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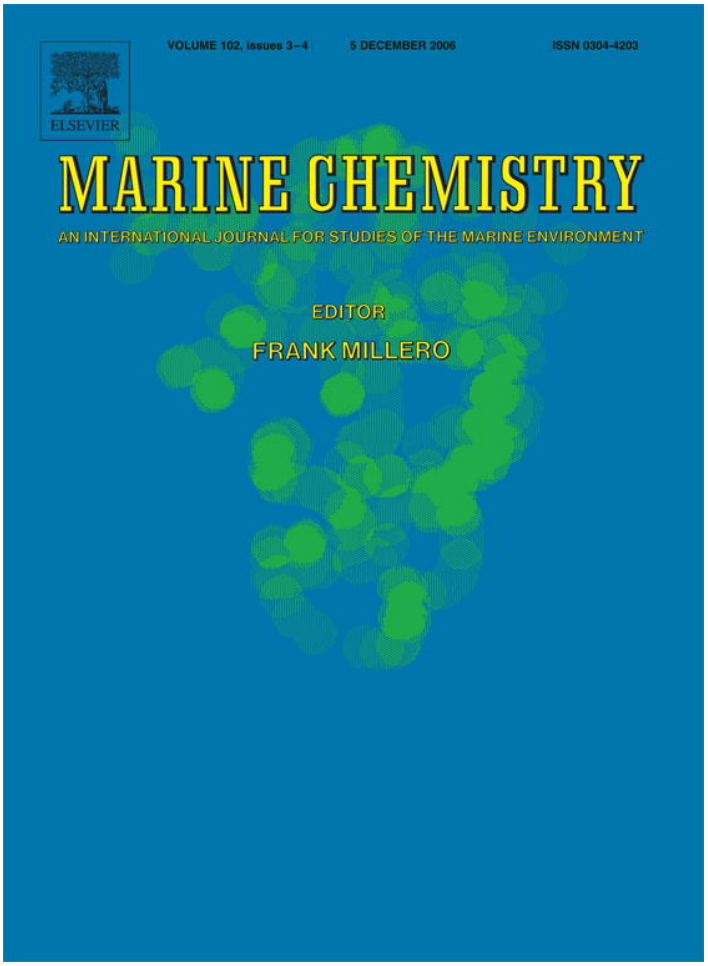

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The impact of resuspension on sediment mercury dynamics, and methylmercury production and fate: A mesocosm study

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Abstract

The objective of this study was to investigate the effects of resuspension on the fate and bioaccumulation of mercury (THg) and methylmercury (MeHg) in shallow estuarine environments, using mesocosms. Two 4-week experiments were conducted in July (Experiment 1) and October (Experiment 2) of 2001 with Baltimore Harbor sediments. Hard clams, *Mercenaria mercenaria*, were introduced into sediments for Experiment 2. Tidal resuspension (4 h on and 2 h off cycles) was simulated, with 3 replicate tanks for each treatment—resuspension (R) and non-resuspension (NR). Sediment cores were collected during the experiments for THg, MeHg, organic content and AVS analyses, and for the determination of methylation/demethylation using Hg stable isotopes (^{199}Hg(II) and CH_3^{199}Hg(II)). Zooplankton samples were collected once a week while clams were taken before and after Experiment 2 for THg and MeHg analyses. Our results suggest that the interplay between Hg methylation and MeHg degradation determines the overall MeHg pool in sediments. Sediment resuspension does not appear to directly impact the Hg transformations but can lead to changes in the association to Hg binding phases, influencing Hg methylation. The bioaccumulation results indicate that sediment resuspension can play an important role in transferring sediment MeHg into organisms.

Keywords: Mercury; Methylmercury; Mercury stable isotopes; Bioaccumulation; Sediment resuspension

1. Introduction

Sediments are the main repository of mercury (Hg) in estuaries (Benoit et al., 1998; Wang et al., 1998) and can be a significant source to the overlying water column via various processes including diffusion, resuspension, and bioturbation (Gagnon et al., 1997; Bloom et al., 1999; Mason and Lawrence, 1999; Mason et al., in press). The mobility and bioavailability of Hg and methylmercury (MeHg) depends upon the nature and concentration of the binding phases in the sediment, which are controlled by sediment redox status. Hg associates primarily with particulate organic matter or iron/manganese oxides through adsorption and coprecipitation reactions in oxidized sediments (Gagnon et al., 1997), with organic matter typically being the overriding controlling solid phase (Miller, 2006). In anoxic sediments, Hg is adsorbed onto and coprecipitated with sulfide minerals (Gobeil and Cossa, 1993; Gagnon et al., 1997; Wang et al., 1998). When

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metal oxides are reduced, Hg can potentially be released into porewater (and eventually to overlying water via diffusion). Hg can also be released as a result of the microbial degradation of organic matter and by chemical dissolution of sulfides due to redox changes during diagenesis.

Under anoxic conditions, dissolved sulfide may precipitate with Fe^{2+} ions, which are released by reduction of iron (hydr)oxides. Iron sulfide can then adsorb and coprecipitate with divalent metals. Many toxic metals, and especially Hg (Miller, 2006) can form highly insoluble sulfide minerals and adsorb/coprecipitate with pyrite and acid volatile sulfide (AVS), making them less bioavailable to aquatic organisms (Allen, 1995). Cooper and Morse (1996) concluded that sulfide-associated trace metals could become more bioavailable following a major oxidation event, such as that caused by dredging, resuspension, and seasonal migration of the redoxcline. During such an oxidation event, metal sulfides may be oxidized and thereby could release the dissolved metals to the overlying water, and thus be a potential source of Hg and other toxic metals to the water column, thereby possibly increasing bioavailability. The released metal could, however, quickly be scavenged or coprecipitated with iron and manganese hydroxides or be complexed by organic matter (Simpson et al., 1998).

As mentioned above, sediment resuspension is one process that can induce a change in sediment redox status, which can be an important factor determining the rate of methylation of Hg in sediments. Hg methylation depends upon environmental factors that control the overall metabolic activity of the methylating organisms (e.g. sulfate reducing bacteria) and the bioavailability of Hg in the matrix where methylation occurs. As the supply of organic carbon enhances Hg methylation rate (Choi and Bartha, 1994), the distribution of methylation activity depends upon the distribution of biodegradable organic matter. In coastal systems, sulfate limitation does not occur. Thus, maximal methylation rates are often observed in biologically active surface sediments near the sediment–water interface (Korthals and Winfrey, 1987; Benoit et al., 2003; Heyes et al., in press) when the supply of organic matter is high and where the redox changes discussed above are likely to be occurring.

While sulfate can stimulate both sulfate reduction and Hg methylation by sulfate reducing bacteria at relatively low sulfate concentrations (Gilmour and Henry, 1991), the higher concentrations of sulfate typically found in estuarine and marine environments can lead to enhanced porewater dissolved sulfide, which has been shown to inhibit Hg methylation (Compeau and Bartha, 1983; Compeau and Bartha, 1987; Gilmour et al., 1998; Benoit et al., 1998). Porewater sulfide levels in estuarine sediments depend not only on the rate of sulfate reduction, and the processes of sulfide oxidation, but also on the iron levels, which dictate the degree of sulfide removal to the solid phase.

Sunderland et al. (2004) found different patterns for MeHg and Hg methylation between two contrasting physical regions: well-mixed sediments (from Passamaquoddy Bay, a semi-enclosed macrotidal estuary at the mouth of the Bay of Fundy) and unmixed sediments (from the St. Croix River, the mouth of a freshwater tributary). MeHg production, measured using Hg stable isotopes, was observed throughout the 15 cm active surface layer of the well-mixed sediments. In contrast, in the unmixed sediments, MeHg concentration and production was observed in a narrowly constrained subsurface layer (2–4 cm sediment). Similar results were observed in the estuarine turbidity maximum (ETM) region of the Hudson River Estuary in that Hg methylation (assayed using Hg stable isotopes) was occurring throughout the upper 25 cm sediment layer (Heyes et al., 2004).

