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Methylmercury Fate in the Hypersaline Environment of the Great Salt Lake: A Critical Review of Current Knowledge

Danielle Barandiaran

Utah State University

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METHYLMERCURY FATE IN THE HYPERSALINE ENVIRONMENT OF THE GREAT SALT LAKE: A CRITICAL REVIEW OF CURRENT KNOWLEDGE

By

Danielle Barandiaran

A paper submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

Soil Science

Approved:

______________________________        ______________________________
Astrid Jacobson                Jeanette Norton
Major Professor               Committee Member

______________________________        ______________________________
Paul Grossl                    Teryl Roper
Committee Member              Department Head

UTAH STATE UNIVERSITY
Logan, Utah

2013
ABSTRACT

Methylmercury Fate in the Hypersaline Environment of the Great Salt Lake: A Critical Review of Current Knowledge

by

Danielle Barandiaran, Master of Science
Utah State University, 2013

Major Professor: Dr. Astrid R. Jacobson
Department: Plants, Soils & Climate

Methylmercury (MeHg) is a highly potent neurotoxic form of the environmental pollutant Mercury (Hg). The processes that are responsible for the conversion of Hg to MeHg are known to be both biotic and abiotic in freshwater systems. Although MeHg contamination is well documented in Great Salt Lake (GSL), the conversion of Hg into MeHg is not well-understood in saline environments much less in hypersaline waters such as GSL. The GSL is a broad, shallow high altitude (1280 m above sea level) lake that is exposed to large amounts of ultraviolet radiation and evaporation, which lead to great volatilization losses of Hg to the atmosphere that may in turn contaminate other bodies of water. In this review biotic and abiotic Hg methylation pathways that are known to occur in marine environments, are investigated to identify the most likely causes for the high amounts of MeHg present in GSL.
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# CONTENTS

**ABSTRACT** ................................................................................................................................. iii

**ACKNOWLEDGMENTS** .................................................................................................................. iv

**LIST OF FIGURES** ......................................................................................................................... vi

**BACKGROUND** ............................................................................................................................. 1

**LITERATURE REVIEW** ................................................................................................................... 4

  - Mercury in the Environment ............................................................................................................... 4
  - Anthropogenic Sources of Hg in the Environment .............................................................................. 5
  - Methyl-mercury Toxicity .................................................................................................................. 7
  - Biogeochemistry of Hg in Aqueous Environments ........................................................................... 10
  - Formation and Fate of $\text{CH}_3\text{Hg}^+$ in the Saline GSL .................................................................... 12
  - Microbial Adaptions to Hg Contaminated Environments .................................................................... 18
  - Challenges to Microbial Hg Methylation/Demethylation Mechanisms in the GSL ....................... 20

**MICROCOSM STUDY** ..................................................................................................................... 23

  - Introduction .................................................................................................................................... 23
  - Materials and Methods ..................................................................................................................... 23
  - Results and Discussion ..................................................................................................................... 26

**SUMMARY AND CONCLUSION** ..................................................................................................... 30

**REFERENCES** ................................................................................................................................. 31
LIST OF FIGURES

Figure 1: Hg concentrations from ice cores extracted from the Upper Fremont Glacier, Wyoming (the data are from Schuster et al. 2002). .................................................................6

Figure 2: Possible fate of Hg in GSL ........................................................................................................16

Figure 3: The distribution of microbial genera at three sites in the GSL. Sulfate reducers

(Deltaproteobacteria, Desulfotomaculum, and Thermodesulfobacteria) are highlighted. The arrow indicates a group of sulfate-reducers in Farmington Bay that are not found in any other part of the lake (Weimer et al., 2008). ........................................21

Figure 4: Salinity in percentage in the south arm of the lake and fraction of total Hg that occurs as CH$_3$Hg$. Total Hg ranges from 6-100 ng L$^{-1}$ (Naftz et al., 2008) .........................22

Figure 5: GSL sampling site marked with orange arrow. .................................................................23

Figure 6: Total microcosm Hg relative to initially added Hg over time. Error bars represent one standard deviation of the mean of triplicate measurements. .........................................................26

Figure 7: Changes in the CH$_3$Hg$^+$/THg ratio in the microcosm solution phases plotted over time. The error bars represent 12% standard error. .........................................................28
METHYLMERCURY FATE IN THE HYPERSALINE ENVIRONMENT OF THE GREAT SALT LAKE: A CRITICAL REVIEW OF CURRENT KNOWLEDGE

BACKGROUND

The Great Salt Lake (GSL) is a terminal inland lake located at the bottom of the Great Basin, UT. It is the remainder of the late Pleistocene Lake Bonneville and is the 4th largest terminal lake, as well as the 2nd most saline lake on the Earth. The lake has no outlet to the ocean and is extremely shallow, with a maximum depth of just 10 meters and a surface area of 4480 km². Likewise, the morphology of the Great Basin is broad and flat and since the climate of the area is arid, this leads to evaporation and great fluctuations in the water levels of the lake as well as an ever-increasing accumulation of salts and minerals (Haul & Langford, 1964).

