# MERCURY STABLE ISOTOPE APPROACHES TO THE STUDY OF MONOMETHYLMERCURY IN THE ENVIRONMENT

By

**SARAH ELIZABETH JANSSEN**

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And approved by

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## ABSTRACT OF THE DISSERTATION

Mercury Stable Isotope Approaches to the Study of Monomethylmercury in the Environment

by SARAH E. JANSSEN

Dissertation Director

John R. Reinfelder

Mercury stable isotopes provide additional insights into the biogeochemical cycling of mercury, but the isotopic composition of MeHg in the environment is understudied. The overall objective of this work was to examine the mercury isotopic composition of MeHg in the microorganisms (anaerobic bacteria) and environmental matrices (anoxic sediment) in which it is formed, in order to understand what controls the isotopic composition of MeHg in aquatic systems.

A system for the quantitative separation of MeHg for isotope analysis that did not cause fractionation of mercury isotopes was built and tested (Chapter 2). Methylmercury from estuarine sediments had $\delta^{202}$Hg values that 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‰). This work provided a reproducible and precise method for measuring MeHg isotopes in complex matrices and also represents the first high precision isotope measurement of MeHg in sediments.

The mercury isotopic composition of MeHg was estimated in fish tissue and compared to sediment MeHg measurements in order to gain insight about feeding locality of white perch and killifish species (Chapter 3). Localized estuarine fish showed similarities between the isotopic composition of MeHg in their tissue and sediment from their capture location. Migratory species were isotopically heavier than sediment MeHg, indicating different feeding areas or mercury sources. This study provides mercury stable isotope data on estuarine species and an improved estimation for isotopic composition of MeHg in fish tissue to aid in source tracking applications.

Mercury isotope fractionation factors during microbial mercury methylation were determined in pure cultures of a sulfate-reducing and an iron-reducing bacterium (Chapter 4). Both bacteria had similar fractionation factors despite differences in methylation rates. The mercury stable isotope composition ($\delta^{202}$Hg) of MeHg produced by these organisms eventually exceeded that of the initial Hg(II) provided, indicating that the organisms accessed an intra- or extracellular pool of Hg that was enriched in $^{202}$Hg. This study is the first to establish both a fractionation factor for methylation in an iron-reducing bacterium and observe an isotopically-enriched pool of bioavailable Hg(II).

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# CHAPTER 1

# GENERAL INTRODUCTION

## 1.1 Mercury in the Environment

Mercury (Hg) is a ubiquitous transition metal that can be found in the Earth's crust, oceans, and atmosphere. The most prominent chemical forms of mercury are gaseous elemental ($Hg^0$), inorganic (Hg(II)) (found complexed to water soluble anions and mineral phases),the  and organometallic species monomethylmercury (MeHg) and dimethylmercury (Clarkson and Magos, 2006). Despite being naturally occurring in the environment from volcanic emissions, hot springs, and other sources, the majority of environmental mercury is from anthropogenic origin. It was estimated that 36% of all Hg atmospheric Hg in the environment is released through natural processes, whereas the other 64% is the result of anthropogenic inputs (Mason and Sheu, 2002). The yearly global estimate of mercury emissions is 2200 tons per annum from emissions (Yin et al., 2010). Earlier in the decade the most common sources of mercury were atmospheric releases from stationary fuel combustion, gold mining, nonferrous metal smelting, and cement production (Pacyna et al., 2010). The majority of Hg released into the atmosphere (95%) is the gaseous $Hg^0$ form, which can be readily oxidized to Hg(II) in the atmosphere and transported to aquatic and terrestrial systems through precipitation (Morel et al., 1998). Detectable concentrations of Hg have been discovered in remote areas, such as the Arctic

and Antarctica, and are attributed to the gaseous distribution of mercury making it a global pollutant (Biswas et al., 2008b; Chaulk et al., 2011; Johnson et al., 2007; Mason and Sullivan, 1999)

### 1.2 The Mercury Biogeochemical Cycle in Aquatic Systems

Once entering aquatic systems, newly formed Hg(II) is reactive and subject to biological and abiotic processes (Harris et al., 2007). This allows for a portion of the Hg (II) to be reduced photochemically or microbially. Aerobic microbes in water and sediment can utilize the mercury resistance (*mer)* operon, which uses a mercuric reductase to reduce Hg (II) to $Hg^0$, as a detoxification mechanism (Amyot et al., 1994; Barkay et al., 2003; Liu et al., 2000). Inorganic mercury that is transported to anoxic zones of sediment is bioavailable to a variety of different anaerobic organisms that can perform processes such as methylation and demethylation.

The production of MeHg is predominantly performed by anaerobic microorganisms with the *hgcA* and *hgcB* genes. These methylating organisms mostly convert Hg(II) to MeHg, but certain species also show the capability of oxidizing $Hg^0$ and further methylating Hg(II) products (Colombo et al., 2013; Hu et al., 2013). Regardless of the production pathway this MeHg production is balanced in the environment by the process of demethylation, either photochemically or biologically mediated. In the euphotic zones MeHg that has been transferred to the water column can be spontaneously degraded by ultraviolet (UV) and visible light; UV radiation has been demonstrated to be the

more prevalent and account for 58-79% of photodemethylation in lake systems (Lehnherr and St. Louis, 2009; Morel et al., 1998). Another important factor controlling the fate of MeHg in aquatic systems is demethylation catalyzed by microbes. Microbes have demonstrated the ability to degrade MeHg utilizing two pathways: reductive demethylation and oxidative demethylation (Barkay et al., 2003; Marvin-DiPasquale and Oremland, 1998; Spangler et al., 1973). In the reductive process MeHg is degraded by predominantly aerobic organisms to form $Hg^0$ and $CH_4$ in oxic waters and sediments. This process is mediated once again by the mercury resistance (*mer*) operon, specifically the organomercurial lyase *MerB,* which cleaves the carbon bond to Hg (Barkay et al., 2003). The other pathway known as oxidative demethylation converts the MeHg to inorganic Hg and $CO_2$ (Marvin-DiPasquale and Oremland, 1998). This process has been shown in anaerobic soils and sediments; however, no pure anaerobic culture has shown consistent demethylation (Bridou et al., 2011; Graham et al., 2012; Schaefer et al., 2004). The net accumulation or destruction of MeHg in a given system is the balance of methylation and demethylation.

Methylmercury that is not degraded is lipophilic and can readily bioaccumulate in tissue. Mercury concentrations will increase with increasing trophic level in both pelagic and benthic food webs; this is termed biomagnification. Studies have shown that in higher trophic level fish >90% of the mercury present is in the MeHg form (Bloom, 1992). Once entering the aquatic food chain mercury can cause biological impairment and change the brain chemistry, hormones, and reproductive success in a range of different organisms

from fish to large mammals (Langford and Ferner, 1999). It has been demonstrated that direct exposure to mercury can degrade dendritic appendages of mammalian neuron cells within minutes, making it a potent neurotoxin (Leong et al., 2001). In addition, in human health mercury species have been linked to developmental issues in children and impairment of cognitive function in adults. Different chemical forms of mercury allow for a multitude of exposure routes, including inhalation of $Hg^0$ and the direct consumption of aqueous species. However, the most prevalent mercury exposure, in developed countries, is the consumption of mercury in fish tissue.

### 1.3 Mercury Methylation in Estuarine Systems

Mercury methylation and the causes behind it are not fully understood in an environmental context. This process takes place in a wide variety of areas including sinking particulate matter the open ocean (Blum et al., 2013; Mason and Sheu, 2002; Mason and Sullivan, 1999), but is most commonly observed in anoxic sediments or soils. In coastal systems MeHg produced in sediment is an important source of Hg to biota (Chen et al., 2014). Recent research in estuarine systems even links the MeHg from in the sediment and particulate matter to MeHg content in lower trophic level organisms (Chen et al., 2014; Kwon et al., 2014). Other research also corroborates this conclusion and has shown that the external supplies of MeHg, such as runoff, do not fully account for MeHg accumulation in biota in brackish water (Gilmour and Henry, 1991). These conclusions have also been drawn in freshwater and oceanic systems, where

concentrations of MeHg in surface water do not correlate with concentrations in fish tissue (Driscoll et al., 2007; Wang and Wong, 2003).

### 1.3.1 The Role of Microorganisms

Methylation can occur through both abiotic and microbial processes. However, it was demonstrated through Hg (II) additions to sediment incubations that the phenomenon of methylation only occurred in non-sterilized sediments (Berman and Bartha, 1986; Jensen and Jernelov, 1969). Over time specific organisms that catalyze the methylation process were identified. All of the known methylators are anaerobic bacteria and belong to four major groups: sulfate reducing bacteria (SRB); iron reducing bacteria (IRB); methanogenic archaea; and firmicutes (Compeau and Bartha, 1985; Gilmour et al., 1992; Gilmour et al., 2013; Parks et al., 2013; Yu et al., 2013). Early research established, through the usage of sulfate reduction inhibitors, that SRB were responsible for 95% of the mercury methylation occurring in high salinity sediment incubations (Compeau and Bartha, 1985). Later research showed that in estuarine and marine sediments the methylation was correlated to parameters such as acid volatile sulfide (AVS), total sulfur, and sulfate reduction rates (Gilmour et al., 1992; King et al., 1999; Schartup et al., 2014). However, other studies showed that mercury methylation was even occurring in estuarine sediments where iron was the predominant electron acceptor (Mehrotra and Sedlak, 2005). Upon further investigation iron reducing genera, *Geobacter* and *Desulfuromonas*, were also shown to methylate mercury, but to a lesser extent in comparison to SRB strains (Kerin et al., 2006). The most recent additions to the group of methylators

are methanogenic archae and firmicutes due to the recent elucidation of the *hgcA* and *hgcB* genes, as well as pure culture work (Gilmour et al., 2013; Parks et al., 2013; Yu et al., 2013).

### 1.3.2. Controls on Methylation in Estuarine Systems

The methylation process in anoxic sediments is of particular concern in rivers, wetlands, and estuaries. These systems are a direct link between terrestrial produced mercury and cycling in the marine environment (Kim et al., 2004). Estuaries have been shown to have varying degrees of methylation due to different diagenetic controls on the microbial community such as organic carbon and acid volatile sulfur (AVS) (Hammerschmidt and Fitzgerald, 2004; Ullrich et al., 2001). Organic carbon can act as an energy source and has been shown to stimulate the growth of methylating bacteria in sediment (Hammerschmidt et al., 2008; Schartup et al., 2013). Another important process to consider when examining methylation is the creation of sulfide during sulfate reduction. The high production of sulfide (AVS) can eventually inhibit microbial growth as well as methylation (Gilmour et al., 1992). Residual sulfide in a system can also negatively impact MeHg production due to the creation solid and dissolved charged HgS species which can be less bioavailable (Drott et al., 2007; Skyllberg, 2008).Temperature can also play an important role in methylation, there is strong evidence showing preferential methylation in warmer summer months and higher demethylation in winter months (Hintelmann and Wilken, 1995; Ullrich et al., 2001). Seasonal variation and spatial differences in sediment

composition are important factors in MeHg production and can cause differences in the extent of MeHg introduced to the food web.

Methylation in estuarine sediments causes increased concentrations of MeHg in fish, specifically in higher trophic level coastal fish (Heyes et al., 2004). MeHg produced in river and estuarine systems can be a significant source to coastal areas, especially in urbanized watersheds (Chen et al., 2012). In addition, estuarine food webs can have a large benthic component due to smaller fish such as killifish and crustaceans (Weis et al., 2003; Weis et al., 1986). Consumption of these benthic species, with high MeHg contents can accelerate biomagnification (Bloom, 1992).

### 1.4 Mercury Stable Isotopes

In recent years the development of high sensitivity mass spectrometers have allowed for the isotopic study of nontraditional elements, such as mercury and other heavy metals. Coupling traditional Hg cold vapor generation with inductively coupled plasma mass spectrometry (MC-ICP-MS) allows for Hg samples, even as low as 5-10ng, to be measured in a variety of matrices (Lauretta et al., 2001). These advances have allowed scientists to study mercury isotope fractionation as well as assign isotopic values to environmental samples for source tracking applications.

Mercury has seven stable isotopes ($^{196}$Hg, $^{198}$Hg, $^{199}$Hg, $^{200}$Hg, $^{201}$Hg, $^{202}$Hg, $^{204}$Hg) that are naturally occurring and participate in physical, chemical, and biological reactions. These isotopes have identical electronic structure, but the

4% mass difference allows for differentiation using MC-ICP-MS techniques. These isotopic pools are capable of undergoing two different types of isotopic fractionations: mass dependent fractionation (MDF) and mass independent fractionation (MIF). The later of the two is further divided into the magnetic isotope effect (MIE), nuclear volume effect (NVE), and UV self-shielding. These fractionation processes, which will partition isotopes between two chemical reservoirs, are the causes behind all the isotopic signatures of Hg found in the environment. Commonly MDF is discussed in regards to Hg isotope $^{202}$Hg; MIF is predominantly observed in odd isotopes $^{199}$ Hg and $^{201}$ Hg, but has also been observed in $^{200}$ Hg.

### 1.4.1. Mass Dependent Fractionation (MDF)

All isotope fractionation takes place on a quantum chemical level and is based off the zero point energies (ZPE) of the isotopes which are derived from molecular vibrational frequencies. Higher mass isotopes have a lower vibrational frequency and ground state (Zeebe and Wolf-Gladrow, 2001). These differences in energies lead to the isotope fractionation that occurs during chemical reactions. When an equilibrium reaction occurs the partitioning between the reactants and products is described as an isotope – exchange between different species or phases. Lower zero point energies result in the molecule being more stable and the bond is less easily broken (requiring more potential energy to dissociate). Heavier isotopes will concentrate in the phase with the strongest bond and the lowest ZPE (Zeebe and Wolf-Gladrow, 2001). It is commonly

observed that heavier isotopes will enrich in the denser or oxidized form of the compound based on the bond strength.

Isotopic differences can also be observed in kinetic reactions, but are only seen when the reaction has not reached completion. If a reaction is allowed to come to completion the isotopic signature of the product will mirror the starting reactants since there is no longer a mass selection (Hayes, 2004). In order for the product to be formed the reactants must overcome the initial activation energy; the differences in activation energies between light and heavy isotopes are estimated once again by ZPE. The activation energy of a molecule containing the light isotope is smaller, due to a higher ZPE, than that of a heavier isotope. Hence light molecules will react faster and preferentially enrich in the product (Johnson et al., 2004). In open systems where the product is removed immediately, the partitioning of isotopes between the two reservoirs can be estimated using the exponential relationship known as the Rayleigh equation. This equation is commonly applied to both kinetic fractionations and equilibrium closed systems (Hayes, 2004).

### 1.4.2. Mass Independent Fractionation (MIF)

Mass independent fractionations do not follow the simple mass rules described in equilibrium and kinetic MDF (Johnson et al., 2004). The most prominent MIF in mercury is MIE and is caused by direct photolysis or secondary reactions from the products of photolysis (Bergquist and Blum, 2009). This phenomenon arises from the different reaction rates of magnetic and non-

magnetic isotopes during spin reactions and usually enriches odd isotopes in the reactants (Zeebe and Wolf-Gladrow, 2001). During photochemical reactions the reactants will enter a quantum chemical singlet state before forming the product. However, odd isotopes with unpaired nuclear spins can undergo spin conversion creating a forbidden electronic transition that cannot form the products and subsequently funnels the odd isotopes back to the reactants (Zheng and Hintelmann, 2010). In the mercury cycle MIE is commonly see in photochemical reduction and photodemethylation (Bergquist and Blum, 2007; Bergquist and Blum, 2009; Zheng and Hintelmann, 2010).

Other MIF exists, but produces smaller changes in the isotope pools in comparison to MIE and is difficult to ascertain in environmental samples. The nuclear volume effect is the displacement of electronic energy due to size and shape differences for the atomic nuclei (Epov et al., 2011; Zheng and Hintelmann, 2010). It is commonly observed at equilibrium that smaller isotopes will partition into the chemical species with lower electron density (Epov et al., 2011). The nuclear radius does not correlate with number of neutrons in isotopic atoms and usually atoms with odd neutrons display a smaller size than expected. In the Hg isotope field NVE reactions are categorized by mass independent trends observed in $Hg^{199}$, $Hg^{200}$, and $Hg^{201}$. These have been seen in equilibrium reactions and some environmental samples such as precipitation (Gratz et al., 2010; Jiskra et al., 2012; Wiederhold et al., 2010). The last type of MIF, UV- self shielding, has only been observed once in Hg isotopes within compact

fluorescent lightbulbs and is stated to only occur in optically thick vapors due to transmittance radiation (Mead et al., 2013).

### 1.4.2. Isotope Notation

The notation used to denote isotopic composition of a substance is known as delta notation (δ) and is expressed as the deviation of an isotopic ratio in comparison to the same ratio in a known standard, in units of per mil (‰) (Eq. 1). The reference standard utilized for Hg isotope calculations is NIST 3133.

$$\delta^{i}E_{X} = \left( \frac{R_{X}{}^{i/j} - R_{STD}{}^{i/j}}{R_{STD}{}^{i/j}} \right) \times 10^{3} \qquad \text{Eq.1}$$

All deviations from MDF, known as MIF, are expressed in the capital delta (Δ) notation and represent the deviation from MDF as predicted by mass ratios (λ).

$$\Delta^{k}E = \delta^{k}E - (\lambda)\,\delta^{i}E \qquad \text{Eq.2}$$

The fractionation factor (α) is used to describe the instantaneous fractionation between two isotope reservoirs for a specific process (Johnson et al., 2004). This is simply defined as the difference in isotopic compositions between two substances, such as reactant and product (Eq. 3). The fractionation factor (α) can also be simplified and expressed as (ε) to represent the fractionation factor in terms of per mil (‰), the same unit as δ (Eq. 4).

$$\alpha_{A-B} = \frac{R_A^{\,i/j}}{R_B^{\,i/j}} \qquad \text{Eq.3}$$

$$\varepsilon_{A-B} = \left(\alpha_{A-B} - 1\right) \times 10^3 \qquad \text{Eq.4}$$

### 1.4.4. Mechanistic Studies of Hg Stable Isotopes

Mercury can go through numerous transformations and the fractionation of Hg stable isotopes during these processes is critical to interpreting natural isotopic compositions. Numerous studies have focused on the kinetic fractionation during reduction, demethylation, and volatilization (Figure 1.1). Mass dependent fractionation of Hg stable isotopes were initially observed in *mer*-mediated reduction of Hg (II) to $Hg^0$ in pure cultures of *E. coli* and environmental samples (Kritee et al., 2007). This study examined a kinetic fractionation process where the product ($Hg^0$) was being removed and not allowed to participate in any addition isotope-exchange reactions. As typically displayed in kinetic MDF reactions the lighter isotopes enriched in the product $Hg^0$, indicated by a more negative $\delta^{202}$ Hg value, in comparison to the starting material. Later studies examining microbial demethylation and methylation showed similar MDF trends with the absence of MIF (Kritee et al., 2009; Perrot et al., 2015; Rodriguez-Gonzalez et al., 2009). However, the two studies that have been performed on the methylation reaction utilize a coupled GC/MC-ICP-MS system with low precision and high variability, warranting further investigation of the methylation reaction (Epov et al., 2008; Rodriguez-Gonzalez et al., 2009). Kinetic studies have also been undertaken to study abiotic processes such as

trans-methylation and dimethylmercury production which show similar magnitude of fractionation factors and the prominence of MDF just like biological studies (Jiménez-Moreno et al., 2013; Malinovsky and Vanhaecke, 2011).

Limited equilibrium studies, which have also shown NVE, have been performed examining mercury thiol and goethite interactions, but yield lower fractionation factors in comparison to the kinetic processes (Jiskra et al., 2012; Wiederhold et al., 2010). Despite the ability to differentiate between these processes based on their fractionation factor the experimental studies of dark reactions have roughly a $\delta^{202}$Hg range of 2 ‰ and are quickly overwhelmed in natural samples that have higher variation due to multiple source materials and multiple Hg transformations. This indicates the total Hg (predominantly inorganic) signatures that are commonly assigned to sediment, water, and biota cannot provide information about the small scale processes occurring, especially since certain products of these reactions ($Hg^0$ and MeHg) are commonly un-measureable.

Other studies examining MIF resulting from photodemethylation and photoreduction are measureable in the environment, unlike the dark reactions previously mentioned (Bergquist and Blum, 2007; Bergquist and Blum, 2009). It was found that photoreduction and photodemethylation enrich odd isotopes in the reactants due to MIE. This process is easily translated to environmental samples and the empirical slopes of Δ199/ Δ201 allow for the differentiation of the photoreduction and photodemethylation (Bergquist and Blum, 2007; Senn et al., 2010). It is observed in the water column that photodemethylation causes the

odd isotopes to be enriched in the remaining MeHg which is subsequently taken up by aquatic organisms; this eventually shows an enrichment of the odd isotopes in aquatic biota tissue. Despite the benefits of odd isotopes for tracking photochemical processes in the water column, MIF signatures are quickly dampened in sediments or soil and the unknowns associated with atmospheric redox makes long range transport modeling of Hg isotopes difficult (Sonke, 2011). However, newer studies examining anomalies in $\Delta^{200}$ Hg may yield useful insight for source tracking and modeling utilizing MIF (Gratz et al., 2010; Lepak et al., 2015).

### 1.4.5. Field Applications of Hg Stable Isotopes

Fractionation processes are difficult to ascertain in environmental samples, but Hg isotopes can also be utilized for source tracking in sediment and soil systems. Success in Hg tracking has been documented in discerning mine waste from the metallic mercury produced in both the San Francisco Bay and the Idrija mine in Solvenia as well as industrial waste in the Pearl River in China (Foucher et al., 2009; Gehrke et al., 2011; Liu et al., 2011). Other studies examining coal ash spills and coal power plant emissions have shown the capability to track Hg sources to a system (Bartov et al., 2012; Sherman et al., 2012). Time records can also be examined using isotopes in sediment cores, studies have shown the onset of industrial activity due to shifts in the isotopic compositions in sediment cores as well as inputs over time from contaminated effluents (Ma et al., 2013; Sonke et al., 2010). If there is a source with an isotopic signature distinct enough from the environmental background signature

then the tracking of Hg contamination and the development of mixing models are possible. The main caveat to these source determination studies is that there is usually only a small range of $\delta^{202}$Hg = -0.2 to -0.9 ‰ for the majority of sediment study sites as shown in Fig. 1.2., specifically if there is a high degree of mixing or multiple source inputs.

Studies of biological tissues from the environment have yielded insight on trophic transfer of Hg isotopes and estimation of Hg sources. The isotopic compositions of $\Delta^{199}$ Hg and $\Delta^{201}$ Hg in biota have allowed for the back calculation of MeHg and Hg pools in the sediment using estimates based off previous lab studies of photochemical reactions (Kwon et al., 2014; Sherman and Blum, 2013). One study applied these calculations and compared $\delta^{202}$ Hg values to determine that sediment, not atmospheric deposition, was the primary Hg source to a freshwater food web (Sherman and Blum, 2013). Other studies using estimates from MIF had similar conclusions from measuring the isotopic compositions of different benthic organisms in estuarine systems and concluded that sediment is a dominant MeHg source (Kwon et al., 2014). In some cases the isotopic compositions (both MDF and MIF) of different groups of fish are distinct enough to make inferences about feeding habits, such as whether the groups feed onshore or offshore (Senn et al., 2010). The decline of MIF signatures in fish tissue collected at depth has even provided isotopic evidence of Hg methylation below the ocean surface mixed layer (Blum et al., 2013). The application of Hg isotopes to controlled field studies have also demonstrated that MIF is absent in trophic transfer, indicating that MIF signatures in biota is from

photochemical reactions from the surrounding environment (Kwon et al., 2012). While MIF may be absent, additional bioaccumulation studies have also shown that isotopic signature derived from food can be subject to incomplete assimilation and MDF can arise due to different ecological stress factors (Kwon et al., 2013). Lastly, a study that separated the inorganic and MeHg pools in animals and fish, using a tissue specific extraction protocol, showed that the MeHg pool was isotopically heavier, supports the idea of MDF due to transformations within the organism (Masbou et al., 2013). The Hg isotopic composition in biological tissue can provide valuable insight into Hg bioaccumulation as well as source tracking applications, but can also be complicated by environmental and physiological factors.

### 1.5 Purpose of Study

Despite the large amount of studies utilizing Hg stable isotopes many do not focus on the MeHg pool, due to analytical difficulties. Techniques that have been used in the literature have produced low precision or limited application for a wide array of matrices. The isotopic composition of critical MeHg pools in the environment, such as sediment, remains unknown and the fractionation of Hg isotopes during methylation is poorly understood. The objective of this study was to apply Hg stable isotopes to examine the isotopic composition of MeHg in complex matrices, such as sediment and pure cultures, utilizing a separation technique that produces higher precision and reproducibility. The specific goals of each chapter were to:

**Chapter 2:** Develop an offline separation system that allows continuous sample introduction to the MC-ICP/MS and test the technique by measuring the isotopic composition of MeHg in sediment.

**Chapter 3:** Study the Hg isotopic composition of THg and MeHg in fish tissue from a contaminated estuary to determine general feeding localities based upon similarities to isotopic composition of sediment MeHg.

**Chapter 4:** Investigate isotopic fractionation of Hg during the production of MeHg in SRB and IRB pure cultures.

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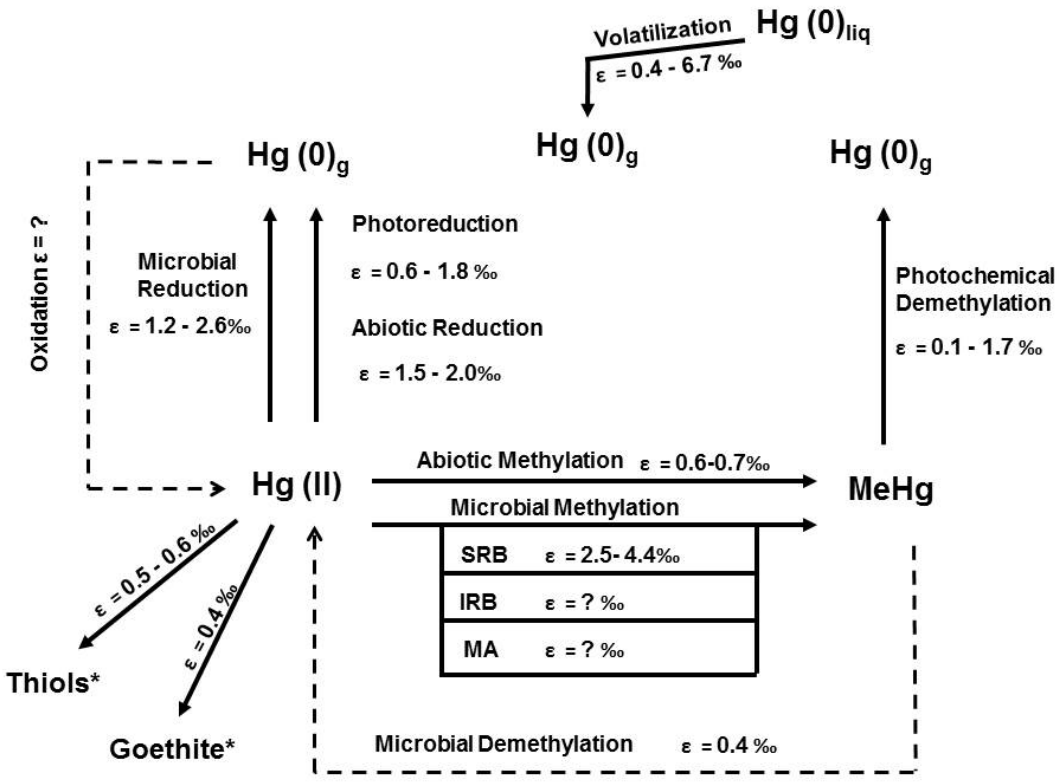


**Figure 1.1.** Fractionation factors, represented as ε, for studied Hg transformations, figure was adapted from Perrot et al. 2015.