The techniques for the measurement of Hg methylation and MeHg demethylation rates, and the associated rate constants, have been improved by the use of stable Hg isotopes combined with Inductively Coupled Plasma-Mass Spectrometer (ICP-MS), as an isotope-specific detector. Advantages over conventional radioactive isotope procedures, used by others (Korthals and Winfrey, 1987; Gilmour and Riedel, 1995; Stordal and Gill, 1995; Hines et al., 2000; Marvin-DiPasquale et al., 2000), are that stable isotopes can be spiked at concentrations near ambient levels of Hg and MeHg due to the high sensitivity of ICP-MS. This results in Hg bioavailability to the methylating organisms being similar to ambient levels and avoids any artifacts associated with the addition of excess Hg (Heyes et al., in press). Additionally, different stable isotopes can be simultaneously added into the same samples for assaying both Hg methylation and MeHg demethylation (Hintelmann and Evans, 1997; Hintelmann et al., 2000; Lambertsson et al., 2001; Sunderland et al., 2004; Heyes et al., 2004, in press).

Although sedimentary dynamics and bioavailability of Hg have been actively studied, there are few studies that have investigated how resuspension affects the fate and bioavailability of Hg in the sediment, its net methylation, and the possible release of Hg and MeHg to the water column. Additionally, most previous studies were conducted on a small scale and/or for a short duration. Therefore, the objective of this study was to investigate the effects of tidal sediment resuspension over a 4-week period on the fate of total mercury (THg) and MeHg and their bioaccumulation, using the new STORM (high

bottom *S*hear realistic water column *T*urbulence *R*esuspension *M*esocosms) 1 m^3 tank facility that we designed and developed (Porter, 1999; Porter et al., 2004a).

The STORM system can simulate both realistic bottom shear stress and water column turbulence levels in a single system, mimicking benthic–pelagic coupling processes realistically, including tidal or episodic sediment resuspension, over long time periods. Two 4-week experiments were conducted in 2001: one in July without clams (Experiment 1) and the other in October with hard clams, *Mercenaria mercenaria* (Experiment 2). Hard clams were chosen because they are suspension feeders, are common in the eastern coastal and estuarine regions of USA (Stanley, 1985) and there is little information on Hg (especially MeHg) bioaccumulation into these organisms. This paper discusses the effects of sediment resuspension on the sedimentary dynamics of THg and MeHg, their fate, transport and bioaccumulation. A companion paper (Kim et al., 2004) discussed the impact of sediment resuspension on water column THg and MeHg dynamics.

2. Material and methods

2.1. Mesocosm and experimental set-up

Muddy sediment from Baltimore Harbor was collected in the spring of 2001 and transferred to a fiberglass holding tank at CBL and prepared for each experiment following techniques developed in Porter (1999) and Porter et al. (2004b). The details of the experimental set-up are described in Kim et al. (2004). Briefly, the sediment was transferred into 6 cylindrical STORM tanks (1 m^2 of surface area and 1 m deep) after defaunation. The sediment was thoroughly mixed and flattened. Filtered ambient water from the mouth of the Patuxent River (a tributary of the Chesapeake Bay, Maryland, USA) was added into the tanks without any disturbance of the sediment layer to a depth of 20 cm above the sediment surface. After a 2-week sediment equilibration period, to re-establish realistic porewater gradients (Porter, 1999), unfiltered ambient water was added to the tanks (as "seed water"). There were 3 resuspension (R) tanks (T1, T2, and T3) and 3 non-resuspension (NR) tanks (T4, T5, and T6) for each experiment. In both systems, water column turbulence intensities were similar. However, high instantaneous bottom shear, which induced sediment resuspension in the R systems, was induced using a specific paddle design, whereas bottom shear velocity was low in the NR systems (Crawford and Sanford, 2001; Porter et al., 2004a). Tidal resuspension (4 h-on and 2 h-off cycles) was maintained over the 4-week period. After Experiment 1, the sediment was transferred back to the holding tank and stored until Experiment 2. Then, the sediment was redistributed into the mesocosm tanks, mixed, and flattened for Experiment 2, as described above.

As mentioned earlier, in Experiment 2, a scaled population of about 50–40 mm long hard clams were placed into the sediment individually by hand after the sediment equilibration period. Five clams were also placed in a plastic basket hanging at 50 cm below the water surface near the wall of each tank (so-called "suspended clams"). Since the clams buried themselves in the sediment and it was not possible to observe them (especially in the R tanks due to turbidity), these suspended clams in the water column helped confirm whether or not the clams were feeding. Given that some negative effects (i.e. inhibition of feeding rate, burrowing, growth and survival of juveniles and adults) can occur to clams exposed to salinities below 15 (Grizzle et al., 2001 and references therein), salinity adjustment was necessary in Experiment 2. The average salinity for all the tanks was approximately 19 throughout the experiment period. The salinity of the input water (Patuxent River water) was around 13.

2.2. Sample collection

Sediment cores for THg, MeHg, and AVS were taken at the start of Experiment 1 (initial sediment cores were incubated in a separate benthic chamber setup, as discussed below), around the mid point of Experiment 1 (day 16), and at the end of the experiment (day 29 for the R tanks and day 36 for the NR tanks) during the "on-cycle" (resuspension actively occurring). The sampling took place a week apart between the R and NR tanks at the end of Experiment 1 as flux experiments were completed in between. While the flux experiment for the R system was conducted, the NR tanks were maintained in the same manner as it had been during Experiment 1. In Experiment 2, sediment cores were collected at the beginning and the end of the experiment (days 25 and 26 for the R and NR tanks, respectively). There was no mid point sampling in Experiment 2.

All the sediment cores, sampling equipment, and containers were acid-cleaned prior to use according to EPA protocols (EPA, 1998). The sediment cores were generally about 9 cm deep, taken in acrylic tubes (3.5 cm diameter and 25 cm long), and sliced immediately at the following intervals: 0–0.5, 0.5–1, 1–2, 2–3, 3–5, and 5–7 cm. The sliced sediment was then quickly stored frozen until analysis. The initial cores were taken from benthic chambers (13.5 cm diameter, 35 cm long). These chambers were set up separately in a flow-through water bath in the dark for initial Hg and AVS measurements so that the sediment surface in the tanks was not disturbed before the

experiments began. The separate cores underwent a 2-week equilibration period indoors in the same manner as the STORM tanks (Kim et al., 2004), representing a similar initial condition as in the tanks.

Clams were shipped on ice from Cherrystone Aqua Farms, Cheriton, Virginia. They were kept in a holding tank with a constant water circulation (filtered water from the mouth of the Patuxent River supplemented with sea salts) until Experiment 2 began. Clams were acclimated, from a salinity of 21, at which they were cultured, to 18–19, our experimental condition (i.e. decreasing salinity by 1 per day). For water quality assurance, levels of ammonia, nitrate, nitrite, salinity, and pH were measured on a daily basis. Algae paste (Aquaculture Supply USA) was fed to clams once a day until the experiment. Ten to 15 clams from the holding tank were sacrificed for initial Hg measurements. Clams were retrieved from all 6 tanks at the end of the experiment. In general, for Hg analysis, tissue samples from 10 to 15 clams in each tank were ground homogeneously and kept frozen until analysis.

Zooplankton samples for Hg analysis were collected during Experiment 2 roughly once a week. A sampling hose attached to a PVC rod was continuously moving in the water column while water was being withdrawn to sample zooplankton as homogeneously as possible. For sampling, a diaphragm pump was used to pump water at a rate of 22 L/min (40 L water from each tank) through polypropylene nets of 210 μm. Then, zooplankton was transferred from the nets to Teflon vials and filtered onto polycarbonate filters. The filters were then stored in Petri dishes, double bagged, and frozen until Hg analysis.

