**Separation of monomethylmercury from estuarine sediments for mercury isotope
analysis**

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Abstract

Estuarine sediments support the production of monomethylmercury (MeHg) which accumulates in aquatic organisms. While natural variation in mercury stable isotope ratios can potentially be used to track sources and transformations of mercury in the environment, the isotopic signature of MeHg in sediments has not been measured directly. The isotopic composition of MeHg has been studied in laboratory experiments and fish using tandem gas chromatography-multicollector inductively coupled plasma mass spectrometry (MC-ICP-MS) systems; however, the precision and sensitivity of this method may be too low for the analysis of many environmental samples including sediments in which MeHg constitutes 1% or less of the total mercury. In this study, we developed an offline separation method for the precise measurement of the Hg isotopic composition of MeHg in estuarine sediments. Separation of MeHg from inorganic species was accomplished by distillation and chemical ethylation-GC, and was followed by gold amalgam trapping to collect and preconcentrate pyrolyzed MeHg, which was then released into an oxidizing solution. MeHg standards processed in this way were collected with an average yield of 97.5%. External precision for all replicate isotope analyses of MeHg process standards was $\pm 0.14\text{‰}$ (2SD, $n=8$) for $\delta^{202}\text{Hg}$ and no detectable fractionation of Hg stable isotopes occurred during the separation. $\delta^{202}\text{Hg}$ values for MeHg separated from estuarine sediments using our approach varied from -0.41 to +0.41‰ and were generally higher, and spatially and temporally more variable, than those for total Hg (-0.21 to -0.48‰).

1. Introduction

Mercury (Hg) is a ubiquitous and toxic trace metal known to bioaccumulate in its organometallic form, monomethylmercury (MeHg). In aquatic ecosystems, up to 90% of Hg in fish is present as MeHg (Bloom, 1992; Grieb et al., 1990). The methylation of Hg in soils and sediment is mediated by anaerobic microorganisms (Compeau and Bartha, 1985; Hamelin et al., 2011; Kerin et al., 2006), and although predictive models of Hg methylation in sediments have been developed (Benoit et al., 1998; Hammerschmidt and Fitzgerald, 2004; Jonsson et al., 2014) the environmental controls on MeHg production remains an active area of research (Schartup et al., 2013).

Mercury stable isotopes are increasingly used to study the sources of Hg to contaminated sediments (Donovan et al., 2013; Foucher and Hintelmann, 2006; Foucher et al., 2009; Gehrke et al., 2007; Liu et al., 2011; Ma et al., 2013; Mil-Homens et al., 2013; Perrot et al., 2010; Sonke et al., 2010) and to characterize the extents of abiotic and biotic transformations of Hg in the environment (Bergquist and Blum, 2007; Jiménez-Moreno et al., 2013; Kritee et al., 2009; Kritee et al., 2007; Perrot et al., 2013; Rodriguez-Gonzalez et al., 2009; Zheng and Hintelmann, 2010). However, few studies have examined the Hg isotopic composition of specific pools of Hg in aquatic systems (Smith et al., 2014; Yin et al., 2013). In particular, analytical challenges have limited the examination of the Hg isotopic composition of MeHg in environmental samples and the isotopic composition of MeHg in marine sediments, where MeHg typically accounts for less than 1% of the total Hg (Hammerschmidt et al., 2004; Mason and Sullivan, 1999; Schartup et al., 2013), has not been measured directly.

Sulfate reducing bacteria in pure culture have been shown to cause significant mass dependent fractionation (MDF) of Hg stable isotopes during Hg methylation resulting in isotopically lighter (depleted in ^{202}Hg) MeHg (Perrot et al., 2015; Rodriguez-Gonzalez et al., 2009). Similar results were obtained in abiotic methylation experiments with methylcobalamin and other methyl group donors (Jiménez-Moreno et al., 2013; Malinovsky and Vanhaecke, 2011; Perrot et al., 2013). In contrast, the Hg isotopic composition of sediment MeHg, inferred from the isotopic analysis of total Hg and the proportion of total Hg present as MeHg in aquatic food chains, indicates that MeHg in sediment is isotopically enriched in ^{202}Hg relative to total Hg (Gehrke et al., 2011; Kwon et al., 2014; Sherman and Blum, 2013). Similarly, MeHg in various aquatic animals with a range of MeHg concentrations has been shown to be enriched in ^{202}Hg relative to total Hg (Masbou et al., 2013). Direct measurement of the isotopic composition of MeHg in sediments is needed to understand these observations and evaluate the extent of isotopic fractionation during Hg methylation and demethylation in the environment.

The Hg isotopic composition of MeHg has been measured by coupling gas chromatography (GC) separation of ethylated Hg species to a multicollector inductively coupled plasma mass spectrometer (MC-ICP-MS) (Dzurko et al., 2009; Epov et al., 2010; Epov et al., 2008). However, chromatographic separation of MeHg may cause up to 0.5‰ variation in $\delta^{202}\text{Hg}$ between the start and end of peak elution (Dzurko et al., 2009) resulting in low precision of isotope ratios reconstructed from the transient signal (Rodriguez-Gonzalez et al., 2009). Analytical precision could be improved through the offline preconcentration of the MeHg prior to isotope analysis. However, conventional MeHg separation systems are designed for a maximum load of only ~2 ng of MeHg

(Liang et al., 1994) whereas at least 10 ng is needed for the highest precision Hg isotope analysis. Overloading GC columns may result in peak broadening or isotopic fractionation (Wehmeier et al., 2003).

The purpose of this study was to develop a procedure for the separation of large quantities of MeHg from estuarine sediment samples for Hg isotope analysis. In order to increase precision relative to online methods, MeHg was separated prior to introduction into the mass spectrometer using a high capacity GC column which could accommodate 10 to 20 ng of MeHg. Elemental Hg from separated MeHg was then collected on gold traps and subsequently desorbed offline for transfer to an oxidizing solution (Gratz et al., 2010; Sun et al., 2013). Since incomplete ethylation (Yang and Sturgeon, 2009) and other analytical steps (Dzurko et al., 2009; Wehmeier et al., 2003) may cause Hg isotope mass biases, the fractionation of Hg isotopes was evaluated for each step of the separation system. With this separation and pre-concentration system, we were able to obtain high precision measurements of the Hg isotopic composition of MeHg from estuarine sediments containing elevated concentrations of inorganic Hg.