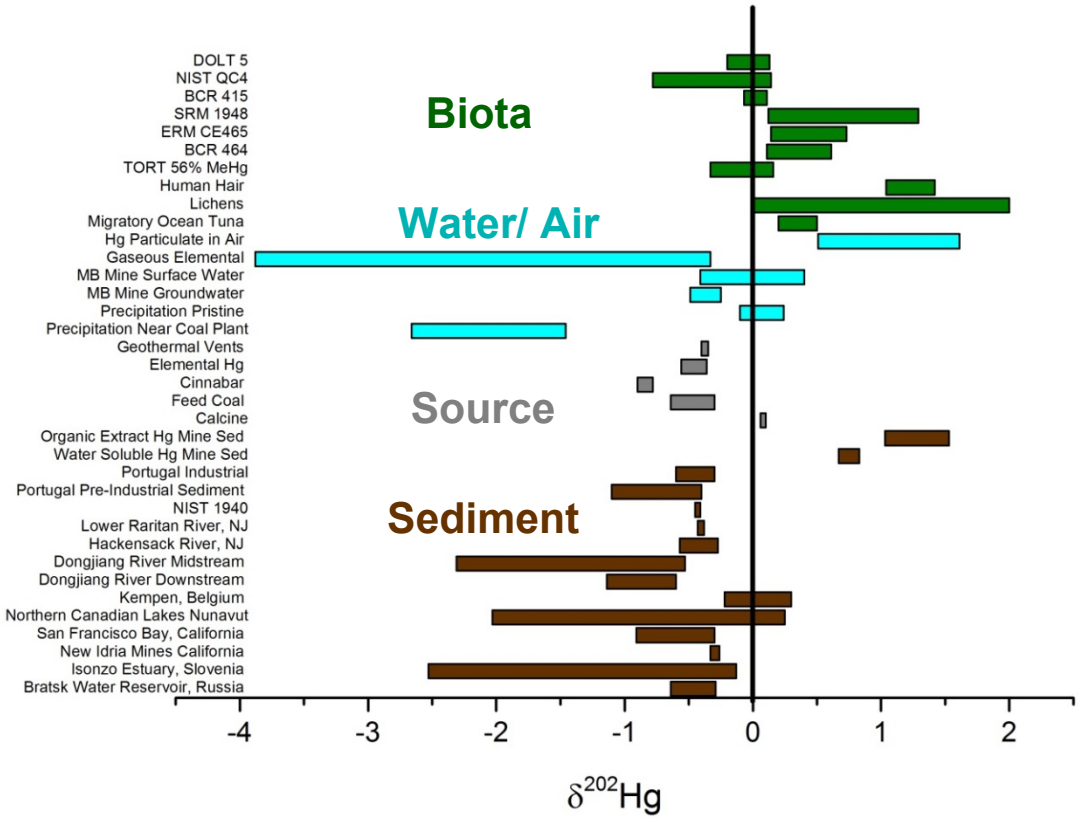


**Figure 1.2.** Ranges of $\delta^{202}Hg$ isotopic compositions in sediment, source materials, water, and biological tissue

# CHAPTER 2

## SEPARATION OF MONOMETHYLMERCURY FROM ESTAURINE SEDIMENTS FOR MERCURY ISOTOPE ANALYSIS

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## ABSTRACT

Estuarine sediments support the production of monomethylmercury (MeHg) and its subsequent accumulation in aquatic organisms. Developments in multicollector inductively coupled plasma mass spectrometry (MC-ICP-MS) have opened the possibility of using the natural variation in Hg stable isotope ratios to track sources and transformations of Hg in the environment, but 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-MC-ICP-MS systems; however, the precision and sensitivity of this method is too low for the analysis of many environmental samples such as 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 ±0.14 ‰ (2SD, n=8) for $\delta^{202}$Hg and no fractionation of Hg stable isotopes occurred during the separation. $\delta^{202}$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 anoxic 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 of 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 were 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 was 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 $\delta^{202}$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. 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 (Supp. Table 2.1). 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 which were 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 (0.8% v/v) and reagent grade potassium chloride (0.2% v/v) were added to each vial. 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 ± 5 μg $g^{-1}$, n = 6) was within 2% of the certified value (132 ± 3 μg $g^{-1}$).

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 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 μg $g^{-1}$ tetraethylborate, ethylation efficiencies averaged 99 ± 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. 2.1). 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 (Supp. Fig. 2.1). 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.

Samples with high inorganic mercury concentrations (>2 μg $g^{-1}$) were 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 (Supp. Table 2.2) (modified from (Demers et al., 2013; 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). Bracketing standards (NIST 3133) were diluted in a $KMnO_4$-$H_2SO_4$ matrix and concentration matched. Blanks of the same $KMnO_4$-$H_2SO_4$ 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 2.1). This standard shows a small degree of MIF with respect to $^{199}$Hg ($\Delta^{199}$ Hg ≈ +0.1), but no significant MIF for the other isotopes. Multiple preparations of UM Almáden and NIST 1944 were used to characterize instrument performance at the University Michigan; standards of UM Almáden and ERM CC580 were additionally run at Rutgers to test instrument performance (Supp. Table 2.3).

## 3. RESULTS

Recoveries of MeHg were determined for individual components of the separation and retrapping system and for the system as a whole (Table 2.1). Samples processed through the desorption system consisting of just the solenoid and permanganate trapping showed a recovery of 102.9 ± 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 ± 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 ± 15.8 % (Table 2.1).

### 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 2.1). Thus, $\delta^{202}$Hg values were not significantly different for MeHg standards passed through the complete separation system (UMich= -0.91 ± 0.12‰ Rutgers = -0.93 ± 0.05‰) or analyzed directly (UMich = -1.07 ± 0.04 ‰, Rutgers = -1.02 ± 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), $\delta^{199}$ Hg was 0.02 ‰ to 0.07 ‰ lower, but not significantly different ($p > 0.05$, unpaired t-test) than MeHg standards analyzed directly. $\delta^{204}$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 $\delta^{204}$Hg in the MeHg standard (1S.D. = 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 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 ± 6.8% and $\delta^{202}$Hg values for separated MeHg (UMich = 0.92 ± 0.02‰,

Rutgers = 0.92 ± 0.16 ‰) were not significantly different than those for unspiked standards ($p > 0.05$, unpaired t-test) (Table 2.1).

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 2.1).

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 2.1; Fig. 2.2). MIF of $^{204}$Hg has not been previously reported for any chemical reactions or environmental matrices, but was observed in compact fluorescent lamps and was 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.

### 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 μg $g^{-1}$ (Table 2.2), which is elevated compared with uncontaminated sediment (0.008 to 0.035 μg $g^{-1}$) (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 ($R^2 = 0.63$, $p < 0.05$).

The isotopic composition of total Hg in the Hackensack and Passaic sediments was fairly uniform (average $\delta^{202}$Hg = -0.36 ± 0.17 ‰, n=8) and very similar to the Hg isotopic composition of the sediment standard reference material NIST 1944 ($\delta^{202}$ Hg = -0.38 ± 0.16 ‰, n=5), which includes sediment collected from various locations within nearby Newark Bay. No MIF of Hg isotopes was observed for total Hg in these sediments (Supp. Table 2.3). For MeHg separated from estuarine sediments, $\delta^{202}$Hg values varied from -0.41 ‰ to +0.41 ‰ and showed a decreasing trend with increasing MeHg concentration (Fig. 2.3). 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 ($p > 0.1$).

## 4. DISCUSSION

The separation and trapping system we developed allows for quantitative (>90%) recovery of 10-30 ng of MeHg with no significant fractionation of Hg isotopes. The variance of $\delta^{202}$Hg measurements for individual MeHg standards (0.02‰ to 0.16‰, 1 SD) was within that of the isotope analysis alone (Bergquist and Blum, 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 $\delta^{202}$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 2.1 and 2.2). 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 when evaluating results (Dzurko et al., 2009). While precision for $\delta^{202}$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 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 $\delta^{202}$Hg value of MeHg from the upstream site HR1 was 0.4‰ lower in samples collected during the spring than winter (Table 2.2). In addition, MeHg from sediment collected at (site HR3A) or just downstream of the Hackensack River-Berry's Creek confluence (site HR4), had lower $\delta^{202}$Hg values in comparison to MeHg from other sites on the Hackensack or Passaic Rivers.

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 $\delta^{202}$Hg values. As a result, the Hg isotopic composition of MeHg in sediments in a given location or season 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 $\delta^{202}$Hg and MeHg concentration, which was independent of total Hg concentration (Fig. 2.3). The isotopic composition of MeHg may therefore be indicative of the relative importance of Hg methylation and demethylation in sediments.

Alternatively, since $\delta^{202}$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 the mouth of Berry's Creek canal and HR4, which had lower $\delta^{202}$Hg values than expected based on their MeHg concentrations. These sites, which are downstream from a Hg-contaminated Superfund Site, may also 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 $\delta^{202}$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, $\delta^{202}$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 $\delta^{202}$Hg values for MeHg from estuarine sediments are from 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 the sources, transformations, and bioaccumulation of Hg in aquatic and terrestrial ecosystems may be examined and tracked.

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**Table 2.1**. Chemical recoveries and Hg isotopic compositions of MeHg standards collected from various stages of the MeHg separation and trapping system

| Process | Analysis | $\delta^{204}Hg$ | 1SD | $\delta^{202}Hg$ | 1SD | $\delta^{201}Hg$ | 1SD | $\delta^{200}Hg$ | 1SD | $\delta^{199}Hg$ | 1SD | $\Delta^{204}Hg$ | $\Delta^{201}Hg$ | $\Delta^{200}Hg$ | $\Delta^{199}Hg$ | 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 ($Hg_i$ 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 | |

**Table 2.2.** Concentrations and $\delta^{202}Hg$ values of total Hg and MeHg in sediment samples from the Hackensack (HR) and Passaic (P) River estuaries. Values are means ± 1 SD (n = 2 to 4). Salinity (Sal) of site water at time of collection is also shown.

| Site | Season | Sal | THg ($\mu g\ g^{-1}$) | 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 |

[a]Analyzed at both the University of Michigan and Rutgers University

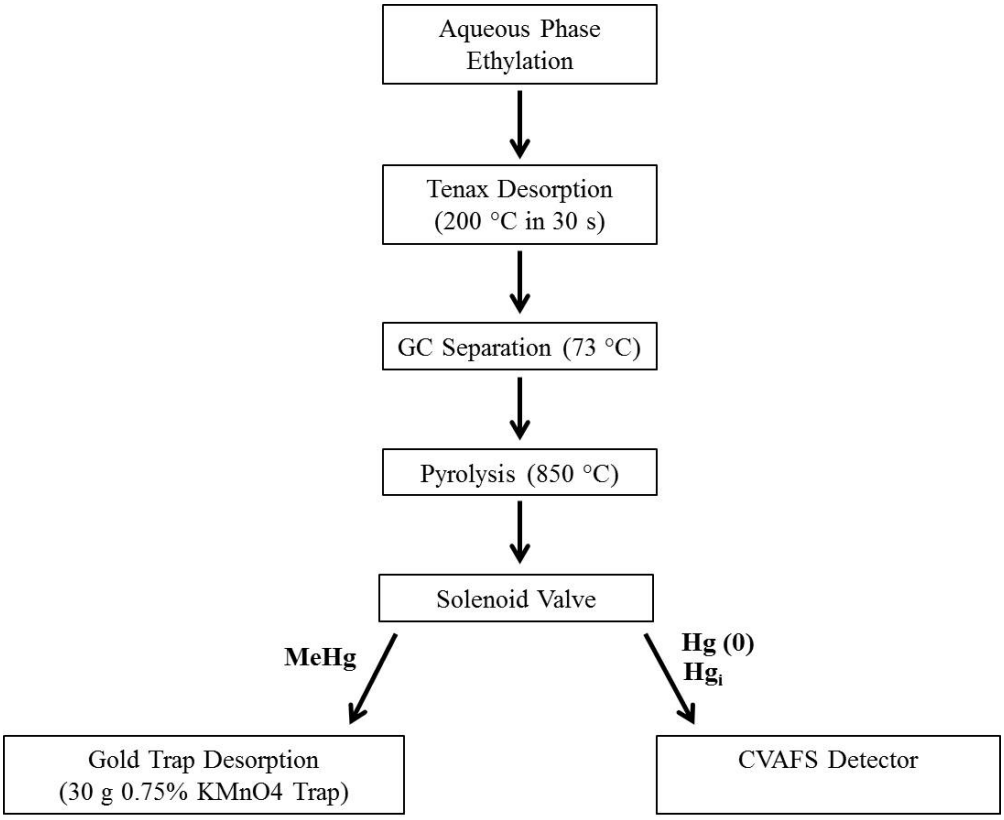



**Fig. 2.1.** Schematic of the MeHg separation and trapping system.

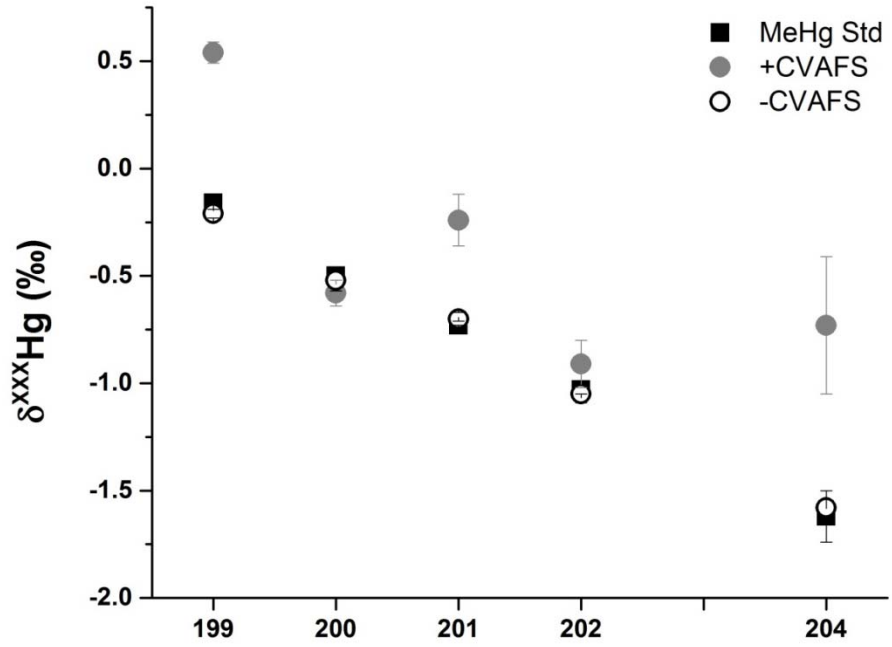



**Fig. 2.2.** Mercury isotopic composition of monomethylmercury (MeHg) standards processed through the separation and trapping system. Values are means ± 1SD for MeHg run at both universities that passed through (●) or bypassed (○) the cold vapor atomic fluorescence spectrometry (CVAFS) detector. Also 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.

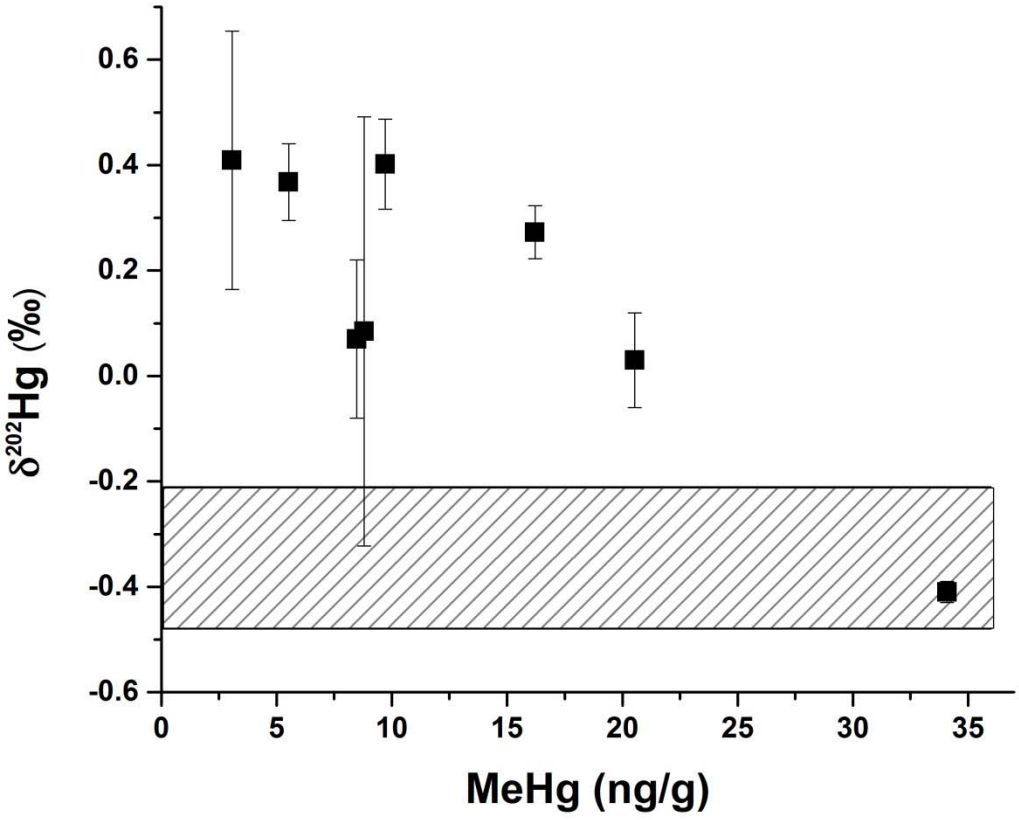



**Fig. 2.3.** Relationship between $\delta^{202}$Hg values for MeHg and the concentration of MeHg in sediments from the Hackensack and Passaic River estuaries. The shaded area represents the range of $\delta^{202}$Hg values for total mercury in the sediment from all sites (average = -0.36 ± 0.17 ‰). 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 (Supp. Table 2.1).

**Supplement Table 2.1** Site information for estuarine sediment samples.

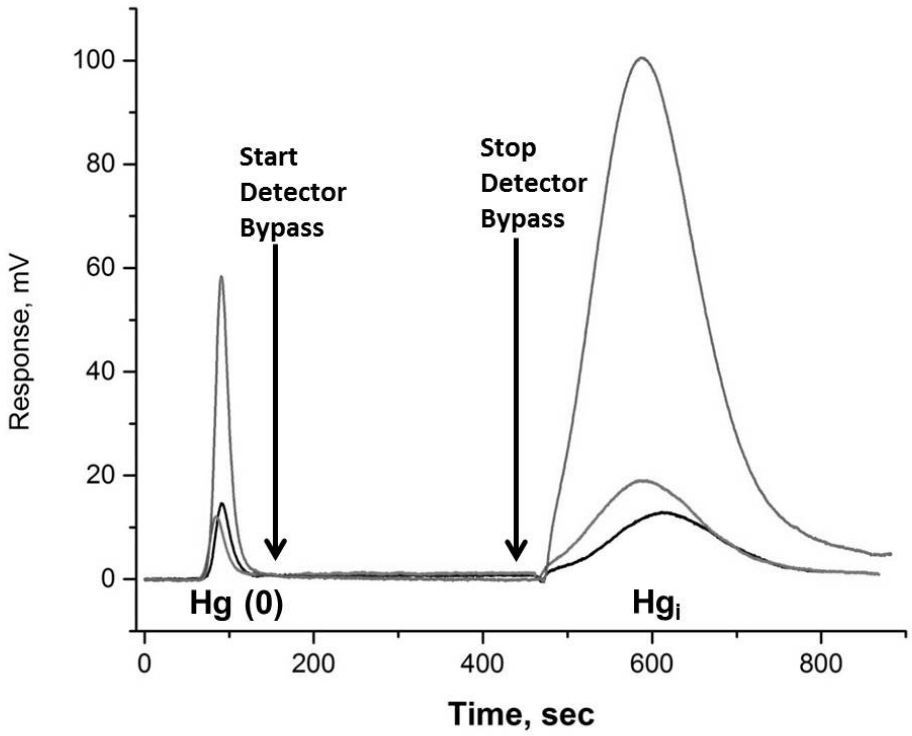
Distance measured from center of Newark Bay (40.6820 N, -74.1270 W).

| Site | Season | Lat | Lon | Distance (km) | Sal | Temp (°C) | OM (%) | pH | Water (%) |
|---|---|---|---|---|---|---|---|---|---|
| HR1 | Jan-13 | 40.8495 | -74.0303 | 25 | 1.92 | 2.3 | 12.4 | 7.7 | 69 |
| HR1 | May-13 | 40.8495 | -74.0303 | 25 | 3.59 | 19.8 | 13.5 | 7.4 | 68 |
| HR2 | Aug-12 | 40.8249 | -74.0340 | 22 | 5.79 | 28.3 | 13.8 | 7.7 | 68 |
| HR3 | Nov-12 | 40.8012 | -74.0651 | 17 | 8.77 | 15.6 | 11.1 | 7.3 | 60 |
| HR3A | Nov-12 | 40.7980 | -74.0753 | 16 | 10.11 | 15.6 | 9 | 7.9 | 55 |
| HR4 | Jan-13 | 40.7765 | -74.0893 | 14 | 8.74 | 2.9 | 10.3 | 7.6 | 59 |
| HR5 | Aug-13 | 40.7428 | -74.0794 | 8.1 | 14.11 | 23.7 | 7.96 | 7.4 | 47 |
| P | Aug-13 | 40.7224 | -74.1222 | 4.8 | 6.55 | 23.1 | 13.6 | 7.7 | 42 |

**Supplement Table 2.2** Mercury stable isotope ratios for MeHg and total Hg from estuarine sediment samples and total Hg in Hg isotope reference standards, Δ values refer to MIF calculations as detailed in Bergquist and Blum (2007)

| | δ204 | Stdev | δ202 | Stdev | δ201 | Stdev | δ200 | Stdev | δ199 | Stdev | Δ204 | Δ201 | Δ200 | Δ199 | n |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **MeHg AVERAGES & 2SD** | | | | | | | | | | | | | | | |
| **HR3a (UMich)** | -0.16 | 0.74 | -0.03 | 0.35 | -0.04 | 0.26 | -0.02 | 0.34 | 0.04 | 0.07 | -0.12 | -0.02 | -0.01 | 0.04 | 2 |
| **HR3a (RU)** | | | 0.17 | 0.05 | 0.05 | 0.48 | 0.07 | 0.28 | 0.09 | 0.03 | | -0.08 | -0.01 | 0.05 | 2 |
| **HR1W** | 0.07 | 0.17 | 0.03 | 0.18 | 0.02 | 0.12 | 0.02 | 0.20 | 0.00 | 0.23 | 0.02 | 0.00 | 0.01 | -0.01 | 2 |
| **HR1SP (Umich)** | -0.57 | | -0.38 | | -0.28 | | -0.28 | | 0.02 | | 0.00 | 0.01 | -0.09 | 0.12 | 1 |
| **HR1SP (RU)** | | | -0.42 | 0.03 | -0.26 | 0.08 | -0.17 | 0.02 | 0.06 | 0.10 | | 0.05 | 0.04 | 0.17 | 2 |
| **HR2** | 0.45 | 0.26 | 0.27 | 0.10 | 0.33 | 0.31 | 0.16 | 0.18 | 0.05 | 0.07 | 0.04 | 0.12 | 0.02 | -0.02 | 2 |
| **HR3** | 0.45 | 0.11 | 0.40 | 0.17 | 0.31 | 0.34 | 0.16 | 0.09 | 0.10 | 0.10 | -0.15 | 0.00 | -0.05 | 0.00 | 2 |
| **HR4** | 0.10 | 1.10 | 0.08 | 0.81 | 0.02 | 0.59 | 0.02 | 0.32 | 0.06 | 0.26 | -0.03 | -0.05 | -0.02 | 0.04 | 2 |
| **HR5** | 0.21 | 0.08 | 0.37 | 0.15 | 0.32 | 0.13 | 0.21 | 0.11 | 0.19 | 0.15 | -0.34 | 0.04 | 0.02 | 0.09 | 2 |
| **PASS** | 0.37 | 0.91 | 0.41 | 0.49 | 0.24 | 0.57 | 0.26 | 0.13 | 0.16 | 0.15 | -0.24 | -0.06 | 0.06 | 0.06 | 2 |
| **THg AVERAGES & 2SD** | | | | | | | | | | | | | | | |
| **HR3a** | -0.41 | 0.23 | -0.21 | 0.16 | -0.15 | 0.11 | -0.08 | 0.10 | 0.06 | 0.10 | -0.09 | 0.01 | 0.02 | 0.11 | 1* |
| **HR1W** | -0.50 | 0.23 | -0.39 | 0.16 | -0.31 | 0.11 | -0.19 | 0.10 | -0.13 | 0.10 | 0.07 | -0.02 | 0.01 | -0.03 | 1* |
| **HR1SP** | | | -0.36 | 0.06 | -0.29 | 0.10 | -0.16 | 0.04 | -0.09 | 0.00 | | -0.02 | 0.02 | 0.00 | 2 |
| **HR2** | -0.50 | 0.23 | -0.36 | 0.16 | -0.27 | 0.11 | -0.18 | 0.10 | -0.09 | 0.10 | 0.05 | 0.01 | 0.00 | 0.00 | 1* |
| **HR3** | -0.57 | 0.23 | -0.35 | 0.16 | -0.31 | 0.11 | -0.19 | 0.10 | -0.10 | 0.10 | -0.06 | -0.05 | -0.01 | -0.01 | 1* |
| **HR4** | -0.66 | 0.23 | -0.48 | 0.16 | -0.33 | 0.11 | -0.21 | 0.10 | -0.12 | 0.10 | 0.05 | 0.03 | 0.03 | 0.00 | 1* |
| **HR5** | | | -0.46 | 0.04 | -0.38 | 0.40 | -0.25 | 0.00 | -0.13 | 0.00 | | -0.03 | -0.02 | -0.02 | 2 |
| **PASS** | | | -0.28 | 0.00 | -0.25 | 0.00 | -0.14 | 0.00 | -0.06 | 0.00 | | 0.00 | 0.01 | 0.01 | 2 |
| **Standards & 2SD** | | | | | | | | | | | | | | | |
| **UM-Almaden (UMich)** | -0.87 | 0.11 | -0.59 | 0.04 | -0.47 | 0.05 | -0.29 | 0.04 | -0.17 | 0.04 | 0.01 | -0.02 | 0.01 | -0.02 | 9 |
| **UM-Almaden (RU)** | | | -0.55 | 0.04 | -0.47 | 0.06 | -0.28 | 0.02 | -0.16 | 0.04 | | -0.06 | -0.01 | -0.02 | 6 |
| **ERM CC580 (RU)** | | | -0.48 | 0.06 | -0.39 | 0.04 | -0.22 | 0.04 | -0.13 | 0.04 | | -0.03 | 0.02 | -0.01 | 5 |
| **NIST 1944 (UMich)** | -0.56 | 0.23 | -0.38 | 0.16 | -0.34 | 0.11 | -0.21 | 0.10 | -0.14 | 0.10 | 0.01 | -0.05 | -0.02 | -0.04 | 5 |

* 2SD estimates are 2SD from the NIST 1944 results.




**Supplement Fig. 2.1.** Cold vapor atomic fluorescence detector signal during MeHg Separation. The GC peak corresponding to MeHg was directed around the detector by means of a solenoid valve after the elution of the Hg (0) peak and before the elution of the Hg(II) peak.

# CHAPTER 3

# IDENTIFICATION OF MERCURY SOURCES TO ESTAURINE FISH IN THE HACKENSACK RIVER, NJ USING MERCURY STABLE ISOTOPES

## ABSTRACT

The consumption of coastal and estuarine fish is an important exposure route for mercury in humans. However, tracking the sources and transfer of inorganic and monomethylmercury (MeHg) to higher trophic level fish species in coastal marine environments remains a challenge. Industrialized estuaries with highly contaminated sediments can have distinct mercury stable isotope signatures for both total Hg and MeHg that may be preserved in resident or migrant fish. Mercury stable isotopes were used in this study to examine Hg sources from sediment and in two important fish species in the Hackensack River estuary, white perch (*Morone americana*) and killifish (*Fundulus heteroclitus*). The Hg mass dependent stable isotope composition, measured as $\delta^{202}$Hg, increased from a value of -0.4 ‰ for total Hg in mudflat sediments to +0.08 ‰ in killifish and +0.21 ‰ in white perch. The observed increasing trend in $\delta^{202}$Hg and the presence of Hg mass independent isotope anomalies ($\Delta^{199}$Hg =0.10-0.40 ‰) in fish tissue indicate the loss of lighter Hg isotopes as a result of photochemical

reduction of Hg (II) to Hg(0) or demethylation of MeHg to Hg(II) with subsequent loss of Hg(0) through volatilization. The isotopic relationship between $\delta^{202}$Hg and $\Delta^{199}$Hg associated with these photochemical losses is well studied and was used to estimate the Hg isotope composition acquired prior to photochemical transformations, most likely representing the food source. Estimated values were then compared to measured isotopic compositions of MeHg and THg in sediments it was determined that benthic killifish had similar isotopic composition of MeHg to that of sediments whereas perch samples deviated from sediment values from the capture locations. Mercury stable isotopes can be a useful tool in future studies of MeHg production and bioaccumulation in the environment, especially in complex systems with multiple sources of mercury.