2.3. Stable isotope spike addition incubation methylation/demethylation assays

Acrylic tubes were used for stable isotope spike addition methylation/demethylation incubation experiments using sediments from only one tank of each system (i.e. T1 for R and T4 for NR tanks in Experiment 1; T2 for R and T5 for NR tanks in Experiment 2). This sampling was made in accordance with other sediment core sampling for Hg and AVS analysis for the initial, mid (Experiment 1 only), and final conditions. Four sediment cores were obtained from each tank and transferred to the laboratory immediately. Hg stable isotope (^{199}Hg(II)), from the Oak Ridge National Laboratory (purity of 92%), stock solution was diluted using the overlying water from the mesocosm tanks and allowed to equilibrate before injection. The goal was to increase the background concentration 20% above that found in Baltimore Harbor sediments (Mason and Lawrence, 1999; Mason et al., 2004). The ^{199}Hg (II) solution (100 μL) was, then, injected into two cores at 1 cm intervals to determine the methylation rate. MeHg stable isotope ($CH_3^{199}Hg$ (II)) was synthesized from the ^{199}Hg (II), using a methylcobalamin reaction followed by methylene chloride extraction. The $CH_3^{199}Hg$ (II) was equilibrated using the overlying water from the mesocosm tanks prior to injection. The target concentration was that estimated to be double the MeHg concentration in situ (Mason and Lawrence, 1999). Then, 100 μL of the $CH_3^{199}Hg$ (II) was injected into the other two cores to obtain the demethylation rate. After a 2 h-incubation at room temperature, the cores were sliced, as described above, and immediately frozen until analysis. All the Hg isotope amended samples were analyzed using ICP-MS as the isotope-specific detector.

2.4. Sample analyses

2.4.1. Total mercury

Sediment samples were split, freeze-dried, and analyzed for total Hg using a Milestone DMA 80 Direct Mercury Analyzer. Biota samples were thawed and digested in 5.0 mL of 3:7 (v/v) acid mixture of concentrated sulfuric acid (18 M)/nitric acid (16 M) in Teflon vials in an oven at 60 °C overnight prior to bromine chloride oxidation (1.5–1 h). Then, excess oxidant was neutralized with 2.0 mL of 2.0 M hydroxylamine hydrochloride prior to analysis (Bloom and Crecelius, 1983). The samples were then reduced by 0.5 mL of 0.5 M stannous chloride, sparged, and the elemental Hg trapped on a gold trap. The gold trap was heated with argon flowing and quantification was done by Cold Vapor Atomic Florescence detection (CVAFS) (Bloom and Fitzgerald, 1988) in accordance with protocols outlined in EPA method 1631 (EPA, 1998). For Hg isotope analysis, samples were prepared in the same manner, as described above, and ICP-MS was used in place of CVAFS (Hintelmann and Evans, 1997; Lambertsson et al., 2001). Standard calibration curves with r^2 of >0.99 for THg were achieved daily. Analysis of standard reference material, estuarine sediment IAEA-405 (3.9–4.3 nmol g^{-1}), typically gave a 90% recovery for CVAFS, and a 83% recovery for ICP-MS. For the DMA, average recovery of standard reference material, marine sediment MESS-3 (0.4–0.5 nmol g^{-1}), was 101%. Analysis of duplicate samples generally yielded a relative percent difference (RPD) of less than 15% for both CVAFS and ICP-MS, and less than 5.0% for DMA. Detection limits were based on 3 standard deviations of the digestion blank measurements. For DMA and CVAFS, the detection limits for THg were 0.1 pmol g^{-1}, respectively. For ICP-MS, the detection limit was 0.5 pmol g^{-1} for sediments amended with the Hg isotopes. All the data presented here are on a dry weight basis.

2.4.2. Methylmercury

Details of the analytical protocols are given elsewhere (Mason et al., 1999; Mason and Lawrence, 1999). Briefly, sediment and biota samples were diluted to 25 mL of deionized water and distilled with 1.0 mL of 9.0 M sulfuric acid and 0.5 mL of 3.0 M potassium chloride solution (Horvat et al., 1993). A 1.0% (w/v) sodium tetraethylborate (0.1 mL) solution was added to the distillate to convert the nonvolatile MeHg to gaseous methylethylmercury (Bloom, 1989). The volatile adduct was then purged from solution and recollected on a solid phase column (Tenax) at room temperature. The methylethylmercury was thermally desorbed from the column and separated by a gas chromatographic (GC) column. All Hg species were converted to elemental Hg in a pyrolytic column at the GC outlet and analyzed by CVAFS. Samples for $CH_3^{199}Hg$ (II) were distilled in the same manner, as described above, and analyzed by ICP-MS in place of CVAFS (Hintelmann and Evans, 1997; Lambertsson et al., 2001). A calibration curve with an r^2 of >0.99 was achieved on a daily basis. Analysis of duplicate samples typically gave a RPD of less than 20%. Detection limits were based on 3 standard deviations of the distillation blank measurements. For CVAFS, detection limits for MeHg were 0.02 pmol g^{-1} for sediments and 0.005 pmol g^{-1} for biota. For ICP-MS, the detection limit for $CH_3^{199}Hg$ (II) was 0.009 pmol g^{-1} for sediment samples. For both ICP-MS and CVAFS, analysis of IAEA-405 (25–30 pmol g^{-1}) generally gave a 90% recovery. Spike recoveries yielded 87% for CVAFS, and 94% for ICP-MS. The data were not corrected for less than 100% spike recovery. All data are reported on a dry weight basis.

2.4.3. Acid volatile sulfide (AVS) and percent organic matter

A subsample of sediment was weighed and added into a tared 3 neck flask. The flasks were attached immediately to a gas manifold with nitrogen gas purging in order to minimize exposure to oxygen. Van Griethuysen et al. (2002) found that losses of AVS due to air-sample contact (less than 15 min) did not occur. While gassing, 20 mL of 6.0 M deoxygenated HCl was added into each flask. The samples then were distilled for 1.5–2 h at room temperature under nitrogen gas flow. Sulfide volatilized during distillation was collected in traps filled with SAOB (sulfide antioxidant buffer) solution (EPA, 1996). Total sulfide in the traps was measured using an ion specific sulfide electrode (Baumann, 1974; Allen et al., 1991; EPA, 1996). A calibration curve with an r^2 of at least 0.99 was achieved daily. Analysis of duplicate samples yielded a RPD of less than 20%. Spike recovery averaged 95%. The detection limit was 0.4 μM. Percent organic content in each interval of sediment samples was determined as loss on ignition to 550 °C overnight.

2.4.4. Statistical analysis

In order to examine correlation between two variables, Pearson's product-moment correlation coefficient (r) and associated significant probability (P) were obtained for the data. All the statistical results were reported as significant at a level of $p<0.05$. We used JMP®, version 4 by SAS institute Inc., Cary, NC, USA for all the statistical analyses. Repeated measures ANOVA was tested using SAS PROC MIXED to see if there was a significant effect on Hg concentration in biota between treatments (R vs. NR) as well as between each time point within the treatments.

3. Results and discussion

3.1. Water column data

The concentrations and distributions, and changes with time, for Hg, MeHg and ancillary parameters in the dissolved and particulate phases in the water column were discussed in detail in Kim et al. (2004). A brief summary of these data are included here (Table 1). As seen in the table, the concentrations of dissolved Hg and MeHg were very similar between the R and NR tanks within an experiment. In contrast, particulate Hg levels were significantly higher in the R tanks and particulate MeHg levels were significantly higher in the NR tanks, so that there was a strong difference in %MeHg for particulate between the R and NR treatments. The differences in particulate Hg and MeHg can be attributed mainly to differences in the Hg and MeHg burdens in sediment compared to living tissue (zooplankton have a much higher %MeHg than sediment, and a lower Hg concentration, as discussed below) and

Table 1
A summary of the water column data from Experiments 1 and 2 (Kim et al., 2004)

Parameter	Experiment 1		Experiment 2	
	R	NR	R	NR
TSS (mg L^{-1})	150±27	10±0.2	63±22	4.5±0.6
Chl *a* (μg L^{-1})	24±2	13±0.9	6.7±0.3	3.6±0.1
Part. Hg (nmol g^{-1})	2.3±0.1	1.1±0.05	2.3±0.2	1.4±0.05
Part. MeHg (pmol g^{-1})	11±2.0	34±5.0	6.0±1.0	26±5.0
%MeHg	0.3±0.09	3.1±2.0	0.3±0.2	2.1±1.7
Total diss. Hg (pM)	5.5±1.0	5.5±1.0	8.0±0.5	6.0±0.3
Diss. MeHg (pM)	0.3±0.2	0.3±0.1	0.2±0.05	0.2±0.05
%MeHg	5.9±12	4.9±8.3	1.8±1.7	2.9±5.2

Data are shown as the average and standard deviation of 3 replicate tanks.

the same is likely true for phytoplankton. Interestingly, chlorophyll *a* (Chl *a*) was higher in the R tanks even given the high turbidity of these systems, reflecting the impact of resuspension on nutrient dynamics (Porter et al., in review-a).