The lake can actually be thought of as 2 separate bodies of water because it is divided by a railroad causeway constructed in 1959 that greatly impacted the salt concentrations between the two sections (Stephens 1990). The southern part is 3 times more saline than the oceans while the northern part of the lake is over 10 times more saline (USGS, 2006). Furthermore, the causeway stopped the once normal mixing of the south arm water creating a monimolimnion, commonly referred to as the deep brine layer (DBL), which is also anoxic (Naftz, 2008). The semi-permeable causeway allows for water to move bi-directionally from the north to the south and vice versa but because more than 90% of GSL’s freshwater inputs are found to the south of the railroad causeway and the DBL flows easily through openings in the causeway, the southern portion of the lake is far less saline than the northern portion.
GSL gets a majority of its water inflow from 3 rivers, the Bear River, the Weber River and the Jordan River. These rivers start out in the highlands with water chemistry dominated by bicarbonate minerals before entering the basins and picking up other minerals such as chlorides and sulfates from various industrial and farming uses (Haul & Langford, 1964). Despite the previous sampling reports there is still relatively little known about the full composition and anthropogenic influences of the minerals that are deposited into the lake (Naftz et al., 2008). There are, however, data that link the combustion of coal to an increase in atmospheric deposition of Hg and since the 19th century, there has been an increase in atmospheric deposition of Hg seen in sediment cores (Beijer et al., 1979). In the 1860s coal and other minerals were discovered by early Mormon pioneers just 40 km away from Salt Lake City and mining operations began soon thereafter (Gwynn, 2002). Smelting of gold, silver, copper, zinc and lead consumed the majority of the coal that was mined in the 1860’s, however, as mineral mining and smelting became less cost effective than the burning of coal for energy the demand from energy companies for the low-sulfur coal increased. By the 1970’s and 1980’s energy companies had bought several of Utah’s coal mines for their own purposes.

The United States Environmental Protection Agency (EPA) Clean Air Act, passed in 1990, put Hg on a list of toxic pollutants that is to be controlled to the greatest possible extent and mandated that Hg emissions be studied; prior to the Clean Air Act, there were no laws or regulations on Hg emissions coming from industries such as coal-fired power plants and mines. Also in the 1990s, the EPA recognized that they did not have enough information to adequately characterize the condition of surface water in the US and began a series of statistically-based surveys to collect, among other parameters, screening-level data on chemical concentrations,
including Hg, in the waters and chemical residues in fish tissue (EPA, 1997). The somewhat surprising result of the two national EPA studies was that anthropogenic derived Hg was found in every water-body tested and in all fish.

Notably absent from the EPA’s national lake survey was Great Salt Lake. However, at that time the Utah State Geologic Survey (USGS) was measuring the levels of selenium in the lake (mid-1990’s) and thought that there might be high levels of Hg too. Remarkably, they found some of the highest levels of Hg measured up to that point in time (Naftz et al., 2008) Concentrations of Hg were measured at 24 ng L\(^{-1}\) with some lake samples surpassing 30 ng L\(^{-1}\). This study also found that between 31-60% of total Hg was in the form of methyl mercury (CH\(_3\)Hg\(^+\)) particularly in the DBL (Figure 2). In contrast, whole water samples from Maryland reservoirs contained between 0.007 and 0.493 ng L\(^{-1}\) CH\(_3\)Hg\(^+\) (Mason & Sveinsdottir, 2003).

The finding that a significant fraction of total Hg in GSL is in the form of CH\(_3\)Hg\(^+\) was surprising because it had been previously demonstrated that salinity inhibits the methylation of Hg (Compeau & Bartha, 1984). Measured levels of CH\(_3\)Hg\(^+\) in GSL (Naftz et al., 2008) appear to contradict that evidence (Blum & Bartha, 1980; Compeau & Bartha, 1983, 1984, 1987; Olson & Cooper, 1974). This leads to the question: How is CH\(_3\)Hg\(^+\) being produced in such hypersaline waters?

The main purpose of this thesis is to critically review the research that has been conducted on the biogeochemistry of Hg methylation in saline environments with the purpose of assessing whether there is enough evidence to determine if the methylation of Hg is due primarily to biotic or abiotic reactions in GSL.
LITERATURE REVIEW

Mercury in the Environment

Hg in its inorganic form is a relatively rare metal in the Earth’s crust; however, both natural and anthropogenic influences release it from the crust or rocks into the atmosphere thereby contributing to the global Hg cycle (Risher & De Rosa, 2007). Once introduced to the natural environment, Hg begins cycling through various compartments, speciation, states of toxicity, and bioavailability. For example, atmospheric Hg may be deposited on water where it then may bind to sediments, precipitate as a mercury sulfide, or be transferred through the benthic food chain moving from either the water column or sediments through several plant and animal species ultimately ending in a more concentrated form of Hg at the top of the food chain. Once in the food chain, Hg’s threat to humans and higher order mammals and birds increases. Volatilization occurs readily from surface waters when in the Hg$^0$ oxidation state and this plays an important role in the global Hg cycle (Mason et al., 1994).

Due to an increase in the number of industrial plants and emission processes, which resulted in increasing air pollution that reached an all-time high by the mid-1980’s (commonly referred to as the industrial maximum of the US) (Shuster et al., 2002), the EPA passed the clean air act, the first mandate by the government to monitor and track coal-power plant emissions. In 1997, EPA defined Hg emissions as follows:

- **Natural mercury emissions** -- the mobilization or release of geologically bound mercury by natural processes, with mass transfer of mercury to the atmosphere;
- **Anthropogenic mercury emissions** -- the mobilization or release of geologically bound
mercury by human activities, with mass transfer of mercury to the atmosphere; or

- **Re-emitted mercury** -- the mass transfer of mercury to the atmosphere by biologic and geologic processes drawing on a pool of mercury that was deposited to the earth's surface after initial mobilization by either anthropogenic or natural activities.