## 1. INTRODUCTION

Estuaries and river ecosystems play an important role in the aquatic fate of mercury (Hg) by acting as a direct link between terrestrial sources and the marine environment (Kim et al., 2004). Elevated sedimentation rates in estuarine systems cause a high retention of Hg from upstream sources, atmospheric deposition, and industrial point sources (Benoit et al., 1998; Mason and Lawrence, 1999). The large inorganic Hg pools in these systems can lead to the process of methylation by a variety of iron-reducing, sulfate-reducing, and methanogenic microorganisms resulting in the production of the neurotoxic methylmercury (MeHg) form (Compeau and Bartha, 1985; Hamelin et al., 2011). External sources of MeHg, such as freshwater wetlands and terrestrial soils can also increase MeHg loads to estuarine and coastal areas. Regardless of its source, once in the marine environment, MeHg can bioaccumulate in estuarine and coastal fish, eventually reaching highly elevated levels greater than 1 $\mu g\ g^{-1}$ MeHg (Heyes et al., 2004).

The production of MeHg and its subsequent bioaccumulation in aquatic food webs is the subject of extensive research, but tracking the source and route of Hg to accumulation into fish tissue remains a challenge. MeHg concentrations in surface waters and sediments do not always correlate with those found in the local biota (Driscoll et al., 2007; Wang and Wong, 2003), making it difficult to identify sources of Hg to food webs. Recent work has demonstrated the potential

use of Hg stable isotopes for source identification of Hg in aquatic biota. Studies of natural fish populations have demonstrated that coastal and estuarine species can have strikingly different Hg isotopic compositions, which potentially reflect their food source, feeding locality (such as depth and range), or extent of MeHg demethylation before transfer to the food web (Blum et al., 2013; Senn et al., 2010). The Hg isotopic composition of MeHg that entered a particular aquatic food web can be estimated based on the extent of photochemically-driven, mass independent fractionation (MIF) of Hg in consumers (Kwon et al., 2014; Sherman and Blum, 2013). Recent direct measurements of the isotopic composition of MeHg in estuarine sediments are generally consistent with such estimates and provide critical information needed to connect MeHg in aquatic animals with environmental sources (Janssen et al., 2015).

It has been demonstrated that Hg isotopes do not fractionate during bioaccumulation in freshwater fish species (Kwon et al., 2012; Kwon et al., 2013) While *in vivo* Hg methylation in fish is possible, this process is considered slow and is not thought to be a major contributor to MeHg concentrations in fish tissue (Wang et al., 2013). In addition, it has been shown that MeHg in fish tissue rapidly equilibrates to the isotopic signature of consumed food (Kwon et al., 2013), so migratory species can be accounted if they are feeding in an area for an extended amount of time. These findings allow for the direct comparison of MeHg in tissue and sediment, which can also give further insight to where the organisms obtained the MeHg. When drawing conclusions from fish tissue isotopic composition, factors such as poor diet or health must be accounted for

since they have been shown to cause the incomplete assimilation of MeHg from food to fish tissue under laboratory conditions (Kwon et al., 2013). Previous surveys have examined Hg stable isotopes in a multitude of different fish species, but little work has addressed small-scale spatial variation within an estuarine system (Gehrke et al., 2011), and none have compared the estimates of MeHg in tissue to direct sediment values.

In this study we examined the concentrations and isotopic composition of Hg species in sediment and two species of fish, killifish and white perch from a contaminated, urban estuary in northeastern New Jersey, USA. In addition, we examined the Hg isotopic composition of sediment and killifish from an uncontaminated estuary in southeastern New Jersey and in black sea bass from coastal waters of the inner Middle Atlantic Bight. This study focused on variations of Hg concentrations and isotopic compositions in fish across trophic levels and feeding habitats.

## 2. METHODS

### 2.1 Sample collection and dissection

All samples were collected under a scientific collecting permit issued by the New Jersey Department of Environmental Protection. Adult and juvenile white perch (*Morone americana*) were collected by cast net from six sites along 16 km of the highly urbanized Hackensack River estuary in northeastern New Jersey, USA (Fig. 3.1) between August and October, 2013. Sites HR1-HR3 are designated as upstream locations, BC, HR3a, and HR4 are within the influence

of the Hg-contaminated Berry's Creek Study area, and sites HR5 and HR6 are downstream sites. Only juvenile white perch were caught at the most downstream site, HR6. Killifish (*Fundulus heteroclitus*) were collected from one site in the Hackensack River estuary (HR4) and at one site within Berry's Creek (TG) using un-baited steal minnow traps in August, 2013 and July, 2014. Killifish were also collected from tide pools at a reference site along the northern border of the Great Bay estuary near the Rutgers Marine Field Station in Tuckerton, New Jersey. For comparison with Hackensack River white perch, black sea bass (*Centropristis striata)* were collected by rod and reel from the inner Middle Atlantic Bight 1 km east of the Great Bay site.

All fish were euthanized according to guidelines set by the American Veterinary Medical Association (2013). Perch and Black Sea Bass were euthanized using 500 mg/L tricane methanesulfonate in the field, individually bagged and transported to the laboratory on ice. Killifish were collected and transported live in a 5 gallon polyethylene bucket filled with estuarine water. Individual killifish were then euthanized in the laboratory by a 15 minute immersion in tricane methanesulfonate.

Whole fish were rinsed with deionized water and placed in acid cleaned glass trays. Dissections were performed in a class 100 laminar flow hood with stainless steel tools, which were first cleaned with 70 % ethanol and then with 1 % hydrochloric acid before each dissection. After scale removal, muscle tissue was collected using a 7 mm diameter dermal punch from large fish and as fillets from fish less than 15 cm in length. Tissue samples were transferred to acid

cleaned polypropylene tubes and freeze-dried for 12-24 hours (Freezone 7, Labconco). Dried samples were ground and homogenized with a Teflon mortar and pestle.

Sediment was collected at each site using a pole mounted Ekman grab sampler. Sediment samples were transferred to acid cleaned mason jars using a stainless steel scoop and transported to the laboratory on ice. After each use, the sediment grab was rinsed with ambient water and no significant carry-over was observed (<0.5 ng). Within 24 hours of collection, sediment samples were homogenized using a Teflon spatula and 10-20 g was immediately freeze-dried for further analysis.

Surface water samples were collected in acid cleaned Teflon bottles, double-bagged, and stored on ice during transport to the laboratory. Suspended particles from the Hackensack River were collected by filtering 300-800 mL of site water using an acid cleaned polypropylene funnel and 0.45 µm acid-cleaned filters (Supor 47 mm, Pall Corporation) in the laboratory. Filters were transferred to Teflon vials and stored frozen until analysis.

**2.2 Mercury analysis**

Methylmercury in fish tissue was leached from 10-20 mg subsamples of dried muscle using 4 N nitric acid (Trace Metal Grade, Fisher) (Hintelmann and Nguyen, 2005). Analysis was performed by cold vapor atomic fluorescence spectroscopy (CVAFS) following isothermal gas chromatographic separation of ethylated derivates according to Liang et al. (1994) using a Brooks Rand MERX-

M analyzer. Digestion efficiency was tested using the TORT-2 standard reference material (lobster hepatopancreas, National Research Council of Canada). The average measured concentration of methylmercury in TORT-2 ($0.144 \pm 0.014 \mu g\ g^{-1}$, n=6) was very similar to the certified value ($0.152 \pm 0.013\ \mu g\ g^{-1}$) indicating recoveries of approximately 95%. All procedural and reagent blanks were below the detection limit of 5 pg of MeHg.

Prior to analysis of total mercury, fish tissue and suspended particle samples were subjected to heat assisted acid leaching in an ultrasonic bath. Subsamples of dried fish muscle (100-200 mg) and filters were transferred to 30 mL Teflon screw cap containers and dissolved with a 4:1 hydrochloric and nitric acid mixture (Trace Metal Grade Fisher Scientific). Dissolved samples were then sonicated for 24 hours at 60 °C to break down organic matter. Sample digests were diluted up to 20 mL with ultra-pure water and preserved with a sufficient volume (at least 100 µL) of 0.2 N bromine monochloride to achieve a pale yellow color. Total Hg concentrations of fish and sediment digests were analyzed by CVAFS using a Brooks Rand MERX-T analyzer. The average recovery of total Hg for TORT-2 was $0.32 \pm 0.01\ \mu g\ g^{-1}$ (n=6) which is within the certified range ($0.27 \pm 0.06\ \mu g\ g^{-1}$). Procedural blanks where below 6 pg.

Sediment samples for total mercury analysis were digested in aqua regia at room temperature. Aliquots of dried sediment (0.5 g) were weighed into glass digestion vessels and leached with a 4:1 mixture (by volume) of hydrochloric acid and nitric acid (Fisher, Trace Metal Grade). Selective extractions were performed

on sediment from each sampling locations using the method detailed in Bloom et al. (2003). Samples were analyzed by CVAFS as described above. Recoveries of total Hg from the CC580 standard reference material (estuarine sediment, European Reference Materials) yielded 130.5 ± 4.7 $\mu g\ g^{-1}$ (n=4, certified value = 132 ± 3 $\mu g\ g^{-1}$). Sediment methylmercury analysis was performed using distillation (Horvat et al., 1993) followed by aqueous phase ethylation coupled to isothermal GC and CVAFS (Tekran 2500). Relative percent difference for duplicate MeHg samples was ≤ 12% and concentration spike recovery averaged 98 ± 14% (n = 7).

**2.3 Mercury isotope analysis**

Mercury isotope measurements were performed at Rutgers University using a ThermoScientific Neptune Plus, multi-collector inductively coupled plasma mass spectrometer (MC-ICP-MS). Sample introduction and instrument settings are shown in Table C1 (Appendix C). Faraday cup detectors were set to measure $^{198}Hg$, $^{199}Hg$, $^{200}Hg$, $^{201}Hg$, $^{202}Hg$, $^{203}Tl$, and $^{205}Tl$; isotope abundances of $^{196}Hg$ and $^{204}Hg$ were not quantified.

Online sample reduction was performed using a Cetac HGX-200 hydride generation system coupled to a Cetac Aridus II desolvating nebulizer. Mercury in solution was reduced with 3% (w/w) tin chloride using the HGX-200 gas-liquid separator prior to introduction into the mass spectrometer. A 20 $ng\ g^{-1}$ solution of thallium chloride was used for mass bias correction and was introduced into the sample line of the MC-ICP-MS using the Aridus II. Elemental mercury was carried from the gas-liquid separator to the MC-ICP-MS with high purity argon to

which trace amounts (5 mL $min^{-1}$) of ultra-high purity nitrogen gas was added to improve signal stability. For each sample, 100 ratios (2 blocks of 50 ratios) were collected and outliers greater than 10% of the average were removed using the Neptune software.

All sample extracts and standard solutions for isotope analysis were diluted to a total Hg concentration of 5 ng Hg $g^{-1}$ with ultra-pure water and were run in duplicate. Duplicates were not performed on all black sea bass samples due to limited sample mass and low mercury concentrations in the tissue. Bracketing standards of NIST 3133 were matrix- and concentration-matched to the samples. Before introduction to the gas liquid separator, samples and standards were neutralized with 15% (w/w) hydroxylamine hydrochloride. Accuracy was determined using a monomethylmercury inter-calibration sample analyzed at the University of Michigan and the UM Almáden standard. Analysis of the monomethylmercury standard at Rutgers gave comparable results ( $\delta^{202}$Hg of -1.02 ± 0.04 ‰, ; $\Delta^{199}$Hg = 0.11 ± 0.05 ‰, n=10) to measurements made at the University of Michigan ($\delta^{202}$Hg = -1.06 ± 0.05 ‰; $\Delta^{199}$Hg = 0.08 ± 0.03 ‰ n = 3). Measurements of the UM Almáden standard ($\delta^{202}$Hg of -0.55 ± 0.04 ‰, n=6) also agreed well with reported measurements ($\delta^{202}$Hg of -0.54 ± 0.08 ‰) (Blum and Bergquist, 2007). External reproducibility of the measurements was determined by the measurement of the ERM CC580 (estuarine sediment) in every analysis batch ($\delta^{202}$Hg value of -0.49 ± 0.07‰, $\Delta^{199}$Hg value of -0.02 ± 0.03, n = 10).

Methylmercury in sediment for mercury isotope analysis was separated using a modified GC separation and trapping method that was previously described in detail (Janssen et al. 2015).

### 2.4 Carbon and nitrogen isotope analysis

Measurements of $\delta^{13}C$ and $\delta^{15}N$ in fish tissue, sediments, and suspended particles were performed using a Eurovector elemental analyzer attached to a GV Instruments IsoPrime isotope ratio mass spectrometer. An in-house standard mix was calibrated against NBS-22 and was used to adjust the stable isotope values to V-PBD using the certified value of $\delta^{13}C$ = -30.03 ‰. Nitrogen was calibrated against IAEA N1 (ammonium sulfate) and adjusted to the isotopic composition of $N_2$ in air using the certified value of $\delta^{15}N$ = 0.4 ‰. Replicate samples for carbon and nitrogen isotope ratios varied by < 0.2 ‰.

## 3. RESULTS

### 3.1 Mercury Concentration and Speciation

The Hackensack River and its tributary Berry's Creek have been subject to legacy contamination dating back to at least 1974 as a result of Hg processing activities. Within the vicinity of the original point source adjacent to Berry's Creek, concentrations of Hg in sediments were previously recorded as high as 900 $\mu g\ g^{-1}$ (Cardona-Marek et al., 2007). In addition, there are currently 50 to 60 active industrial discharges, 3 coal fired utility boilers, 7 sewage treatment plants, and 32 combined sewer outflows within the Meadowlands district surrounding the

Hackensack River (Kiviat and MacDonald, 2002). We found sediments throughout the entire tidal portion of the Hackensack River to be highly contaminated with average total Hg concentrations of 3 to 4 $\mu g\ g^{-1}$ (Fig 3.2 & ) compared to background concentrations in the fresh water portion of the estuary above the Oradell dam (0.12 ± 0.02 $\mu g\ g^{-1}$, n=3). Total Hg concentrations in sediment were somewhat variable seasonally and showed a slightly decreasing trend downstream from stations HR2 to HR5. Sediments at the confluence of Berry's Creek and the Hackensack River (station HR3a) had a higher average total Hg concentration ($p < 0.05$) than sediments just upstream (HR3) or downstream (HR4). Based on selective extractions methods (Bloom et al., 2003), 80% to 90% of sedimentary Hg was strongly bound to sulfide (Supplement Fig. 3.1).

Despite small spatial variations in total Hg, MeHg concentrations in the Hackensack River varied with distance upstream from Newark Bay (Fig. 3.2). In the spring and summer, MeHg concentrations increased upstream with maximum values found 18 km above Newark Bay (station HR1). In the fall and winter however, maximum MeHg concentrations were found in the middle of the estuary 10 to 13 km above Newark Bay (stations H3, H3a, and H4).

Elevated concentrations of THg and MeHg were also measured in Hackensack River fish. White perch collected from the Hackensack River were grouped by developmental age (adult or juvenile) and capture location (upstream or downstream). Killifish and black sea bass were identified by capture location only. Total Hg was found to be the highest in adult perch from the Hackensack

(0.56-0.59 μg $g^{-1}$, n=18) and Berry's Creek killifish (0.29 μg $g^{-1,}$ n=3). Juvenile perch and killifish displayed similar total Hg concentrations, 0.13 and 0.19 μg $g^{-1}$ respectively, but are still elevated in comparison to Great Bay killifish (0.06 μg $g^{-1}$) and bass (0.09 μg $g^{-1}$). MeHg concentrations in killifish were 0.09 μg $g^{-1}$ for the Hackensack River and 0.21 μg $g^{-1}$ for Berry's Creek, respectively, which are 1.5 and 5 times higher than MeHg in killifish from the Great Bay reference site (Table 3.1). Hackensack perch are also shown to be 5 times higher than the Eastern United States average Hg concentration in perch species (0.14 μg $g^{-1}$) (Karimi et al., 2012). While no concentration difference for MeHg or THg were observed between upstream and downstream perch, adult fish had 5 times higher MeHg concentrations (0.35 -0.36 μg $g^{-1}$) than juveniles captured at the same location (0.07 -0.08 μg $g^{-1}$). Concentrations of MeHg in adult perch were higher than those in adult black sea bass from the inner Mid-Atlantic Bight, whereas the juveniles showed similarities in both THg and MeHg to bass.

No significant differences in percent total Hg as MeHg (%MeHg) were observed among juvenile and adult perch from upstream or downstream collection sites. The highest %MeHg (76%, n=3) was observed in killifish from Berry's Creek which was higher than that (50%, n=12) in killifish caught in the main body of the Hackensack River near the confluence with Berry's Creek. Methylmercury ranged from 60-70% in adult and juvenile perch with no significant difference between the ages ($p>0.05$, unpaired *t*-test).

### 3.2 Carbon and Nitrogen Isotopes

Perch species have been shown to switch between pelagic and benthic prey depending on the environment and age (Pothoven and Hook, 2015; Weis, 2005); whereas killifish are known to be predominant benthic feeders (Samaritan and Schmidt; Weis et al., 2011; Weis et al., 2003). Nitrogen isotope ratios ($\delta^{15}N$) for downstream perch were on average 4-5‰ higher in comparison to upstream individuals as seen in Fig.3.3. Differences in $\delta^{15}N$ values between upstream and downstream perch were not reflected in the concentrations of total Hg or MeHg or % MeHg, which usually increase with trophic level (Gilmour et al., 1998; Hammerschmidt and Fitzgerald, 2006; Watras et al., 1998; Watras and Bloom, 1992). Hackensack killifish which were captured at HR3 show a similar trend in $\delta^{15}N$ to perch samples (Table 3.1). This indicates that there was little trophic difference between the two species in this estuary, despite upstream and downstream designations. Trophic variations may result from difference in the $\delta^{15}N$ baseline for different portions of the river. In addition, suspended particulate matter in the Hackensack River was more enriched in $^{15}N$ in comparison to sediment, indicating that $^{15}N$-enriched wastewater effluent may affect baseline $\delta^{15}N$ levels in the estuary.

No significant difference ($p>0.05$, unpaired *t*-test) is observed in carbon isotopes ($\delta^{13}C$ ) between species or capture locations in the Hackensack. Most of the individuals plotted in Fig. 3.3 show similar $\delta^{13}C$ compositions to that of sediment, specifically the upstream perch and HRK samples. In the Hackensack

River estuary, $\delta^{13}C$ values for suspended particulate matter were 4‰ to 5‰ lower than those of sediment.

### 3.3 Mercury Stable Isotopes: Sediment

Mercury stable isotopes were used to examine the sediment pool and assign possible Hg sources to the Hackensack estuary (Table 3.2). Sediments upstream of the confluence with Berry's Creek show a very narrow range of $\delta^{202}Hg$ values (-0.35‰ ± 0.06‰). Lower $\delta^{202}Hg$ values (-0.45 ± 0.13‰) were observed in downstream sites closer to Newark Bay and are significantly different than upstream values ($p<0.05$). Mercury in sediment from the Berry's Creek site (BC-TG), which is near a known mercury source to the Hackensack River estuary, had a slightly higher $\delta^{202}Hg$ value (-0.30 ± 0.05‰), than in the rest of the system. While these spatial variations indicate that Hg from Berry's Creek is diluted with $^{202}Hg$-depleted Hg from downstream, a simple mixing pattern is not apparent in a $^{202}Hg$-inverse concentration plot, but does indicate that there are two isotopic end members for the system (Supplement Figure 3.2).

Suspended particulate matter had a higher average $\delta^{202}Hg$ value (-0.28 ± 0.14‰) than sediment throughout the Hackensack River estuary (-0.35 to -0.45‰). This indicates that Hg in suspended particulate matter in the estuary may be sourced primarily from Berry's Creek than other sources. Sediment from the Great Bay estuary had a $\delta^{202}Hg$ value of -0.23 ± 0.07. No significant mass independent fractionation was observed in any sediment THg or particulate matter, which indicates a strong anthropogenic signal ($\Delta^{199}Hg$, $\Delta^{201}Hg < 0.1$‰).

Methylmercury isotope compositions for the Hackensack were examined previously and showed a more dynamic range than THg samples ranging from $\delta^{202}$Hg =0.4 to -0.4‰ (Janssen et al., 2015). Samples for MeHg from Berry's Creek Tide Gate displayed an isotopic composition of $\delta^{202}$Hg = -0.19 ± 0.11‰ (n=2) with no significant MIF.

### 3.4 Mercury Stable Isotopes: Fish Tissue

Perch specimens did not show significant variations in $^{202}$Hg stable isotopic composition between upstream and downstream collections ($p>0.05$) as observed in $\delta^{15}$N measurements (Table 3.2). The ranges of juvenile perch ($\delta^{202}$Hg = 0.35- 0.42 ‰) were higher in $\delta^{202}$Hg than adults ($\delta^{202}$Hg =0.19- 0.24‰) in the Hackensack, but a higher standard deviation was observed in juvenile specimens. Killifish collected from the HR site displayed an average $\delta^{202}$Hg value of 0.08 ± 0.12‰ which was lower than that for adult and juvenile perch, but higher than that for killifish from Berry's Creek. Berry's Creek killifish were more depleted in $^{202}$Hg in comparison to the HR killifish or perch, despite having a similar $\delta^{15}$N (Table 3.2 and Fig. 3.6).

### 3.5 Fish Tissue Mass Independent Fractionation (MIF)

All fish collected from the Hackensack and Berry's Creek estuaries have Hg that is enriched in $\Delta^{199}$Hg and $\Delta^{201}$Hg indicating mass-independent fractionation of Hg stable isotopes (Table 3.2). The relatively small extents of MIF in these fish likely reflect low levels of photochemical Hg transformations in this highly turbid estuary. While $\Delta^{199}$Hg values in perch and killifish varied from

0.17 to 0.32‰, they do not vary systematically with species, capture location, or, in the case of the white perch, developmental stage. Great Bay killifish show similar extents of MIF to killifish and perch collected in the Hackensack River, but black sea bass had a greater extent of MIF than all of the estuarine fish. A York regression model was performed on $\Delta^{199}Hg$ vs. $\Delta^{201}Hg$, which describe the relative enrichment of $^{199}Hg$ with respect to $^{201}Hg$ during mass-independent fractionation. Samples from the Hackensack River had a slope of 1.13 ± 1.40 and Berry's Creek estuaries and 1.22 ± 0.04 for fish from the Great Bay and the coastal Middle Atlantic Bight (Fig. 3.5). There is no significant difference between the slopes of the two populations based upon the large error associated with slope from the York regression for the Hackensack fish; both slopes are indicative of the magnetic isotope effect that is commonly observed as a result of the photochemical degradation of MeHg and photochemical reduction (Bergquist and Blum, 2007). Fish tissue from the Hackensack showed that photoreduction is a prominent pathway for MIF in this system in addition to photodemethylation. In contrast, a slope of 1.21 for fish from the Great Bay and the coastal Middle Atlantic Bight is similar to that observed in oceanic fish (Senn et al., 2010) and indicates that photodemethylation of MeHg may be more important in this more pristine ecosystem.

### 3.6 Estimation of MeHg Composition in Fish Tissue & Sediments

In order to estimate the mass-dependent isotopic composition of Hg ($\delta^{202}Hg$) in fish tissue prior to photochemical transformations, the following relationship between $\Delta^{199}Hg$ and $\delta^{202}Hg$, weighted for the proportion of inorganic

and MeHg in the fish, was used to account for both photodemethylation and photoreduction in the water column.

$$\delta^{202}Hg_{FISH} = \frac{\Delta^{199}Hg_{FISH}}{\left(f_{MeHg} \times 4.79\right) + \left(\left(1 - f_{MeHg}\right) \times 1.18\right)} \quad \text{Eq.1}$$

In Eq. 1, $f_{MeHg}$ is the fraction of MeHg in the fish, and 4.79 and 1.18 are the slopes of the relationships between $\Delta^{199}$Hg and $\delta^{202}$Hg for photodemethylation and Hg(II) photoreduction, respectively, calculated from the results of Bergquist and Blum in experiments with 10 mg $L^{-1}$ DOC (Bergquist and Blum, 2007). The results for $\delta^{202}Hg_{fish}$ represent the isotopic composition of THg (inorganic Hg plus MeHg) in the fish prior to any photochemical reaction. The mass-dependent isotopic composition of MeHg was estimated using the proportion of MeHg in the fish and an isotope mass balance assuming that the isotopic composition of inorganic Hg in the fish prior to any photochemical reaction was similar to that of the sediment at each capture location according to Eq. 2.

$$\delta^{202}Hg_{FISH} = \left(f_{MeHg} \times \delta^{202}Hg_{MeHg}\right) + \left(\left(1 - f_{MeHg}\right) \times \delta^{202}Hg_{Hg}\right) \quad \text{Eq.2}$$

Estimates of the isotopic composition of MeHg in fish prior to photochemical reactions (Table 3.3) showed that MeHg in Hackensack River and Berry's Creek fish was enriched in $^{202}$Hg in comparison to the total Hg in fish tissue (Table 3.2). In contrast, fish collected from the Great Bay and coastal Middle Atlantic Bight reference sites displayed a depletion of $^{202}$Hg in MeHg relative to total Hg in these fish. Localized killifish in the Hackensack displayed similarities between the estimated $\delta^{202}$Hg in tissue and the directly measured

values of MeHg in sediment (Table 3.3 and Fig 3.4). However, fish tissue MeHg in HR and BC killifish was slightly depleted in $^{202}Hg$ (≈0.1 ‰) in comparison to the sediment at the capture location. The estimates of isotopic composition of perch species did not resemble any directly measured values of sediment MeHg or THg in the Hackensack estuary.

## 4. DISCUSSION:

The Hackensack River is a highly industrialized system with THg averaging 3.8 ± 1.2 μg $g^{-1}$ (n=49) in sediments from the estuarine portion. These values are an order of magnitude higher than other well studied coastal regions such as Long Island Sound (0.35 μg $g^{-1}$) (Hammerschmidt and Fitzgerald, 2004) and Chesapeake Bay (0.16 μg $g^{-1}$) (Hollweg et al., 2009). Studies in the Hackensack River have consistently shown that both THg and MeHg are highly elevated in comparison to other estuarine sites in the Northeastern United States (Kwon et al., 2014; Schartup et al., 2014). The isotopic signatures of sediment related to the Hackensack and Berry's Creek area do not greatly differ from other contaminated sites. The range of $\delta^{202}Hg$ in the Hackensack sediment spans from (-0.30 to -0.45 ‰) which falls within the measurements of mining-contaminated sediments in Slovenia (Foucher et al., 2009), San Francisco Bay (Gehrke et al., 2011), and urbanized watersheds in China (Liu et al., 2011) as well as others. The small range of THg isotopic compositions for most industrialized sediments makes it difficult to discern different Hg processing signatures.

Biota tissue is also highly impacted by these high sediment Hg concentrations; adult perch in this study were shown to have 5 times higher MeHg than the national average (Karimi et al., 2012). Mercury in killifish, specifically from Berry's Creek, have also been studied for over 30 years and have shown high THg and MeHg contents that correspond to this study (Chen et al., 2014; Kwon et al., 2014; Weis et al., 2003; Weis et al., 1981; Weis et al., 1986). Mercury concentrations in perch and killifish tissue, despite being elevated, show a high degree of uniformity between upstream and downstream samples in the Hackensack (Table 3.1). Fish examined in this study show similar isotopic compositions to previously studied coastal species (Kwon et al. 2014; Senn et al., 2010). Coastal fish show larger ranges in $\delta^{202}$Hg than oceanic species, but also lower extents of MIF represented as $\Delta^{199}$Hg (Fig. 3.6). This variation in isotopic composition allows for the differentiation of Hackensack perch and killifish from previously studied oceanic and coastal species. However, due to the limited studies of estuarine fish it remains unknown whether the enrichment of $\delta^{202}$Hg and low $\Delta^{199}$Hg values are constant in other estuarine and coastal systems.