3.2. Overall sediment trends

Table 2 shows average THg and MeHg (% MeHg in parenthesis) from the sediment cores taken in the tanks which were not used for the methylation/demethylation assays (T2, 3, 5, and 6) in Experiment 1. Overall, as expected, there was little variation in THg concentrations with depth and over time between the R and NR tanks. The range of THg in the surface sediment in these experiments agreed well with concentrations previously measured in Baltimore Harbor by Mason and Lawrence (1999) in the vicinity of the sample collection site (average of 2.3 nmol g^{-1}). THg concentration in Experiment 2 shows a similar trend with that of Experiment 1 (Table 3). As seen in Table 2, MeHg concentration appeared to be higher in the top 2 cm sediment than down core in Experiment 1 (except the NR final cores). Overall, MeHg concentration and % MeHg near the sediment surface in the R tanks appeared to be slightly higher, compared to the NR tanks in Experiment 1. In Experiment 2, however, a similar pattern was observed in MeHg concentration between the R and NR tanks (Table 3). The fraction of the Hg as MeHg was generally low (<0.5% of THg concentration) for both experiments, which was similar to % MeHg in estuarine sediments found by others (Gobeil and Cossa, 1993; Gagnon et al., 1996; Benoit et al., 1998; Heyes et al., 2004), and to that measured in Baltimore Harbor previously (Mason and Lawrence, 1999).

Tables 2 and 3 show the average AVS concentration and standard deviation from the 3 replicate tanks within each treatment. AVS concentration fell within the lower range of AVS values found in surface sediments (top 2 cm) of Baltimore Harbor (e.g. AVS concentration was mostly <100 μmol g^{-1} but was as high as 800 μmol g^{-1}) (Mason and Lawrence, 1999). Overall, increasing AVS with depth can be explained by sulfate reduction, which is a major pathway for the oxidation of organic matter in

Table 2
Average concentrations of THg, MeHg, AVS, and % organic content in Experiment 1

	Depth (cm)	THg[a] (nmol g^{-1})	MeHg[a] (pmol g^{-1})	AVS[b] (μmol g^{-1})	% Organic matter[b]
Initial	0–0.5	2.8	3.6 (0.13)	36	12
	0.5–1	2.9	4.1 (0.14)	132	12
	1–2	2.9	4.7 (0.16)	114	13
	2–3	3.0	4.3 (0.14)	81	13
	3–5	3.0	4.7 (0.16)	122	13
Mid-R	0–0.5	2.8	4.6 (0.17)	12±2.6	12±0.6
(day 16)	0.5–1	2.8	4.7 (0.17)	34±4.1	12±1.0
	1–2	2.9	3.4 (0.12)	42±21	12±0.1
	2–3	2.9	2.5 (0.09)	69±20	12±0.4
	3–5	2.7	3.9 (0.15)	94±10	12±0.1
	5–7	2.8	3.4 (0.12)	94	12±0.8
Final-R	0–0.5	2.8	3.9 (0.14)	31±5.5	13±0.5
(day 29)	0.5–1	2.9	2.9 (0.10)	57±8.2	13±0.9
	1–2	2.8	5.0 (0.17)	66±17	13±0.4
	2–3	2.9	2.1 (0.07)	85±12	13±0.6
	3–5	2.9	1.8 (0.06)	85±14	12±0.04
	5–7	3.0	3.0 (0.10)	103±17	12±0.1
Mid-NR	0–0.5	2.8	4.7 (0.17)	18±6.0	13±0.3
(day 16)	0.5–1	2.9	2.7 (0.09)	49±5.9	12±0.5
	1–2	2.8	2.6 (0.09)	58±12	12±0.1
	2–3	2.7	1.9 (0.07)	60±4.2	12±0.2
	3–5	2.7	0.6 (0.02)	75±30	12±0.2
	5–7	2.8	3.4 (0.12)	69±10	12±0.5
Final-NR	0–0.5	2.8	2.1 (0.08)	8.0±7.2	15±0.3
(day 36)	0.5–1	2.9	2.4 (0.08)	31±12	14±0.7
	1–2	2.9	2.9 (0.10)	53±7.1	13±0.3
	2–3	3.1	2.9 (0.09)	71±5.0	13±0.2
	3–5	2.9	2.6 (0.09)	72±8.7	13±0.1
	5–7	2.8	3.9 (0.14)	82±11	12±0.4

[a] THg and MeHg data from non-isotope spiked cores in the R tanks (T2,3) and the NR tanks (T5,6). Percent MeHg are in parentheses.
[b] AVS and % organic content are shown in average of 3 replicate tanks and standard deviation. See the text for details.

Table 3
Average concentrations of THg, MeHg, AVS, and % organic content in Experiment 2

	Depth (cm)	THg[a] (nmol g^{-1})	MeHg[a] (pmol g^{-1})	AVS[b] (μmol g^{-1})	% Organic matter[b]
Initial	0–0.5	2.7	–	39	12
	0.5–1	2.9	5.0 (0.17)	86	12
	1–2	3.1	4.4 (0.14)	75	13
	2–3	3.1	4.6 (0.15)	72	13
	3–5	3.0	3.3 (0.12)	94	13
	5–7	3.1	3.6 (0.10)	94	12
Final-R	0–0.5	2.9	4.3 (0.15)	28±9.4	13±0.3
(day 25)	0.5–1	3.0	4.0 (0.14)	66±19	13±0.3
	1–2	3.0	3.1 (0.10)	55±13	13±0.1
	2–3	3.1	3.0 (0.10)	79±16	13±0.3
	3–5	3.1	4.1 (0.13)	82±25	13±0.1
	5–7	3.1	3.3 (0.11)	90±20	13±0.2
Final-NR	0–0.5	2.8	4.2 (0.15)	24±1.0	14±0.3
(day 26)	0.5–1	2.9	3.6 (0.12)	57±13	13±0.1
	1–2	2.9	2.7 (0.09)	58±11	13±0.2
	2–3	2.9	3.0 (0.10)	73±13	13±0.3
	3–5	2.9	3.5 (0.12)	81±6.2	13±0.3
	5–7	3.0	3.8 (0.13)	85±3.4	12±0.2

[a] THg and MeHg data from non-isotope spiked cores in the R tanks (T1,3) and the NR tanks (T4,6). Percent MeHg are in parenthesis.
[b] AVS and % organic content are shown in average of 3 replicate tanks and standard deviation. See the text for details.

anoxic estuarine sediments (Van Den Berg et al., 1998; Lin et al., 2002). In contrast, percent organic content showed little difference between the treatments over time and with depth for both Experiments 1 and 2 (Tables 2 and 3).

The overall results showed no distinct seasonal difference in sediment MeHg concentration between Experiments 1 and 2. As mentioned earlier, Hg methylation is driven by environmental factors (e.g. microbial activity, sulfate/sulfide levels, and sediment organic content). Given similar ranges of AVS and % sediment organic content between the two experiments, a significant difference in Hg methylation was not expected. Additionally, average temperatures of water column were not markedly different (25 and 20 °C in Experiments 1 and 2, respectively; Kim et al., 2004), indicating that microbial activity due to temperature changes was not large. Similarly, Heyes et al. (2004) found for the Hudson River (NY) sediment that MeHg concentration was comparable between June and October, but the Hg methylation rates in June were higher by a factor of four, compared to February.

3.3. Mercury isotope incubation experiments

Fig. 1 presents % methylation of the duplicate cores in both Experiments 1 and 2. In Experiment 1, it appeared that average % methylation (solid line) in the R tank on day 16 was relatively high at the top 0–1 cm sediment, compared to the NR tank (dotted line) (Fig. 1A). At the end of Experiment 1, average % methylation in the R tank was mostly lower down core than the NR tank, except the top 0.5 cm sediment (Fig. 1B). In Experiment 2, average % methylation in both R and NR tanks showed a similar pattern, being higher near the surface (0–2 cm) than down core (Fig. 1C). However, it should be noted that concentrations were variable between the duplicate cores within each tank, making it difficult to discern any effect of sediment resuspension on Hg methylation above that of natural variability.