However, it was not until March 2005 that the EPA put a cap on Hg emissions from coal-powered plants, reducing the overall anthropogenic source of atmospheric Hg (EPA, 2005).

**Anthropogenic Sources of Hg in the Environment**

Anthropogenic influences such as mining have led to the release of thousands of tons Hg into the atmosphere (Salomons, 1995). From the time of the Phoenicians and Carthaginians (~2700 BCE) until the widespread adoption of the cyanidation method between the 1880s and 1990s, Hg was used in mining to amalgamate and concentrate precious metals such as gold and silver because of the simplicity of the process and the relative ease of separating Hg from the metals (Lacerda & Salomons, 1998). In the late 19th century, the US was a major gold producer using the Hg amalgamation method. It is estimated that the US alone consumed nearly 70,000 tons of Hg from 1850-1900 (Lacerda & Salomons, 1998). Since the amalgamation process is not efficient, significant amounts of the Hg used are released to the environment (Nriagu, 1993). It is estimated that 60-65% Hg used in mining is released into the atmosphere and approximately 35% enters soils and waters often leaching from contaminated tailings (Lacerda, 1997). Although the amalgamation method is now a minor source of direct Hg emissions in the US, globally, it amounts to 1000 metric tons of annual Hg releases from at least 70 countries predominantly in Asia, Africa and South America (Randall & Chattopadhyay, 2013). The result is that, mining
currently contributes to 24% of global Hg emissions (Natural Resources Defense Council, 2013).

The debate over whether atmospheric Hg deposition occurs on local, regional, or global scales is ongoing (Mason et al., 1994). One of the methods to study the aggregated effects of global to local scale events on Hg deposition is to measure Hg concentrations in persistent ice cores together with estimates of ice accumulation rates to derive annual Hg deposition rates (Vandal et al., 1991). Comparing the rates to historic events can also help to separate out the relative contributions of natural and anthropogenic sources of atmospheric Hg. Schuster et al. (2002) worked on ice-cores from the Upper Fremont Glacier in Wyoming, which is the closest persistent (glacial) ice to the GSL and only such record of mid-latitude deposition in North America. Given the high elevation of the glacier (4100 m) and its remote location, Hg concentrations in the ice cores result predominantly from regional and global inputs. Schuster et al. (2002) found that over the last 270 years anthropogenic inputs contributed 52%, volcanic events 6%, and background sources 42%. A 20-fold increase in total Hg over pre-industrial levels occurred during the mid-1980s and is referred to as the industrial maximum (Figure 1). The estimates from the Upper Figure 1: Hg concentrations from ice cores extracted from the Upper Fremont Glacier, Wyoming (the data are from Schuster et al. 2002).
Fremont Glacier compare favorably with United Nations figures that state coal-fired power plants are currently the largest source of Hg pollution, accounting for 50% of annual Hg emissions in the US (30 metric tons) and 46% of global emissions (850 metric tons) (AMAP/UNEP, 2008). Although US emissions of Hg have decreased by 65% over the last 20 years, the atmospheric concentration of Hg has continued to increase by 1.5% per year in the Northern Hemisphere, with the anthropogenic flux being about 2.5-fold larger than the natural one (Slemr & Langer, 1992).

It is now widely accepted that the source of most of the Hg in aquatic environments originates as atmospheric deposition (Hall et al., 1995). However, since there is no one point-source for the Hg, both atmospheric deposition and inflows from streams and other water flows into the lake have also been studied extensively over the past few years. Naftz et al. (2009) conducted several studies to differentiate between watercourse inputs and atmospheric sources of Hg in the GSL. They found that atmospheric deposition of Hg was the most common input to the GSL at 32 kg year\(^{-1}\) as opposed to the 6 kg year\(^{-1}\) originating from riverine deposition.

**Methyl-mercury Toxicity**

**History.** As early as the late 1800’s Doctors in London described ill neurological effect from constant Hg exposure at a time when elemental Hg was a common ingredient in many medicines ranging from laxative to antiseptics and many others. In the early 1900’s Hg was also linked to other neurological impairments and in the 1960’s organic CH\(_3\)Hg\(^+\) was linked to the mass poisoning in Japan near Minimata Bay.

When Hg gets converted into CH\(_3\)Hg\(^+\) it becomes a potent neurotoxin (Mergler et al.,
2007). The EPA has separate criteria for Hg and \( \text{CH}_3\text{Hg}^+ \) because having one or two methyl groups attached to the Hg makes it lipophilic and able to bind to proteins as a thiolate complex – most likely with cysteine (Harris et al., 2003). \( \text{CH}_3\text{Hg}^+ \) is the only known Hg compound that is bioaccumulated and biomagnified in the food chain and represents up to 95% of total Hg in top predators (Celo et al., 2006).

Human exposure to \( \text{CH}_3\text{Hg}^+ \) can result in severe central nervous system disturbances including, ataxia, brain atrophy and slurred speech, which contribute to diseases such as Minamata Disease, named for the mass poisoning in the 1953-1960 from eating contaminated fish in the Minamata Bay, Japan. The effects on the central nervous system are sometimes fatal and are amplified in early prenatal development. Since \( \text{CH}_3\text{Hg}^+ \) bioaccumulates in the aquatic biota it poses a threat to any organism that consumes fish from Hg contaminated water.