Mercury concentrations and bulk Hg isotopic compositions in both fish tissue and sediment measured from the Hackensack cannot definitively establish the feeding localities of killifish and perch in the system. Estimations of $\delta^{202}$Hg of MeHg in fish tissue were performed and compared to previously studied sediment MeHg values from the Hackensack (Janssen et al. 2015), which have been shown to have a more dynamic range than THg in sediments. Previous

work has calculated the isotopic composition of Hg in fish tissue prior to photochemical reactions (Kwon et al., 2014; Sherman and Blum, 2013), but were not modified to incorporate the process of photoreduction and account for the % of MeHg in the fish tissue. The fish in this estuary are commonly ≤ 70% MeHg and it can be inferred that if photochemical processes impact the MeHg portion of the tissue, Hg (II) may also be effected. It was found that the incorporation of photoreduction makes the final estimate of $\delta^{202}$Hg, on average, isotopcially lighter by 0.01 to 0.08 ‰ than just correcting for just photodemethylation (Table 3.3). After these corrections it was observed that the estimate of isotopic composition for MeHg in killifish tissue strongly resembles the directly measured MeHg values in sediment; whereas the estimates for perch did not display this trend.

Analysis and estimates of isotopic compositions of MeHg in sediments and killifish in the Hackensack support the conclusion that the Hg sources of these fish are obtained from their local environment and that the species stay localized into specific areas of the estuary. Killifish are known to feed on primarily detritus and benthic derived food sources and have been show to ingest sediment (Weis et al., 1981; Weis et al., 1986). The conclusion can be made that the sediment is an important source of Hg to these species as shown in previous studies (Kwon et al. 2014; Chen et al. 2014). Despite the similarities between sediment and tissue isotopic compositions of MeHg; fish tissue was still isotopically lighter in $^{202}$Hg by approximately 0.1 ‰. A plausible explanation for this anomaly in both HR and BC killifish may be due to the fact the top most layer

of sediment, where benthic organisms are feeding, is also isotopically lighter than the bulk measurements of sediment MeHg sampled at a 10 cm depth (Janssen et al. 2015). Spatial segregation of lighter MeHg to the top most layer of sediment may be spurred by diffusion of the MeHg or bioturbation in the sediment. Further investigation of the isotopic compostiion of MeHg at different sediment depths must performed to further examine this phenomenom.

The top most layer of sediment may also be influenced by photochemical effects specifically near the exposed banks of the river where killifish were captured. No significant MIF was observed in the bulk sediment sampled, but the upper 1-2 cm of the sediment that are exposed to sunlight at tidal changes may be subject to these photochemical processes. The subsequent MIF signature may also be diluted when sampling both the top and deeper layers of sediments for MeHg. Hackensack and Berry's creek killifish both displayed lower $\Delta^{199}$Hg values than previously studied organsms (Fig. 3.6) which indicate that their photochemical exposure is lower than other species, which is expected from benthic feeders. Additionally, the greater extent of MIF in the Hackensack killifish ($\Delta^{199}$Hg = 0.32) in comparison to the BC killifish ($\Delta^{199}$Hg = 0.18) can be attributed to the fact that the Hackensack specimens are in the tidal portion of the estuary whereas the BC samples were captured near a tide gate. This may indicate that the HR killifish food source and sediment has higher photochemical exposure due to the tidal nature of the estuary, whereas the area above the tide gate does not have this effect. However, other factors such as the consumption

of phytoplankton and cyanobacteria, which may undergo or be exposed to MIF, cannot be excluded.

Estimates for migratory white perch indicate that the $\delta^{202}$Hg of MeHg in these fish (0.45 to 0.83‰) was higher than that for MeHg in sediments throughout the Hackensack River (-0.4 to +0.4‰). Based on this result, there are a few options to explain the variation observed in the perch tissue. Perch are a migratory species in the Hackensack and the isotopic composition of their tissue may not fully reflect that of their current food source. Previous studies examining Hg transfer from food sources to fish have shown that dietary rates greatly outweigh any small scale fractionation in the tissue, but there is an extended period of time needed to fully assimilate the isotopic composition of the new food source (Kwon et al., 2012; Kwon et al., 2013; Wang et al., 2013). It is possible that the perch species have not fully shifted over to an isotopic composition resembling their Hackensack food sources and still show some remnant signature of their previous habitat. This complicates further studies examining food sources for migratory species and needs to be accounted for in future studies.

Additionally, perch feed on both pelagic and benthic sources throughout the entire river, unlike previously examined killifish. The fact that the perch are more diverse in their feeding habits indicates that they could be consuming an isotopically heavier pool of MeHg near or directly related to the sediment. It is known that the dark fractionation process of microbial demethylation will make the remaining MeHg isotopically heavier (Kritee et al., 2009). A food source that

has higher demethylation rates may exhibit this isotopic enrichment of $^{202}$Hg in MeHg pool. Recent studies have shown that the process of demethylation can occur in growing rice plants (Xu et al., 2015), which may be relatable to the plants, such as phragmites and spartina, in this system.

In summary, the source of MeHg in estuarine fish can be identified using the Hg speciation and isotopic compositions of fish tissue. Migratory species, such as perch, make source apportionment using Hg isotopes more difficult due to their wide range of feeding habitats and possible re-equilbration of Hg isotopes in tissue as they enter and subsequently leave feeding locations. Regardless, our estimations of the isotopic composition of MeHg in Hackensack River-Berry's Creek perch are enough to conclude that Hackensack sediment is not their main source of MeHg, as it appears to be for killifish. The utilization of Hg stable isotopes to examine and estimate pools of MeHg in the environment will continue to be a critical factor in identifying and monitoring Hg sources and bioaccumulation in aquatic food webs.

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**Table 3.1:** Mercury concentrations and carbon and nitrogen isotope ratios in fish from the Hackensack River and Great Bay, NJ. Sample include killifish from Berry's Creek (BC), the Hackensack River (HR), and Great Bay (GB), juvenile (j) and adult (a) white perch from upstream (UHR) and downstream (DHR) sites in the Hackensack River, and black sea bass from the inner Middle Atlantic Bight (MAB).

| | MeHg ($\mu g\ g^{-1}$)* | 1SD | THg ($\mu g\ g^{-1}$)* | 1SD | %MeHg** | 1SD | $\delta^{13}C$, ‰ | 1SD | $\delta^{15}N$, ‰ | 1SD | Length (mm) | 1SD | Wet wt (g) | 1SD | n |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| BC-killifish | 0.21 | 0.06 | 0.29 | 0.13 | 75.8 | 16.3 | -27.53 | 0.72 | 14.94 | 1.19 | 74 | 8 | 10 | 4 | 3 |
| HR-killifish | 0.09 | 0.03 | 0.19 | 0.07 | 50.0 | 17.0 | -24.12 | 1.68 | 13.41 | 1.32 | 56 | 23 | 46 | 30 | 12 |
| UHR-perch(j) | 0.08 | 0.02 | 0.13 | 0.07 | 70.3 | 33.6 | -25.20 | 2.19 | 10.53 | 0.21 | 78 | 23 | 8 | 5 | 3 |
| UHR-perch(a) | 0.35 | 0.20 | 0.56 | 0.25 | 63.1 | 26.3 | -24.50 | 1.43 | 10.74 | 1.24 | 233 | 31 | 238 | 101 | 15 |
| DHR-perch(j) | 0.07 | 0.03 | 0.13 | 0.09 | 60.0 | 23.8 | -21.94 | 0.74 | 15.94 | 2.10 | 56 | 13 | 4 | 2 | 5 |
| DHR-perch(a) | 0.36 | 0.13 | 0.59 | 0.04 | 62.0 | 20.4 | -21.36 | 0.48 | 16.99 | 1.55 | 208 | 21 | 135 | 46 | 3 |
| GB-killifish | 0.04 | 0.01 | 0.06 | 0.02 | 60.3 | 25.1 | -16.70 | 0.18 | 12.12 | 0.55 | 73 | 8 | 7 | 1 | 4 |
| MAB-black sea bass | 0.04 | 0.03 | 0.09 | 0.06 | 44.6 | 26.5 | -17.80 | 0.34 | 13.84 | 0.19 | 288 | 25 | 301 | 81 | 5 |

*All concentrations are represented in wet weight

** % MeHg is the average of all individuals in a group

**Table 3.2:** Mercury Isotopic Compositions in Fish Tissue and Sediment from the Hackensack River estuary and Great Bay estuary, NJ, USA. Samples are as described in Table 1.

**Biota THg Isotopes**

| | $\delta^{202}$Hg | 2SD | $\Delta^{199}$Hg | 2SD | $\Delta^{201}$Hg | 2SD | n |
|---|---|---|---|---|---|---|---|
| BC-killifish | -0.24 | 0.24 | 0.18 | 0.02 | 0.11 | 0.04 | 3 |
| HR-killifish | 0.08 | 0.12 | 0.32 | 0.12 | 0.24 | 0.10 | 12 |
| UHR-perch(j) | 0.35 | 0.16 | 0.17 | 0.12 | 0.10 | 0.14 | 3 |
| UHR-perch(a) | 0.24 | 0.16 | 0.24 | 0.10 | 0.17 | 0.08 | 17 |
| DHR-perch(j) | 0.42 | 0.17 | 0.26 | 0.11 | 0.16 | 0.14 | 3 |
| DHR-perch(a) | 0.19 | 0.02 | 0.21 | 0.02 | 0.15 | 0.04 | 3 |
| GB-killifish | -0.25 | 0.24 | 0.33 | 0.12 | 0.17 | 0.04 | 3 |
| MAB-black sea bass | -0.69 | 0.64 | 0.65 | 0.08 | 0.46 | 0.10 | 5 |

**Sediment THg Isotopes**

| | $\delta^{202}$Hg | 2SD | $\Delta^{199}$Hg | 2SD | $\Delta^{201}$Hg | 2SD | n |
|---|---|---|---|---|---|---|---|
| BC-TG | -0.30 | 0.05 | 0.00 | 0.03 | -0.02 | 0.04 | 2 |
| HR Upstream | -0.36 | 0.12 | 0.00 | 0.03 | -0.03 | 0.06 | 24 |
| HRa (BC-Con) | -0.35 | 0.13 | 0.00 | 0.05 | -0.02 | 0.02 | 8 |
| HR Downstream | -0.45 | 0.13 | 0.00 | 0.05 | -0.02 | 0.05 | 17 |
| HR SPM | -0.28 | 0.14 | 0.01 | 0.06 | -0.01 | 0.06 | 6 |
| Tuckerton (TB)* | -0.23 | 0.07 | 0.06 | 0.06 | 0.00 | 0.06 | 1 |

* 2SD estimates are based on measurements of ERM CC580

**Table 3.3:** Estimations of MeHg Isotopic Composition in Fish Tissue and Comparison to $\delta^{202}$Hg of THg and MeHg in Sediment at Capture Location

| | **$\delta^{202}$Hg of MeHg in Tissue, ‰** Photodemeth+Photo-reduction | **$\delta^{202}$Hg of MeHg in Tissue, ‰** Photodemeth only | **$\delta^{202}$Hg of THg in Sediments, ‰ *** | **$\delta^{202}$Hg of MeHg in Sediments, ‰** |
|---|---|---|---|---|
| DHR-perch (a) | 0.48 | 0.51 | -0.45 | 0.08-0.41 |
| DHR-perch (j) | 0.52 | 0.56 | -0.45 | 0.08-0.41 |
| UHR-perch (a) | 0.48 | 0.51 | -0.36 | -0.41-0.27 |
| UHR-perch (j) | 0.59 | 0.60 | -0.36 | -0.41-0.27 |
| BC-killifish | -0.28 | -0.27 | -0.3 | -0.19 |
| HR-killifish | 0.30 | 0.37 | -0.35 | 0.40 |
| GB-killifish | -0.39 | -0.34 | -0.28 | ND |
| MAB-black sea bass | -1.71 | -1.49 | -0.28 | ND |

*Values from Janssen et al. 2015 (see chapter 2)

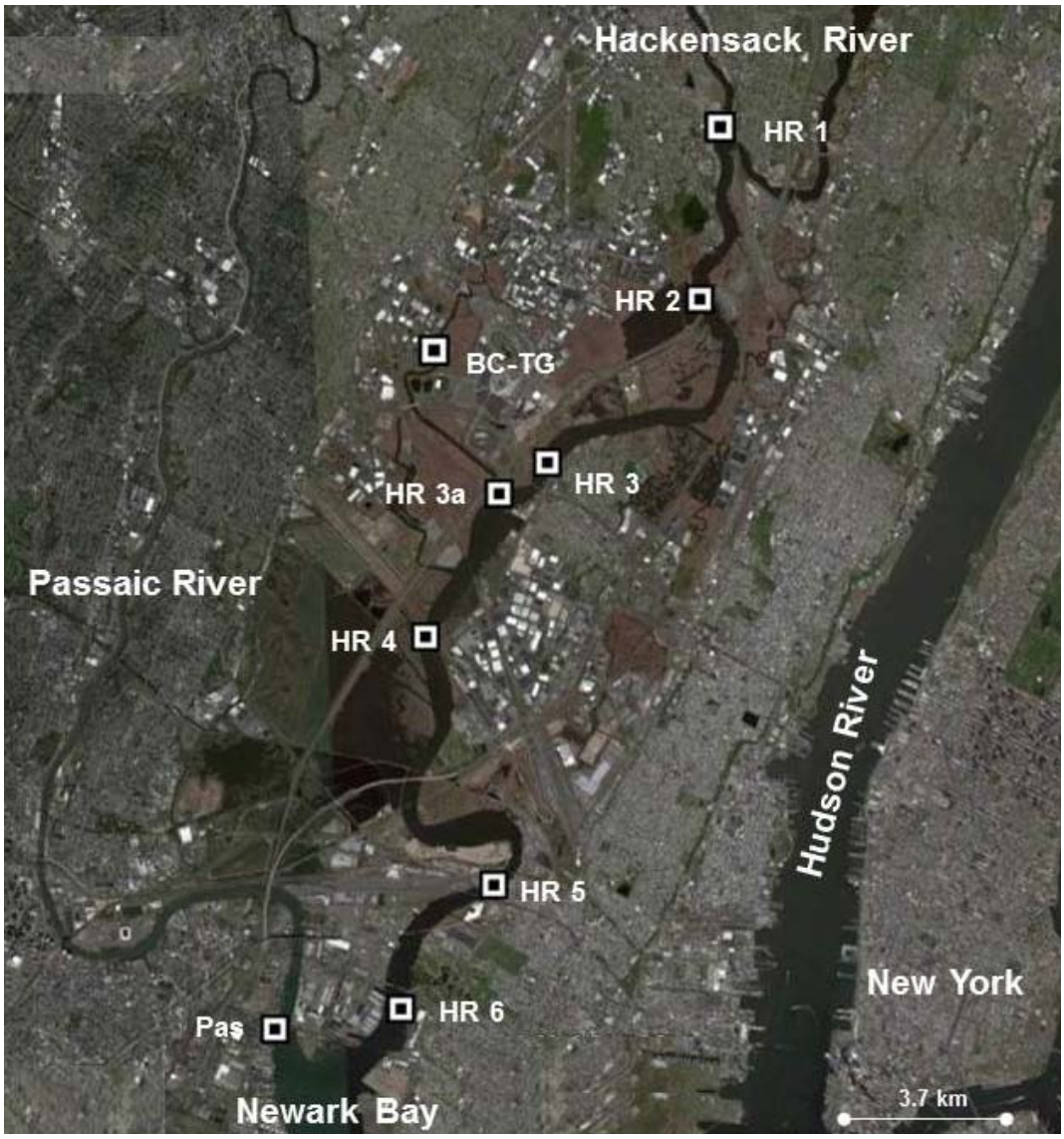



**Fig. 3.1:** Site map of sediment and fish collection sites on the Hackensack River, NJ. Sediment samples were collected from all sites. Perch were collected from sites HR1-HR5; killifish were captured at the Berry's Creek Tide Gate (BC-TG) and the shoreline between HR3 and HR4.

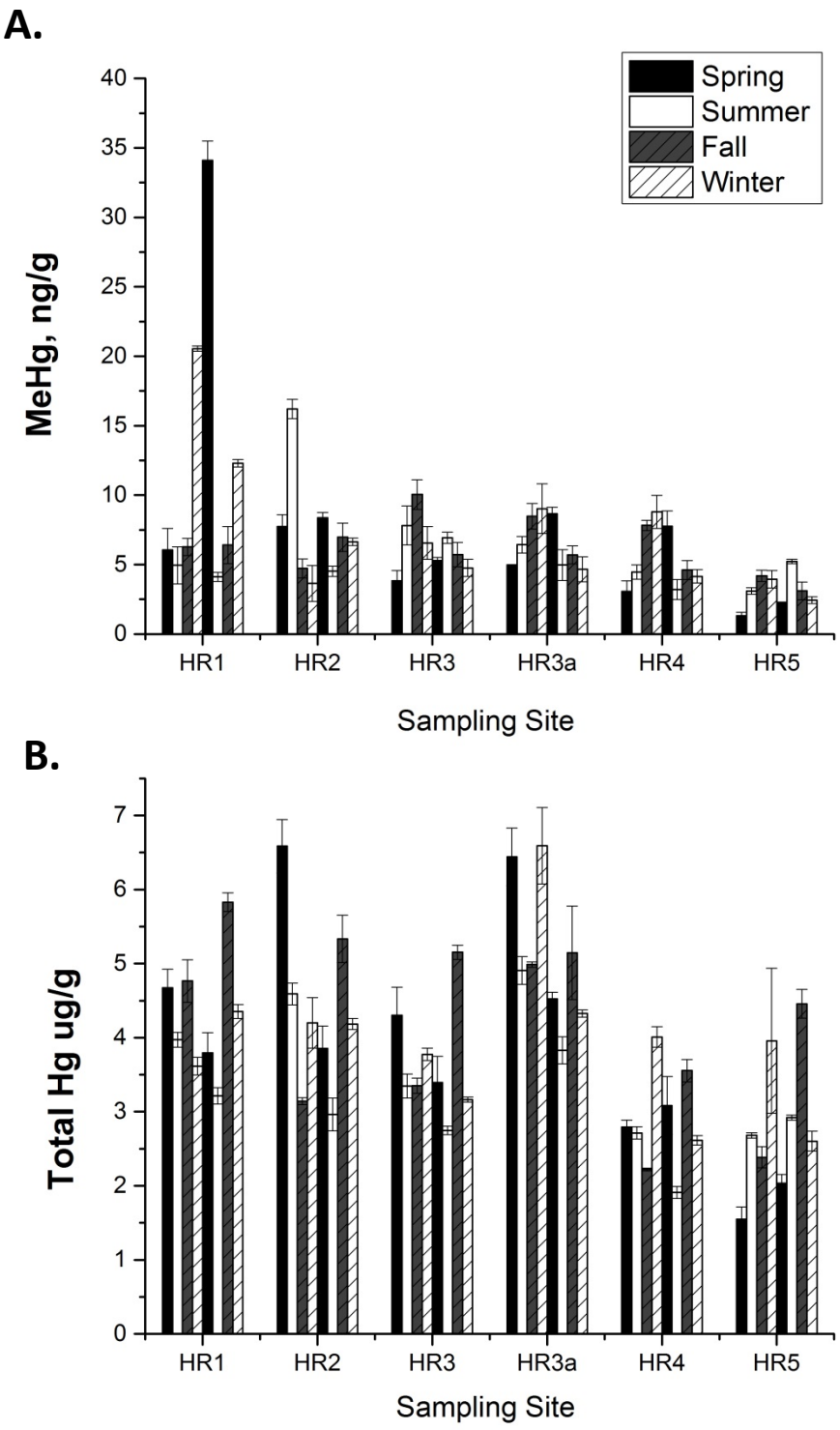



**Fig. 3.2:** a) Seasonal MeHg and b) total Hg concentrations in sediments from the Hackensack River from 2012-2014. Error bars represent uncertainty between field duplicates.

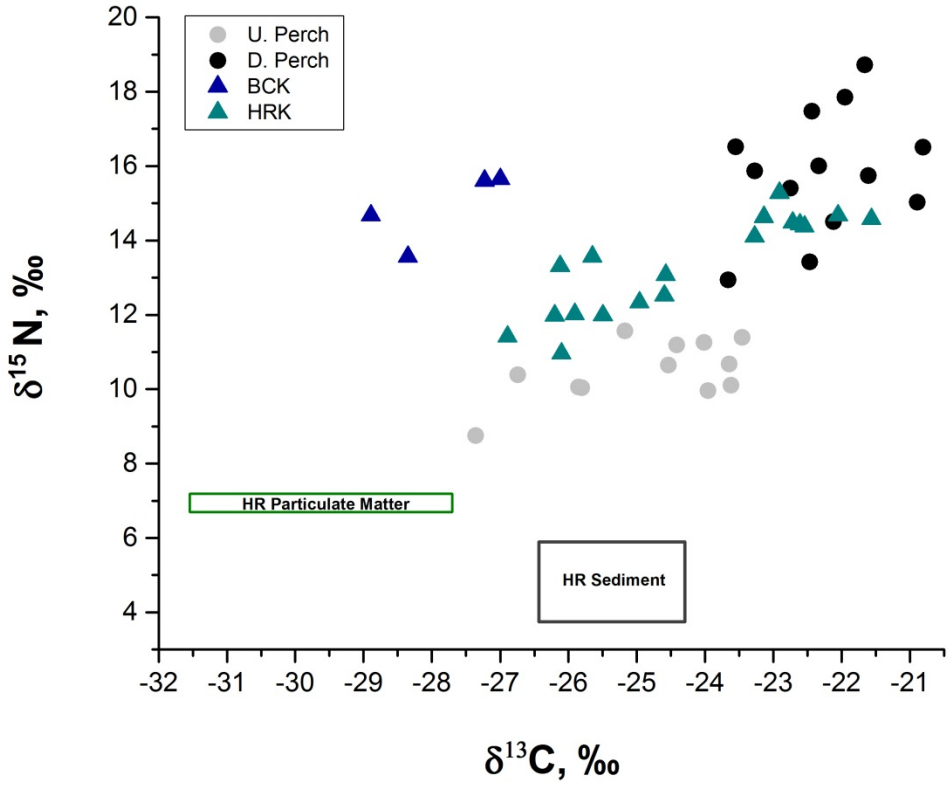



**Fig. 3.3:** Distributions of $\delta^{13}C$ and $\delta^{15}N$ values for fish, sediment, and suspended organic matter from the Hackensack River. No differentiation is observed between $\delta^{15}N$ for killifish and perch. Trophic differences are observed within perch species based upon feeding locality with downstream perch displaying a more enriched signature in comparison to upstream individuals. Values for $\delta^{13}C$ in the sediment indicate that it is the most plausible base for the estuarine fish studied

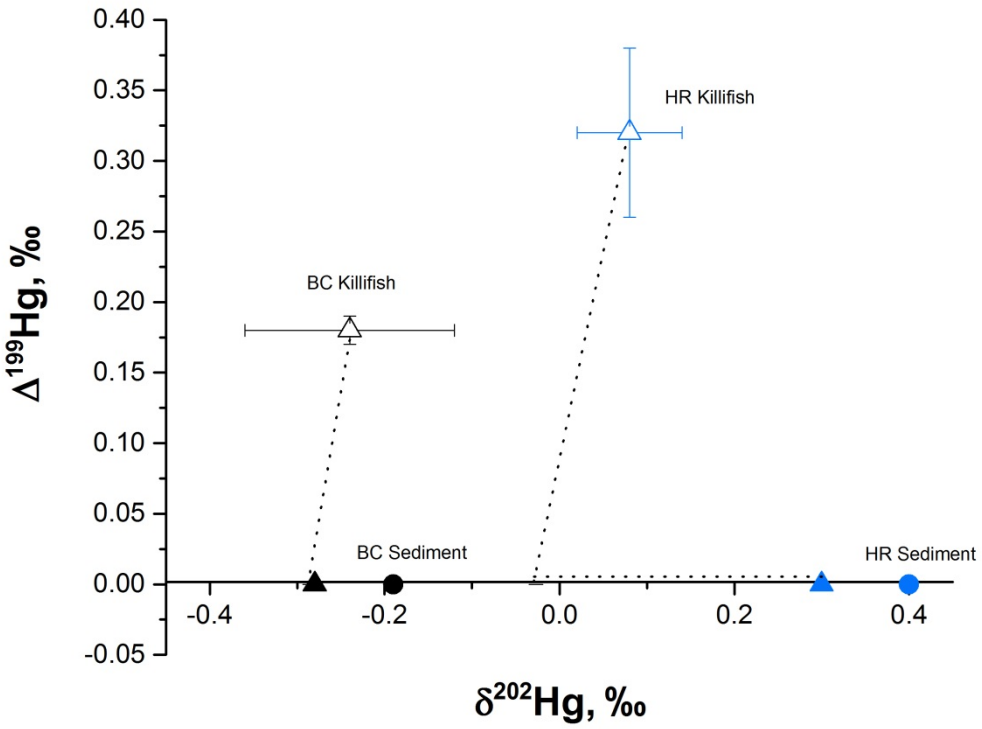



**Fig. 3.4:** Comparison of MeHg estimates of $\delta^{202}$Hg in killifish tissue to directly measured sediment values. Dashed lines represent the correction for photochemical processes as well as % MeHg in the tissue. The final estimated composition (filled ▲), is plotted along with the $\delta^{202}$Hg of sediment MeHg at the capture location (filled ●). It is observed that the fish tissue estimates of $\delta^{202}$Hg for MeHg are isotopically lighter (≈ 0.1 ‰) than directly measured sediment from the capture locations.

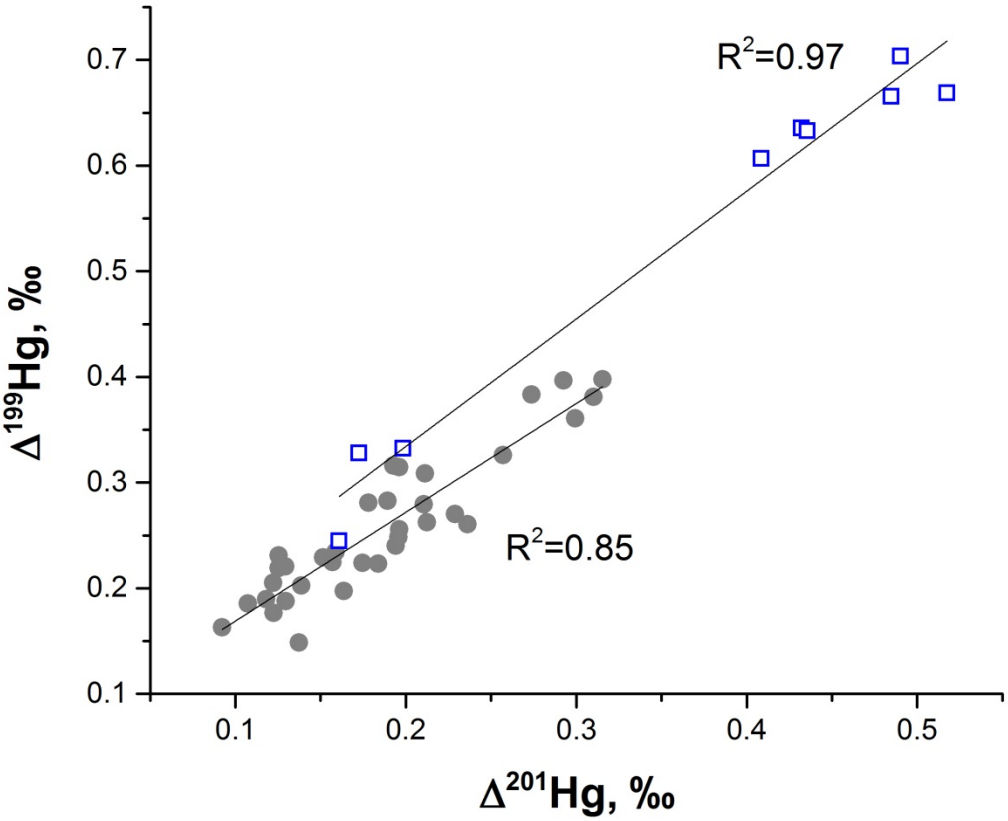



**Fig. 3.5:** Relationship between $\Delta^{199}$Hg and $\Delta^{201}$Hg in Hackensack River estuary (●) and Great Bay (□) fish. The slope for Hackensack fish is 1.13 and for Great Bay fish is 1.21. These slopes are between the bounds set in controlled experiments for photodemethylation (1.3) and photoreduction (≈1.0) (Bergquist and Blum, 2007). This indicates that the species in this study may be a composite of both processes.