Given total suspended solids in the water column (average of 150 mg L^{-1} for Experiment 1) (Table 1) and a sediment density of 1.5 g cm^{-3} (Wainright and Hopkinson, 1997; Chang, 1999), the amount of sediment suspended to the water column is estimated to be comparable to less than 0.5 mm of the sediment. Thus, the direct physical impact of resuspension on sediment geochemistry is small and any impact must be due to secondary effects. Heyes et al. (2004) similarly concluded from the Hudson River data that sediment resuspension likely influenced Hg methylation through a secondary effect, as only a small amount of sediment material was frequently resuspended.

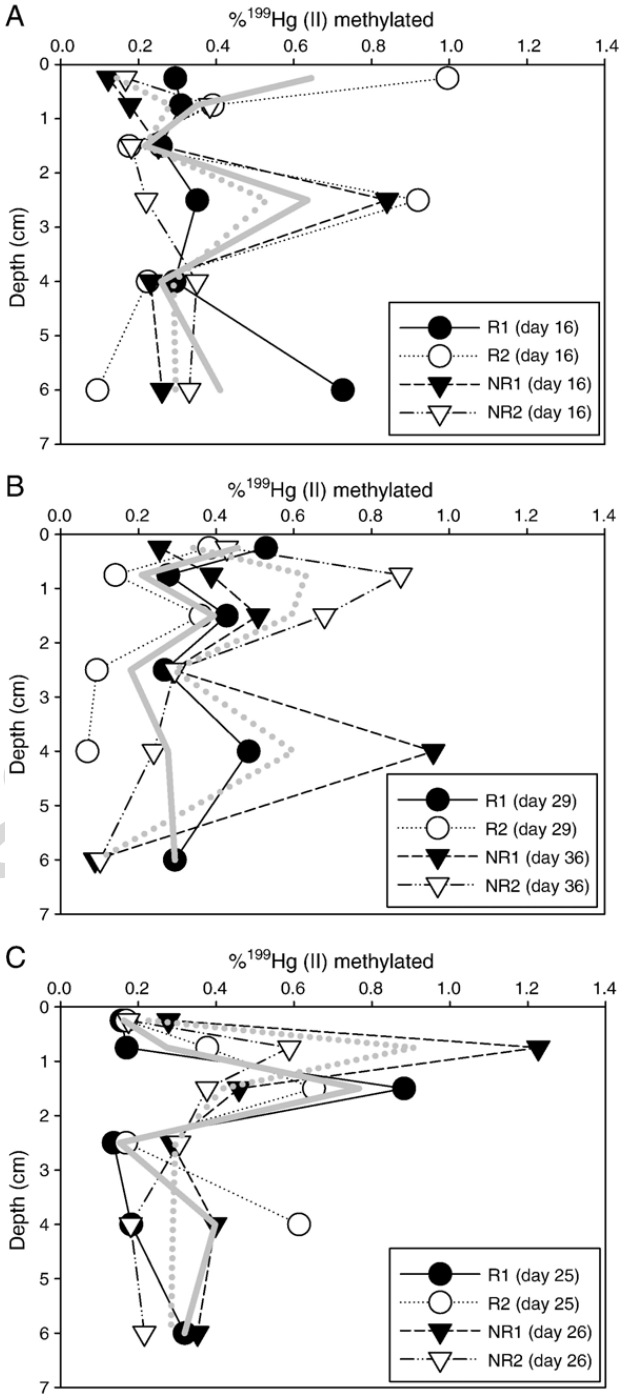

Fig. 1. Percent methylation in sediment cores: (A) mid cores (Experiment 1); (B) final cores (Experiment 1); (C) final cores (Experiment 2). Average of duplicate cores are presented in solid line (R tank) and dotted line (NR tank), respectively.

In addition, biological mixing may have influenced MeHg dynamics. Macroinfauna (e.g. polychaetes, amphipods) densities evolved in the NR tanks, but not the R tanks, over the course of the two experiments (especially in Experiment 1), even though both experiments began with defaunated sediments (Porter et al., in review-a). These communities developed from larval

planktonic stages that must have been present in the seed water for the tanks, added at the beginning of the experiment. Also, in Experiment 2, clams were introduced into the sediment for the bioaccumulation study. Thus, the effects of macroinfauna abundance on Hg methylation need to be considered.

Fig. 2 shows the relationship between the amount of $CH_3^{199}Hg$ (II) produced in the 2 h incubation and the sediment MeHg concentration. There was a significantly positive correlation between $CH_3^{199}Hg$ (II) produced and in situ MeHg concentration (r=0.8 and 0.6 for Experiments 1 and 2, respectively). This supports the notion that in situ MeHg concentration is a good indicator of Hg methylation rates, as found by others in estuarine sediments (Heyes et al., 2004, in press; Sunderland et al., 2004). Overall, the amount of $CH_3^{199}Hg$ (II) produced was significantly and negatively correlated with AVS concentration (r=−0.5), suggesting that increased Hg methylation was coupled with the oxidation of AVS, which was probably changing Hg bioavailability to the methylating organisms. The effect could be due to the release of Hg into solution as a result of AVS oxidation (Miller, 2006), or due to the changing speciation of Hg in porewater as a result of the changing sulfide levels and enhanced bioavailability of Hg to methylating bacteria at low sulfide levels (Benoit et al., 2003), or both factors. Although there was no attempt to analyze dissolved Hg species and sulfate/sulfide concentration in the porewater, this is the likely explanation for the negative relationship between AVS and $CH_3^{199}Hg$ (II) produced.

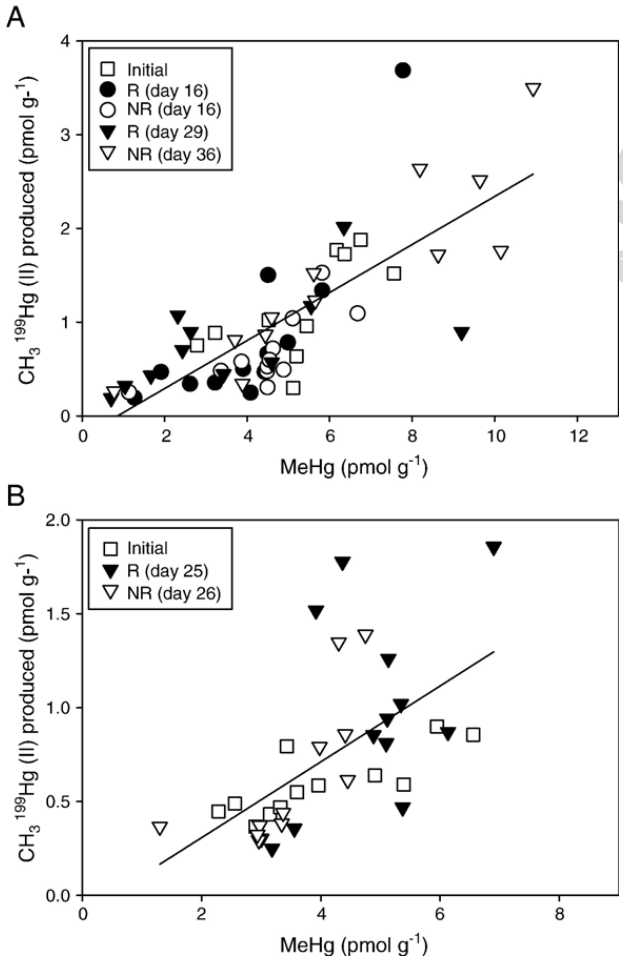

Fig. 2. The correlations between $CH_3^{199}Hg$ (II) produced in 2 h and sediment MeHg in (A) Experiment 1 and (B) Experiment 2.