In 2004, the Food and Drug Administration (FDA) released a public advisory warning for pregnant women or those planning to become pregnant to limit their fish and shellfish intake per week, warning that the benefits from eating fish are not seen when toxic levels of \( \text{CH}_3\text{Hg}^+ \) are consumed (FDA & EPA, 2004). After this advisory was issued there was public concern over the Hg concentration of common fish found in supermarkets. A study conducted in Illinois found that some locally-caught fish had higher concentrations of toxic \( \text{CH}_3\text{Hg}^+ \) than what the FDA had previously reported, for instance, swordfish that was originally reported by the FDA to have 0.97 ppm actually contained 1.26 ppm (Burger & Gochfeld, 2006).

**\( \text{CH}_3\text{Hg}^+ \) toxicity concerns relevant to the GSL.** Because GSL is hypersaline, there are no fish that inhabit the lake except in Willard Bay, a manmade freshwater reservoir completed in 1964 that only spans 40 km\(^2\) at the north eastern arm of the lake. However, the GSL plays an
important role in the migration of several species of waterfowl (Aldrich & Paul, 2002) and has been classified as a Western Hemisphere Shorebird Reserve (Caudell & Conover, 2006). The birds primarily feed on the brine shrimp (*Artemia franciscana*), which are also a multi-million dollar industry for the state of Utah and contribute up to 45% of the world’s supply of brine shrimp (DNR, 2011). Migratory birds, whose reproductive success can be affected by elevated concentrations of Hg (Vest et al., 2009) are at great risk from the consumption of the brine shrimp from GSL. In 2005, the Utah Division of Water Quality put three duck species on human consumption advisory because the level of Hg was found to be at dangerous levels by the Utah Division of Wildlife Resources (UDWR); this made Utah the first state to have a consumption advisory put on waterfowl.

Shorebirds, being a significant part of the ecology of GSL, and a stopping/nesting spot for more than a third of the Western United States populations (Paul & Manning 2002), are adversely affected by CH$_3$Hg$^+$. Reproductive stress and reduction is seen in avian species mostly due to a thinning of eggshells and a reduction in egg production (Lundholm, 1995). In the previous study, CH$_3$Hg$^+$ was administered daily and after just 9 days it was observed that egg production had completely stopped and after 15 days the birds were also displaying signs of neurotoxicity.

While the hypersaline GSL does not pose a risk to humans from eating contaminated fish, it has been proposed that the system is a potential risk as a source for other surrounding bodies of water due to the volatilization of Hg/ CH$_3$Hg$^+$ from the GSL and its subsequent re-deposition in nearby streams and lakes (Naftz et al., 2009). This mechanism of Hg mobilization is of particular interest in Utah because most of the lakes and rivers throughout the state are fresh water, and
home to fish that the public do catch and eat.

Biogeochemistry of Hg in Aqueous Environments

In aqueous environments Hg is influenced by pH, redox conditions and the types and amounts of organic matter and inorganic substances present, which lead to different Hg species and fates. In natural waters around the world Hg exists as elemental Hg\(^0\), inorganic ionic Hg (+1 or +2), inorganic complexes (e.g., HgCl\(^+\)), methylated Hg (e.g., CH\(_3\)Hg\(^+\) or (CH\(_3\))\(_2\)Hg), bound to organic ligands in the form of soluble organomercurial compounds, or sorbed to suspended particles or sediment surfaces (Ullrich et al., 2001). Hg tends to preferentially bind to thiol and other reduced sulfur-containing functional groups rather than the acidic phenolic and carboxylic groups that dominate soluble OM. This is because Hg is a soft metal cation with highly polarizable electrons in its outer shell. As such, it has a preference for soft anions or ligands such as sulfur, nitrogen, and the less electronegative halides (Stumm & Morgan, 1996).

Hg biogeochemistry is complex in aqueous systems, which makes it difficult to predict its fate. For example, reduced sediments may act as both a sink and secondary source of Hg and CH\(_3\)Hg\(^+\) (Covelli et al., 1999). Although sediment:water partitioning coefficients vary broadly, they are estimated to be on the order of 10\(^4\)-10\(^5\) for Hg and 10\(^3\)-10\(^5\) for CH\(_3\)Hg\(^+\) (Stordal et al., 1996; Coquery et al., 1997; Lawson et al., 2001; Ullrich et al., 2001). Seasonal variations in Hg and CH\(_3\)Hg\(^+\) sorption and/or release from sediments may be related to redox effects, but may also be related to changes in DOM. For example, while reduced conditions favors the formation of insoluble HgS that settles into the sediments, the same conditions favor Hg desorption from sediments. High DOM concentrations can also solubilize Hg from HgS due to the complexation
of Hg with thiol (-SH) groups in the DOM. In contrast, oxic conditions, high pH, and low
temperatures favor sediment sorption of Hg and CH$_3$Hg$^+$ (Ullrich et al., 2001). These
observations result from numerous biogeochemical interactions involving not only Hg, but also
conditions favoring Fe and Mn oxide and oxyhydroxide dissolution and precipitation in the
sediments (Benoit et al, 1999; Ullrich, 2001).

Through photodegradation, organic Hg species at the surface of natural water bodies are
reduced to volatile elemental Hg$^0$ (Nelson et al., 1973). Up to 30 % of the dissolved Hg that is
present in ocean and freshwaters is found in the form of Hg$^0$ (Mason & Fitzgerald, 1993; Vandal
et al., 1991). It tends to accumulate at the surface of the water bodies where it is more highly
concentrated than in the atmosphere (Xiao et al., 1990). Since the species is highly volatile,
much of the Hg$^0$ at the surface of natural water bodies is lost back to the atmosphere. Diurnal
temperature changes as well as large seasonal temperature variability account for large fluxes in
Hg concentrations in the surface water with higher summer or daytime temperatures leading to
greater losses of Hg to the atmosphere.

