg to the methylating organisms in the mid-Bay is likely limited by their formation. Therefore Hg–S complexation could account for the low MeHg concentrations seen at this location, despite having the Bay's highest SRR. Similarly, Marvin-DiPasquale and Agee (2003) showed a decoupling of Hg methylation rate and SRR for sites in the San Francisco Bay delta region, when sulfide built up in the summer at these sites. Because HgS^0 is the predicted dominant Hg ligand at sulfide concentrations below current analytical detection limits (~0.1 μM), it is not known if low sulfide condition inhibits methylation in the Hudson River sediment or if other factors, discussed above, limit the build up of MeHg in this environment.

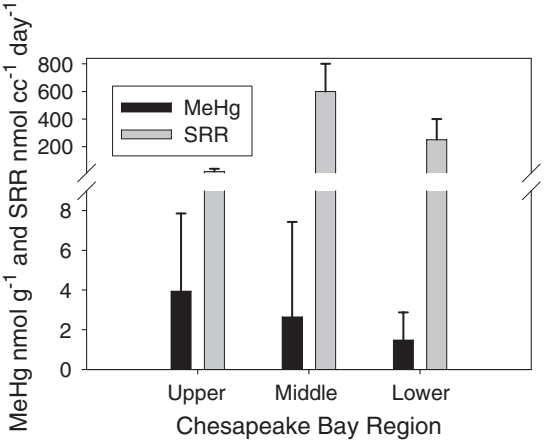

Fig. 9. Methylmercury (MeHg) concentration and sulfate reduction rates (SRR) in the Chesapeake Bay.

While Hg methylation is inhibited at high levels of sulfide, relatively high concentrations of MeHg are found in salt marshes (King et al., 2002). However, a closer examination of the SRR between these salt marsh sites and the Chesapeake Bay, and the Hudson River (Santore, personal communication), show that the sites studied by King et al. (1999, 2001) do not have high rates relative to the mid and lower Chesapeake Bay; nor are the Hg methylation rates as high as those found in the Hudson River (Heyes et al., 2004) or Long Island Sound (Hammerschmidt and Fitzgerald, 2004). It is not known how rapidly MeHg is demethylated in such environments and this may account for the relatively high MeHg concentrations. Additionally, the microbial community assemblage may differ markedly between the Chesapeake Bay and Hudson River sediments and salt marshes, as microbial composition is clearly an important factor in Hg methylation (King et al., 1999, 2001; Devereux et al., 1996).

Our understanding of the demethylation mechanism is bias toward contaminated sites, where the mer operon is perhaps dominant, and the driving force behind demethylation in more pristine environments remains unclear. Overall, while there may be a higher overall burden of Hg in an estuary, as measured as a sediment concentration, due to anthropogenic and other inputs, the existing spatial and temporal data discussed here clearly indicate that microbial activity and speciation of Hg are as least as important as total Hg concentration in determining the net Hg concentration in these sediments. The relationship between MeHg production and MeHg concentration appears to be strong for large estuaries and lakes, where other sources/contributions of MeHg are limited, and in situ production dominates as the main MeHg source. In such systems, there is a strong relationship between MeHg in sediments and in the overall burden in the water column. Thus, MeHg concentration within such a system is strongly dependent on its production and destruction in the sediment.

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References

Baeyens, W., Leermakers, M., Papina, T., Saprykin, A., Brion, N., Noyen, J., De Geiter, M., Elskens, M., Goyens, L., 2003. Bioconcentration and biomagnification of mercury and methylmercury in North Sea and Scheldt Estuary fish. Arch. Environ. Contam. Toxicol. 45, 498–508.

Barkay, T., Miller, S.A., Summers, A.O., 2003. Bacterial mercury resistance from atoms to ecosystems. FEMS Microbiol. Rev. 27, 355–384.

Benoit, J.M., Gilmour, C.C., Mason, R.P., Heyes, A., 1999. Sulfide controls on mercury speciation and the bioavailability in sediment pore waters. Environ. Sci. Technol. 33, 951–957.

Benoit, J.M., Mason, R.P., Gilmour, C.C., Aiken, G.R., 2001. Constants for mercury binding by dissolved organic carbon isolates from the Florida Everglades. Geochim. Cosmochim. Acta 65, 4445–4451.