Fig. 3 shows % demethylation of the duplicate cores for both Experiments 1 and 2. Overall, % demethylation was much greater than % methylation as found by others, using Hg stable isotopes techniques, in freshwater and estuarine environments (Hintelmann et al., 2000; Sunderland et al., 2004; Heyes et al., in press). Additionally, compared to % methylation, there was much less variability in % demethylation between the duplicate cores. On day 16 in Experiment 1, both R and NR tanks showed little trend with depth, although there was one core showing much less % demethylation than the other in the NR tank (Fig. 3A). In the final cores, while % demethylation in the R tank was lower in the top sediment (0–1 cm) than down core, the NR tank showed little variation with depth (Fig. 3B). In Experiment 2, however, a similar pattern was observed between the R and NR tanks, being relatively lower in the top 0.5 cm sediment and remaining constant with depth (Fig. 3C).

Based on our results, rate constants for Hg methylation and MeHg demethylation can be determined using kinetic approaches suggested by Hintelmann et al. (2000). The rate constants are calculated using the following equation (Hintelmann et al., 2000), which treats both methylation and demethylation as pseudo first order reactions. The net rate of $CH_3^{199}Hg$ (II) production from added ^{199}Hg (II) is:

$$d[CH_3^{199}Hg(II)]/dt = k_1[^{199}Hg(II)] - k_2[CH_3^{199}Hg(II)] \quad (1)$$

where

k_1= methylation rate constant (day^{-1}),
k_2= demethylation rate constant (day^{-1}),
[^{199}Hg (II)]= concentration of added ^{199}Hg ($nmol\ g^{-1}$),
[$CH_3^{199}Hg$ (II)]= concentration of $CH_3^{199}Hg$ (II) ($nmol\ g^{-1}$) produced.

For a short-term assay (e.g. 2 h), the second term in Eq. (1) is assumed to be negligible because changes in [$CH_3^{199}Hg$ (II)] are small enough in the early stages. It is also assumed that all other factors affecting Hg methylation are constant over the incubation time. Thus,

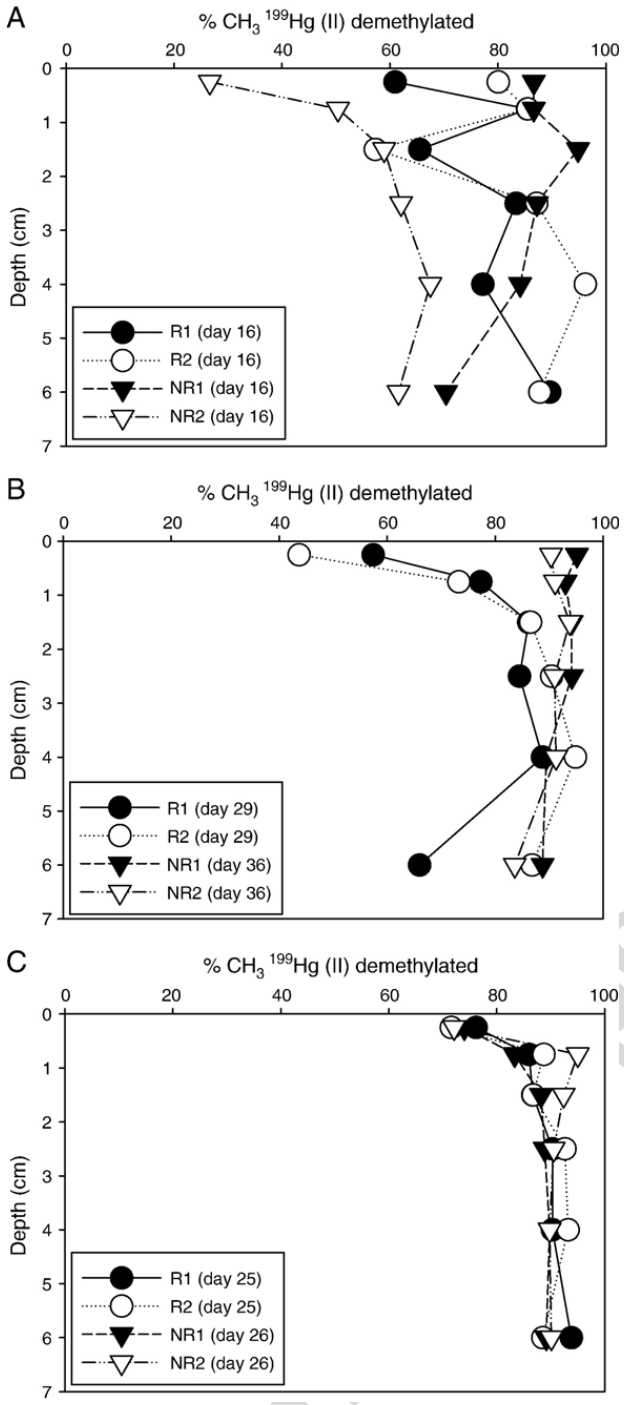

Fig. 3. Percent demethylation in sediment cores: (A) mid cores (Experiment 1), (B) final cores (Experiment 1) and (C) final cores (Experiment 2).

after integrating Eq. (1), the methylation rate constant is obtained as below:

$$k_1 = [CH_3^{199}Hg(II)]/([^{199}Hg(II)]t) \quad (2)$$

In a similar way, the demethylation rate constants were calculated from Eq. (1). At the beginning of the incubation experiment after addition of $CH_3^{199}Hg$ (II), as there is no excess [^{199}Hg (II)], then by integration,

$$k_2 = (1/t)\ln([CH_3^{199}Hg(II)]_0/[CH_3^{199}Hg(II)]) \quad (3)$$

where $[CH_3^{199}Hg\ (II)]_0$ is the initial concentration of $CH_3^{199}Hg$ (II) in the sediment.

Overall, demethylation rate constants were 2–3 orders of magnitude higher than methylation rate constants. Additionally, the results from isotope spike incubation experiments appear to be reasonably consistent with ambient Hg pools in sediments. That is, at a steady state, if equal fractions of added spike isotopes are bioavailable for transformation, then Eq. (1) leads to [MeHg]/[Hg]= k_1/k_2. This relationship was tested by examining the ratios of in situ THg and MeHg concentrations to that of the rate constants. The ratios were of the same order of magnitude for both the R and NR tanks (Table 4), suggesting that the MeHg isotope added must be similarly available in sediments for demethylation as inorganic Hg is for methylation.

Moreover, the characteristic time to equilibrium ($1/(k_1 + k_2)$) was 1–3 h, suggesting that MeHg turnover occurred very rapidly in the sediment. Thus, the methylation process seems to play an important role in determining MeHg concentration in sediments as, in the absence of continuous methylation, bioavailable MeHg would be rapidly depleted. Our preliminary MeHg mass balance for these systems (Kim et al., 2004) suggested that net MeHg production within the mesocosm tanks was higher in the R tanks than in the NR tanks. While the mass balance indicated an overall net formation of MeHg in the R tanks, the results for the NR tanks were equivocal given the errors associated with the mass balance estimates.

Table 5 presents ratios of the methylation and demethylation rate constants and in situ MeHg/Hg concentrations across ecosystems for comparison. As seen in Table 5, the ratio of the methylation and demethylation rate constants are generally in good agreement with the ratios of in situ Hg and MeHg concentrations, similar to the results from this study. It should, however, be noted that since the stable isotope ($CH_3^{199}Hg$ (II) or ^{199}Hg (II)) was added in solution, there is the possibility, even though it was prequilibrated, that the spiked isotope could have been more quickly methylated/demethylated when it was injected into cores, compared to the in situ Hg or MeHg. Others have professed similar concerns that the rates determined from such short term rate measurements (e.g. Hintelmann et al., 2000; Benoit et al., 2003) may be higher than those occurring in situ. However, the results of this study suggest that the rate constants for methylation and demethylation measured using stable isotope spike experiments provide a good measure of the potential for transformation within a sediment. Thus, it is reasonable to use these rate constants

Table 4
Comparison of methylation and demethylation rate constants with ratios of in situ MeHg and THg concentrations for sediments from Experiments 1 and 2