In its inorganic form elemental Hg was thought to be unavailable to microbes; however,
Alfonso de Magalhaes & Tubino (1995) demonstrated that metallic Hg can be oxidized to Hg (II)
compounds in aqueous solutions (especially in the presence of Cl ions), forming Hg salts that are
then biotically methylated. Their finding suggests that biotic methylation can occur in the
presence of Cl. A study of Swedish lakes by Lee and Iverfeldt (1991) found concentrations of
total Hg in the range of 0.2-80 ngL$^{-1}$. Approximately 40% of the Hg was present as CH$_3$Hg$^+$.
This is also true for the hypersaline GSL where total Hg was measured to be in the range of 7 ng
L$^{-1}$ – >100 ng L$^{-1}$ with CH$_3$Hg$^+$ comprising 31-60% of the Hg species found in the deep brine
layer (Naftz et al., 2008).

Until recently, it was thought that the formation of CH$_3$Hg$^+$ in aquatic environments was largely the result of biological methylation processes involving sulfate-reducing bacteria. More recent evidence is revealing that under certain conditions abiotic reactions may significantly contribute to CH$_3$Hg$^+$ formation just as abiotic photodegradation is a significant mechanism for the degradation of CH$_3$Hg$^+$ (Celo et al., 2006). Similarly, recent studies of biotic Hg methylation suggest that microbes other than sulfate reducing bacteria may play a role in CH$_3$Hg$^+$ formation (Ullrich et al., 2001). These processes will be discussed further in the following section that focuses on CH$_3$Hg$^+$ formation in saline waters as found in the GSL.

**Formation and Fate of CH$_3$Hg$^+$ in the Saline GSL**

**Abiotic Hg methylation and demethylation.** The abiotic methylation of Hg in aquatic environments has been demonstrated both in the laboratory and naturally occurring waters and may play an important or even primary role in CH$_3$Hg$^+$ production (Craig & Moreton, 1985; Bellama et al., 1988; Gilmour & Henry, 1991; Weber, 1993). The abiotic methylation of the Hg$^{2+}$ ion was first demonstrated in the early 1970s by researchers mimicking the biotic methylation of Hg by methylcobalamin (a form of vitamin B$_{12}$) in the laboratory in simple aqueous systems (Bertilsson & Neujahr, 1971; Imura et al., 1971, DeSimone et al., 1973). As seen in equation 1, the reaction involves the transfer of a methyl group from cobalt to Hg and is quantitative (Celo et al., 2006).

\[
MeCblH_2O + Hg^{2+} + H_2O \rightarrow Cbl(H_2O)_2^+ + CH_3Hg^+ \quad [eq. 1]
\]

This reaction, however, occurs in low pH waters with low Cl concentrations. Celo et al. (2006)
demonstrated that in solutions containing 1.0 M Cl, the methylation reaction was completely shut down. Cl concentrations in the GSL range from approximately 1.5–5.6 M (Rupke & McDonald, 2012) suggesting that this reaction is not a source of CH$_3$Hg$^+$ in the high Cl, high pH waters of the GSL.

Craig & Rapsomaikis (1985) later demonstrated the potential for metals to undergo two-step oxidative addition reactions to form methyl-metal compounds. Since they involve an oxidation step, the reactions are more likely to occur in the oxygenated water column than anoxic sediments (Craig & Rapsomaikis, 1985). Unlike the methylcobalamin reactions, the two-step oxidative addition reaction involves metal-ligand complexes. Such reactions are therefore highly likely in natural systems where metals are generally complexed with a variety of ligands (e.g., humic and fulvic matter, amino acids, sulfur ligands, chloride, etc.). The first step of the reaction involves the formation of monomethyl-metal-ligand complexes and the second step follows an oxidative pathway to form various methyl-compounds as seen in equation 2 with Hg as the metal (Craig & Rapsomaikis, 1985; Hamasaki et al., 1995; Randal & Chattopadhyay, 2013).

\[
CH_3I + HgY_2 \xrightarrow{slow} [CH_3HgY_2^+ \cdot I^-] \xrightarrow{fast} CH_3HgY_2I
\]  
[eq. 2]
In this reaction Y represents the ligand and CH$_3$I an alkyl halide (Craig & Rapsomaikis, 1985). The most important Hg complexing ligands present in the GSL are considered to be dissolved organic matter (DOM), hydroxyls, sulfur, and chloride (Craig & Rapsomaikis, 1985; Buffle, 1988; Ravichandran, 2004).

Two methyl halides that have been shown to methylate mercury are methyltin (MeSn) and methyliodide (MeI). Although there is no available research indicating that GSL water or sediment has been analyzed for these compounds, it is probable that they are present in the lake.
MeSn was used for years as a stabilizer in polymer production, as glass coating catalyst, an agrochemical fungicide, insecticide, bacteriocide, and timber preservative, and an antifouling agent in paint (Dahab et al., 1990; Ranke, 2002). As a result of its widespread use, many natural waters are contaminated with the compound. In addition, MeSn can form naturally in aqueous environments by numerous types of reactions including oxidative addition and nucleophilic attack (Celo et al., 2006). Furthermore, concentrations of MeSn tend to be high in highly saline waters (Hamasaki et al., 1995). Jewett et al. (1978) proposed a bimolecular transmethylation reaction between mono-, di-, and trimethyl tin complexes and the mercuric ion as shown in equation 3.

\[
Me_nSn(IV) + Hg(II) \rightarrow Me_{n-1}Sn(IV) + MeHg(II) \quad [\text{eq. 3}]
\]

Celo et al. (2006) found this reaction to be quantitative even in aqueous solutions at pH 10 containing 1.0 M Cl. The chloride ion is thought to aid in the electrophilic transalkylation (Kashin et al., 1979). Since these reactions seem to require the presence of chloride and their rates increase with pH, MeₙSn could very likely play an important role in the methylation of Hg in the GSL (Celo et al., 2006).