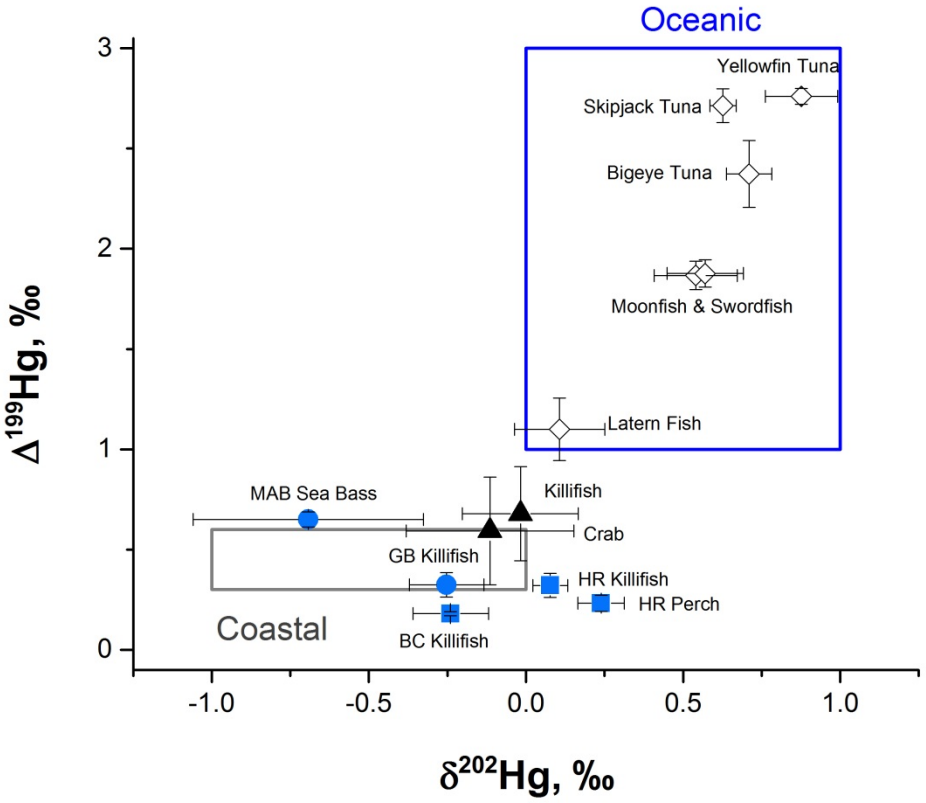
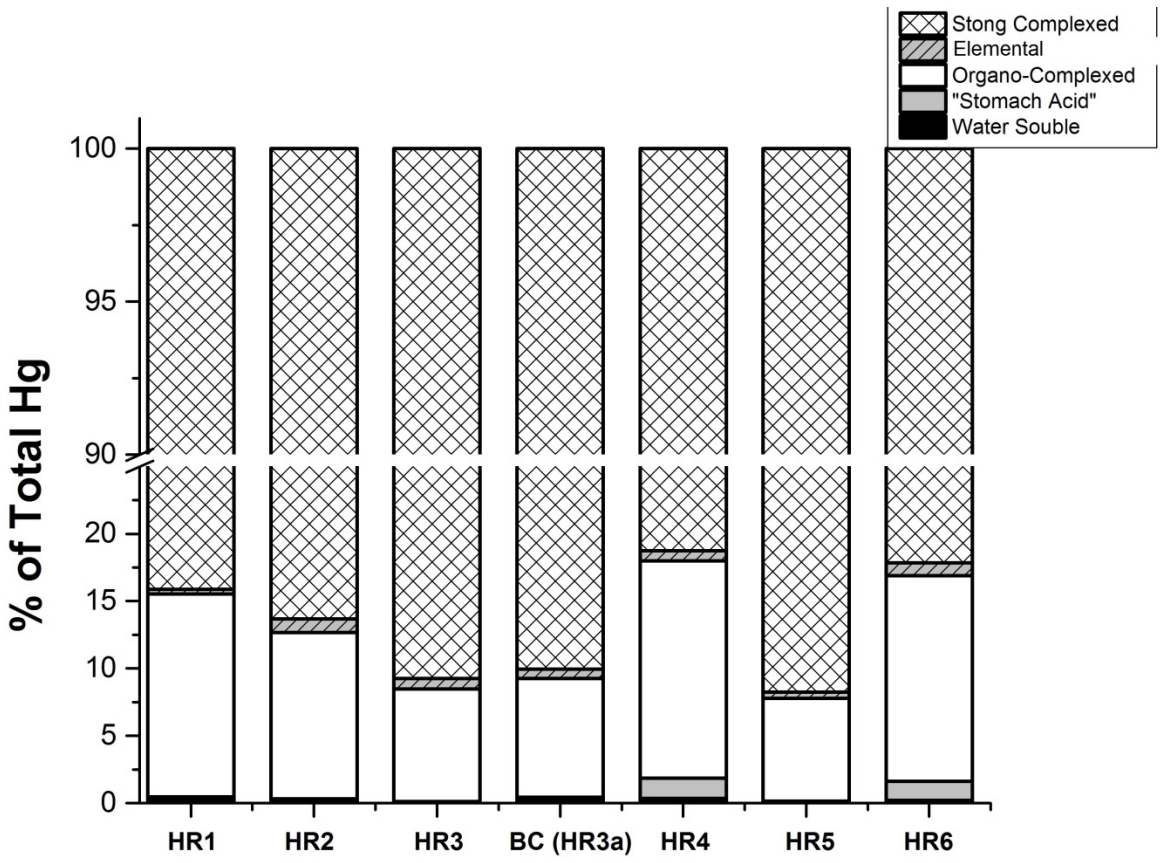
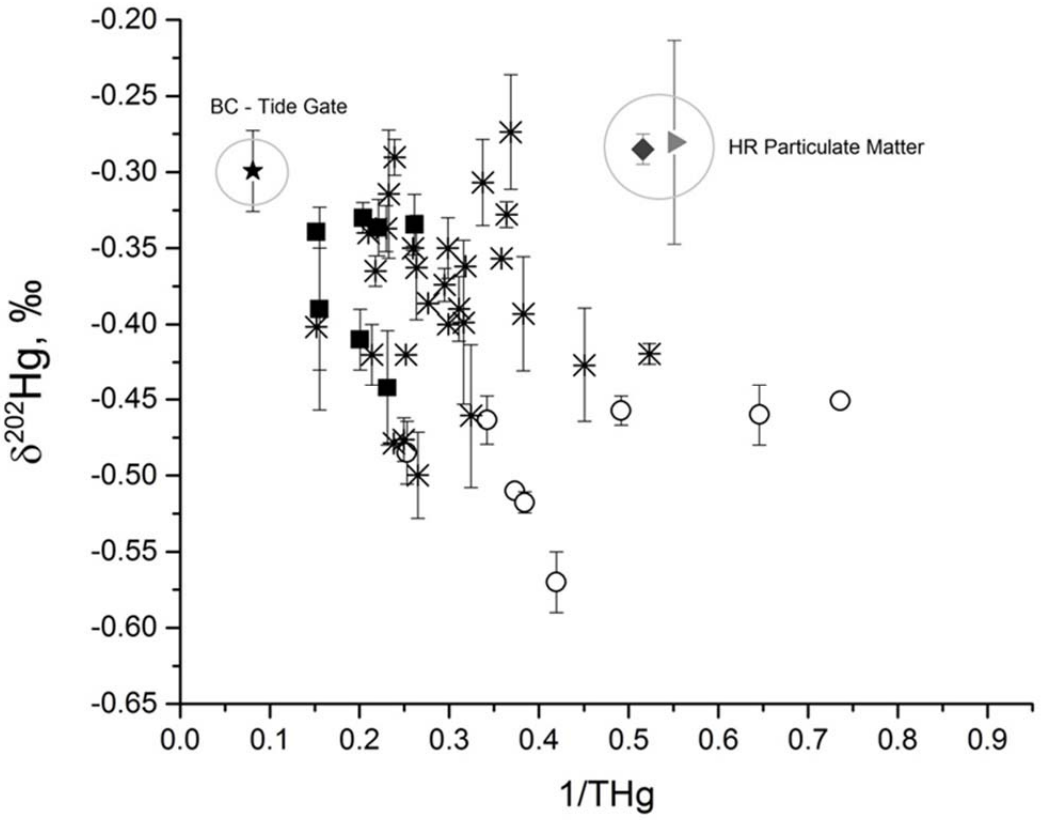



**Fig. 3.6:** Comparison of $\delta^{202}Hg$ and $\Delta^{199}Hg$ in coastal and oceanic fish from this study (blue symbols), Kwon et al. 2014 (black symbols), and Blum et al. 2013 (open symbols). Constraints for the coastal and oceanic boxes are set from the Senn et al. 2010 study. Coastal species are shown to have lower extent of MIF ($\Delta^{199}Hg$), but a larger range of $\delta^{202}Hg$ compositions (-1.0 to 0.25 ‰).




**Supplement Fig. 3.1:** Speciation of total Hg in Hackensack sediments using the selective extraction protocol (Bloom et al., 2003). The sum of all the fractions extracted were ≥ 90% of the total value measured. Strongly complexed Hg species presumably bound to sulfide or Fe/Mn minerals are the most prominent in the sediments. It is concluded that despite the highly elevated concentrations on Hg in the Hackensack River only a small water soluble and organically bound portions (<2%) are available for microbial methylation, demethylation, and reduction.




**Supplement Fig. 3.2:** Isotopic composition of total Hg in the Hackensack River sediments. Black symbols represent sites HR1-HR3 including the BC confluence; open circles represent sites HR5-HR6 closer to Newark Bay. Sediment values for $\delta^{202}$ Hg indicate that there is a slight depletion in $^{202}$Hg at downstream sites in comparison to HR1-HR3. However, the differences are not significant enough to perform a mixing model. The isotopic composition of particulate matter collected from all sampling sites strongly resembles the $\delta^{202}$Hg values on upstream sediment sites; indicating that upstream sediment is a major contributor to the particulate matter found throughout the river.

# CHAPTER 4

# FRACTIONATION OF MERCURY STABLE ISOTOPES DURING MICROBIAL METHYLMERCURY PRODUCTION BY IRON- AND SULFATE- REDUCING BACTERIA

Submitted to *Environmental Science and Technology*

## ABSTRACT

The biological production of monomethylmercury (MeHg) in soils and sediments is an important factor controlling mercury (Hg) accumulation in aquatic and terrestrial food webs. Analytical advances have allowed for the examination of the fractionation of Hg stable isotopes during photochemical and microbial transformations. However, the fractionation of Hg isotopes during biologically mediated Hg methylation has not been fully examined due to limitations on the quantitative separation of MeHg from complex matrices. In this study we assessed the extent of Hg stable isotope fractionation during Hg methylation in non-growing cultures of the bacteria *Geobacter sulfurreducens* PCA and *Desulfovibrio desulfuricans* ND132. In both organisms, Hg stable isotope ratios of directly analyzed MeHg showed mass-dependent discrimination against $^{202}$Hg relative to $^{198}$Hg (lower $\delta^{202}$Hg values), but no mass-independent fractionation

(MIF) of Hg stable isotopes during Hg methylation. Despite differences in methylation rates and phylogenetic classification, *G. sulfurreducens* PCA and *D. desulfuricans* ND132 had similar kinetic reactant/product Hg fractionation factors ($\alpha_{r/p}$ = 1.0009 and 1.0011, respectively). Unexpectedly, after both organisms methylated 34% to 36% of the inorganic Hg to which they had been exposed, accumulated MeHg acquired an isotopic composition 0.4‰ greater than the initial value of reactant inorganic Hg. This indicates that a $^{202}$Hg-enriched pool of inorganic Hg was preferentially utilized as a substrate for methylation by these organisms. In addition, the change in sign of Hg isotope fractionation during later stages of methylation by *D. desulfuricans* indicates that multiple intra- and/or extracellular pools provided substrate inorganic Hg for methylation in this organism. Our results show that the Hg stable isotopic composition of microbially-produced MeHg provides a window into the intracellular compartmentalization of Hg(II) in methylating microorganisms and may be useful in identifying bioavailable Hg in natural systems.

## 1. INTRODUCTION

Methylmercury (MeHg) is a neurotoxic form of mercury that readily bioaccumulates in food webs which can lead to elevated risk of human exposure. The process of mercury methylation, which converts inorganic Hg(II) into MeHg, is mediated by anaerobic microorganisms in a variety of different ecological niches including saturated soils, wetlands, and sediments. Within these matrices, mercury methylators with a variety of metabolisms have been identified in pure culture studies and in environmental samples by molecular techniques and incubations with specific inhibitors and stimulators: sulfate reducing bacteria (SRB); iron reducing bacteria (IRB); dehalogenating bacteria; fermentative bacteria; and methanogenic archaea (Compeau and Bartha, 1985; Gilmour et al., 1992; Gilmour et al., 2013; Parks et al., 2013; Yu et al., 2013). Despite their phylogenetic variation, all of the known methylators contain two specific genes, *hgcA* and *hgcB*, which are mandatory for the production of MeHg (Parks et al., 2013). The production of MeHg in aquatic systems is connected to SRB based on correlations with bulk sediment parameters such as acid volatile sulfide (AVS), total sulfur, and sulfate reduction rates (Gilmour et al., 1992; King et al., 1999; Schartup et al., 2014). However, the importance of other δ proteobacteria, such as IRB in low sulfate sediments and freshwater systems has also been shown (Fleming et al., 2006). Pure culture work with IRB has also shown that they can methylate mercury at comparable rates to SRB(Gilmour et al., 2013; Schaefer and Morel, 2009). The rate of methylation is dependent on the cellular metabolism, rate of Hg uptake, and bioavailable pools which can vary greatly

between strains and higher order taxonomic groups of microorganisms (Gilmour et al., 2013; Schaefer et al., 2011). Despite current knowledge, the exact contribution of different microbial taxa to MeHg pools as well as methylation rates in the environment remains unclear.

The field of Hg stable isotopes has allowed for the identification of isotopically distinct pools of Hg, such as those in fish tissue and sediment, and limited source apportionment (Gehrke et al., 2011; Janssen et al., 2015; Ma et al., 2013; Masbou et al., 2013; Sherman and Blum, 2013). In addition, Hg isotope fractionation, the partitioning of Hg isotopes between reactant and product pools during kinetic or equilibrium reactions, has been examined (Bergquist and Blum, 2007; Bergquist and Blum, 2009; Wiederhold et al., 2010). Investigations of mercury isotope fractionation aim to develop applications of Hg stable isotopes as biogeochemical proxies and have examined a variety of Hg transformations including photochemical reduction/demethylation (Bergquist and Blum, 2007), abiotic methylation (Jiménez-Moreno et al., 2013; Malinovsky and Vanhaecke, 2011), volatilization (Ghosh et al., 2013), sorption (Jiskra et al., 2012; Wiederhold et al., 2010), and microbial transformations (Kritee et al., 2009; Kritee et al., 2007).

Previous Hg isotope studies examined microbially-mediated reduction of Hg(II) and reductive demethylation in resistant organisms with the *mer* operon (Kritee et al., 2009; Kritee et al., 2007). Only a few studies have examined the fractionation of Hg stable isotopes during biological Hg methylation (Perrot et al., 2015; Rodriguez-Gonzalez et al., 2009) despite its central role in Hg

biogeochemistry, mostly due to analytical challenges. Microbial studies are particularly challenging since, unlike animal tissues, the majority of Hg present in microbial cultures is in the inorganic form, which must be separated from the MeHg for isotopic analysis.

Online techniques, utilizing transient GC signals for multicollector inductively coupled plasma mass spectrometry (MC-ICP/MS)(Dzurko et al., 2009; Epov et al., 2008), have been used in the past to estimate Hg isotope fractionation factors for Hg methylation by SRB (Perrot et al., 2015; Rodriguez-Gonzalez et al., 2009). Important insight can be obtained from this work, but there are some caveats associated with this approach. Online separation and analysis of MeHg produces a lower external precision in the delta values in comparison with preconcentration and continuous sample introduction (Janssen et al., 2015). In addition to analytical differences low MeHg yields in previous studies of microbial Hg methylation complicated the approximation of fractionation factors (Kritee et al., 2013).

Given the wide variety of organisms capable of producing MeHg, it is critical to future systematic isotope studies to understand whether the fractionation of Hg isotopes is strictly due to the reaction catalyzed by the cobalamin-binding protein HgcA, which is common to all Hg methylating organisms, or if Hg isotope fractionation varies across phylogenetic groups and different metabolisms. It has been shown that metabolic differences within the same species of SRB do not influence the fractionation of Hg stable isotopes during methylation (Perrot et al., 2015), but it is unknown whether MeHg

produced by different organisms such as IRB show a significant shift in isotope ratios in comparison to SRB due to their phylogenetic differences as well as rates of methylation.

Our work examines the kinetic fractionation of Hg stable isotopes during Hg methylation by pure cultures of the widely studied SRB and IRB strains, *Desulfovibrio desulfuricans* ND132 and *Geobacter sulfurreducens* PCA, respectively. These organisms represent two distinct, yet environmentally relevant groups of organisms associated with the biogeochemical cycling of Hg. If different types of methylating microbes impact different MeHg isotopic signatures, Hg isotope fractionation may allow future differentiation of pathways leading to formation of environmental pools of MeHg, specifically MeHg found in sediment and fish tissue (Masbou et al., 2013). Microbially driven Hg isotope fractionation may also prove useful for deciphering multi-step Hg transformations within cells and the bioavailability of various forms of Hg(II) in the environment.

## 2. METHODS

### 2.1 Microorganisms and culture conditions

Strain *Geobacter sulfurreducens* PCA (ATCC 51573) was obtained from the American Type Culture Collection, and *Desulfovibrio desulfuricans* ND132 was obtained from C. Gilmour. *G. sulfurreducens* PCA was cultured under fumarate-reducing (40 mM) conditions with acetate (10 mM) as an electron donor and carbon source at 30°C (Schaefer and Morel, 2009). Strain ND132 was initially grown in a modified medium for *D. vulgaris* with lactate (60 mM) as the

electron donor and sulfate (30 mM) as the acceptor at 30°C (Zane et al., 2010). Cultures of *D. desulfuricans* ND132 were later starved and inoculated into a low sulfate media amended with pyruvate/fumarate prior to methylation experiments. *G. sulfurreducens* PCA and *D. desulfuricans* ND132 were grown under a nitrogen atmosphere.

Cell biomass was quantified using the Bradford protein assay (Bio-Rad Laboratories) in order to calculate specific methylation rates for the cultures. Aliquots of cell cultures (1 mL) were taken from methylation assay vials during sampling. The cultures were centrifuged for 10 mins at 9000 x g to remove the supernatant and pellets were stored frozen at -20 °C until analysis. Cells were resuspended in 1 mL of 10 mM TRIS buffer and sonicated for 3 mins in 10 sec intervals. Samples and BSA (Bovine Serum Albumin) standards were mixed with the dye reagent and measured via UV-Vis spectrophotometry (Thermo Scientific Genesys 10S).

**2.2 Mercury Methylation Experiments**

Mercury methylation experiments were performed using washed cells. All manipulations of the strains during the washing procedure were performed in an anaerobic chamber (Coy Laboratory Products Inc.) under a gas mixture of 97% $N_2$ and 3% $H_2$. Cells were harvested at exponential phase, centrifuged for 7 min. at 7400 x g, and washed three times in fresh assay buffer. Assay buffer for *G. sulfurreducens* PCA and *D.desulfuricans* ND132 consisted of 10 mM MOPS (pH = 6.8) amended with acetate/fumarate or pyruvate/fumarate, respectively. Prior to

the start of the methylation experiments, 10 μM cysteine was equilibrated with 50 nM mercuric nitrate (NIST 3133) in 100 mL of assay buffer in a stoppered serum bottle for one hour at 30°C. Aliquots of washed cells (2.5 mL to 3.5 mL) were then added to the assay vials to a final density ($OD_{660}$) of 0.05 for *G. sulfurreducens* PCA and 0.07 for *D. desulfuricans* ND132 (2.7 ± 0.16 and 5.9 ± 0.64 mg protein $mL^{-1}$, n=3, respectively). Cells were incubated with Hg(II) for 1-24 hours at 30°C. At designated time points, Hg methylation was stopped by freezing the entire contents of individual bottles. Bottles from all experiments remained frozen until MeHg analysis.

The Hg isotopic composition of cellular and dissolved phase Hg was examined in duplicate incubations of *G. sulfurreducens* PCA. Cell partitioning was not examined in *D. desulfuricans* ND132, since previous work showed that with 10 μM cysteine, 95-100% of inorganic mercury is associated with the cells (Schaefer et al., 2011). Samples were filtered using a Teflon syringe filter holder with 0.2 μm polycarbonate and represent the sum of intra and extra cellular Hg. Both filtrate and filters were stored in Teflon vials and digested with bromine chloride to a final concentration of 0.06 N.

The extent of demethylation was determined in separate experiments that employed similar protocols to methylation assays except that 10 nM of monomethylmercury chloride (Brooks Rand Labs) was added to assays with 10 μM cysteine in place of $Hg(NO_3)_2$ (NIST 3133).

**2.3 Mercury Analysis**

Samples were distilled prior to methylmercury analysis using a Tekran 2750 gas manifold and heating unit according to EPA method 1630. The only modification made to the distillation procedure was the addition of cupric sulfate (1 M) in place of 1% APDC to mitigate interferences from remnant sulfide in the samples (Olson et al., 1997). Distillation blanks were all below 0.4 pM and MeHg concentration spike recoveries were within EPA guidelines (112.7 ± 5.5 %, n = 8).(U.S.EPA, 2001) To assess the extent of abiotic methylation during distillation assay bottles containing media, heat inactivated cell cultures, and 50 nM mercuric nitrate were acidified and also distilled. For both types of media, abiotic methylation produced 1.4 to 2.6 pM which is negligible in comparison to the concentrations of MeHg produced by live cultures and collected for isotopes (2 to 36 nM). Distilled samples were derivatized using aqueous phase ethylation and analyzed by gas chromatography coupled to cold vapor atomic fluorescence spectroscopy (GC-CVAFS) on a Tekran 2700 (Liang et al., 1994).

For total mercury analysis, 5-10 mL aliquots were removed from the original assay or growth bottle and placed into acid cleaned Teflon digestion vials. Samples were oxidized using bromine monochloride (BrCl), to a final concentration of 0.01 N. Total mercury analysis was performed at least 24 hours after BrCl addition. Samples were diluted in ultra-pure water and excess BrCl was neutralized using 2 M hydroxylamine hydrochloride prior to sample introduction. Mercury analysis was performed using tin chloride (0.45 M)

reduction followed by cold vapor atomic absorption spectroscopy (CVAAS) on a Hydra AA (Teledyne-Leeman Labs). Procedural blanks were all below 0.1 nM.

**2.4 Mercury Isotope Analysis**

Methylmercury isotope samples were distilled and separated from the inorganic matrix using a modified GC separation technique (Janssen et al., 2015). Preconcentration onto gold traps was performed prior to desorption into a 0.06 M potassium permanganate solution according to previously described methods (Janssen et al., 2015). To ensure that no fractionation occurred during processing, MeHg standards (Brooks Rand Methylmercury Chloride) of known isotopic composition were processed and collected during each batch. No significant fractionation was observed for processed MeHg standard ($\delta^{202}$Hg = -0.94 ± 0.13 ‰, n = 7) in comparison to directly analyzed MeHg standard ($\delta^{202}$Hg = -0.97 ± 0.06 ‰) (Supplement Table 4.1). Total mercury isotope samples were diluted to appropriate concentrations (2.5 to 5 ppb) prior to mass spectrometry.

All samples for mercury isotope analysis were analyzed using a Thermo-Fisher Neptune multicollector inductively coupled plasma mass spectrometer (MC-ICP-MS) at Rutgers University according to previously published protocols (Bergquist and Blum, 2007; Biswas et al., 2008a). Bracketing standards (NIST 3133) were concentration and matrix matched prior to analysis. Standards for preconcentrated MeHg samples were diluted in a $KMnO_4$- $H_2SO_4$ matrix and standards for THg samples were diluted into a BrCl matrix (<0.005 N). Prior to introduction into the mass spectrometer samples and bracketing standards were

reduced online with 3% (w/w) tin chloride using a CETAC HGX-200 cold vapor system. Reference standards UM Almáden and ERM CC580 were also prepared multiple times and analyzed during each MC-ICP-MS run to assess precision and instrument performance (see Supplement Table 4.1). Nomenclature for Hg isotopic compositions follows that suggested by Blum and Bergquist (2007).

## 3. RESULTS AND DISCUSSION

### 3.1 Mercury Methylation and Demethylation

In the presence of 50 nM cysteine-bound Hg(II), *D. desulfuricans* ND132 and *G. sulfurreducens* PCA methylated 60% to 36% of the inorganic Hg to which they were exposed (Fig. 4.1, Supplement Tables 4.2 and 4.3). Biological replicates of methylation assays for individual organisms, performed on different days, showed similar methylation rates and MeHg yields. The methylation rate for *G. sulfurreducens* PCA over the first 6 h was 0.51 ± 0.05 nmol Hg $mg^{-1}$ protein $h^{-1}$. After 6 h, methylation decreased and the concentration of MeHg remained relatively constant (18.1 to 18.7 nM) over the next 42 h. Under these experimental conditions, the maximum yield of MeHg for *G. sulfurreducens* PCA was 18 nM MeHg or approximately 36% of the 50 nM inorganic Hg originally added to each bottle. *D. desulfuricans* ND132 methylated mercury at a rate of 0.96 ± 0.13 nmol Hg $mg^{-1}$ protein $h^{-1}$, which is 1.8 times faster than *G. sulfurreducens* PCA. At this rate, *D. desulfuricans* ND132 produced 39 nM of MeHg over 24 h or about 60%.

Previous studies addressed the potential effects of Hg recycling by MeHg demethylation on the isotopic fractionation of Hg during the examination of biological MeHg production. Although MeHg degradation by *D. desulfuricans* ND132 has been observed in some (Bridou et al., 2011; Gilmour et al., 2011), but not all (Graham et al., 2012) previous studies, in the present study, no net demethylation ($p > 0.05$) was observed in abiotic controls or in live incubations of either bacterium when exposed to 10 nM MeHg (Table 4.1). Thus, within the analytical uncertainty of MeHg concentration measurements, we observed unidirectional Hg methylation and no demethylation.

### 3.2 Mercury Stable Isotopic Composition of Microbially Produced MeHg

Inorganic Hg added to the methylation assays had an initial isotopic composition ($\delta^{202}$Hg) of 0.00 ± 0.03‰ (n= 10). Over the course of these incubations, the concentration of total Hg (MeHg plus Hg(II)) did not vary by more than 20% and the isotopic composition of total Hg remained identical to that of the initial value (Supplement Tables 4.2 and 4.3) confirming Hg isotope mass balance.