		Depth (cm)	k_1 ($\times 10^{-2}$) (d^{-1})	k_2 (d^{-1})	k_1/k_2 ($\times 10^{-3}$)	$[MeHg]_{tot}$ /$[Hg]_{tot}$ ($\times 10^{-3}$)
Experiment 1 (Mid-day 16)	R	0–0.5	7.7	15	5.1	7.1
		0.5–1	4.2	23	1.8	5.2
		1–2	2.6	12	2.3	1.0
		2–3	7.6	23	3.3	1.4
		3–5	3.1	29	1.1	1.3
		5–7	4.9	26	1.9	1.5
	NR	0–0.5	1.7	14	1.2	2.8
		0.5–1	3.4	8.4	4.0	3.6
		1–2	2.6	23	1.1	2.5
		2–3	6.4	18	3.5	4.2
		3–5	3.5	18	2.0	3.4
		5–7	3.6	13	2.7	4.1
Experiment 1 (Final-days 29 and 36)	R	0–0.5	5.5	8.6	6.4	2.1
		0.5–1	2.5	17	1.5	4.1
		1–2	4.7	24	2.0	2.2
		2–3	2.2	25	0.9	2.1
		3–5	3.3	31	1.1	0.7
		5–7	3.5	19	1.9	1.6
	NR	0–0.5	4.1	32	1.3	2.9
		0.5–1	7.6	31	2.5	4.7
		1–2	7.1	34	2.1	3.8
		2–3	3.5	32	1.1	4.3
		3–5	7.2	28	2.6	3.2
		5–7	1.1	24	0.5	1.3
Experiment 2 (Final-days 25 and 26)	R	0–0.5	2.0	16	1.2	3.6
		0.5–1	3.3	25	1.3	3.7
		1–2	9.2	24	3.8	2.8
		2–3	1.8	30	0.6	3.8
		3–5	4.8	30	1.6	3.6
		5–7	3.8	30	1.3	4.1
	NR	0–0.5	2.7	16	1.7	4.4
		0.5–1	7.1	29	3.8	6.8
		1–2	5.0	28	1.8	4.5
		2–3	3.5	27	1.3	2.9
		3–5	3.5	27	1.3	3.6
		5–7	3.4	27	1.3	3.6

Data are shown in average of duplicate cores.

to compare across ecosystems the propensity of a particular environment to methylate Hg or demethylate MeHg. The comparison shows that while the rate constant determined for Hg methylation in the mesocosms is somewhat higher than those measured in other environments, it is the demethylation rate constant that is substantially higher than that found by others using other methods and in other ecosystems (Table 5).

3.4. Impact of resuspension on Hg methylation/demethylation

The results of this study show that in situ MeHg concentration is a good indicator of Hg methylation activity and the ratio of in situ THg and MeHg concentrations are in good agreement with the ratio of the methylation and demethylation rate constants. The negative relationship between AVS and $CH_3^{199}Hg$ (II) produced suggests that sediment resuspension can have an impact by changing the association of Hg with binding phases, thereby influencing Hg methylation. As mentioned above, sulfate reducing bacteria are the primary agents for methylating Hg. Maximal methylation occurs in environments where sulfate is sufficient to stimulate sulfate reduction and, as a result, Hg methylation, but where there is relatively low sulfide, so that methylation is not limited (Choi and Bartha, 1994; Benoit et al., 1998, 2003). Additionally, bioavailability of Hg is an important factor in Hg methylation. Benoit et al. (1999a) developed a chemical equilibrium model to test the hypothesis that the bioavailability of Hg to sulfate reducing

Table 5
Comparison of methylation and demethylation rate constants with ratios of in situ MeHg and THg concentrations across ecosystems for the upper sediments

Location type and method[a]	k_1 ($\times 10^{-2}$) (day^{-1})	k_2 (day^{-1})	k_1/k_2 ($\times 10^{-3}$)	$[MeHg]_{tot}/[Hg]_{tot}$ ($\times 10^{-3}$)	References
Hudson River (E/S)	0.009	0.64	0.1	2.0	Heyes et al. (in press)
Bay of Fundy (E/S)	0.2	0.2	10	3.3	Sunderland et al. (2004)
San Pablo Bay (EW/R)	1.4	0.3	56	18	Marvin-DiPasquale et al. (2003)
115 Wetland (FW/S)	3.2	5.0	6.4	78	Heyes (unpublished)
Ontario Lakes (L/S)	1.4	0.5	30	24	Hintelmann et al. (2000)
Everglades (W/S+R)	1–4	0.04	250–1000	1–40	Benoit et al. (2003)/Marvin-DiPasquale and Oremland (1998)[b]
Lake Sediment (L/R)	0.2	1–3	0.7–1	–	Pak and Bartha (1998)

[a] Type: E=Estuarine; L=Lake; F=Freshwater; W=Wetland. Method: S=Stable isotopes used; R=Radioisotopes used.
[b] Methylation study used stable isotopes; demethylation study used radioisotopes.

bacteria is a function of the concentration of neutral Hg sulfide complexes that can readily diffuse across the bacterial membrane. The model results suggest that as sulfide increases, the dominant Hg speciation changes from neutral dissolved Hg complexes (e.g. HgS^0 (aq)) to charged sulfide complexes. Octanol–water partition experiments (Benoit et al., 1999b) and culture experiments (Benoit et al., 2001) supported this hypothesis. More recent studies (Miller, 2006) indicate that the relationship is not straightforward as there is an interaction between neutral Hg sulfide complexes and organic matter in solution that reduces the overall neutral Hg sulfide concentration below that predicted by earlier models, such as Benoit et al. (1999a). Overall, sediment resuspension can enhance methylation by decreasing sulfide levels, but it may also limit methylation if sediments become too oxic by limiting the activity of sulfate reducing bacteria.

The demethylation of MeHg has received relatively less attention until recently, compared to Hg methylation. MeHg demethylation can proceed by biotic and abiotic pathways. Abiotic pathways such as photodegradation have been reported in the water column (Weber, 1993; Sellers et al., 1996). Although environmental factors controlling demethylation rates are not yet fully understood, Marvin-DiPasquale et al. (2000) found that reductive demethylation (RD), which yields Hg^0 as the main product, was a major pathway in extremely contaminated sediments (i.e. ppm levels of THg concentration), while oxidative demethylation (OD) was dominant in less contaminated sediments (natural environments). In addition, both sulfate reducing bacteria and methanogens have been shown to be the primary agents for OD (Oremland et al., 1991, 1995; Pak and Bartha, 1998). Thus, in environments where OD dominates, the end product, Hg(II) may be re-methylated or associated with reduced sulfur species. Our study suggests that recycling of Hg appears to be important and the balance between methylation and demethylation ultimately determines MeHg concentration in sediments.

As mentioned earlier, a macroinfaunal community unexpectedly developed in the NR tank, not the R tanks, over the course of the experiments. It was found that polychaete larvae were significantly higher in the water column of the R tanks, compared to the NR tanks, but they were not able to settle due to the continual sediment resuspension (Porter et al., in review-a). Additionally, in Experiment 2, the combination of introducing clams to the sediment, and macrofauna that developed in the NR tanks, may be an explanation for similar trends in Hg methylation and MeHg demethylation between the R and NR tanks. Overall, invertebrate activity could have stimulated sediment mixing in the NR tanks, mimicking through biological processes the impact of physical mixing in the R tanks. Sunderland et al. (2004) showed that physical mixing in the Bay of Fundy resulted in a relatively deep active sediment layer (15 cm) in the oxic-transitory range. They observed that MeHg production occurred throughout the active sediment layer, which was facilitated by anoxic organic rich "mottles" or "pockets". Thus, depending on macrofauna abundance and their movement, the presence of organisms may result in similar sediment conditions that can be favorable to Hg methylation. There is, however, very little information on the impact of infaunal abundance on MeHg production and demethylation. As one example, Benoit et al. (in press) showed a definite impact of macrofauna burrow density on MeHg distribution in sediments of Boston Harbor. Studies are, therefore, needed to further investigate the effects of infaunal abundances in the sediment on Hg methylation and MeHg demethylation.