2. Materials and Methods

2.1. Sediment collection

Sediment samples for MeHg separation and isotope analysis were collected over five seasons from August 2012 to August 2013 along a salinity gradient ($S = 2$ to 14) in the Hackensack River estuary and at one site in the Passaic River estuary ($S = 6.6$), New Jersey, USA (Table S1). Surface sediment (to approximately 10 cm depth) was

collected from a small boat using a pole-mounted Ekman grab and subsamples were transferred to 500 mL acid-cleaned glass jars with a stainless steel scoop. Sediment-filled jars were placed in plastic bags and stored on ice immediately after collection and during transport to the laboratory. Sediment samples were stored at 4°C for no longer than 3 days prior to freeze-drying and extraction. All sampling tools were rinsed with ambient surface water between samples. After sampling, equipment was leached in de-ionized water and tested for mercury carryover. The amount of total Hg in these leachates was typically less than 0.005 ng g⁻¹ of Hg in the 100 mL wash.

2.2. Preparation of sediment for Hg and MeHg analysis

Samples for total Hg analysis were prepared by acid extraction according to the appendix to EPA Method 1631B (U.S.EPA, 1999). Approximately 0.5 to 1 g of dry sediment was weighed into glass flasks fitted with Teflon caps. A small volume (10 mL) of a 4:1 mixture of hydrochloric and nitric acids (Trace Metal Grade, Fisher Scientific) was added to each flask and they were then incubated at room temperature for 24 hours. Sample digests were preserved with 0.07 N bromine monochloride and diluted to 40 mL with ultra-pure water.

Methylmercury was separated from sediment by distillation according to Horvat et al. (1993). Briefly, 0.5 g of dry sediment was weighed into Teflon distillation vials and 30 mL of ultra-pure water was added. Trace metal grade sulfuric acid and reagent grade potassium chloride were added to final concentrations of 0.8% v/v and 0.2% v/v, respectively. Distillation lines were attached to 50 mL Teflon receiving vessels containing 5 mL of ultra-high purity water and purged with ultra-high purity Ar (60 mL

min⁻¹). The distillation was performed at 125 °C in a custom aluminum heating block and was run for approximately four hours until 75% of the original volume was distilled. Method recovery was tested using a 10 ng g⁻¹ MeHgCl spiking solution prepared from a 1 µg g⁻¹ stock (Brooks Rand Labs); sediment recovery spikes (2 ng MeHg) ranged between 80-110% (mean = 90.6%, 1 SD = 8%, n = 15). While these spiked sediments were used to evaluate MeHg recovery, they were not analyzed for Hg isotopes since they contained too little MeHg. All distillation blanks were below 0.03 ng of Hg per distillate (mean = 0.025, 1 SD = 0.016, n = 16). Relative percent difference was calculated for duplicate samples and averaged 8.4% (n = 47). Samples were analyzed within two days of distillation. For samples with low MeHg concentrations, multiple distillates were pooled.

2.3. Sample analysis and separation

Total Hg was analyzed by tin chloride reduction, cold vapor atomic absorbance spectrometry (CVAAS) according to EPA Method 245.1 using a Hydra AA Mercury Analyzer (Teledyne-Leeman Labs) (U.S.EPA, 1994). A 0.1 to 0.5 mL aliquot of the sediment digest was added to Teflon sample tubes and excess BrCl was reduced with 0.1 mL of 15% (w/w) hydroxylamine hydrochloride (certified ACS grade, Fisher Scientific). Samples were reduced online with 10 % (w/w) tin chloride (certified ACS, Fisher Scientific). Method performance was verified using the European Reference Material (ERM) CC580 (estuarine sediment). Our average measured value for ERM CC580 ($130 \pm 5 \mu\text{g g}^{-1}$, n = 6) was within 2% of the certified value ($132 \pm 3 \mu\text{g g}^{-1}$).

Methylmercury was analyzed by cold vapor atomic fluorescence spectrometry (CVAFS) of distillates following isothermal gas chromatographic (GC) separation of ethylated derivatives according to Liang et al. (1994). Distillates (25-30 mL) were added to glass impingers and ethylated with sodium tetraethylborate (8 ppm final concentration; Alfa Aesar). Ethylation yields are known to vary with the concentration of tetraethylborate and reaction time (Liang et al., 1994). Since ethylation can cause large mass-dependent fractionation of Hg at yields below 90% (Yang and Sturgeon, 2009), we tested various ethylation reagent concentrations to achieve the highest possible chemical recovery. The efficiency of ethylation was tested by oxidizing a portion of the ethylated and purged distillate with bromine monochloride (2% w/w) for 24 hours and measuring total Hg as described above. We found, consistent with Liang et al. (1994), that with $8 \mu\text{g g}^{-1}$ tetraethylborate, ethylation efficiencies averaged $99 \pm 0.3\%$ ($n=12$) and the amount of Hg remaining in the impingers was comparable to equipment blanks. Ethylated products were purged from solution with 4.5 L of ultra-high purity nitrogen, collected on 100 mg Tenax TA 60/80 traps (Supelco Analytical), and subsequently introduced into a GC column prepared with 15% OV-3 Chromosorb W-AW 60/80 (Ohio Valley Specialty Chemical).

2.4. Collection of total Hg for Hg isotope analysis

For isotope analysis of total Hg in estuarine sediment, dried sediment samples were weighed into ceramic boats and combusted in a custom made, two-stage furnace as described previously (Gehrke et al., 2011; Tsui et al., 2012). A stream of Hg-free O_2

carried released Hg(0) into an oxidizing trap filled with 24 g of 1% (w/w) potassium permanganate. Samples were analyzed for concentration using a Nippon Instruments MA-2000 CVAAS. Before isotope analysis, samples were reduced with tin chloride and preconcentrated into a smaller potassium permanganate trap (5.5-6.5 g) using Hg-free air with an automatic sample changer (Nippon Instruments, SC-3) to final concentrations of 5 ng Hg g⁻¹.