Benoit, J., Gilmour, C.C., Heyes, A., Mason, R.P., Miller, C., 2003. Geochemical and biological controls over methylmercury production and degradation in aquatic ecosystems. In: Chai, Y., Braids, O.C. (Eds.), Biogeochemistry of Environmentally Important Trace Elements, ACS Symposium Series #835American Chemical Society, Washington, DC, pp. 262–297.

Birch, G.F., Taylor, S.E., 2000. Distribution and possible sources of organochlorine residues in sediments of a large urban estuary, Port Jackson, Sydney. Aust. J. Earth Sci. 47, 749–756.

Bloom, N.S., 1989. Determination of picogram levels of methylmercury by aqueous phase ethylation, followed by cryogenic gas chromatography with cold vapour atomic fluorescence detection. Can. J. Fish Aquat. Sci. 46, 1131–1140.

Bloom, N.S., Gill, G.A., Cappellino, S., Dobbs, C., Mcshea, L., Driscoll, C., Mason, R., Rudd, J., 1999. Speciation and cycling of mercury in Lavaca Bay, Texas, sediments. Environ. Sci. Technol. 33, 1–13.

Choi, S.-C., Bartha, R., 1994. Environmental factors affecting mercury methylation in estuarine sediments. Bull. Environ. Contam. Toxicol. 53, 805–812.

Choi, S.-C., Chase, T. Jr., Bartha, R., 1994. Metabolic pathways leading to mercury methylation in *Desulfovibrio desulfricans* LS. Appl. Environ. Microbiol. 60, 4072–4077.

Conaway, C., Squire, S., Mason, R.P., Flegal, A.R., 2003. Mercury speciation in the San Francisco bay estuary. Mar. Chem. 80, 199–225.

Devereux, R., Winfrey, M.R., Winfrey, J., Stahl, D.A., 1996. Depth profile of sulfate reducing bacterial ribosomal RNA and mercury methylation in an estuarine sediment. FEMS Microbiol. Ecol. 20, 31–32.

Ekstrom, E.B., Morel, F.M.M., Benoit, J.M., 2003. Mercury methylation independent of the acetyl coenzyme A pathway in sulfate-reducing bacteria. Appl. Environ. Microbiol. 69, 5414–5422.

Fujiki, M., Tajima, S., 1992. The pollution of Minamata Bay by mercury. Water Sci. Technol. 25, 133–140.

Gill, G.A., Fitzgerald, W.F., 1987. Picomolar measurements of mercury in seawater and other materials using stannous chloride reduction and 2-phase gold amalgamation with gas phase detection. Mar. Chem. 20, 227–243.

Gilmour, C.C., Riedel, G.S., 1995. Measurement of Hg methylation in sediments using high specific-activity ^{203}Hg and ambient Incubation. Water, Air, Soil Pollut. 80, 747–756.

Gilmour, C.C., Henry, E.A., Mitchell, R., 1992. Sulfate stimulation of mercury methylation in freshwater sediments. Environ. Sci. Technol. 26, 2281–2287.

Gilmour, C.C., Riedel, G.S., Coates, J.S., Lovley, D., 1996. Abstract. Amer. Soc. Microbiol., New Orleans.

Gilmour, C.C., Riedel, G.S., Ederington, M.C., Bell, J.T., Benoit, J.M., Gill, G.A., Stordal, M.C., 1998. Methylmercury concentrations and production rates across a trophic gradient in the northern Everglades. Biogeochemistry 40, 327–345.

Hammerschmidt, C.R., Fitzgerald, W.F., 2004. Geochemical controls on the production and distribution of methylmercury in nearshore marine sediments. Environ. Sci. Technol. 38, 1487–1495.

Heyes, A., Miller, C., Mason, R.P., 2004. Mercury and methylmercury in the Hudon River sediment. Impact of tidal resuspension on partitioning and methylation. Mar. Chem. 90, 75–89.

Hines, M.E., Horvatt, M., Fagneli, J., Bonzongo, J.C.J., Batkay, T., Major, E.B., Scott, K.J., Bailey, E.A., Warwick, J.J., Lyons, W.B., 2000. Mercury biogeochemistry in the Idrija River, Slovenia, from the above the mine into the Gulf of Trieste. Environ. Res. 83, 129–139.