3.5. Mercury bioaccumulation

THg and MeHg concentrations in zooplankton were measured only during Experiment 2. THg in zooplankton

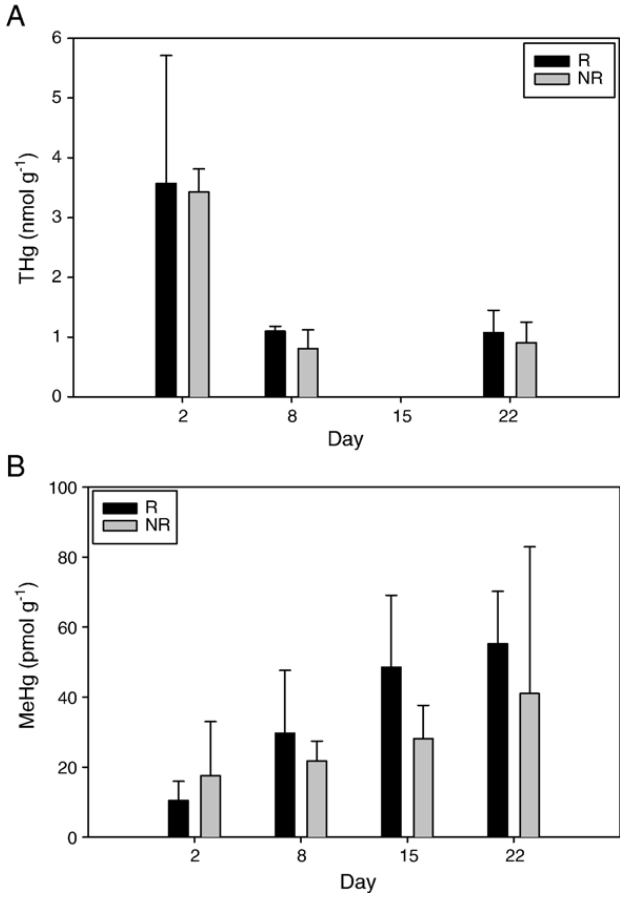

Fig. 4. Average concentrations of (A) THg in zooplankton and (B) MeHg in zooplankton (Experiment 2). Error bars show standard deviations of 3 replicate tanks.

is shown in Fig. 4A. Unfortunately, zooplankton samples were not collected for THg on day 15. THg concentration was not significantly different between the two treatments (R vs. NR) and there was no significant interaction found between time and the treatments. However, THg concentration in zooplankton decreased significantly after day 2 and then remained relatively constant. Recall that in the beginning of the experiment ambient water from the Patuxent River, mixed with high strength seawater, was added to the tanks, and the zooplankton in this water was the source of zooplankton in the mesocosms, and that the first sampling was made on day 2 of the experiment. Thus, zooplankton THg likely represented the concentration and reflected conditions in the Patuxent River for the first week until zooplankton growth, uptake and depuration lead to the concentration being reflective of the in situ concentrations during the experiments. A sharp decrease in THg also appeared to be linked to a zooplankton biomass increase. Zooplankton biomass (organisms>210 μm) on day 8 increased about a factor of two in the R tanks and a factor of 3.5 in the NR tanks, compared to day 2 (Porter et al., unpublished data). Similarly, Chen and Folt (2005) found that zooplankton density was negatively correlated with Hg concentration in zooplankton.

As seen in Fig. 4B, the average concentration of MeHg increased over time in both systems. Overall, MeHg concentration in zooplankton was relatively higher in the R tanks, compared to the NR tanks. There was no significant interaction found between time and the treatments. In the R tanks, MeHg in zooplankton increased significantly on day 15 and 22, compared to day 2. However, the MeHg increase in the NR tanks was not significantly different over time. Changes in zooplankton concentration did not mirror water column MeHg concentration that was relatively constant over time, and that did not significantly differ between experimental systems (Table 1).

The concentration of THg and MeHg in clams is presented in Table 6. There was no significant difference found in THg and MeHg concentration between the treatments and no significant interaction between time and the treatments was also found. Additionally, there was no significant difference in THg and MeHg concentration between the suspended and the sediment clams within each treatment. Compared to the initial concentration, MeHg concentration in clams significantly increased in both the R and NR tanks. A similar pattern was observed with the suspended clams. MeHg in clams accounted for 71±12% of THg concentration, on average. There are very few data available on MeHg or % MeHg concentration in clams in field populations. MeHg, however, generally accounts for 20–80% of THg in invertebrates while it is 10–30% in plants and 80–100% in fish and higher predators (Claisse et al., 2001; Mason and Benoit, 2003). For example, our result falls within the range of % MeHg in oysters (*Crassostrea gigas*) and mussels (*Mytilus* spp.) from the French Coast (11–88% with a median of 43%) (Claisse et al., 2001).

As mentioned above, average water temperature during the course of Experiment 2 was 20 °C and salinity for all the tanks was 19 (Kim et al., 2004). Within these ranges of temperature and salinity, there are generally no detrimental effects on pumping rate and growth of the

Table 6
Average concentration of THg and MeHg in clams, *Mercenaria mercenaria*, with standard deviation of 3 replicate tanks at the beginning and the end of Experiment 2

	THg (nmol g^{-1})	MeHg (nmol g^{-1})	% MeHg
Initial	0.18	0.13	72
R tanks	0.25±0.04	0.16±0.006	65±11
S[a]–R tanks	0.24±0.08	0.18±0.06	74±12
NR tanks	0.23±0.04	0.15±0.01	64±5.9
S[a]–NR tanks	0.20±0.1	0.17±0.05	86±10

[a] Suspended clams in the R and NR tanks (see the text for details).

clams (Grizzle et al., 2001 and references therein.). Actually, the R tanks seem to be beneficial to feeding of clams in that Chl *a* concentration was higher and there was less zooplankton biomass (i.e. less competition), compared to the NR tanks. In a successive experiment in July of 2002, a clam gape monitor (Porter et al., in review-b) was developed and used to measure clam gape activity (closing/opening) during the experiment and it was found that clams in the R tanks were open 62% of time at TSS levels of 106 mg L^{-1}, a similar amount to the clams in the NR tanks, suggesting that hard clams were coping with the turbid environment and were actively feeding. This observation is in contrast to what had been previously found (Bricelj and Malouf, 1984; Turner and Miller, 1991). Our overall data from a number of experiments (Kim, 2004; Bergeron, 2005) support the observations of the clam gape experiments that clams were feeding at high turbidity as the ratio of phytoplankton (Chl *a*) to POM was lower with increasing clam density, indicating that phytoplankton was removed by the clams.

Riisgård et al. (2003) found that filter feeding bivalves reduced their opening state and finally ceased filtering within a few hours when algal concentrations fell below 0.5 μg L^{-1}. Chl *a* in Experiment 2 was above this value and was significantly higher in the R tanks than NR tanks, averaging 6.7±0.3 and 3.6±0.1 μg L^{-1}, respectively (Table 1). Under the experiment conditions, it is therefore likely that filtration rates of clams in both R and NR tanks were similar. Clams in the R tanks were in the turbid environment where filtration rates may have been negatively affected but food concentration was relatively higher (compared to the NR tanks). In contrast, clams in the NR tanks likely faced food limitation due to a lower standing stock of phytoplankton.

A bioaccumulation modeling study showed that overall biomass and MeHg burden in biota were highly sensitive to varying phytoplankton production and the filtration rate of filter feeders, whose biomass was dominant in the system (Kim, 2004). In addition, increasing biomass resulted in a decrease in MeHg burden in biota (dilution effect). In support of this observation, Pickhardt et al. (2002) found that an algal bloom reduced MeHg uptake by zooplankton because increasing algal density decreased MeHg accumulation, resulting in lower dietary inputs to zooplankton. Other studies have found similar results in that contaminant burden in algae decreased as biomass increased (Ashley, 1998; Winkels et al., 1998). Additionally, the modeling study showed that sediment resuspension played an important role in transporting the elevated MeHg to the water column, resulting in a higher MeHg burden in biota. Similarly, other studies have showed that sediment resuspension contributed to increasing concentrations of contaminants (e.g. PCB, Hg) in benthic organisms such as bivalves and polychaetes due to the transfer from the sediment to the water column (Sunderland et al., 2004; Charles et al., 2005).

4. Summary

Our results suggest that sediment resuspension plays a role in Hg methylation by changing the association of Hg with sediment binding phases. However, other factors, such as macrofauna abundance, may be as important as physical mixing in controlling conditions that are favorable to Hg methylation. Demethylation rate constants were found to be similar between the R and NR systems, suggesting that continual Hg methylation was required to maintain the MeHg pool in sediments. In addition, sediment resuspension can play a large role in transferring sediment MeHg to organisms in shallow water systems.

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