2.5. Collection of MeHg for Hg isotope analysis

We developed a method for the quantitative separation of MeHg from sediment samples using a modified GC separation system coupled to a chemical re-trapping system (Fig. 1). Prior to applying this method to environmental samples, a MeHgCl standard (Brooks Rand Laboratories, Seattle, WA) of known isotopic composition was analyzed without sediment to evaluate isotopic fractionation of Hg during MeHg separation and re-trapping. For collection of MeHg, laboratory standards and sediment samples were distilled, distillates were ethylated and purged, and liberated MeHg was collected on Tenax traps as described above. Ethylated MeHg from Tenax traps was then introduced at a flow rate of 30 mL min⁻¹ into a 14 cm long Teflon preparatory column (1.3 cm ID) packed with 15% OV-3 Chromosorb W-AW 60/80 and heated to 73-75 °C. GC-separated derivatives then passed through a pyrolysis column and a Tekran 2500 CVAFS detector. A solenoid valve was placed between the pyrolysis column and the detector to divert MeHg-derived Hg(0) to a 100 mg gold bead trap (Brooks Rand) for the duration of the MeHg peak, which started 180 s after the start of Tenax desorption and

lasted for 220 s (Fig. S1). Bypassing the CVAFS detector was necessary as it was a potential source of Hg contamination (likely from peak carryover) and mass independent Hg isotopic fractionation (see below). The amount of MeHg that could be separated using our preparatory column with yields greater than 95% ranged from 1 to 20 ng.

Fig. 1. Schematic of the MeHg separation and trapping system.

High inorganic mercury concentrations ($>2 \mu\text{g g}^{-1}$) in samples was found to interfere with ethylation as previously observed (Liang et al., 2004). For sediment samples with high concentrations of inorganic Hg, subsamples (1-5 mL) of distillates

were ethylated separately and later pooled onto a single gold trap following GC separation.

Mercury collected on gold traps was desorbed offline using a temperature-controlled chemical trapping system. Gold traps were heated slowly from room temperature (25 °C) to 500 °C over 3.5 hours using an automatic temperature controller (Cole Parmer Digi-sense) programmed with 11 temperature ramping segments (Table S2) (modified from Demers et al., (2013) and Gratz et al., (2010)). The heating coil was powered with a 140 V transformer set to 20% voltage. During desorption, gold traps were flushed with ultra-high purity Ar at a flow rate of 8 mL min⁻¹ which carried released Hg into a glass impinger containing 30 mL of an oxidizing solution of 0.75% potassium permanganate (w/w, ACS grade Fisher Chemical), 5% trace metal grade sulfuric acid, and 5% trace metal grade nitric acid. Trapped Hg samples were stored in glass vials with Teflon-lined caps prior to isotope analysis. Procedural and reagent blanks for the chemical trapping system were 0.44 ± 0.06 ng of Hg per trap (n = 6). Trapped MeHg samples were preconcentrated to final concentrations that ranged from 1.3 to 5 ng Hg g⁻¹.

2.6. Mercury isotope analysis

Mercury isotope ratios were analyzed at the University of Michigan using a Nu Instruments multicollector inductively coupled plasma mass spectrometer (MC-ICP-MS) and follow-up measurements were made at Rutgers University using a Thermo-Fisher Neptune according to previously published protocols (Bergquist and Blum, 2007; Biswas et al., 2008). Nomenclature for Hg isotopic compositions follows that suggested

by Blum and Bergquist (2007). Bracketing standards (NIST 3133) were diluted in a $\text{KMnO}_4\text{-H}_2\text{SO}_4$ matrix and concentration matched. Blanks of the same $\text{KMnO}_4\text{-H}_2\text{SO}_4$ solution were additionally employed to perform On-Peak-Zero measurements before the standard and sample analysis and subtracted from the analyte signals during processing. Prior to introduction into the mass spectrometer, samples were reduced online with 3% (w/w) tin chloride using a custom built gas-liquid separator. Samples analyzed at Rutgers University utilized a CETAC HGX-200 that was based on the University of Michigan design. The isotopic composition of the Brooks Rand MeHgCl standard was measured at the University of Michigan and at Rutgers (Table 1). This standard shows a small degree of MIF with respect to ^{199}Hg ($\Delta^{199}\text{Hg} \approx +0.1$), but no significant MIF for the other isotopes. Multiple preparations of UM Almadén and NIST 1944 were used to characterize instrument performance at the University Michigan; standards of UM Almadén and ERM CC580 were additionally run at Rutgers to test instrument performance (Supplement Table 3).

3. Results

3.1. Chemical recoveries of MeHg standards during separation and retrapping

Recoveries of MeHg were determined for individual components of the separation and retrapping system and for the system as a whole (Table 1). Samples processed through the desorption system consisting of just the solenoid and

Table 1. Chemical recoveries and Hg isotopic compositions of MeHg standards collected from various stages of the MeHg separation and trapping system