Although MeI has been used to recover metals from naturally occurring ores and scrap, anthropogenic inputs of methyliodide to the aquatic environments are generally small relative to natural sources (Craig et al., 1998; Scarrat & Moore, 1999). The largest inputs of MeI come from marine organisms such as algae, fungi, and seaweed, which produce MeI as a defense compound or byproduct in the production or breakdown of larger defense compounds (Celo et al., 2006). In the open ocean concentrations of MeI range from 1.2 to 235 ng L⁻¹, but in coastal areas where biomass production is more intense, concentrations of MeI can be several thousand times higher
Methylation by MeI typically only occurs with reduced forms of metals and has not been observed with Hg(II) (Celo et al., 2006). However, Hg(0) can be methylated by MeI presumably by oxidative addition as seen in equation 4 (Maynard, 1932; Celo et al., 2006).

\[
Hg(0) + MeI \rightarrow MeHgI
\]  

[Ceq. 4]

Celo et al. (2006) found that this is a pseudo-first order reaction that depends linearly on the methyl iodide concentration and occurs at all pH, ionic strength conditions and chloride concentrations. This suggests that MeI methylation of Hg may occur in the lake, particularly if the concentration of Hg\(^0\) is high.

The abiotic demethylation of CH\(_3\)Hg\(^+\) via light energy (Sellers et al., 1996) is known to contribute significantly to Hg cycling in natural waters (Ullrich et al., 2001; Celo et al., 2006). Because the GSL lies at such high altitude, averaging 1280 meters above sea level (Arnow, 1985), the lake is exposed to high levels of ultra violet radiation, which under normal circumstances would allow for a higher rate of loss of Hg to the atmosphere, however, salinity inhibits CH\(_3\)Hg\(^+\) photodegradation (Black et al., 2012). On the other hand, Costas and Liss (2000) observed the photoreduction in seawater as a function of dissolved organic carbon (DOC) and found that the reaction increased with increasing concentrations of DOC and light intensity, and decreasing wavelength. Thus, in the GSL, CH\(_3\)Hg\(^+\) photoreduction would be both negatively affected by salinity and positively affected by high levels of ultraviolet radiation and DOC concentrations.

**Microbial Hg Methylation and Demethylation.** Although abiotic oxidative complexation plays a role in Hg methylation, biological processes are generally thought to be far
more important in terms of their overall contribution to $\text{CH}_3\text{Hg}^+$ (Figure 2), with sulfate-reducing bacteria in the sediments being the most important source of all (Compeau & Bartha, 1985; Benoit et al., 2003; Chen et al., 2008). However, more recently iron-reducing bacteria (Fleming et al., 2006; Kerin et al., 2006) and methanogens (Hamelin et al., 2011) have been shown to also methylate Hg in anoxic environments. And there is mounting evidence that bacteria exist that can methylate Hg in oxic environments such as snow and surface sea waters (Montperrus et al., 2007; Lehnerr et al., 2011). Clearly, the number, types, and environments of microbes that methylate Hg are much greater than previously thought.

Figure 2: Possible fate of Hg in GSL

An exciting recent discovery by Parks et al. (2013) has shown that two genes, hgcA and hgcB control anoxygenic Hg methylation. So far 52 bacteria and archaea for which genomes
have been sequenced, possess the hgcAB cluster – including a psychrophile, thermophile, and human intestinal methanogen (Sonke et al., 2013).

Interestingly, methylcobalamin, the first compound shown to methylate Hg abiotically, also acts as a methyl group donor in the biological methylation (Hamasaki et al., 1995). Traditionally, the methylation of Hg by microbes was thought to be distinctively different from abiotic processes in that it required the Hg be free or un-complexed for uptake into the cell, and was thus limited by concentrations of the mercuric ion. This would have made Hg highly unavailable in the highly saline waters of the GSL. However, more recent work suggests that dissolved, neutrally-charged complexes such as HgCl$_2^0$ are being taken up by microbes (Benoit et al., 1999). This is because microbial uptake involves diffusive transport of Hg across membranes, and the membranes are known to be more permeable to uncharged species than ions (Ullrich et al., 2001).

Current thinking is that Hg bioavailability is controlled by the concentration of HgCl$_2$ in oxic marine waters, and by HgS$^0$, Hg(SH)$_2^0$, or polysulfide HgS$_n^0$ in anoxic waters (Benoit et al., 1999; Ullrich et al., 2001). The concentrations of the dissolved sulfides is especially important because low concentrations seem to enhance methylation; whereas, high concentrations inhibit both the amount and rate of CH$_3$Hg$^+$ formation (Andren & Harriss, 1975; Benoit et al., 1999; Ullrich et al., 2001).

Although DOM ligands reduce the quantity of free Hg in natural waters, in sulfide-limited waters it has been demonstrated that once bacteria take up Hg, DOM enhances the bacteria’s ability to methylate it (Jackson, 1989). Furthermore, once CH$_3$Hg$^+$ has been formed, DOM facilitates its movement within the water column and plays a role in increasing Hg
concentrations in lakes (Miskimmin, 1991). CH$_3$Hg$^+$ may be lost from waters and sediments through biological demethylation (Nascimento & Chartone-Souza, 2003).