Hintelmann, H., Evans, R.D., 1997. Application of stable isotopes in environmental tracer studies — measurements of monomethyl–mercury (CH_3Hg^+) by isotope dilution and detection of species transformation. Fresenius' J. Anal. Chem. 358, 378–385.

Hintelmann, H., Wilken, R.-D., 1995. Levels of total mercury and methylmercury compounds in sediments of the polluted Elbe River: influence of seasonally and spatially varying environmental factors. The Sci. Total Environ. 166, 1–10.

Hintelmann, H., Keppel-Jones, K., Evans, R.D., 2000. Constants of mercury methylation and demethylation rates in sediments and comparison of tracer and ambient mercury availability. Environ. Toxicol. Chem. 19, 2204–2211.

Horvat, M., Bloom, N.S., Liang, L., 1993. Comparson of distillation with other current isolation methods for the determination of methyl mercury compounds in low level environmental samples. Part 1. Sediments. Anal. Chim. Acta 281, 135–152.

Kannan, K., Smith, R.G. Jr., Lee, R.F., Windom, H.L., Heitmuller, P.T., Macauley, J.M., Summers, J.K., 1998. Distribution of total mercury and methyl mercury in water, sediment and fish from south Florida estuaries. Arch. Environ. Contam. Toxicol. 34, 109–118.

Kerhig, H.A., Pinto, F.N., Moreira, I., Malm, O., 2003. Heavy metals and methylmercury in a tropical coastal estuary and mangrove in Brazil. Org. Geochem. 34, 661–669.

Kim, E.-H. 2004. The Importance of Physical Mixing and Sediment Chemistry and Methylmercury Biogeochemical Cycling and Bioaccumulation within Shallow Estuaries. Ph.D. Thesis, University of Maryland, College Park. 286 pp.

Kim, E.-H., Mason, R.P., Porter, E.T., Soulen, H.L., 2004. The effect of resuspension on the fate of total mercury and methyl mercury in

a shallow estuarine ecosystem: a mesocosm study. Mar. Chem. 86, 121–137.

Kim, E.-H., Mason R.P., Porter, E.T., Soulen, H.L., in press. The impact of resuspension on sediment mercury dynamics, and methylmercury production and fate: a mesocosm study. Mar. Chem.

King, J.K., Saunders, F.M., Lee, R.F., Jahnke, R.A., 1999. Coupling mercury methylation rates to sulfate reduction rates in marine sediments. Environ. Toxicol. Chem. 18 (7), 1362–1369.

King, J.K., Kostka, J.E., Frischer, M.E., Saunders, F.M., Jahnke, R.A., 2001. A quantitative relationship that demonstrates mercury methylation rates in marine sediments are based on the community composition and activity of sulfate reducing bacteria. Environ. Sci. Technol. 35, 2491–2496.

King, J.K., Harmon, S.M., Fu, T.T., Gladden, J.B., 2002. Mercury removal, methylmercury formation, and sulfate-reducing bacteria profiles in wetland mesocosms. Chemosphere 46, 859–870.

Korthals, E.T., Winfrey, M., 1987. Seasonal and spatial variations in mercury methylation and demethylation in an oligotrophic lake. Appl. Environ. Microbiol. 53, 2397–2404.

Mason, R.P., Lawrence, A.L., 1999. Concentration, distribution, and bioavailability of mecury and methylmercury in sediments of Baltimore Harbor and Chesapeake Bay, Maryland, USA. Environ. Tox. Chem. 18, 2438–2447.

Mason, R.P., Fitsgerald, W.F., Morel, F.M.M., 1994. The biogeochemical cycling of elemental mercury—anthropogenic influences. Geochim. Cosmochim. Acta 58, 3191–3198.

Mason, R.P., Lawson, N.M., Lawrence, A.L., Leaner, J.L., Lee, J.G., Sheu, G.-R., 1999. Mercury in the Chesapeake Bay. Mar. Chem. 65, 77–96.

Martin-Doimeadios, R.C.R., Tessier, E., Amouroux, D., Guyoneaud, R., Duran, R., Caumette, P., Donard, O.F.X., 2004. Mercury methylation/demethylation and volatilization pathways in estuarine sediment slurries using species specific enriched stable isotopes. Mar. Chem. 90, 107–123.