Process	Analysis	$\delta^{204}\text{Hg}$	1SD	$\delta^{202}\text{Hg}$	1SD	$\delta^{201}\text{Hg}$	1SD	$\delta^{200}\text{Hg}$	1SD	$\delta^{199}\text{Hg}$	1SD	$\Delta^{204}\text{Hg}$	$\Delta^{201}\text{Hg}$	$\Delta^{200}\text{Hg}$	$\Delta^{199}\text{Hg}$	n	% MeHg Recovery
MeHg Standard (Brooks Rand)	UMich	-1.61	0.08	-1.07	0.04	-0.75	0.02	-0.52	0.05	-0.20	0.04	-0.01	0.05	0.02	0.07	3	NA
	Rutgers	--	--	-1.02	0.02	-0.73	0.03	-0.51	0.03	-0.17	0.04	--	0.04	0.01	0.08	11	
Distillation	UMich	-1.43	--	-0.94	--	-0.71	--	-0.45	--	-0.05	--	-0.03	-0.01	0.02	0.18	1	93.5 ± 4.0
	Rutgers	--	--	-1.04	0.04	-0.73	0.06	-0.50	0.06	-0.17	0.05	--	0.05	0.02	0.10	6	
Ethylation/Tenax/Pyrolysis	UMich	-1.60	--	-1.10	--	-0.78	--	-0.54	--	-0.24	--	0.05	0.05	0.01	0.04	1	92.2 ± 2.0
	Rutgers	--	--	-0.98	0.02	-0.73	0.01	-0.49	0.01	-0.24	0.00	--	0.00	0.00	0.00	2	
Complete System (With Detector)	UMich	-0.85	0.41	-0.94	0.12	-0.30	0.17	-0.57	0.06	0.51	0.08	0.55	0.41	-0.10	0.75	6	113.8 ± 15.8
	Rutgers	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	
Complete System (No Detector)	UMich	-1.39	0.18	-0.91	0.12	-0.61	0.09	-0.48	0.08	-0.15	0.05	0.01	0.08	-0.04	0.05	3	97.5 ± 7.2
	Rutgers	--	--	-0.93	0.05	-0.68	0.06	-0.41	0.06	-0.19	0.06	--	0.02	0.06	0.04	5	
Complete System (Hg Spike)	UMich	-1.37	0.09	-0.92	0.02	-0.60	0.08	-0.36	0.00	-0.12	0.03	0.00	0.09	0.10	0.11	2	93.7 ± 6.8
	Rutgers	--	--	-0.92	0.16	-0.67	0.12	-0.43	0.02	-0.13	0.06	--	0.02	0.03	0.10	2	

permanganate trapping showed a recovery of $102.9 \pm 8.6 \%$ ($n= 7$). For the entire separation system, including distillation, tenax traps, isothermal GC, pyrolysis column, solenoid valve, and permanganate retrapping (but bypassing the CVAFS detector) the average recovery of mercury introduced as MeHg was $97.5 \pm 7.2\%$. Similar individual recoveries were obtained for other components of the system, except the CVAFS detector, which when included resulted in recoveries of $113 \pm 15.8 \%$ (Table 1).

3.2. Hg isotopic composition of MeHg standards during separation and retrapping

The complete MeHg separation and trapping system including distillation, but bypassing the CVAFS detector, showed no significant fractionation of Hg stable isotopes (Table 1). Thus, $\delta^{202}\text{Hg}$ values were not significantly different for MeHg standards passed through the complete separation system (UMich= $-0.91 \pm 0.12\%$, Rutgers = $-0.93 \pm 0.05\%$) or analyzed directly (UMich = $-1.07 \pm 0.04\%$, Rutgers = $-1.02 \pm 0.02\%$) ($p > 0.05$, unpaired t-test). Similarly, for MeHg passed through complete or truncated separation systems (bypassing both the GC column and the CVAFS detector), $\delta^{199}\text{Hg}$ was 0.02% to 0.07% lower, but not significantly different ($p > 0.05$, unpaired t-test) than MeHg standards analyzed directly. $\delta^{204}\text{Hg}$ values obtained using the complete or truncated separation systems differed from those for the MeHg standard by 0.01% and 0.22% , respectively. However, analytical precision for $\delta^{204}\text{Hg}$ in the MeHg standard ($1\text{SD} = 0.08\%$) is such that this variation is not significant ($p > 0.05$, unpaired t-test).

To examine if the presence of inorganic Hg had an effect on the isotopic composition of the MeHg standard separated using our system, inorganic Hg (NIST 3133) was spiked into MeHg standards in a 5:1 (inorganic Hg:MeHg) ratio prior to distillation. Recoveries of MeHg for inorganic Hg-spiked standards averaged $93.7 \pm 6.8\%$ and $\delta^{202}\text{Hg}$ values for separated MeHg (UMich = $0.92 \pm 0.02\text{‰}$, Rutgers = $0.92 \pm 0.16\text{‰}$) were not significantly different than those for unspiked standards ($p > 0.05$, unpaired t-test) (Table 1).

Following the practice of previous studies, we used distillation of MeHgCl for the initial separation of MeHg from environmental samples because this technique results in high recoveries and has fewer processing steps compared with chemical extraction (Liang et al., 2004). Although distillation can have its drawbacks, including artifact MeHg formation and chromatographic interference from large quantities of inorganic Hg(II) (Liang et al., 2004; Horvat et al., 1993), these were not observed in the MeHg spike recoveries in the present study. Moreover, and as previously observed (Dzurko et al., 2009; Yang and Sturgeon, 2009), distillation of MeHgCl showed no significant fractionation of Hg isotopes ($p > 0.05$, unpaired t-test) (Table 1).

While no mass-dependent or mass-independent fractionation of ^{200}Hg or ^{202}Hg occurred when Hg vapor passed through the CVAFS detector, significant mass-independent fractionation (MIF) of ^{199}Hg , ^{201}Hg , and ^{204}Hg was observed (Table 1; Fig. 2). MIF of ^{204}Hg was previously reported in precipitation (Demers et al 2013) and in compact fluorescent lamps and was tentatively attributed to self-shielding effects (Mead et al., 2013). While analytical uncertainty with respect to ^{204}Hg cannot be ruled out, the observed MIF of ^{199}Hg , ^{201}Hg , and ^{204}Hg may have resulted from the

photochemical oxidation of elemental Hg or photoreduction of oxidized Hg deposited on the walls of the detector cell, but further experiments are needed to fully understand these processes.

Fig. 2. Mercury isotopic composition of monomethylmercury (MeHg) standards processed through the separation and trapping system. Values are means $\pm$ 1SD for MeHg run at both universities that passed through (●) or bypassed (○) the cold vapor atomic fluorescence spectrometry (CVAFS) detector. Also shown is the isotopic composition of the MeHg standard (MeHg STD) prior to separation (squares). For samples passed through the cold vapor atomic fluorescence detector, significant mass independent fractionation of ^{199}Hg , ^{201}Hg , and ^{204}Hg ($p < 0.01$, unpaired t-test) was observed.