For both bacterial strains, $\delta^{202}$Hg values of directly analyzed MeHg were initially lower than reactant Hg(II) by 0.5‰ to 0.7‰, consistent with mass-dependent discrimination against $^{202}$Hg relative to $^{198}$Hg during Hg methylation (Fig 4.2). No mass independent fractionation (MIF) of Hg stable isotopes was observed during Hg methylation by either *G.sulfurreducens* PCA or *D.desulfuricans* ND132 (Supplement Tables 4.2 and 4.3). As Hg methylation

proceeded, $\delta^{202}$Hg values of MeHg increased as expected with the depletion of $^{198}$Hg in reactant Hg(II). After 20% to 25% of Hg(II) was methylated, $\delta^{202}$Hg values of MeHg became higher than that of the initially added inorganic Hg(II) eventually exceeding the starting value by nearly 0.4‰. In *G. sulfurreducens* PCA, Hg methylation stopped after 36% of added Hg(II) was methylated (Fig 4.1) and the $\delta^{202}$Hg of MeHg had reached its highest value (0.38‰). In *D. desulfuricans* ND132, however, methylation continued until about 60% of added Hg(II) was methylated (40% remaining as inorganic Hg(II)). As the fraction of unmethylated Hg(II) remaining in incubations of *D. desulfuricans* ND132 decreased from 66 to 40%, the $\delta^{202}$Hg of MeHg produced by this strain decreased from 0.35‰ to approximately 0‰.

The production of MeHg with higher $\delta^{202}$Hg values than the Hg(II) initially added indicates that the intracellular pool of substrate Hg(II) for methylation in both strains was enriched in $^{202}$Hg relative to total Hg(II) in the incubations. This was confirmed in short-term experiments with *G. sulfurreducens* PCA in which cellular Hg was enriched in $^{202}$Hg by 0.1 to 0.28‰ relative to initial Hg(II) (Supplement Fig. 4.2). In *G. sulfurreducens* PCA, the proportion of Hg that was methylated (36%) exceeded the fraction of total Hg associated with cells (11 to 23%, Supplement Fig. 4.1). Thus, the intracellular pool of substrate Hg(II) for methylation in *G. sulfurreducens* PCA must have been supplied with Hg(II) from outside the cell. At the concentrations of Hg(II) used in these incubations and in the presence of excess cysteine, the speciation of extracellular Hg(II) was most likely overwhelmingly dominated by cysteine complexes (Cardiano et al., 2011).

The isotopic enrichment of intracellular Hg(II) may therefore have resulted from the fractionation of Hg isotopes during the exchange of Hg(II) among its various cysteine complexes or among intracellular and extracellular pools during Hg(II) transport.

In contrast to *G. sulfurreducens* PCA, *D. desulfuricans* ND132 cells accumulate (inside and on the cell surface) most (>90%) of the Hg(II) to which they are exposed (Schaefer et al., 2011). Thus, the partitioning of Hg(II) among subcellular compartments was likely responsible for the isotopic enrichment of the substrate pool of Hg(II) in this bacterium. In this case, the observed decrease in $\delta^{202}$Hg values of MeHg after *D. desulfuricans* ND132 had methylated 35% of added Hg(II) indicates that a second pool of cellular Hg that was depleted in $^{202}$Hg relative to Hg(II) at the start of the experiment supplied substrate Hg(II) once the pool enriched with the heavier isotopes was consumed.

### 3.3 Mercury Isotope Enrichment Factors

Instantaneous mercury isotope enrichment factors (ε) for bacterial Hg methylation were estimated using $\delta^{202}$Hg values for microbially produced MeHg and the fraction of remaining (unmethylated) Hg(II). Results were fit to a linear fractionation model for a closed system with accumulated product (Hoefs, 2004; Valley, 1986)

$$\delta_p = \delta_i + (f_r) \times \varepsilon_{p/r} \quad (1)$$

in which $\delta_p$ corresponds to the $\delta^{202}$Hg values of accumulated MeHg, $\delta_i$ is the initial $\delta^{202}$Hg of substrate Hg(II), $f_r$ is the fraction of remaining Hg(II), and $\varepsilon_{p/r}$ is

the product-reactant mercury isotope enrichment factor. Enrichment factors were estimated with respect to the intracellular pool of Hg(II) that was a substrate for methylation by rescaling this "bioreactive fraction" of remaining Hg(II) to 0%. For *G. sulfurreducens* PCA, the bioreactive fraction of Hg(II) was estimated as the fraction of initially added Hg(II) consumed when methylation stopped (36%). Since Hg isotope fractionation may have continued, had Hg methylation by *G. sulfurreducens* PCA not stopped at 36%, the size of the bioreactive pool of Hg(II) and the estimated enrichment factor for *G. sulfurreducens* PCA, should be considered minimum values. For *D. desulfuricans* ND132, the bioreactive fraction of Hg(II) was estimated as the fraction of initially added Hg(II) consumed by the time the $\delta^{202}$Hg of MeHg had stopped increasing (34%).

Product-reactant mercury isotope enrichment factors ($\varepsilon_{p/r}$) calculated using Eq. 1 were -0.92‰ for *G. sulfurreducens* PCA and -1.1‰ for *D. desulfuricans* ND132, which correspond to reactant/product fractionation factors ($\alpha_{r/p}$) of 1.0009 and 1.0011, respectively (Supplement Table 4.4). Our results gave similar enrichment factors for both linear and non-linear (Rayleigh distillation) fractionation models, although no curvature was observed in the $\delta^{202}$Hg vs. $f_r$ plots as would be expected for a distillation, and slightly higher coefficients of determination were obtained for the linear model. Linear fractionation of stable isotopes is observed during reversible reactions in which products and reactants reach a state of pseudo-equilibrium (e.g. acid-base or mineral dissolution reactions), or during irreversible processes when a proximate pool of substrate exchanges with and is partially buffered by a larger pool of reactant (e.g. sulfate

reduction (Canfield, 2001)) such that isotopic enrichment of the reactant pool proceeds more slowly than would occur if the substrate pool were isolated and finite. Assuming that the final step of Hg methylation in *G. sulfurreducens* PCA and *D. desulfuricans* ND132 is irreversible (Table 4.1), the apparent linear fractionation of Hg stable isotopes during methylation we observed indicates that there is a second pool of bioreactive Hg(II). This secondary pool may have exchanged with, and partially buffered, the proximate pool of substrate Hg(II) in these bacteria, consistent with the patterns of $\delta^{202}$Hg values in produced MeHg described above.

The Hg isotope fractionation factors we estimated for Hg methylation by iron and sulfate-reducing bacteria are higher than those for abiotic Hg methylation ($\alpha_{r/p}$ = 1.0006-1.0007 (Malinovsky and Vanhaecke, 2011) although Jiménez-Moreno et al. (2013) recently measured an $\alpha_{r/p}$ of 1.004 for Hg methylation by reaction with methylcobalamin, but two to four times lower than previously reported fractionation factors for another sulfate-reducing bacterium, *Desulfovibrio dechloracetivorans,* grown under fermentative (1.0026 – 1.0044) (Rodriguez-Gonzalez et al., 2009) or sulfate-reducing conditions (1.0025) (Perrot et al., 2015). Biologically-catalyzed transformations of Hg, including demethylation of MeHg and Hg(II) reduction were previously shown to have fractionation factors in the range of 1.0003 to 1.0015 (Kritee et al., 2009; Kritee et al., 2007; Kritee et al., 2013). Differences between the fractionation factors determined in this study and those measured previously may arise from differences in the extents or rates of Hg(II) methylation. For example, the lower

rates of Hg methylation observed by Perrot et al. (2015) , compared to the present study, may have resulted in higher Hg isotope fractionation than those observed here. In addition, the fractionation factors reported by Perrot et al. (2015) were based on the isotopic composition of Hg(II), not MeHg, and assumed that all added Hg(II) was bioreactive, which our results suggest (Fig 4.2) may not be the case. Both studies highlight the challenges associated with modelling Hg isotope fractionation in complex biological media with multiple and exchangeable intra- and extracellular forms of Hg(II) .

Our results indicate that different taxonomic groups of Hg methylating bacteria have similar Hg isotope fractionation factors. A similar conclusion was reached regarding metabolic differences within the same species of bacteria (Perrot et al., 2015). This indicates that the fractionation of Hg isotopes may be dominated by an enzymatic step of the methylation process that is common among diverse anaerobic microorganisms, presumably that is catalyzed by the gene products of *hgcA* and *hgcB* (Gilmour et al., 2013). However, all strains tested to date are gram-negative bacteria that belong to *Deltaproteobacteria*. With the discovery that gram-positive bacteria (Gilmour et al., 2013) and methanogenic archaea methylate, further testing may change this picture. Similarities in fractionation factors notwithstanding, major differences in the cellular accumulation of Hg(II) by *G.sulfurreducens* PCA and *D. desulfuricans* ND132 indicate that these organisms access distinct intra- and extracellular pools of Hg(II) prior to methylation.

In addition to biological effects, the extracellular speciation of Hg(II) will also likely affect the isotopic composition of biologically produced MeHg. For example, it has been shown that Hg(II) bound to thiol (Wiederhold et al., 2010) or iron oxyhydroxide particles (Jiskra et al., 2012) has lower $\delta^{202}$Hg compared with aqueous chloro- or hydroxide complexes. How the formation of these and other species of Hg(II) in natural systems affect Hg bioavailability and isotope fractionation in Hg methylating microorganisms has yet to be examined.

In our experiments, cells were provided with a relatively high concentration of bioavailable Hg(II) so that the rate of biological Hg methylation would not be limited by the supply of Hg(II) and that the maximum extent of isotope fractionation associated with this transformation would be observed. Apparent product-reactant $^{202}$Hg enrichment factors ($\varepsilon_{p/r}$) for Hg methylation estimated as the y-intercepts of un-rescaled $\delta^{202}$Hg-$f_r$ relationships were -0.58‰ for *G. sulfurreducens* PCA and -0.87‰ for *D. desulfuricans* ND132. These represent the maximum isotopic fractionation between MeHg and total dissolved Hg(II) that may be observed in natural environments, such as sediment porewaters. However, in the environment, the limitation of Hg methylation by the low bioavailability of Hg(II) (Benoit et al., 2001; Benoit et al., 1999; Drott et al., 2007) could result in lower extents of kinetic fractionation and the production of isotopically enriched MeHg, as was recently observed in a tidal estuary (Janssen et al., 2015).

In summary, our results show that 1) the intracellular pool of substrate Hg(II) in Hg methylating anaerobic bacteria is isotopically enriched relative to

total Hg(II), 2) multiple pools of intra- and extracellular Hg(II) contribute bioreactive Hg for methylation, and 3) Hg stable isotopes provide a useful tool to study the intracellular compartmentalization of Hg(II) and the link between biochemical Hg methylation and Hg(II) bioavailability.

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**Table 4.1:** MeHg concentrations in cultures of *G. sulfurreducens* and *D. desulfuricans* incubated with 10 nM MeHg and 10 µM cysteine. Abiotic controls contained media plus MeHg and cysteine, but no cells. Standard deviation represents biological triplicates.

| | ***G. sulfurreducens*** **(PCA)** | ***D. desulfuricans*** **(ND132)** |
|---|---|---|
| Time, hours | MeHg Conc, nM | MeHg Conc, nM |
| 0 | 9.2 ± 0.6 | 9.7 ± 0.6 |
| 1 | 8.1 ± 0.5 | 11.0 ± 2.0 |
| 2 | 8.4 ± 1.6 | 8.6 ± 0.7 |
| 3 | 8.7 ± 0.4 | 11.1 ± 1.4 |
| 6 | 8.1 ± 2.0 | 10.9 ± 0.1 |
| 24 | 8.2 ± 1.8 | 9.6 ± 2.2 |
| Abiotic (24) | 8.2 ± 0.5 | 10.2 ± 0.2 |

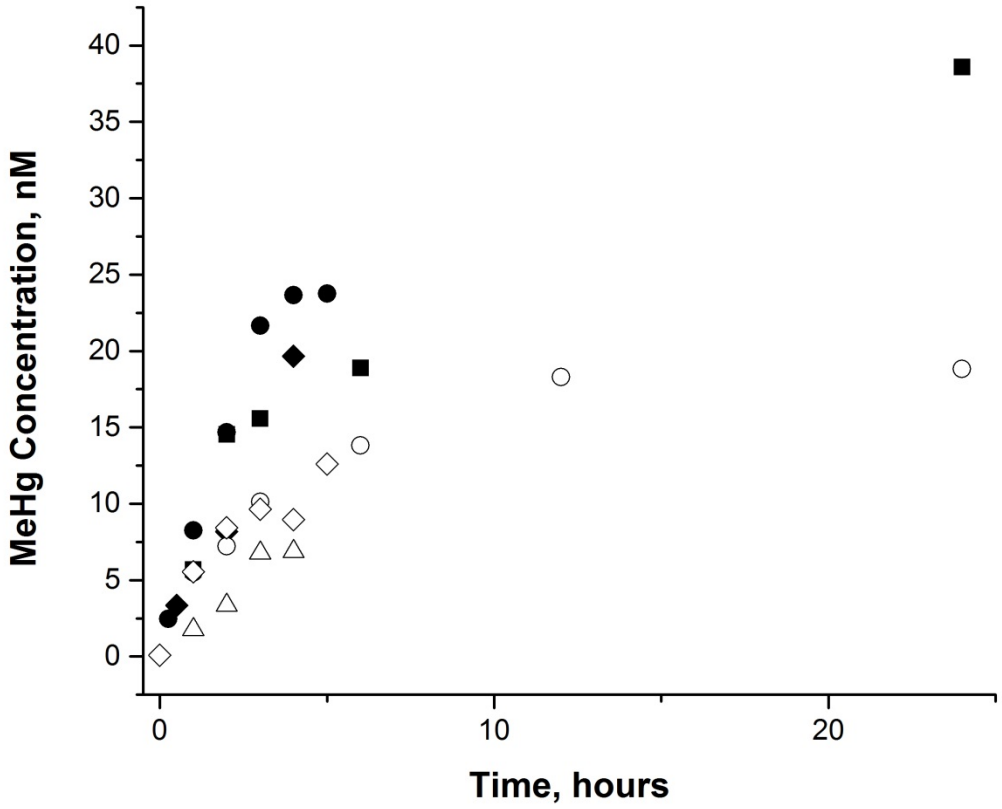



**Fig. 4.1**: MeHg production by *G. sulfurreducens* (open symbols) and *D. desulfuricans* (solid symbols) in washed cell assays with 10 µM cysteine and 50-60 nM mercuric nitrate (NIST 3133). Each point represents a separate assay bottle and symbol shapes correspond to biological replicates performed on different days. The average analytical uncertainty (2SD) for MeHg measurements was 2.6 nM.

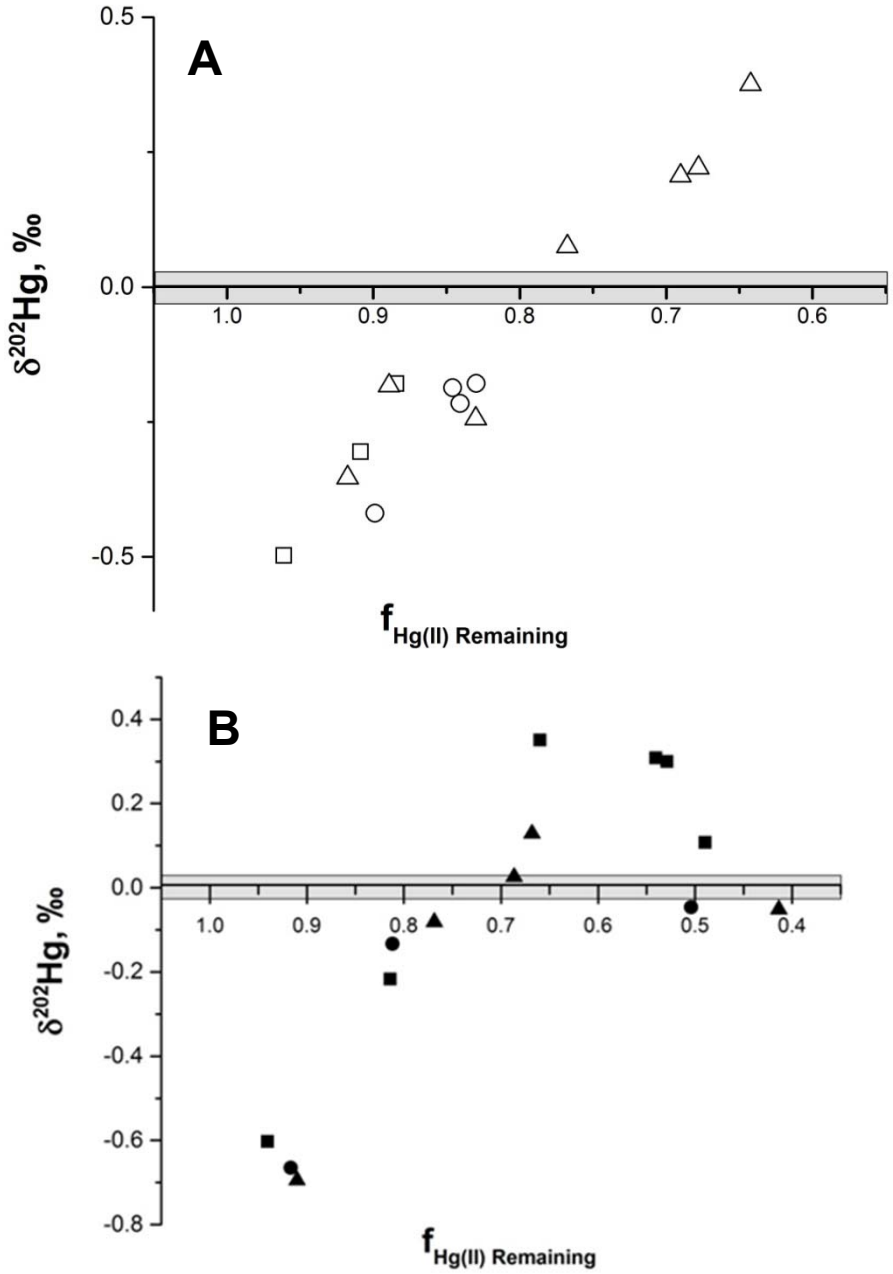
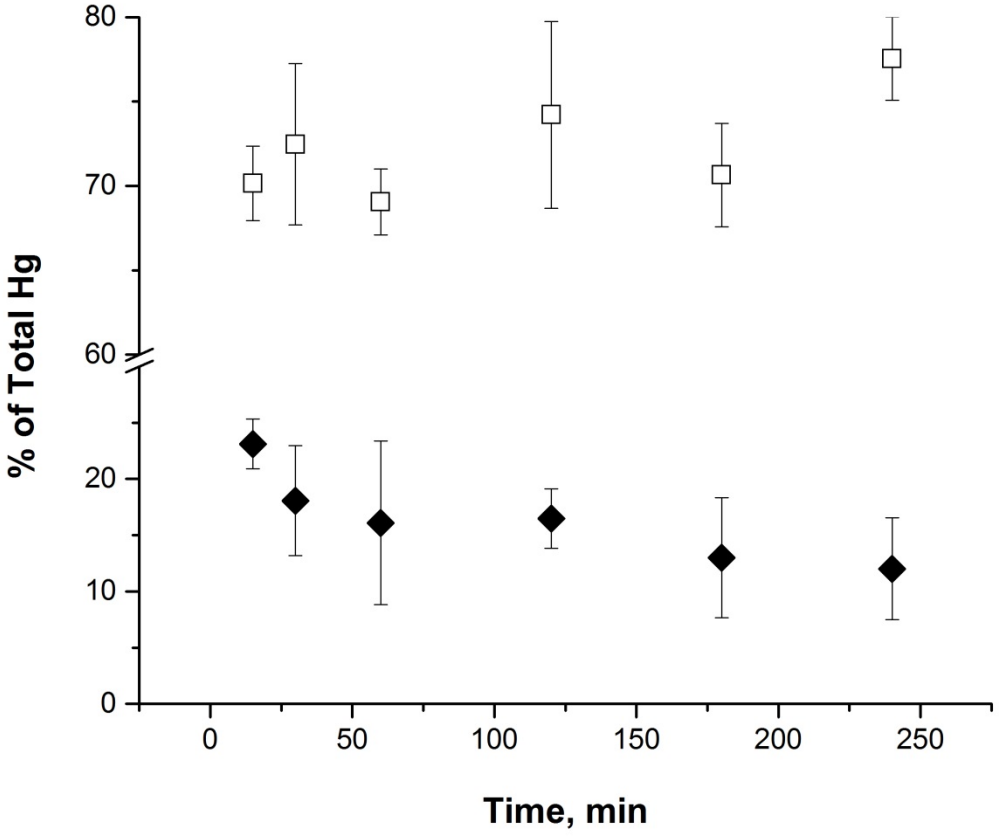
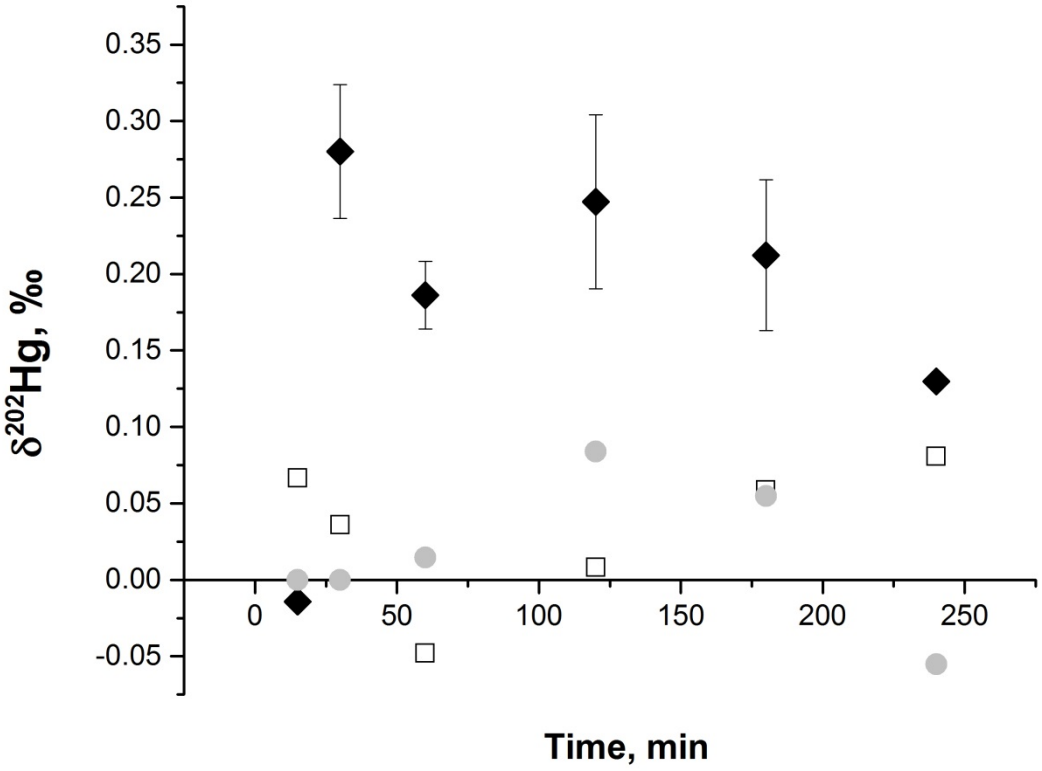



**Fig. 4.2**: Mass-dependent fractionation of $^{202}$Hg in MeHg produced by (A) *G. sulfurreducens* and (B) *D. desulfuricans* in washed cell assays with 10 µM cysteine and 50-60 nM mercuric nitrate (NIST 3133). $\delta^{202}$Hg values for MeHg are plotted with respect to the fraction of remaining total inorganic Hg(II). Gray bars represent the analytical precision associated with the isotopic composition of inorganic Hg (NIST 3133) supplied to the organism at the start of the experiment. Values are for indvidual incubation bottles and symbol shapes correspond to biological replicates performed on different days.




**Supplement Fig. 4.1:** Percentage of cell associated (♦) or dissolved (□) Hg in washed cell assays of *G. sulfurreducens* with 10 μM cysteine and 70 nM mercuric nitrate (NIST 3133). Note that samples from this experiment were analyzed for the Hg isotopic composition of total Hg (Fig. S2), but were not used for the analysis of MeHg concentration or MeHg isotopes. Over the course of this experiment, the concentration of total Hg varied by less than 1.6%. Losses of dissolved Hg to bottle walls or syringes during filtering were on the order of 12%. Error bars indicate the propagation of error associated with measurements of the selected pool and THg in biological replicates.




**Supplement Fig. 4.2:** Isotopic composition of total Hg in washed cell assays of *G. sulfurreducens* with 10 µM cysteine and 70 nM mercuric nitrate (NIST 3133). Values are for cell bound (filtered, ♦), dissolved (filtrate, □), and total Hg (inorganic plus MeHg, unfiltered, ●). Error bars for cell-bound total Hg represent 1SD of biological replicates. The analytical uncertainty for $\delta^{202}$Hg in the media matrix is ±0.08 ‰ (2SD) as determined by replicate anlayses (n = 8) of the UM Almáden standard.

# CHAPTER 5

# CONCLUSION

This study served to elucidate the Hg isotopic composition of MeHg in natural sediments and pure culture. Furthermore, the isotopic composition of both MeHg and THg were utilized to identify MeHg sediment sources to biota in the Hackensack River, NJ. The measurement of the isotopic composition of MeHg in matrices with high inorganic Hg is a constant challenge. Previous measurements of MeHg isotopic composition required matrices with a high % MeHg (Masbou et al., 2013) or the direct measurement of aqueous phase ethylation products coupled to MC-ICP/MS via gas chromatography column (Dzurko et al., 2009; Epov et al., 2010; Rodriguez-Gonzalez et al., 2009). These approaches were unsuitable for matrices such as sediment and pure cultures due to the MeHg only accounting for 0.1- 2 % of the total Hg.

The first objective of this work was to enhance the sensitivity of MeHg isotope measurements utilizing an offline concentration system. This system was modeled off the traditional aqueous phase ethylation and GC separation analysis protocol for MeHg, with additional collection of pyrolyzed MeHg onto gold traps and further desorption into a potassium permanganate solution. This approach showed no significant fractionation and high recoveries of Hg isotopes as long as the CVAFS detector was bypassed. This preconcentration process provided higher precision in MC-ICP/MS measurements than previous online techniques (Dzurko et al., 2009; Epov et al., 2008).

Natural sediments, from the Hackensack and Passaic rivers, were the first samples separated by the system and showed a dynamic range of isotopic composition for MeHg in comparison to THg measurements. It was observed that $\delta^{202}$Hg values for MeHg increased downstream, suggesting that this general spatial trend may be a factor of physical mixing with a lighter MeHg being produced upstream and transported downstream. However, the isotopic composition of MeHg in sediment may also be impacted by the biological production and degradation of MeHg by microorganisms as well as the bioavailable pools of Hg(II). The isotopic compositions from the Hackensack-Passaic system are the first measurements of MeHg in sediment and represent an important pool in the Hg biogeochemical cycle that has been understudied in the isotope community.

In aquatic systems, sediments are considered an important pool of both Hg(II) and MeHg and are a major contributor to MeHg accumulation in estuarine biota (Chen et al., 2014; Kwon et al., 2014). A field study of Hackensack was undertaken to determine if the isotopic composition of MeHg in the sediment could be linked to the isotopic compositions in fish tissue as a proxy for feeding habitat. Samples of perch and killifish from the Hackensack River displayed relatively homogenous Hg concentrations as well as similar $\delta^{15}$N and $\delta^{13}$C values indicating similar trophic designations. Slopes associated with odd isotopes $\Delta^{199}$Hg and $\Delta^{201}$Hg showed that the photochemical processes responsible for MIF in the Hackensack River are likely a mix of both photodemethylation and photoreduction. To overcome the complexity of different photochemical effects as

well as varying % MeHg in the tissue, we modified the calculations found in Sherman and Blum (2013) and Kwon et al. (2014). The estimation of the isotopic composition of MeHg in fish tissue was based upon a weighted slope to correct for both photochemical processes and a mass balance to account for the fact that the fish tissue is < 100% MeHg. Using these corrections and comparing to the direct isotope measurements of sediment MeHg it was observed that MeHg compositions in killifish tissue were similar to sediment values. However, migratory perch deviated from the sediment isotopic compositions of capture locations. This case study demonstrated that a systematic approach is needed in both direct measurement of isotopic composition and the estimation calculations of MeHg in fish tissue.