Similar to the differences between biotic and abiotic Hg methylation, the biological demethylation of Hg is a very different process from the abiotic photo-degradation of CH$_3$Hg$^+$. For instance, the biological demethylation of Hg occurs in the dark and is a dominant mechanism in anaerobic, saline sediments (Oremland et al., 1991).

**Microbial Adoptions to Hg Contaminated Environments**

Elevated levels of dissolved organic carbon (DOC) increase the diversity of the microbial community in aquatic environments. The GSL has a high concentration of DOC due in large part to decaying matter from the nearby wetlands, and to the considerable population of brine shrimp in the lake (Naftz et al., 2008). Most of the Hg that is deposited in aquatic systems is a substrate for microbial activity and the biotic conversion of inorganic mercury in the Hg$^0$ form to the highly toxic CH$_3$Hg$^+$ (Beijer & Jernelov, 1979). The microbial communities responsible for sulfate reduction, and methylation of mercury are primarily anaerobic and live in anoxic lake-bottom sediments (King et al., 1999).

Bacteria have adapted methods to detoxify the surrounding environment and survive in areas of high Hg (Robinson & Tuovinen, 1984). Through the metabolism of sulfur, sulfate-reducing bacteria produce CH$_3$Hg$^+$ as a byproduct (Compeau & Bartha, 1985). Normally sulfate-reducing bacteria use methylcobalamin to synthesize acetate from CO$_2$, however, Hg acts as a competing methyl group acceptor, thus resulting in CH$_3$Hg$^+$ (Choi & Bartha, 1993). Although microbial formation of CH$_3$Hg$^+$ is not as effective at removing Hg from their environments as the
precipitation of HgS or the volatilization of dimethylmercury, CH$_3$Hg$^+$ is relatively non-toxic to microbes because in this form it tends to become bound to clay particles including suspended clay that end up being ingested by aquatic animals.

Another way that microbes have adapted to environments with high Hg concentrations is by producing enzymes that detoxify Hg, converting toxic forms to relatively harmless mercury through enzymatic reduction via the *mer* operon (Komura & Izaki, 1971; Osborn et al., 1997; Silver, 1996; Summers, 1986). The *mer* operon is a group of mercury-resistance genes that includes *merR*, a gene encoding a DNA-binding protein that controls expression of a number of other genes. Mercuric resistance genes, especially *merA*, are distributed over a diverse group of bacteria and archaea. The *mer* operon can be located chromosomally between transposons and on plasmids. When the operon is flanked by transposons or a plasmid, the genes may become widely distributed via horizontal gene transfer, which is promoted by severe, growth-inhibitory conditions. This makes the GSL an ideal environment to promote gene transfer of *mer* operon-containing transposons and plasmids.

There are two types of *mer* resistance genes: *merA*, which encodes a narrow-spectrum phenotype that confers resistance to only inorganic mercury salts, and *merB*, which encodes a broad-spectrum phenotype that expresses resistance to organomurcurials, such as CH$_3$Hg$^+$, and mercury salts (Bogdanova et al., 1998; Misra, 1992; Silver & Phung, 1996). Bacteria that are resistant to both Hg in its ionic form and CH$_3$Hg$^+$ code for proteins that regulate mercury transport (MerA, MerP, MerT) and mercury degradation (MerA and MerB). MerB (organomercurial lyase) is crucial to remediation of CH$_3$Hg$^+$-contaminated waterways because of its ability to convert CH$_3$Hg$^+$ to Hg$^{2+}$. MerA (mercuric ion reductase) reduces the resulting Hg$^{2+}$

to less toxic elemental mercury (Nascimento & Chartone-Souza, 2003). After Hg$^{2+}$ is reduced to Hg$^{0}$ in an NADPH-dependent reaction, the non-toxic Hg$^{0}$ volatilizes from the cell (Schottel, 1978).

**Challenges to Microbial Hg Methylation/Demethylation Mechanisms in the GSL**

Microbial Hg methylation was originally thought to be inhibited by saline water (Olson & Cooper, 1974; Blum & Bartha, 1980; Compeau & Bartha, 1983, 1984, 1987). Now we know that it occurs in marine and estuarine environments (Weber, 1993). However, the combined influence of factors controlling the process, such as methylation, demethylation, and CH$_3$Hg$^+$ mobilization, are still poorly understood (Chen et al., 2008). In addition, the effects of total water column concentrations of Hg and CH$_3$Hg$^+$ on Hg methylation in the sediments is poorly understood due in part to the difficulty in measuring their low concentrations in seawater. Thus Hg methylation in marine environments remains an area of active study.

Although marine and in particular estuarine waters are better models of Hg biogeo cycling in the GSL than freshwater systems, there are still major differences between the two saline systems. For example, GSL is hypersaline with salt concentrations 3.5 – 8 times greater than those in the ocean (Gwynn, 2002). Levels of UV-light reaching the lake are about 15% higher than those at sea level (Gwynn, 2002). Sulfates concentrations in the hypersaline water are 7.2 % dry weight, similar to typical ocean concentrations of 7.7% (Rupke & McDonald, 2012).
Dissolved sulfides in the lake range from $< 0.1$ to $1.4$ mg L$^{-1}$ (Naftz et al., 2008; Wurtsbaugh & Jones, 2012) with continuous inputs from the surrounding area. By comparison, dissolved sulfide concentrations in the water column outside the Santa Barbara Basin averaged 3 nM (96 ng L$^{-1}$) in the 0-600 m depth range and up to 15 nM (480 ng L$^{-1}$) below 400 m within the basin (Kuwabara et al., 1999). Pore water concentrations of dissolved sulfide at the site ranged from approximately 0.001 – 100 μM (0.032 – 3200 μg L$^{-1}$) – more than an order of magnitude lower than the concentrations observed in the GSL. Finally, Brandt et al. (2000) reported that the anoxic DBL of the GSL has some of the highest levels of sulfates and rates of sulfate reduction measured in the natural environment. The high levels of sulfate in the GSL suggest that as in coastal marine, sulfate reducing bacteria could play a major role in methylating Hg in the GSL, thus increasing its bioavailability.