3.3. Concentrations and Hg isotopic compositions of total Hg and MeHg from estuarine sediments

The concentration of total Hg in sediment samples from the Hackensack and Passaic River estuaries varied from 2.3 to 5.0 $\mu\text{g g}^{-1}$ (Table 2), which is elevated compared with uncontaminated sediment (0.008 to 0.035 $\mu\text{g g}^{-1}$) (Gilmour et al., 1992). The concentration of MeHg in these sediments varied from 3 to 34 ng g^{-1} and accounted for between 0.1 and 0.3% of the total Hg. Total Hg concentrations were not correlated with either site salinity or sediment organic matter content ($p > 0.1$), but the concentrations of MeHg in the Hackensack River were inversely correlated with site salinity ($R^2 = 0.67$, $p < 0.05$) and were positively correlated with sediment organic matter contents (Table S1; $R^2 = 0.63$, $p < 0.05$).

The isotopic composition of total Hg in the Hackensack and Passaic sediments was fairly uniform (average $\delta^{202}\text{Hg} = -0.36 \pm 0.17\text{‰}$, $n=8$) and very similar to the Hg isotopic composition of the sediment standard reference material NIST 1944 ($\delta^{202}\text{Hg} = -0.38 \pm 0.16\text{‰}$, $n=5$), which includes sediment collected from various locations within nearby Newark Bay (Parris et al., 1995). No MIF of Hg isotopes was observed for total Hg in these sediments (Table S3). For MeHg separated from estuarine sediments, $\delta^{202}\text{Hg}$ values varied from -0.41‰ to $+0.41\text{‰}$ and showed a decreasing trend with increasing MeHg concentration (Fig. 3). No MIF of ^{199}Hg or ^{201}Hg was observed in sediment-bound MeHg. The Hg isotopic compositions of total Hg and MeHg were not correlated with site salinity or sediment organic matter content ($p > 0.1$).

Table 2. Concentrations and $\delta^{202}\text{Hg}$ values of total Hg and MeHg in sediment samples from the Hackensack (HR) and Passaic (P) River estuaries. Values are means $\pm$ 1 SD (n = 2 to 4). Salinity (Sal) of site water at time of collection is also shown.

Site	Season	Sal	THg ($\mu\text{g g}^{-1}$)	THg- ^{202}Hg (‰)	MeHg (ng g^{-1})	MeHg- ^{202}Hg (‰)	% MeHg
HR1	Jan-13	1.92	3.62 ± 0.12	-0.39 ± 0.00	20.5 ± 0.19	0.03 ± 0.09	0.57
HR1	May-13	3.59	3.80 ± 0.27	-0.36 ± 0.03	34.1 ± 1.4	-0.41 ± 0.02^a	0.90
HR2	Aug-12	5.79	4.59 ± 0.15	-0.36 ± 0.01	16.2 ± 0.69	0.27 ± 0.05	0.35
HR3	Nov-12	8.77	3.35 ± 0.05	-0.35 ± 0.02	9.71 ± 1.6	0.40 ± 0.09	0.29
HR3A	Nov-12	10.11	4.98 ± 0.04	-0.21 ± 0.09	8.48 ± 1.2	0.07 ± 0.15^a	0.17
HR4	Jan-13	8.74	4.01 ± 0.14	-0.48 ± 0.01	8.80 ± 1.2	0.08 ± 0.41	0.22
HR5	Aug-13	14.11	2.92 ± 0.03	-0.46 ± 0.02	5.22 ± 0.17	0.37 ± 0.07	0.18
P	Aug-13	6.55	2.31 ± 0.37	-0.28 ± 0.00	3.06 ± 0.01	0.41 ± 0.25	0.13

^aAnalyzed at both the University of Michigan and Rutgers University

Fig. 3. Relationship between $\delta^{202}\text{Hg}$ values of MeHg and the concentration of MeHg in sediments from the Hackensack and Passaic River estuaries. The range of $\delta^{202}\text{Hg}$ values for total mercury in the sediment (hatched area) from all sites (average = $-0.36 \pm 0.17\text{‰}$). With the exception of sites 3A and 4, MeHg concentrations in sediments decreased from the most upstream sites in the Hackensack River to those closest to Newark Bay (Table S1).

Discussion

The separation and trapping system we developed allows for quantitative (>90%) recovery of 10-20 ng of MeHg with no significant fractionation of Hg isotopes. The variance of $\delta^{202}\text{Hg}$ measurements for individual MeHg standards (0.02‰ to 0.16‰, 1 SD) was within that of the isotope analysis alone (Blum and Bergquist, 2007). Similar levels of precision were obtained for odd mass Hg isotopes and no significant mass-dependent or mass-independent fractionation was observed during the separation and trapping of MeHg. For MeHg separated from estuarine sediments, the variability of $\delta^{202}\text{Hg}$ values for the majority of samples analyzed was relatively low (< 0.1‰) and similar to that for MeHg standards passed through the separation system (Tables 1 and 2). Thus our method is suitable for the analysis of Hg isotope ratios in MeHg from experimental or environmental samples and the evaluation of mass-dependent or mass-independent fractionation of Hg isotopes during the synthesis or transformation of MeHg.

An important difference between our method and those used previously to measure Hg isotopes in MeHg is that, in our method, inorganic Hg(II) derived from MeHg is concentrated in a homogeneous aqueous solution prior to reduction and introduction into the MC-ICP-MS. Our system therefore avoids the Hg isotopic fractionation that occurs when the transient MeHg peak produced during chromatographic separation is introduced into the MC-ICP-MS directly and the necessity to correct for fractionation during data reduction (Dzurko et al., 2009). While precision for $\delta^{202}\text{Hg}$ (2SD = 0.14‰, based on all analyses run at UMich and RU) is improved relative to that obtained with online introduction techniques (2SD = 0.18 to

0.56‰) (Dzurko et al., 2009; Epov et al., 2008; Rodriguez-Gonzalez et al., 2009), the absolute accuracy of either approach for environmental or experimental samples remains to be fully evaluated. The online approach, which does not require re-trapping, may be most useful for experimental samples in which the ratio of inorganic Hg to MeHg is lower than that in many environmental samples.