The field measurements of MeHg and THg isotopes in biological tissue and sediments are important for the future progression of Hg isotopes as tools for environmental monitoring. However, the isotope fractionation associated with the methylation provides insight on how these environmental pools are formed. The last objective of this work was to determine the fractionation factor during methylation for two Hg methylating microorganisms, *Geobacter sulfurreducens* PCA and *Desulfovibrio desulfuricans* ND132. Previous work examining isotopic changes during methylation used bacteria capable of only low MeHg yields and predominantly measured Hg(II) using previously mentioned online techniques which leaves many unanswered questions about the fractionation process. This work showed that fractionation factors were similar between the two organisms despite difference in methylation rates and phylogeny. However, it was also

observed that these organisms will preferentially methylate heavier Hg(II) in comparison to the bulk Hg(II) provided, causing the production of MeHg 0.4‰ heavier than the starting material. This anomaly indicates the two δ-proteobacteria tested have access to different pools of Hg(II) either extra- or intracellularly. The process of methylation is more complicated than initially presumed in previous studies and indicates that different bioavailable pools of Hg may be preferentially utilized during the uptake of Hg(II). Understanding the process of methylation from a mechanistic standpoint will allow for a better understanding of the formation of MeHg pools in environmental matrices, such as sediment.

**Suggestions for Future Studies**

This work represents a starting point for the implementation of direct isotopic measurement of MeHg species using offline separation techniques. Methylmercury is an extremely important pool in the Hg biogeochemical cycle that can now be further examined utilizing Hg isotopes for both field and laboratory studies.

Future field studies examining the isotopic composition of MeHg need to account for different types of MeHg inputs as well as lower net MeHg production, specifically in estuarine systems. The isotopic composition of MeHg should be established in wetlands and areas of high terrestrial runoff, since these have been noted to be important sources of MeHg to aquatic systems (Jonsson et al., 2014; Tsui et al., 2012). From our results in the Hackensack, we believe that

there is an upstream source of MeHg that causes higher MeHg concentrations in winter and spring months and the wide range of isotopic compositions. These sources need to be examined because; different diagenetic controls on methylation may produce isotopic compositions of MeHg that were not observed in the Hackensack study. Sediments that have lower net MeHg concentrations, such as Tuckerton and downstream site (HR6) in this study, should also be examined in the future as a direct comparison to highly productive sites for Hg methylation. Further analytical challenges will have to be addressed before examining these types of sites, specifically high percentages of organic matter and low MeHg concentrations. Different preconcentration techniques, such as organic extractions and bulk acid leaching, will need to be tested for separation efficiency when examining wetland and terrestrial sites. Additionally, samples with ≤1 ng $g^{-1}$ MeHg require a scale up of separation processes and appropriate tests to ensure that fractionation does not occur during modification of the separation system, specifically during aqueous phase ethylation.

The establishment of the MeHg isotopic composition in sediment allows for more in depth bioaccumulation studies. A systematic study of an estuary like the Hackensack would also require the establishment of the isotopic composition of MeHg in particulate matter, which is more representative of a pelagic food source. Once again, low MeHg concentrations would need to be addressed in a similar manner to low MeHg sediments. Measurement of the particulate MeHg pool would also allow us to establish whether any isotopic changes occur to this pool related to resuspension or diffusion of MeHg from the sediment. If benthic

(sediment) and pelagic (particulate matter) sources of MeHg are established using Hg isotopes, this will allow for more precise determination of Hg sources for different fish species. Better estimates of MeHg isotopic composition in fish tissue can also be determined using the MeHg isotope extraction procedure (Masbou et al., 2013) paired with the corrections for photodemethylation mentioned here and in previous studies (Kwon et al., 2014; Sherman and Blum, 2013). Studies aiming to examine the bioaccumulation and cycling of MeHg in aquatic environments should aim to assess major pools of MeHg in the system, specifically in sediment, particulate matter, and biological tissue. Due to the labor intensive nature of isotope analysis of MeHg, comprehensive studies examining multiple pools should aim for smaller study areas.

The process of methylation also warrants further investigation in both the field and in laboratory studies. The elucidation of bioavailable pools of Hg(II) for Hg methylating microorganisms remains a particular challenge. The sediment THg and MeHg isotopes examined in this study represent bulk parameters and not the specific bioavailable pools available to methylating microorganisms. Examination of isotopic compositions of Hg and MeHg in porewater may provide better proxies for these bioavailable pools in the environment. Laboratory studies should also be implemented to understand the formation of different pools of Hg(II) during uptake in pure cultures, as observed in Chapter 4. A heavier pool of Hg(II) was preferentially methylated, but it is unknown whether this is an intra- or extracellular phenomenon. Washed cell uptake assays (Schaefer and Morel, 2009; Schaefer et al., 2011) may be useful in understanding the isotopic

composition of dissolved, loosely cell associated, and intracellular pools of Hg(II). The bioavailability of Hg (II) should also be tested in non-methylating organisms (Szczuka et al., 2015) to determine if these isotopically heavier pools are still created in the absence of methylation.

Lastly, fractionation of Hg isotopes during methylation needs to be addressed in the other classes of Hg methylators. Fractionation factors have been established for three sulfate-reducing and one iron- reducing bacteria (Perrot et al., 2015; Rodriguez-Gonzalez et al., 2009). However, methanogenic archaea are largely different from previously studied δ-proteobacteria and can be important Hg methylators in sulfate and iron limited freshwaters (Yu et al., 2013). In addition, the identification of gram positive organisms with the *hgcA* and *hgcB* genes means there is another group of Hg methylators with diverse metabolisms that need to be examined (Gilmour et al., 2013; Parks et al., 2013). Isotope studies examining fractionation during methylation should be undertaken with the methanogen, *Methanospirillum hungatei*. This organism is easily cultured under laboratory conditions and has known rates of methylation (Yu et al., 2013). An additional benefit to examining fractionation in *M. hungatei* is the fact that the organism can be grown in coculture and participates in syntrophic interactions with SRB. A fractionation factor and subsequent isotopic compositions during methylation in a coculture may be a better comparison to environmental systems than just monocultures (Yu, 2011). In order to completely examine the fractionation of Hg isotopes during biological methylation, gram positive organisms should be investigated as well. Gram positive organisms containing

the *hgcA* and *hgcB* are diverse and extremely understudied. Organisms can be compared via metabolism in this group (SRB, IRB, fermentative, and dehalogenating) in order to verify that metabolic difference do not induce differences in the fractionation factor for methylation. Studies utilizing these gram positive bacteria will have to first establish the rates and extent of Hg methylation prior to performing isotope experiments. There are more analytical challenges associated with both the methanogens and gram positive bacteria, specifically lower MeHg yields and the inability to perform resting cell assays. However, with careful manipulation, these experiments can be performed and compared directly to the fractionation factors for methylation obtained from the δ-proteobacteria.

The work presented in this dissertation is the starting point for continual advancement in MeHg isotope analysis. All of the suggested research requires additional modifications in sample preparation and separation of MeHg for isotope analysis. The elucidation of the isotopic composition of MeHg pools in the environment and laboratory studies will aid in our understanding of the Hg biogeochemical cycle as well as provide insight when utilizing Hg isotopes for source tracking applications.

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# APPENDIX A: Hackensack Sampling Data

**Table A1:** Fish and Sediment Collection Locations

| FISH | Lat | Long |
|---|---|---|
| DHR-perch (a) | 40.7418 | -74.0794 |
| DHR-perch (j) | 40.7418 | -74.0794 |
| UHR-perch (a) | 40.8494 | -74.0303 |
| UHR-perch (j) | 40.8494 | -74.0303 |
| BC-killifish | 40.8189 | -74.0863 |
| HR-killifish | 40.8023 | -74.0659 |
| GB-killifish | 39.5077 | -74.3383 |
| MAB-black sea bass | 39.5164 | -74.1148 |

| SEDIMENT | Lat | Long |
|---|---|---|
| HR1 | 40.8494 | -74.0303 |
| HR2 | 40.8247 | -74.0355 |
| HR3 | 40.8023 | -74.0659 |
| HR3a | 40.7980 | -74.0753 |
| HR4 | 40.7779 | -74.0904 |
| HR5 | 40.7418 | -74.0794 |
| GB | 39.5077 | -74.3383 |

**Table A2:** Physical Characteristics and Hg Concentrations for Biota Samples from the Hackensack River and Tuckerton Bay, NJ

| Tag ID | MeHg (ug/g ww) | THg (ug/g ww) | % MeHg | Length, mm | Weight, g |
|---|---|---|---|---|---|
| **BC Killifish** | | | | | |
| BC1_006 | 0.16 | 0.16 | 94.7 | 76.0 | 9.8 |
| BC2_010 | 0.19 | 0.28 | 66.9 | 56.0 | 4.6 |
| BC7_014 | 0.27 | 0.42 | 65.9 | 62.0 | 6.2 |
| **HR Killifish** | | | | | |
| HK2_002 | 0.08 | 0.11 | 73.8 | 63.0 | 66.0 |
| HK3_004 | 0.08 | 0.13 | 62.3 | 111.0 | 82.0 |
| HK4_006 | 0.13 | 0.15 | 84.5 | 42.0 | 66.0 |
| HK7_008 | 0.06 | 0.18 | 30.7 | 33.0 | 61.0 |
| HK13_010 | 0.06 | 0.18 | 32.5 | 34.0 | 60.0 |
| HK16_012 | 0.05 | 0.13 | 40.1 | 51.0 | 72.0 |
| HK17_014 | 0.06 | 0.15 | 42.2 | 50.0 | 66.0 |
| HK18_016 | 0.11 | 0.35 | 30.1 | 26.0 | 55.0 |
| CON_029 | 0.12 | 0.22 | 56.4 | 62.0 | 5.4 |
| CON_031 | 0.10 | 0.20 | 50.9 | 68.0 | 6.7 |
| CON _033 | 0.11 | 0.24 | 47.0 | 63.0 | 6.0 |
| CON_035 | 0.10 | 0.21 | 49.1 | 64.0 | 6.1 |
| **Tuk Killifish** | | | | | |
| TUK1_018 | 0.03 | 0.07 | 41.1 | 75.0 | 6.5 |
| TUK3_020 | 0.03 | 0.09 | 36.4 | 81.0 | 8.3 |
| TUK4_022 | 0.04 | 0.05 | 83.9 | 75.0 | 6.6 |
| TUK11_024 | 0.04 | 0.05 | 80.0 | 62.0 | 5.0 |
| **Tuk BSB** | | | | | |
| 1498_004 | 0.02 | 0.06 | 30.5 | 287.0 | 277.0 |
| D141_006 | 0.03 | 0.04 | 84.9 | 280.0 | 254.0 |
| 1088_008 | 0.01 | 0.06 | 14.1 | 299.0 | 350.0 |
| D011_010 | 0.04 | 0.07 | 51.5 | 252.0 | 200.7 |
| D0152_012 | 0.08 | 0.18 | 41.9 | 321.0 | 407.0 |
| **HK Perch Downstream Adult** | | | | | |
| 2.1_004 | 0.51 | 0.61 | 82.6 | 183.0 | 78.9 |
| 2.2_006 | 0.33 | 0.54 | 61.4 | 206.0 | 135.6 |
| 2.4_004 | 0.26 | 0.61 | 41.8 | 235.0 | 191.0 |
| **HK Perch Upstream Adult** | | | | | |
| 3.6_014 | 0.73 | 0.92 | 79.7 | 283.0 | 401.1 |
| 5.1_022 | 0.34 | 1.06 | 32.1 | 286.0 | 445.8 |
| 6.3_026 | 0.47 | 0.96 | 48.8 | 267.0 | 289.2 |
| 6.5_030 | 0.45 | 0.63 | 71.3 | 270.0 | 378.6 |
| 5.6_034 | 0.27 | 0.30 | 92.2 | 201.0 | 121.8 |
| 3.1_008 | 0.13 | 0.44 | 28.9 | 206.0 | 164.1 |
| 3.3_012 | 0.34 | 0.44 | 78.2 | 240.0 | 260.5 |
| 3.5_016 | 0.13 | 0.40 | 33.2 | 217.0 | 182.9 |
| 4.9_020 | 0.56 | 0.57 | 98.7 | 212.0 | 142.4 |
| 5.2_026 | 0.22 | 0.35 | 62.4 | 184.0 | 108.2 |
| 5.4_030 | 0.14 | 0.37 | 37.8 | 242.0 | 222.6 |
| 5.5_034 | 0.24 | 0.49 | 50.4 | 205.0 | 185.7 |
| 6.6_038 | 0.72 | 0.77 | 94.2 | 253.0 | 309.3 |
| 6.7_042 | 0.35 | 0.35 | 100.4 | 215.0 | 230.9 |
| 6.8_046 | 0.16 | 0.41 | 38.3 | 219.0 | 170.6 |
| **HK Upstream Juv.** | | | | | |
| 4.6_018 | 0.07 | 0.23 | 32.0 | 56.0 | 2.2 |
| 4-5J_012 | 0.07 | 0.07 | 96.0 | 93.0 | 10.1 |
| 5-1J_016 | 0.04 | 0.07 | 53.9 | 90.0 | 10.3 |
| **HR Downstream Juv.** | | | | | |
| 1-41_004 | 0.04 | 0.09 | 47.2 | 62.0 | 3.2 |
| 1-32_014 | 0.06 | 0.12 | 49.9 | 57.0 | 2.0 |
| 1-25_020 | 0.06 | 0.11 | 48.9 | 66.0 | 3.4 |

**Table A3:** Isotopic Compositions for Biota Samples from the Hackensack River and Tuckerton Bay, NJ

| Tag ID | δ199 | δ200 | δ201 | δ202 | Δ199 | Δ200 | Δ201 |
|---|---|---|---|---|---|---|---|
| **BC Killifish** | | | | | | | |
| BC1_006 | 0.15 | -0.04 | 0.02 | -0.14 | 0.19 | 0.03 | 0.13 |
| BC2_010 | 0.11 | -0.09 | -0.06 | -0.21 | 0.16 | 0.01 | 0.09 |
| BC7_014 | 0.09 | -0.19 | -0.18 | -0.38 | 0.19 | 0.00 | 0.11 |
| **HR Killifish** | | | | | | | |
| HK2_002 | 0.39 | 0.11 | 0.38 | 0.11 | 0.36 | 0.05 | 0.30 |
| HK3_004 | 0.42 | 0.19 | 0.40 | 0.17 | 0.38 | 0.10 | 0.27 |
| HK4_006 | 0.27 | 0.03 | 0.24 | 0.02 | 0.27 | 0.02 | 0.23 |
| HK7_008 | 0.40 | 0.09 | 0.36 | 0.06 | 0.38 | 0.06 | 0.31 |
| HK13_010 | 0.31 | 0.06 | 0.17 | -0.04 | 0.32 | 0.08 | 0.19 |
| HK16_012 | 0.42 | 0.14 | 0.38 | 0.08 | 0.40 | 0.09 | 0.32 |
| HK17_014 | 0.27 | 0.05 | 0.26 | 0.03 | 0.26 | 0.03 | 0.24 |
| HK18_016 | 0.42 | 0.09 | 0.37 | 0.10 | 0.40 | 0.04 | 0.29 |
| CON_029 | 0.27 | 0.08 | 0.28 | 0.12 | 0.24 | 0.02 | 0.19 |
| CON_031 | 0.32 | 0.03 | 0.24 | 0.04 | 0.31 | 0.01 | 0.21 |
| CON _033 | 0.31 | 0.07 | 0.28 | 0.12 | 0.28 | 0.01 | 0.19 |
| CON_035 | 0.28 | 0.03 | 0.27 | 0.10 | 0.26 | -0.02 | 0.20 |
| **Tuk Killifish** | | | | | | | |
| TUK1_018 | 0.29 | -0.04 | 0.07 | -0.13 | 0.33 | 0.03 | 0.17 |
| TUK3_020 | 0.20 | -0.09 | 0.02 | -0.18 | 0.25 | 0.00 | 0.16 |
| TUK4_022 | 0.32 | -0.14 | -0.08 | -0.30 | 0.39 | 0.02 | 0.14 |
| TUK11_024 | 0.23 | -0.19 | -0.10 | -0.40 | 0.33 | 0.01 | 0.20 |
| **Tuk BSB** | | | | | | | |
| 1498_004 | 0.44 | -0.30 | -0.10 | -0.67 | 0.61 | 0.04 | 0.41 |
| D141_006 | 0.36 | -0.53 | -0.40 | -1.11 | 0.64 | 0.02 | 0.43 |
| 1088_008 | 0.45 | -0.45 | -0.27 | -1.01 | 0.71 | 0.05 | 0.49 |
| D011_010 | 0.53 | -0.14 | 0.14 | -0.39 | 0.63 | 0.06 | 0.44 |
| D0152_012 | 0.60 | -0.09 | 0.31 | -0.28 | 0.67 | 0.05 | 0.52 |
| **Downstream Adult** | | | | | | | |
| 2.1_004 | 0.24 | 0.07 | 0.30 | 0.18 | 0.20 | -0.02 | 0.16 |
| 2.2_006 | 0.27 | 0.10 | 0.30 | 0.19 | 0.22 | 0.01 | 0.16 |
| 2.4_004 | 0.27 | 0.11 | 0.28 | 0.20 | 0.22 | 0.01 | 0.13 |
| **HK Perch Upstream Adult** | | | | | | | |
| 3.6_014 | 0.39 | 0.17 | 0.43 | 0.31 | 0.31 | 0.01 | 0.20 |
| 5.1_022 | 0.31 | 0.14 | 0.42 | 0.33 | 0.22 | -0.02 | 0.17 |
| 6.3_026 | 0.27 | 0.16 | 0.35 | 0.28 | 0.20 | 0.02 | 0.14 |
| 6.5_030 | 0.31 | 0.15 | 0.39 | 0.32 | 0.23 | -0.01 | 0.15 |
| 5.6_034 | 0.28 | 0.20 | 0.36 | 0.32 | 0.20 | 0.04 | 0.12 |
| 3.1_008 | 0.25 | 0.08 | 0.27 | 0.12 | 0.22 | 0.02 | 0.18 |
| 3.3_012 | 0.29 | 0.10 | 0.33 | 0.18 | 0.25 | 0.01 | 0.20 |
| 3.5_016 | 0.21 | 0.14 | 0.33 | 0.26 | 0.15 | 0.01 | 0.14 |
| 4.9_020 | 0.34 | 0.03 | 0.30 | 0.05 | 0.33 | 0.00 | 0.26 |
| 5.2_026 | 0.24 | 0.10 | 0.26 | 0.19 | 0.19 | 0.00 | 0.12 |
| 5.4_030 | 0.32 | 0.13 | 0.37 | 0.21 | 0.26 | 0.03 | 0.21 |
| 5.5_034 | 0.25 | 0.15 | 0.32 | 0.26 | 0.19 | 0.02 | 0.13 |
| 6.6_038 | 0.35 | 0.16 | 0.41 | 0.27 | 0.28 | 0.03 | 0.21 |
| 6.7_042 | 0.33 | 0.14 | 0.34 | 0.22 | 0.28 | 0.03 | 0.18 |
| 6.8_046 | 0.30 | 0.18 | 0.37 | 0.32 | 0.22 | 0.02 | 0.13 |
| **HK Upstream Juev.** | | | | | | | |
| 4.6_018 | 0.305 | 0.136 | 0.371 | 0.283 | 0.234 | -0.006 | 0.159 |
| 4-5J_012 | 0.213 | 0.207 | 0.360 | 0.318 | 0.133 | 0.048 | 0.121 |
| 5-1J_016 | 0.255 | 0.228 | 0.359 | 0.442 | 0.143 | 0.007 | 0.027 |
| **HR Downstream Juev.** | | | | | | | |
| 1-41_004 | 0.47 | 0.23 | 0.55 | 0.56 | 0.33 | -0.05 | 0.13 |
| 1-32_014 | 0.30 | 0.14 | 0.45 | 0.36 | 0.21 | -0.04 | 0.18 |
| 1-25_020 | 0.32 | 0.17 | 0.44 | 0.35 | 0.23 | 0.00 | 0.18 |

**Table A4:** Hg Concentrations and Chemical Parameters for Sediment from the Hackensack River, NJ

| Season | Site | THg, μg $g^{-1}$ | 1 SD | MeHg ng $g^{-1}$ | 1 SD | Organic Matter, % | Organic Nitrogen, % | Organic Carbon, % | Acid Volatile Sulfide, μmol $g^{-1}$ | Temp, C° | pH | Sal |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| SP12 | HR-1 | 4.7 | 0.2 | 6.1 | 1.5 | 14.0 | 0.5 | 5.6 | 0.1 | 19.9 | 7.4 | 2.2 |
| | HR-2 | 6.6 | 0.4 | 7.7 | 0.9 | 14.1 | 0.4 | 5.7 | 1.7 | 19.5 | 7.3 | 3.7 |
| | HR-3 | 4.3 | 0.4 | 3.8 | 0.7 | 13.3 | 0.4 | 5.4 | BD | 19.4 | 7.3 | 5.9 |
| | BC | 6.4 | 0.4 | 5.0 | 0.0 | 12.7 | 0.3 | 5.2 | 4.7 | 19.3 | 7.3 | 7.4 |
| | HR-4 | 2.8 | 0.1 | 3.1 | 0.8 | 11.2 | 0.3 | 4.1 | 0.5 | 19.1 | 7.3 | 8.8 |
| | HR-5 | 1.5 | 0.2 | 1.3 | 0.3 | 7.7 | 0.2 | 2.7 | 6.2 | 19.2 | 7.4 | 13.1 |
| SU12 | HR-1 | 4.0 | 0.1 | 4.9 | 1.3 | 12.1 | 0.4 | 4.8 | 6.1 | 28.4 | 7.5 | 4.2 |
| | HR-2 | 4.6 | 0.1 | 16.2 | 0.7 | 13.8 | 0.4 | 5.5 | 14.6 | 28.3 | 7.7 | 5.8 |
| | HR-3 | 3.3 | 0.2 | 7.8 | 1.4 | 10.2 | 0.3 | 4.4 | 45.0 | 27.8 | 7.7 | 8.5 |
| | BC | 4.9 | 0.2 | 6.4 | 0.6 | 10.7 | 0.3 | 4.5 | 16.6 | 27.9 | 7.6 | 10.2 |
| | HR-4 | 2.7 | 0.1 | 4.5 | 0.5 | 8.6 | 0.2 | 3.8 | 27.9 | 28.1 | 7.5 | 12.0 |
| | HR-5 | 2.7 | 0.0 | 3.1 | 0.2 | 9.4 | 0.2 | 3.9 | 3.8 | 28.4 | 7.4 | 17.1 |
| FA12 | HR-1 | 4.8 | 0.3 | 6.3 | 0.6 | 14.0 | 0.4 | 5.1 | 15.7 | 15.5 | 7.4 | 4.5 |
| | HR-2 | 3.1 | 0.0 | 4.7 | 0.7 | 15.0 | 0.4 | 5.0 | 12.2 | 16.0 | 7.3 | 6.2 |
| | HR-3 | 3.4 | 0.1 | 9.7 | 1.6 | 11.1 | 0.3 | 4.7 | 15.3 | 15.6 | 7.3 | 8.8 |
| | BC | 5.0 | 0.0 | 8.5 | 0.9 | 9.0 | 0.2 | 3.6 | 39.4 | 15.6 | 7.4 | 10.1 |
| | HR-4 | 2.2 | 0.0 | 7.8 | 0.4 | 16.5 | 0.2 | 3.4 | 8.3 | 15.5 | 7.4 | 11.4 |
| | HR-5 | 2.4 | 0.1 | 4.2 | 0.4 | 10.9 | 0.2 | 3.4 | 51.4 | 15.7 | 7.5 | 14.3 |
| WI13 | HR-1 | 3.6 | 0.1 | 20.5 | 0.2 | 12.4 | 0.5 | 6.0 | 8.6 | 2.3 | 7.7 | 1.9 |
| | HR-2 | 4.2 | 0.3 | 3.6 | 1.3 | 11.4 | 0.4 | 5.2 | 31.8 | 3.9 | 7.5 | 2.8 |
| | HR-3 | 3.8 | 0.1 | 6.6 | 1.2 | 9.1 | 0.1 | 0.9 | 8.4 | 3.0 | 7.5 | 5.1 |
| | BC | 6.6 | 0.5 | 9.0 | 1.8 | 12.3 | 0.1 | 1.2 | 4.9 | 2.9 | 7.5 | 6.9 |
| | HR-4 | 4.0 | 0.1 | 8.8 | 1.2 | 10.3 | 0.1 | 1.1 | 15.6 | 2.9 | 7.6 | 8.7 |
| | HR-5 | 4.0 | 1.0 | 4.0 | 0.6 | 9.9 | 0.1 | 0.9 | 37.3 | 4.6 | 7.7 | 13.1 |
| SP13 | HR-1 | 3.8 | 0.3 | 34.1 | 1.4 | 13.5 | 0.4 | 4.1 | 20.8 | 19.8 | 7.4 | 3.6 |
| | HR-2 | 3.9 | 0.3 | 7.4 | 1.5 | 13.0 | 0.3 | 4.7 | 4.2 | 19.0 | 7.3 | 5.2 |
| | HR-3 | 3.4 | 0.4 | 5.3 | 0.2 | 11.9 | 0.3 | 4.4 | 10.6 | 18.8 | 7.5 | 7.9 |
| | BC | 4.5 | 0.1 | 8.7 | 0.5 | 12.9 | 0.4 | 5.5 | 8.1 | ND | ND | 9.1 |
| | HR-4 | 3.1 | 0.4 | 7.8 | 1.1 | 15.6 | 0.4 | 6.5 | 4.1 | 17.2 | 7.4 | 10.3 |
| | HR-5 | 2.0 | 0.1 | 2.2 | 0.1 | 8.1 | 0.2 | 3.5 | 6.6 | 16.1 | 7.5 | 13.6 |
| SU13 | HR-1 | 3.2 | 0.1 | 4.1 | 0.3 | 8.3 | 0.2 | 3.8 | 17.0 | 24.2 | 8.2 | 4.5 |
| | HR-2 | 3.0 | 0.2 | 4.8 | 0.6 | 12.2 | 0.4 | 5.4 | 19.8 | 23.5 | 8.1 | 6.3 |
| | HR-3 | 2.7 | 0.1 | 6.9 | 0.4 | 9.9 | 0.3 | 3.9 | 4.4 | 23.5 | 8.1 | 8.2 |
| | BC | 3.8 | 0.2 | 5.0 | 1.1 | 9.6 | 0.3 | 4.2 | 5.3 | 23.4 | 8.1 | 8.1 |
| | HR-4 | 1.9 | 0.1 | 3.2 | 0.7 | 8.1 | 0.2 | 3.3 | 6.5 | 23.6 | 8.0 | 11.1 |
| | HR-5 | 2.9 | 0.0 | 5.2 | 0.2 | 8.0 | 0.2 | 4.5 | 5.8 | 23.7 | 7.9 | 14.1 |
| | HR-6 | 1.4 | 0.1 | 0.9 | 0.1 | 6.8 | 0.2 | 4.0 | 4.4 | 24.9 | 7.8 | 14.9 |
| FA13 | HR-1 | 5.8 | 0.1 | 6.4 | 1.3 | 12.2 | 0.5 | 5.7 | 9.5 | 8.4 | 7.5 | 7.6 |
| | HR-2 | 5.3 | 0.3 | 7.0 | 1.0 | 12.3 | 0.4 | 5.4 | 5.1 | 8.4 | 7.5 | 8.9 |
| | HR-3 | 5.2 | 0.1 | 5.7 | 0.9 | 10.2 | 0.3 | 4.1 | 3.0 | 8.2 | 7.6 | 13.7 |
| | BC | 5.1 | 0.6 | 5.7 | 0.6 | 9.4 | 0.3 | 4.2 | 6.3 | ND | ND | 15.0 |
| | HR-4 | 3.6 | 0.2 | 4.6 | 0.7 | 9.6 | 0.3 | 4.2 | 5.3 | 8.2 | 7.7 | 16.4 |
| | HR-5 | 4.5 | 0.2 | 3.1 | 0.6 | 9.3 | 0.2 | 4.6 | 5.9 | 8.9 | 7.7 | 19.9 |
| WI14 | HR-1 | 4.4 | 0.1 | 12.3 | 0.3 | 12.2 | 0.4 | 4.6 | ND | 3.9 | 7.4 | 3.9 |
| | HR-2 | 4.2 | 0.1 | 6.6 | 0.3 | 11.4 | 0.3 | 5.0 | ND | 2.5 | 7.5 | 5.5 |
| | HR-3 | 3.2 | 0.0 | 4.8 | 0.6 | 8.8 | 0.2 | 3.9 | ND | 1.0 | 7.5 | 8.6 |
| | BC | 4.3 | 0.0 | 4.7 | 0.9 | 9.4 | 0.3 | 4.0 | ND | ND | ND | 10.0 |
| | HR-4 | 2.6 | 0.1 | 4.2 | 0.5 | 9.3 | 0.2 | 3.9 | ND | 2.1 | 7.6 | 11.4 |
| | HR-5 | 2.6 | 0.1 | 2.4 | 0.2 | 9.1 | 0.2 | 4.1 | ND | 1.9 | 7.7 | 15.4 |