Marvin-DiPasquale, M., Agee, J.L., 2003. Microbial mercury cycling in sediments of the San Francisco Bay-Delta. Estuaries 26, 1517–1528.

Marvin-DiPasquale, M., Capone, D.G., 1998. Benthic sulfate reduction along the Chesapeake Bay central channel: I. Spatial trends and controls. Mar. Ecol. Prog. Ser. 168, 213–228.

Marvin-DiPasquale, M., Agee, J., McGowan, C., Oremland, R.S., Thomas, M., Krabbenhoft, D., Gilmour, C.C., 2000. Methyl-mercury degradation pathways: a comparison among three mercury-impacted ecosystems. Environ. Sci. Technol. 34, 4908–4916.

Marvin-DiPasquale, M., Boynton, W.R., Capone, D.G., 2003. Benthic sulfate reduction along the Chesapeake Bay central channel. II. Temporal controls. Mar. Ecol. Prog. Ser. 260, 55–70.

Maryland Department of the Environment, 2003. Assessment of Impacts from the Hart-Miller Island Dredge Material Containment Facility, Maryland. Year 17 Rechnical report July 1998–April 2000. 109 Pages. Technical and Regulatory Services, Maryland Department of the Environment. 1800 Washinton Blvd. Suite 540. Baltimore MD 21230-1718.

Myers, G.J., Davidson, P.W., Cox, C., Shamlaye, C.F., Palumbo, D., Cernichiiari, E., Sloan-Reeves, J., Wilding, G.E., Kost, J., Huang, L.S., Clarkson, T.W., 2003. Prenatal methylmercury exposure from ocean fish consumption in the Seychelles child development study. Lancet 361, 1686–1692.

Pak, K.R., Bartha, R., 1998. Mercury methylation and demethylation in anoxic lake sediments and by strictly anaerobic bacteria. Appl. Environ. Microbiol. 64, 1013–1017.

Ravichandran, M., 2004. Interactions between mercury and dissolved organic matter. A review. Chemosphere 55, 319–331.

Regnell, O., Ewald, G., Lord, E., 1997. Factors controlling temporal variation in methyl mercury levels in sediment and water of a seasonally stratified lake. Limnol. Oceanogr. 42, 1784–1795.

Roden, E.E., Tuttle, J.H., 1993. Inorganic sulfur cycling in the mid and lower Chesapeake Bay sediments. Mar. Ecol. Prog. Ser. 93, 101–118.

Schaefer, J.K., Jagi, J., Reinfelder, J., Cardona, T., Ellickson, K.M., Tel-Or, S., Barkay, T., 2004. Role of the bacterial organomercury lyase (MerB) in controlling methylmercury accumulation in mercury contaminated natural waters. Environ. Sci. Technol. 38, 4304–4311.

Stordal, M.C., Gill, G.A., 1995. The determination of mercury methylation rates using 203-Hg radiotracer technique. Water, Air, Soil Pollut. 80, 725–734.

Sunderland, E.M., Gobas, F.A.P.C., Heyes, A., Branfereun, B.A., Bayer, A.K., Cranston, R.E., Parsons, M.B., 2004. Speciation of mercury in well-mixed estuarine sediments. Mar. Chem. 90, 91–105.

Tomiyasu, T., Nagano, A., Yonehara, N., Sakamoto, H., Rifardi,, Oki, K., Akagi, H., 2000. Mercury contamination in the Yatsushiro Sea, south-western Japan: spatial variations in sediment. Sci. Tech. Environ. 257, 121–132.

Vanzoest, R., Vaneck, G.T.M., 1993. Historical input and behaviour of PAH's of Hexachlorobenzine, Polychlorinated-Biphenyls, Polycyclic Aromatic-Hydrocarbons in 2 dated sediment cores from the Scheldt Estuary, SW Netherlands. Mar. Chem., 103–4495.

Warner, K.A., Roden, E.E., Bonzongo, J.-C., 2003. Microbial mercury transformation in anoxic freshwater sediments under iron-reducing and other electron-accepting conditions. Environ. Sci. Technol. 37, 2159–2165.

Winfrey, M.R., Rudd, J.W.M., 1990. Environmental factors affecting the formation of methylmercury in low pH lakes. Environ. Toxicol. Chem. 9, 853–869.