The Hackensack and Passaic Rivers provided an ideal system to test our separation and pre-concentration system for Hg isotope analysis of MeHg from estuarine sediments containing elevated concentrations of inorganic Hg. The Hg isotopic composition of total Hg in sediments from the Hackensack and Passaic River estuaries was relatively homogenous indicating that sediment-bound inorganic Hg (>99% of total Hg) in these tidally connected waterways is well mixed. However, the Hg isotopic composition of MeHg from Hackensack River estuary sediments varied seasonally and over small spatial scales. For example, the $\delta^{202}\text{Hg}$ value of MeHg from the upstream site HR1 was 0.4‰ lower in samples collected during the spring than winter (Table 2). In addition, MeHg from sediment collected at (site HR3A) and just downstream of the Hackensack River-Berry's Creek confluence (site HR4), had lower $\delta^{202}\text{Hg}$ values than in MeHg from samples collected just upstream or downstream (Tables 2 and S1).

Prior studies of abiotic and biological Hg methylation showed that MDF of Hg isotopes occurs with lighter isotopes enriched in the methylated product (Jiménez-Moreno et al., 2013; Malinovsky and Vanhaecke, 2011; Perrot et al., 2013; Perrot et al., 2015; Rodriguez-Gonzalez et al., 2009). Similarly, the microbial demethylation of MeHg by organomercury lyase (MerB) in mercury-resistant aerobic bacteria results in a

discrimination against heavier Hg isotopes (Kritee et al., 2007). Although the Hg isotopic fractionation associated with oxidative demethylation of MeHg (catalyzed by anaerobic microorganisms such as those living in estuarine sediments) has not been measured, it too likely results in the production of inorganic Hg (II) with lower $\delta^{202}\text{Hg}$ values. As a result, the Hg isotopic composition of MeHg in sediments may be controlled by the relative rates of Hg methylation and demethylation and their Hg isotope fractionation factors. For example, in sediments where demethylation is more important than methylation or has a higher fractionation factor, the steady-state pool of MeHg is expected to be enriched in ^{202}Hg relative to inorganic Hg and vice versa. We observed an inverse relationship between $\delta^{202}\text{Hg}$ and MeHg concentration, which was independent of the Hg isotopic composition and concentration of total Hg (Fig. 3). The isotopic composition of MeHg in sediments may therefore be indicative of the relative importance of Hg methylation and demethylation.

Alternatively, since $\delta^{202}\text{Hg}$ values for MeHg generally increased downstream within the Hackensack-Passaic system, the observed spatial trend may represent the transport of MeHg depleted in ^{202}Hg from an upstream source (or vice versa) rather than *in situ* production and degradation. This may also be the case for MeHg collected near (site HR3A) and downstream (site HR4) of the mouth of Berry's Creek canal, which had lower $\delta^{202}\text{Hg}$ values than expected based on their MeHg concentrations. These sites, which are downstream from a Hg-contaminated Superfund Site, may receive MeHg depleted in ^{202}Hg .

Other factors such as the isotopic composition of inorganic Hg that is bioavailable to Hg methylating microorganisms might also play a role in the Hg isotopic composition

of MeHg in these sediments. For example, a small, bioavailable portion of inorganic Hg in sediments could affect the isotopic composition of microbially-produced MeHg if it was isotopically distinct from the much larger pool of non-bioavailable inorganic Hg without measurably altering the isotopic composition of total Hg. It was recently shown that Hg adsorbed on iron oxyhydroxide (goethite) is depleted in ^{202}Hg relative to dissolved species (Jiskra et al., 2012), potentially creating a pool of dissolved inorganic Hg that is enriched in ^{202}Hg and available to microorganisms. However, in estuarine sediments, organically-bound inorganic Hg was found to have lower $\delta^{202}\text{Hg}$ values than the presumably less bioavailable sulfide-bound Hg (Wiederhold et al., 2015). Finally, another factor that may explain the range of MeHg isotopic compositions we observed are differences in Hg fractionation factors among the dominant species of microbes catalyzing Hg methylation and demethylation in the environment.

In freshwater and estuarine aquatic food webs, $\delta^{202}\text{Hg}$ values for MeHg were estimated to be approximately 0.6‰ higher than those for total Hg in local sediments (Gehrke et al., 2011; Kwon et al., 2014; Sherman and Blum, 2013). This is consistent with our direct observation that $\delta^{202}\text{Hg}$ values for MeHg from estuarine sediments are 0.3‰ to 0.8‰ higher than those for total Hg and indicates that the enrichment of MeHg in ^{202}Hg occurs primarily in the environment prior to entry into the food web.

The method we developed for the analysis of the Hg isotopic composition of MeHg from environmental samples extends the application of Hg isotopes to many new environmental settings and areas of research and provides a new tool with which to study and track the sources, transformations, and bioaccumulation of Hg in aquatic and terrestrial ecosystems.