**Table A5:** Isotopic Compositions for THg in Sediments from the Hackensack River, NJ

| ID | Season | δ199 | δ200 | δ201 | δ202 | Δ199 | Δ200 | Δ201 |
|---|---|---|---|---|---|---|---|---|
| BC West Riser 1 | SU2013 | -0.09 | -0.15 | -0.24 | -0.32 | -0.01 | 0.01 | 0.00 |
| BC West Riser 2 | SU2013 | -0.06 | -0.12 | -0.24 | -0.28 | 0.01 | 0.02 | -0.03 |
| Tuckerton | SU2013 | 0.00 | -0.08 | -0.17 | -0.23 | 0.06 | 0.04 | 0.00 |
| Passaic 1 | SU2013 | -0.07 | -0.13 | -0.25 | -0.28 | 0.00 | 0.01 | -0.04 |
| HK 6 Bay | SU2013 | -0.15 | -0.23 | -0.34 | -0.42 | -0.04 | -0.02 | -0.02 |
| HR1 | WI2014 | -0.09 | -0.15 | -0.27 | -0.33 | -0.01 | 0.02 | -0.02 |
| HR2 | WI2014 | -0.07 | -0.14 | -0.23 | -0.30 | 0.00 | 0.01 | -0.01 |
| HR3 | WI2014 | -0.12 | -0.22 | -0.34 | -0.44 | -0.01 | -0.01 | -0.01 |
| HR4 | WI2014 | -0.13 | -0.22 | -0.33 | -0.42 | -0.02 | 0.00 | -0.02 |
| HR5 | WI2014 | -0.16 | -0.26 | -0.40 | -0.51 | -0.03 | 0.00 | -0.02 |
| HR3 (BC Con) | WI2014 | -0.13 | -0.22 | -0.30 | -0.42 | -0.03 | -0.02 | 0.02 |
| HR1 | SU2013 | -0.08 | -0.19 | -0.29 | -0.40 | 0.02 | 0.02 | 0.01 |
| HR2 | SU2013 | -0.07 | -0.15 | -0.26 | -0.33 | 0.02 | 0.02 | -0.01 |
| HR3 | SU2013 | -0.07 | -0.17 | -0.26 | -0.33 | 0.02 | 0.00 | -0.01 |
| HR4 | SU2013 | -0.10 | -0.20 | -0.30 | -0.41 | 0.00 | 0.01 | 0.01 |
| HR5 | SU2013 | -0.13 | -0.25 | -0.39 | -0.45 | -0.02 | -0.02 | -0.05 |
| HR3 (BC Con) | SU2013 | -0.11 | -0.19 | -0.28 | -0.35 | -0.03 | -0.01 | -0.02 |
| HR1 | SP2013 | -0.10 | -0.18 | -0.33 | -0.39 | 0.00 | 0.02 | -0.04 |
| HR2 | SP2013 | -0.09 | -0.18 | -0.29 | -0.35 | 0.00 | 0.00 | -0.03 |
| HR3 | SP2013 | -0.10 | -0.21 | -0.32 | -0.38 | 0.00 | -0.02 | -0.03 |
| HR4 | SP2013 | -0.07 | -0.20 | -0.32 | -0.43 | 0.03 | 0.02 | 0.00 |
| HR5 | SP2013 | -0.11 | -0.21 | -0.35 | -0.45 | 0.00 | 0.01 | -0.01 |
| HR3 (BC Con) | SP2013 | -0.09 | -0.18 | -0.29 | -0.35 | 0.00 | -0.01 | -0.03 |
| HR1 | FA2013 | -0.09 | -0.16 | -0.25 | -0.33 | 0.00 | 0.01 | 0.00 |
| HR2 | FA2013 | -0.12 | -0.19 | -0.32 | -0.38 | -0.02 | 0.00 | -0.03 |
| HR3 | FA2013 | -0.07 | -0.17 | -0.27 | -0.34 | 0.01 | 0.00 | -0.01 |
| HR4 | FA2013 | -0.10 | -0.21 | -0.37 | -0.43 | 0.01 | 0.01 | -0.05 |
| HR5 | FA2013 | -0.11 | -0.24 | -0.37 | -0.49 | 0.01 | 0.00 | -0.01 |
| HR3 (BC Con) | FA2013 | -0.15 | -0.22 | -0.35 | -0.42 | -0.05 | -0.01 | -0.03 |
| HR1 | WI2013 | -0.13 | -0.19 | -0.31 | -0.39 | -0.03 | 0.01 | -0.02 |
| HR2 | WI2013 | -0.13 | -0.25 | -0.41 | -0.48 | -0.01 | -0.01 | -0.05 |
| HR3 | WI2013 | -0.13 | -0.26 | -0.37 | -0.50 | 0.00 | -0.01 | 0.00 |
| HR4 | WI2013 | -0.12 | -0.21 | -0.33 | -0.48 | 0.00 | 0.03 | 0.03 |
| HR5 | WI2013 | -0.13 | -0.28 | -0.38 | -0.48 | -0.01 | -0.03 | -0.01 |
| HR3 (BC Con) | WI2013 | -0.09 | -0.19 | -0.29 | -0.34 | -0.01 | -0.02 | -0.03 |
| HR1 | SU2012 | -0.13 | -0.27 | -0.38 | -0.37 | -0.04 | -0.09 | -0.11 |
| HR2 | SU2012 | -0.09 | -0.18 | -0.27 | -0.36 | 0.00 | 0.00 | 0.01 |
| HR3 | SU2012 | -0.08 | -0.12 | -0.27 | -0.27 | -0.01 | 0.02 | -0.07 |
| HR4 | SU2012 | -0.09 | -0.15 | -0.24 | -0.27 | -0.02 | -0.01 | -0.03 |
| HR5 | SU2012 | -0.08 | -0.26 | -0.45 | -0.51 | 0.05 | 0.00 | -0.07 |
| HR3 (BC Con) | SU2012 | -0.09 | -0.15 | -0.29 | -0.33 | 0.00 | 0.01 | -0.04 |
| HR1 | SP2012 | -0.13 | -0.21 | -0.34 | -0.42 | -0.03 | 0.00 | -0.03 |
| HR2 | SP2012 | -0.08 | -0.16 | -0.29 | -0.40 | 0.02 | 0.04 | 0.01 |
| HR3 | SP2012 | -0.09 | -0.15 | -0.31 | -0.31 | -0.01 | 0.00 | -0.08 |
| HR4 | SP2012 | -0.06 | -0.14 | -0.33 | -0.36 | 0.03 | 0.04 | -0.06 |
| HR5 | SP2012 | -0.09 | -0.23 | -0.34 | -0.46 | 0.02 | 0.00 | 0.01 |
| HR3 (BC Con) | SP2012 | -0.12 | -0.19 | -0.35 | -0.39 | -0.02 | 0.00 | -0.05 |
| HR1 | FA2012 | -0.04 | -0.05 | -0.16 | -0.23 | 0.02 | 0.06 | 0.01 |
| HR2 | FA2012 | -0.12 | -0.18 | -0.34 | -0.36 | -0.02 | 0.00 | -0.07 |
| HR3 | FA2012 | -0.10 | -0.19 | -0.31 | -0.35 | -0.01 | -0.01 | -0.05 |
| HR4 | FA2012 | -0.10 | -0.22 | -0.35 | -0.43 | 0.01 | -0.01 | -0.03 |
| HR5 | FA2012 | -0.15 | -0.27 | -0.46 | -0.57 | -0.01 | 0.02 | -0.03 |
| HR3 (BC Con) | FA2012 | 0.06 | -0.08 | -0.15 | -0.21 | 0.11 | 0.02 | 0.01 |

**Table A6:** Isotopic Compositions for THg of Particulate Matter from the Hackensack River, NJ

**Particulate Matter**

| Tag ID | δ199 | δ200 | δ201 | δ202 | Δ199 | Δ200 | Δ201 |
|---|---|---|---|---|---|---|---|
| HK 1 | -0.12 | -0.17 | -0.30 | -0.34 | -0.03 | 0.00 | -0.05 |
| HK 2 | -0.08 | -0.10 | -0.22 | -0.27 | -0.01 | 0.04 | -0.01 |
| HK 3 | -0.05 | -0.07 | -0.17 | -0.24 | 0.01 | 0.05 | 0.01 |
| HK 4 | -0.05 | -0.09 | -0.17 | -0.23 | 0.01 | 0.03 | 0.01 |
| HK 5 | -0.09 | -0.19 | -0.33 | -0.39 | 0.01 | 0.00 | -0.04 |
| HK BC | 0.00 | -0.10 | -0.16 | -0.21 | 0.05 | 0.01 | 0.00 |

## APPENDIX B: Hg Methylation Tables

**Table B1:** Mercury stable isotope ratios for a MeHg stock solution (Brooks Rand methylmercury chloride) analyzed as total Hg and as separated MeHg, and the Hg isotope reference standards ERM CC580 and UM Almáden.

| MeHg Stock Standard (analyzed as THg) | | | | | | | |
|---|---|---|---|---|---|---|---|
| | $\delta^{199}Hg$ | $\delta^{200}Hg$ | $\delta^{201}Hg$ | $\delta^{202}Hg$ | $\Delta^{199}Hg$ | $\Delta^{200}Hg$ | $\Delta^{201}Hg$ |
| | -0.11 | -0.46 | -0.67 | -0.99 | 0.14 | 0.04 | 0.08 |
| | -0.17 | -0.54 | -0.76 | -1.04 | 0.09 | -0.01 | 0.03 |
| | -0.18 | -0.48 | -0.68 | -0.95 | 0.06 | -0.01 | 0.03 |
| | -0.13 | -0.42 | -0.69 | -0.9 | 0.1 | 0.03 | -0.01 |
| AVE | -0.14 | -0.48 | -0.7 | **-0.97** | 0.1 | 0.01 | 0.03 |
| 1SD | 0.04 | 0.05 | 0.04 | **0.06** | 0.03 | 0.03 | 0.04 |
| MeHg Trap Standards (analyzed as separated MeHg) | | | | | | | |
| | $\delta^{199}Hg$ | $\delta^{200}Hg$ | $\delta^{201}Hg$ | $\delta^{202}Hg$ | $\Delta^{199}Hg$ | $\Delta^{200}Hg$ | $\Delta^{201}Hg$ |
| | -0.21 | -0.45 | -0.71 | -0.92 | 0.02 | 0.01 | -0.02 |
| | -0.12 | -0.42 | -0.66 | -0.86 | 0.09 | 0.01 | -0.02 |
| | -0.13 | -0.34 | -0.65 | -0.93 | 0.11 | 0.12 | 0.05 |
| | -0.26 | -0.51 | -0.86 | -1.12 | 0.02 | 0.05 | -0.02 |
| | -0.18 | -0.26 | -0.45 | -0.71 | 0 | 0.1 | 0.08 |
| | -0.17 | -0.41 | -0.66 | -0.96 | 0.07 | 0.07 | 0.07 |
| | -0.19 | -0.57 | -0.78 | -1.08 | 0.08 | -0.02 | 0.03 |
| | -0.15 | -0.48 | -0.7 | -0.95 | 0.08 | 0 | 0.02 |
| AVE | -0.18 | -0.43 | -0.68 | **-0.94** | 0.06 | 0.04 | 0.02 |
| 1SD | 0.05 | 0.1 | 0.12 | **0.13** | 0.04 | 0.05 | 0.04 |
| ERM CC580 | | | | | | | |
| | $\delta^{199}Hg$ | $\delta^{200}Hg$ | $\delta^{201}Hg$ | $\delta^{202}Hg$ | $\Delta^{199}Hg$ | $\Delta^{200}Hg$ | $\Delta^{201}Hg$ |
| | -0.13 | -0.28 | -0.48 | -0.53 | 0 | -0.01 | -0.08 |
| | -0.14 | -0.26 | -0.42 | -0.53 | 0 | 0.01 | -0.02 |
| | -0.1 | -0.14 | -0.35 | -0.4 | 0 | 0.07 | -0.05 |
| | -0.13 | -0.21 | -0.39 | -0.48 | -0.01 | 0.03 | -0.02 |
| | -0.08 | -0.19 | -0.33 | -0.44 | 0.03 | 0.03 | 0 |
| AVE | -0.12 | -0.22 | -0.39 | **-0.48** | 0 | 0.02 | -0.03 |
| 1SD | 0.03 | 0.06 | 0.06 | **0.06** | 0.02 | 0.03 | 0.03 |
| UM Almáden | | | | | | | |
| | $\delta^{199}Hg$ | $\delta^{200}Hg$ | $\delta^{201}Hg$ | $\delta^{202}Hg$ | $\Delta^{199}Hg$ | $\Delta^{200}Hg$ | $\Delta^{201}Hg$ |
| | -0.16 | -0.26 | -0.45 | -0.52 | -0.03 | 0 | -0.06 |
| | -0.11 | -0.24 | -0.4 | -0.48 | 0.01 | 0.01 | -0.04 |
| | -0.15 | -0.28 | -0.5 | -0.53 | -0.02 | -0.01 | -0.09 |
| | -0.19 | -0.31 | -0.45 | -0.57 | -0.05 | -0.02 | -0.02 |
| | -0.14 | -0.27 | -0.47 | -0.59 | 0.01 | 0.02 | -0.03 |
| | -0.12 | -0.25 | -0.41 | -0.56 | 0.02 | 0.03 | 0.01 |
| | -0.17 | -0.29 | -0.48 | -0.6 | -0.02 | 0.01 | -0.04 |
| | -0.17 | -0.29 | -0.48 | -0.6 | -0.02 | 0.01 | -0.04 |
| AVE | -0.15 | -0.27 | -0.46 | **-0.56** | -0.01 | 0.01 | -0.04 |
| 1SD | 0.03 | 0.02 | 0.03 | **0.04** | 0.02 | 0.02 | 0.03 |

**Table B2:** Concentrations and mercury isotope ratios of MeHg and total Hg in incubations of *Geobacter sulfurreducens* PCA. Each line corresponds to an individual assay bottle.

***Geobacter sulfurreducens* PCA**

| | Concentrations | | | | MeHg Isotopes | | | | | | | THg Isotopes | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time, hr | % MeHg | MeHg, nM | THg, nM | $\delta^{202}Hg$ | $\delta^{201}Hg$ | $\delta^{200}Hg$ | $\delta^{199}Hg$ | $\Delta^{201}Hg$ | $\Delta^{200}Hg$ | $\Delta^{199}Hg$ | $\delta^{202}Hg$ | $\delta^{201}Hg$ | $\delta^{200}Hg$ | $\delta^{199}Hg$ | $\Delta^{201}Hg$ | $\Delta^{200}Hg$ | $\Delta^{199}Hg$ |
| Exp 1 | 1 | 3.9 | 1.3 | 33.1 | -0.5 | -0.32 | -0.19 | -0.1 | 0.05 | 0.06 | 0.02 | 0.1 | 0.07 | 0.04 | 0.07 | 0 | -0.01 | 0.04 |
| | 3 | 9.1 | 3.7 | 40.2 | -0.31 | -0.17 | -0.11 | 0 | 0.06 | 0.04 | 0.07 | 0.1 | 0.1 | 0.06 | 0.03 | 0.02 | 0.02 | 0.01 |
| | 4 | 11.5 | 4.9 | 42.8 | -0.18 | -0.12 | -0.05 | 0.09 | 0.02 | 0.04 | 0.13 | -0.13 | -0.11 | -0.09 | -0.04 | -0.01 | -0.03 | 0 |
| Exp 2 | 1 | 10.1 | 4.3 | 42 | -0.42 | -0.31 | -0.2 | -0.1 | 0.01 | 0.01 | 0.01 | 0.02 | 0.04 | -0.02 | 0.22 | 0.02 | -0.03 | 0.22 |
| | 2 | 15.4 | 6.9 | 44.5 | -0.19 | -0.15 | -0.04 | -0.01 | -0.01 | 0.05 | 0.04 | -0.13 | 0.07 | -0.18 | 0.05 | 0.16 | -0.12 | 0.08 |
| | 3 | 15.9 | 6.5 | 40.6 | -0.22 | -0.17 | -0.09 | -0.04 | -0.01 | 0.02 | 0.01 | -0.07 | 0.21 | 0.01 | 0.03 | 0.26 | 0.05 | 0.05 |
| | 4 | 17 | 7.4 | 43.4 | -0.18 | -0.12 | -0.03 | 0.08 | 0.02 | 0.06 | 0.12 | -0.01 | 0.06 | 0.05 | 0.19 | 0.06 | 0.05 | 0.19 |
| Exp 3 | 1 | 8.2 | 5.5 | 66.7 | -0.35 | -0.19 | -0.11 | 0.02 | 0.07 | 0.07 | 0.11 | -0.04 | 0.02 | -0.04 | 0.04 | 0.05 | -0.02 | 0.05 |
| | 2 | 11.1 | 7.2 | 65.1 | -0.18 | -0.06 | 0 | 0.02 | 0.08 | 0.09 | 0.07 | -0.01 | -0.09 | -0.15 | -0.16 | -0.08 | -0.14 | -0.16 |
| | 3 | 17 | 10.1 | 59.5 | -0.24 | -0.19 | -0.01 | -0.15 | 0 | 0.11 | -0.09 | 0.08 | -0.04 | -0.13 | -0.05 | -0.1 | -0.17 | -0.07 |
| | 4 | 23.2 | 13.8 | 59.4 | 0.08 | 0.03 | 0.09 | 0.08 | -0.03 | 0.05 | 0.06 | -0.09 | -0.11 | 0.06 | -0.11 | -0.04 | 0.1 | -0.09 |
| | 12 | 31 | 18.3 | 59 | 0.21 | 0.2 | 0.21 | 0.1 | 0.04 | 0.11 | 0.05 | 0.04 | -0.03 | 0.21 | -0.26 | -0.07 | 0.19 | -0.27 |
| | 24 | 32.2 | 18.8 | 58.5 | 0.22 | 0.18 | 0.04 | 0 | 0.02 | -0.07 | -0.05 | 0.09 | 0.19 | 0.14 | 0.02 | 0.12 | 0.09 | 0 |
| | 48 | 35.8 | 18.1 | 50.7 | 0.38 | 0.3 | 0.28 | 0.14 | 0.01 | 0.1 | 0.05 | 0.05 | 0.12 | 0.04 | 0.02 | 0.08 | 0.02 | 0.01 |

**Table B3:** Concentrations and mercury isotope ratios of MeHg and total Hg in incubations of *Desulfovibrio desulfuricans* ND132. Each line corresponds to an individual assay bottle.

***Desulfovibrio desulfuricans ND132***

| | Concentrations | | | | MeHg Isotopes | | | | | | | THg Isotopes | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time, hr | % MeHg | MeHg, nM | THg, nM | $\delta^{202}Hg$ | $\delta^{201}Hg$ | $\delta^{200}Hg$ | $\delta^{199}Hg$ | $\Delta^{201}Hg$ | $\Delta^{200}Hg$ | $\Delta^{199}Hg$ | $\delta^{202}Hg$ | $\delta^{201}Hg$ | $\delta^{200}Hg$ | $\delta^{199}Hg$ | $\Delta^{201}Hg$ | $\Delta^{200}Hg$ | $\Delta^{199}Hg$ |
| | 0.5 | 6 | 2.47 | 41.43 | -0.6 | -0.43 | -0.19 | -0.16 | 0.02 | 0.12 | 0 | 0 | 0.15 | 0.07 | 0.03 | 0.14 | 0.07 | 0.02 |
| | 1 | 18.6 | 8.26 | 44.47 | -0.22 | -0.13 | -0.04 | -0.06 | 0.03 | 0.07 | 0 | 0.01 | 0.15 | 0.02 | 0.01 | 0.14 | 0.02 | 0.01 |
| Exp 1 | 2 | 34 | 14.69 | 43.17 | 0.35 | 0.25 | 0.22 | 0.09 | -0.01 | 0.05 | 0 | 0.08 | 0.11 | 0.08 | 0.08 | 0.04 | 0.04 | 0.06 |
| | 3 | 47.1 | 21.66 | 46.01 | 0.3 | 0.24 | 0.16 | 0.1 | 0.02 | 0.01 | 0.03 | 0.17 | 0.22 | 0.07 | 0.03 | 0.09 | -0.02 | -0.01 |
| | 4 | 51 | 23.67 | 46.36 | 0.11 | 0.11 | -0.03 | -0.09 | 0.03 | -0.08 | -0.12 | 0.03 | 0.14 | 0.04 | -0.01 | 0.11 | 0.03 | -0.02 |
| | 0.5 | 8.3 | 3.34 | 39.98 | -0.66 | -0.46 | -0.18 | -0.03 | 0.04 | 0.15 | 0.14 | -0.02 | 0.12 | 0.03 | 0.02 | 0.13 | 0.04 | 0.02 |
| Exp 2 | 2 | 18.8 | 8.17 | 43.42 | -0.13 | -0.19 | 0.03 | 0.03 | -0.09 | 0.1 | 0.06 | 0.01 | 0.11 | 0.08 | -0.02 | 0.1 | 0.08 | -0.02 |
| | 4 | 49.6 | 19.66 | 39.63 | -0.05 | -0.1 | -0.04 | -0.04 | -0.07 | -0.01 | -0.02 | 0.06 | 0.02 | -0.01 | 0 | -0.02 | -0.03 | -0.01 |
| | 1 | 9 | 5.58 | 62.07 | -0.69 | -0.46 | -0.31 | -0.18 | 0.06 | 0.04 | 0 | -0.03 | 0.02 | 0 | 0.01 | 0.05 | 0.01 | 0.02 |
| | 2 | 23.1 | 14.54 | 62.81 | -0.08 | -0.07 | 0 | -0.02 | -0.01 | 0.04 | 0 | -0.02 | 0 | 0 | -0.03 | 0.01 | 0.01 | -0.03 |
| Exp 3 | 3 | 31.3 | 15.58 | 49.7 | 0.03 | 0 | 0.03 | 0.03 | -0.02 | 0.02 | 0.02 | -0.02 | -0.02 | -0.02 | -0.02 | -0.01 | -0.01 | -0.01 |
| | 4 | 33.2 | 16.88 | 50.85 | 0.13 | 0.05 | 0.07 | 0 | -0.04 | 0.01 | -0.03 | -0.2 | -0.15 | -0.11 | -0.05 | -0.01 | -0.02 | 0 |
| | 6 | 46 | 23.76 | 51.7 | 0.31 | 0.24 | 0.21 | 0.1 | 0.01 | 0.06 | 0.03 | 0.05 | 0.11 | 0.01 | -0.04 | 0.07 | -0.02 | -0.05 |
| | 24 | 58.6 | 38.58 | 65.81 | -0.05 | -0.02 | -0.02 | 0.05 | 0.02 | 0.01 | 0.06 | 0.05 | 0.03 | 0.05 | 0.02 | -0.01 | 0.03 | 0 |

**Table B4:** Estimates of mercury isotope enrichment and fractionation factors for bacterial Hg methylation based on fits of $\delta^{202}$Hg values for microbially produced MeHg and the fractions of remaining bioreactive (see text) Hg(II) to linear (Eq.1) and non-linear (Rayleigh distillation) fractionation models. The non-linear model for the isotopic composition of accumulated product is $\delta_P = \delta i - \varepsilon p/r\ f_r \ln(f_r)/(1-f_r)$ (Mariotti et al. 1981) in which all terms are the same as those defined for Eq. 1. Reactant/product fractionation factors ($\alpha_{r/p}$) were calculated as $\alpha_{r/p}) = -\varepsilon p/r/10^3 + 1$.

| Closed, Reversible | | Closed, Irreversible | |
|---|---|---|---|
| f | MeHg d202 | [f/(1-f)]*ln(f) | MeHg d202 |
| 0.89 | -0.50 | -0.94 | -0.50 |
| 0.77 | -0.35 | -0.87 | -0.35 |
| 0.74 | -0.31 | -0.86 | -0.31 |
| 0.72 | -0.42 | -0.84 | -0.42 |
| 0.69 | -0.18 | -0.83 | -0.18 |
| 0.68 | -0.18 | -0.82 | -0.18 |
| 0.57 | -0.19 | -0.74 | -0.19 |
| 0.55 | -0.22 | -0.73 | -0.22 |
| 0.52 | -0.24 | -0.71 | -0.24 |
| 0.52 | -0.18 | -0.71 | -0.18 |
| 0.35 | 0.08 | -0.56 | 0.08 |
| 0.13 | 0.21 | -0.31 | 0.21 |
| 0.10 | 0.22 | -0.25 | 0.22 |
| 0.00 | 0.38 | 0.00 | 0.38 |
| | | | |
| Slope ($\varepsilon_{r/p}$): | -0.91 | Slope ($\varepsilon_{p/r}$): | 0.89 |
| Intercept: | 0.34 | Intercept: | 0.45 |
| RSQ: | 0.94 | RSQ: | 0.91 |
| | | | |
| $\alpha_{r/p}$ | 0.9991 | $\alpha_{p/r}$ | 1.00089 |

| | |
|---|---|
| $\alpha_{p/r}$ | 1.00091 |

| | *G. sulfurreducens* **PCA** | | *D. desulfuricans* **ND132** | |
|---|---|---|---|---|
| | Closed Reversible | Closed Irreversible | Closed Reversible | Closed Irreversible |
| Slope ($\varepsilon_{p/r}$): | -0.918 | -0.8868 | -1.1070 | -1.0036 |
| Intercept: | 0.342 | 0.4474 | 0.2366 | 0.3217 |
| RSQ: | 0.9365 | 0.9145 | 0.9293 | 0.8831 |
| **$\alpha_{r/p}$** | **1.0009** | **1.0009** | **1.0011** | **1.0010** |

**REFERENCE**

Marioti, A., Germon, J.C., Hubert, P., Kaiser, P., Letolle, R., Tardieux, A., Tardieux, P., 1981. Experimental determination of nitrogen kinetic isotope fractionation: some principles; illustrations for the denitrification and nitrification processes. Plant and Soil 62, 413–430.

## APPENDIX C: Instrument Parameters

**Table C1:** MC-ICP-MS Operation Parameters and Cup Configuration

| Plasma Parameters | |
|---|---|
| Sample Gas | 0.925-0.928 L/min |
| Add Gas | 0.24-0.28 L/min |
| RF Power | 1200 W |
| **Aridus II Nebulizer** | |
| Sweep Gas Flow | 6.3-6.8 mL/min |
| Spray Chamber | 110 C |
| Desolvator | 160 C |
| **HGX-200 Hydride Generation System** | |
| Solution Uptake Rate | 0.70 mL/min |
| Pump Speed | 13 rpm |

| Cup Configuration | | | | | | |
|---|---|---|---|---|---|---|
| **L3** | **L2** | **L1** | **C** | **H1** | **H2** | **H3** |
| $^{198}Hg$ | $^{199}Hg$ | $^{200}Hg$ | $^{201}Hg$ | $^{202}Hg$ | $^{203}Tl$ | $^{205}Tl$ |

**Table C2:** Temperature controller program for offline Hg desorption.

| Segment | Starting Temp, °C | End Temp, °C | Time, min |
|---|---|---|---|
| 1 | 23 | 100 | 10 |
| 2 | 100 | 125 | 10 |
| 3 | 125 | 150 | 20 |
| 4 | 150 | 175 | 30 |
| 5 | 175 | 200 | 50 |
| 6 | 200 | 225 | 40 |
| 7 | 225 | 250 | 20 |

| | | | |
|---|---|---|---|
| 8 | 250 | 275 | 10 |
| 9 | 275 | 350 | 10 |
| 10 | 350 | 450 | 10 |
| 11 | 450 | 550 | 5 |