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References

- Benoit, J.M., Gilmour, C.C., Mason, R.P., Riedel, G.S., Riedel, G.F., 1998. Behaviour of mercury in the Patuxent River estuary. *Biochem. 40*, 249-264.
- Bergquist, B.A., Blum, J.D., 2007. Mass-dependent and -independent fractionation of Hg isotopes by photoreduction in aquatic systems. *Science* 318, 417-20.
- Biswas, A., Blum, J.D., Bergquist, B.A., Keeler, G.J., Xie, Z., 2008. Natural mercury isotope variation in coal deposits and organic soils. *Environ. Sci. Technol.* 42, 8303-8309.
- Bloom, N.S., 1992. On the chemical form of mercury in edible fish and marine invertebrate tissue. *Can. J. Fish. Aquat. Sci.* 49, 1010-1017.
- Blum, J.D., Bergquist, B.A., 2007. Reporting of variations in the natural isotopic composition of mercury. *Anal. Bioanal. Chem.*, 388: 353-359.
- Compeau, G.C., Bartha, R., 1985. Sulfate-reducing bacteria: principle methylators of mercury in anoxic estuarine sediment. *Appl. Environ. Microbiol.*, 50(2): 498-502.
- Demers, J.D., Blum, J.D., Zak, D.R., 2013. Mercury isotopes in a forested ecosystem: implications for air-surface exchange dynamics and the global mercury cycle. *Global Biogeochem. Cycles*, 27(1): 222-238.
- Donovan, P.M., Blum, J.D., Yee, D., Gehrke, G.E., Singer, M.B., 2013. An isotopic record of mercury in San Francisco Bay sediment. *Chem. Geo.*, 349-350(0): 87-98.
- Dzurko, M., Foucher, D., Hintelmann, H., 2009. Determination of compound-specific Hg isotope ratios from transient signals using gas chromatography coupled to multicollector inductively coupled plasma mass spectrometry (MC-ICP/MS). *Anal. Bioanal. Chem.*, 393(1): 345-55.
- Epov, V.N. et al., 2010. Approach to measure isotopic ratios in species using multicollector-ICPMS coupled with chromatography. *Anal. Chem.*, 82(13): 5652-5662.
- Epov, V.N. et al., 2008. Simultaneous determination of species-specific isotopic composition of Hg by gas chromatography coupled to multicollector ICPMS. *Anal. Chem.*, 80(10): 3530-3538.
- Foucher, D., Hintelmann, H., 2006. High-precision measurement of mercury isotope ratios in sediments using cold-vapor generation multi-collector inductively coupled plasma mass spectrometry. *Anal. Bioanal. Chem.*, 384(7-8): 1470-8.
- Foucher, D., Ogrinc, N., Hintelmann, H., 2009. Tracing mercury contamination from the Idrija mining region (Slovenia) to the Gulf of Trieste using Hg isotope ratio measurements. *Environ. Sci. Technol.*, 43(1): 33-39.
- Gehrke, G.E., Blum, J.D., Marvin-DiPasquale, M., 2011. Sources of mercury to San Francisco Bay surface sediment as revealed by mercury stable isotopes. *Geochim. Cosmochim. Acta*, 75(3): 691-705.
- Gehrke, G.E., Blum, J.D., Meyers, P.A., 2007. Hg accumulation in reducing sediments of the Mediterranean Sea: trace metal and Hg isotope evidence. *Geochim. Cosmochim. Acta*, 71(15): A314-A314.
- Gilmour, C.C., Henry, E.A., Mitchell, R., 1992. Sulfate stimulation of mercury methylation in freshwater sediments. *Environ. Sci. Technol.*, 26: 2281-2287.
- Gratz, L.E., Keeler, G.J., Blum, J.D., Sherman, L.S., 2010. Isotopic composition and fractionation of mercury in Great Lakes precipitation and ambient air. *Environ. Sci. Technol.*, 44(20): 7764-7770.
- Grieb, T.M. et al., 1990. Factors affecting mercury accumulation in fish in the upper Michigan Peninsula. *Environ. Toxicol. Chem.*, 9(7): 919-930.
- Hamelin, S., Amyot, M., Barkay, T., Wang, Y., Planas, D., 2011. Methanogens: principal methylators of mercury in Lake Periphyton. *Environ. Sci. Technol.*, 45(18): 7693-700.
- Hammerschmidt, C.R., Fitzgerald, W.F., 2004. Geochemical controls on the production and distribution of methylmercury in near-shore marine sediments. *Environ. Sci. Technol.*, 38(1487-1495).

- Hammerschmidt, C.R., Fitzgerald, W.F., Lamborg, C.H., Balcom, P.H., Visscher, P.T., 2004. Biogeochemistry of methylmercury in sediments of Long Island Sound. *Mar. Chem.*, 90(1–4): 31–52.
- Horvat, M., Blum, J.D., Liang, L., 1993. Comparison of distillation with other current isolation methods for the determination of methyl mercury in low level environmental samples. *Anal. Chim. Acta*, 281(135–152).
- Jiménez-Moreno, M., Perrot, V., Epov, V.N., Monperrus, M., Amouroux, D., 2013. Chemical kinetic isotope fractionation of mercury during abiotic methylation of Hg(II) by methylcobalamin in aqueous chloride media. *Chem. Geo.*, 336: 26–36.
- Jiskra, M., Wiederhold, J.G., Bourdon, B., Kretzschmar, R., 2012. Solution speciation controls mercury isotope fractionation of Hg(II) sorption to goethite. *Environ. Sci. Technol.*, 46(12): 6654–62.
- Jonsson, S. et al., 2014. Differentiated availability of geochemical mercury pools controls methylmercury levels in estuarine sediment and biota. *Nat. Commun.*, 5: 4624.
- Kerin, E.J. et al., 2006. Mercury methylation by dissimilatory iron-reducing bacteria. *Appl. Environ. Microbiol.*, 72(12): 7919–7921.
- Kritee, K., Barkay, T., Blum, J.D., 2009. Mass dependent stable isotope fractionation of mercury during mer mediated microbial degradation of monomethylmercury. *Geochim. Cosmochim. Acta*, 73(5): 1285–1296.
- Kritee, K., Blum, J.D., Johnson, M.W., Bergquist, B.A., Barkay, T., 2007. Mercury stable isotope fractionation during reduction of Hg(II) to Hg(0) by mercury resistant microorganisms. *Environ. Sci. Technol.*, 41(6): 1889–1895.
- Kwon, S.Y., Blum, J.D., Chen, C.Y., Meattley, D.E., Mason, R.P., 2014. Mercury isotope study of sources and exposure pathways of methylmercury in estuarine food webs in the Northeastern U.S. *Environ. Sci. Technol.*, 48(17): 10089–10097.
- Liang, L., Horvat, M., Bloom, N.S., 1994. An improved speciation method for mercury GC/CVAFS after aqueous phase ethylation and room temperature precollection. *Talanta*, 41(3): 371–379.
- Liang, L. et al., 2004. Re-evaluation of distillation and comparison with HNO₃ leaching/solvent extraction for isolation of methylmercury compounds from sediment/soil samples. *Appl. Organomet. Chem.*, 18(6): 264–270.
- Liu, J., Feng, X., Yin, R., Zhu, W., Li, Z., 2011. Mercury distributions and mercury isotope signatures in sediments of Dongjiang, the Pearl River Delta, China. *Chem. Geo.*, 287(1–2): 81–89.
- Ma, J., Hintelmann, H., Kirk, J.L., Muir, D.C.G., 2013. Mercury concentrations and mercury isotope composition in lake sediment cores from the vicinity of a metal smelting facility in Flin Flon, Manitoba. *Chem. Geo.*, 336: 96–102.
- Malinovsky, D., Vanhaecke, F., 2011. Mercury isotope fractionation during abiotic transmethylation reactions. *Int. J. Mass Spectrom.*, 307(1–3): 214–224.
- Masbou, J., Point, D., Sonke, J.E., 2013. Application of a selective extraction method for methylmercury compound specific stable isotope analysis (MeHg-CSIA) in biological materials. *J. Anal. At. Spectrom.*, 28(10): 1620–1628.
- Mason, R.P., Sullivan, K.A., 1999. The distribution and speciation of mercury in the south and equatorial Atlantic. *Deep Sea Res. Part 2: Top. Stud. Oceanogr.*, 46(5): 937–956.
- Mead, C., Lyons, J.R., Johnson, T.M., Anbar, A.D., 2013. Unique Hg stable isotope signatures of compact fluorescent lamp-sourced Hg. *Environ. Sci. & Technol.*, 47(6): 2542–2547.
- Mil-Homens, M. et al., 2013. Tracing anthropogenic Hg and Pb input using stable Hg and Pb isotope ratios in sediments of the central Portuguese Margin. *Chem. Geo.*, 336: 62–71.
- Parris, R., Cronise, M., Fales, C. 1995. Collection of sediment for SRM 1944. U.S. Dept. of Commerce. ROA 839-04-95-005.

- Perrot, V. et al., 2015. Identical Hg isotope mass dependent fractionation signature during methylation by sulfate-reducing bacteria in sulfate and sulfate-free environment. *Environ. Sci. Technol.*, 49(3): 1365-1373.
- Perrot, V. et al., 2010. Tracing sources and bioaccumulation of mercury in fish of Lake Baikal– Angara River using Hg isotopic composition. *Environ. Sci. & Technol.*, 44(21): 8030-8037.
- Perrot, V. et al., 2013. Successive methylation and demethylation of methylated mercury species (MeHg and DMeHg) induce mass dependent fractionation of mercury isotopes. *Chem. Geo.*, 355: 153-162.
- Rodriguez-Gonzalez, P. et al., 2009. Species-specific stable isotope fractionation of mercury during Hg(II) methylation by an anaerobic bacteria (*Desulfobulbus propionicus*) under dark conditions. *Environ. Sci. & Technol.*, 43(24): 9183-9188.
- Schartup, A.T., Mason, R.P., Balcom, P.H., Hollweg, T.A., Chen, C.Y., 2013. Methylmercury production in estuarine sediments: role of organic matter. *Environ. Sci. & Technol.*, 47(2): 695-700.
- Sherman, L.S., Blum, J.D., 2013. Mercury stable isotopes in sediments and largemouth bass from Florida lakes, USA. *Sci. Total Environ.*, 448: 163-175.
- Smith, R.S. et al., 2014. Small-scale studies of roasted ore waste reveal extreme ranges of stable mercury isotope signatures. *Geochim. Cosmochim. Acta*, 137(0): 1-17.
- Sonke, J.E. et al., 2010. Sedimentary mercury stable isotope records of atmospheric and riverine pollution from two major European heavy metal refineries. *Chem. Geo.*, 279(3-4): 90-100.
- Sun, R., Enrico, M., Heimbürger, L.-E., Scott, C., Sonke, J., 2013. A double-stage tube furnace—acid-trapping protocol for the pre-concentration of mercury from solid samples for isotopic analysis. *Anal. Bioanal. Chem.*, 405(21): 6771-6781.
- Tsui, M.T. et al., 2012. Sources and transfers of methylmercury in adjacent river and forest food webs. *Environ. Sci. Technol.*, 46(20): 10957-64.
- U.S.EPA, 1994. Determination of mercury in water by cold vapor atomic absorption spectrometry. EPA-600-R-94-111.
- U.S.EPA, 1999. Appendix to Method 1631, Revision B: total mercury in tissue, sludge, sediment and soil. EPA-821-R-01-013.
- Wehmeier, S., Ellam, R., Feldmann, J., 2003. Isotope ratio determination of antimony from the transient signal of trimethylstibine by GC-MC-ICP-MS and GC-ICP-TOF-MS. *J. Anal. At. Spectrom.*, 18(9): 1001-1007.
- Wiederhold, J. G. and others 2015. Mercury isotope signatures in contaminated sediments as a tracer for local industrial pollution sources. *Environ. Sci. Technol.* 49, 177-185.
- Yang, L., Sturgeon, R., 2009. Isotopic fractionation of mercury induced by reduction and ethylation. *Anal. Bioanal. Chem.*, 393(1): 377-385.
- Yin, R. et al., 2013. Mercury isotope variations between bioavailable mercury fractions and total mercury in mercury contaminated soil in Wanshan Mercury Mine, SW China. *Chem. Geo.*, 336: 80-86.
- Zheng, W., Hintelmann, H., 2010. Isotope fractionation of mercury during its photochemical reduction by low-molecular-weight organic compounds. *J. Phys. Chem. A*, 114(12): 4246-4253.