Adding further support to this hypothesis is a study by Weimer et al. (2008) who demonstrated with phylochip data that several genera of bacteria able to reduce sulfate occur in the lake (Figure 3), and a map of the spatial distribution of salinity in the GSL next to a spatial map of the fraction of total Hg occurring as CH$_3$Hg$^+$. Figure 3: The distribution of microbial genera at three sites in the GSL. Sulfate reducers (Deltaproteobacteria, Desulfotomaculum, and Thermodesulfo bacteri a) are highlighted. The arrow indicates a group of sulfate-reducers in Farmington Bay that are not found in any other part of the lake (Weimer et al., 2008).
(Figure 4) that shows considerable overlap between high salt concentrations and CH$_3$Hg$^+$ (Naftz et al., 2008). Nevertheless, existing mercury cycling models do not sufficiently describe the complex interfaces between the sulfur cycle and the production of CH$_3$Hg$^+$, in the hypersaline environment of GSL.

Since, unusually high concentrations of CH$_3$Hg$^+$ have been measured both in the GSL and migrating and shore birds frequenting the lake, the mechanisms involved in the formation of CH$_3$Hg$^+$ in the GSL warrant investigation. In particular, the interactions between CH$_3$Hg$^+$ and Hg and the ligands present in GSL need to be studied in order to ascertain whether 1) the abiotic methylation of Hg contributes significantly to the CH$_3$Hg$^+$ concentrations in the lake, or 2) biotic methylation processes control the formation of CH$_3$Hg$^+$ in the hypersaline lake.

Towards that end, I conducted a study of the rate of CH$_3$Hg$^+$ production in the sediment and water column samples from the GSL by setting up microcosms, stopping sulfate reduction at different time points, and then measuring the amount of CH$_3$Hg$^+$ formed in each sample.
MICROCOSM STUDY

Introduction

Microcosms are small, environmentally controlled systems used to model what is happening in the natural environment. To determine if biotic methylation is possible in the hypersaline water of GSL I investigated biotic CH$_3$Hg$^+$ production in GSL sediment and water microcosms by stopping sulfate reduction and then measuring the amount of CH$_3$Hg$^+$ that formed over time. This treatment was compared to CH$_3$Hg$^+$ production in distilled deionized water (negative control) and CH$_3$Hg$^+$ production in untreated GSL sediment and water. I hypothesized that CH$_3$Hg$^+$ production would decrease in the samples treated to eliminate sulfate reduction relative to the untreated sample. No CH$_3$Hg$^+$ production was expected in the negative control.

Materials and Methods

Sample Collection. Sediment samples and the overlying water were collected from the site 41.20645 North and -112.67226 East in the south arm of GSL (Figure 5) in new, 2-L Nalgene bottles that were rinsed with distilled water prior to sample collection. The bottles were filled with sediments and lake water to the brim (no headspace) and kept in a cooler while

Figure 5: GSL sampling site marked with orange arrow.
being transported to the lab in Logan, UT (~ 5 hours) before being stored at 4°C for a week prior to being used in a microcosm.

**Microcosm Set-up.** To maintain the redox state of the Hg all work setting up the microcosms was conducted in a glove box under an N₂ atmosphere. Thirty cm³ of the saturated GSL sediment were transferred into amber-colored serum bottles to prevent UV reduction of the Hg.

Sodium molybdate (Na₂MoO₄) has been shown in other studies to inhibit up to 95% of the microbial activity of methylation by blocking sulfate reduction (Compeau & Bartha, 1985; Krämer & Cypionka, 1989). Therefore I set the microcosms up with experimental parameters of 1) 10 mL of Na₂MoO₄, 75 mL of lake water, and 30 cm² of sediment (+Mo), 2) 85 mL of lake water and 30 cm² of saturated sediment (control), and 3) a negative control (neg control) of only 10 mL sterile saline water (3.0 M NaCl) and 90 mL lake water with no added sediments. Lake water was collected at the same time and location as the sediments. The bottles were then sealed with a butyl rubber stopper and aluminum crimper cap under a N₂ atmosphere. These microcosms were pre-incubated at 30°C (3 hours) before being injected with 1 mL of HgCl₂ (5000 ng L⁻¹) resulting in final Hg concentrations of approximately 580 ng L⁻¹ in the +Mo and control bottles and 500 ng L⁻¹ in the neg control bottle due to the addition. In order to stop all Hg reduction (biotic and abiotic), 10 mL of a 20% zinc acetate solution, purged with N₂ gas, (Smith and Klug 1981) was added to the bottles at time points: 24 h, 48 h, 1 week and 2 weeks. Immediately after the addition of zinc acetate, the water and sediments from the microcosms were decanted into plastic Nalgene centrifuge bottles and centrifuged at 10000 x g for 20 min at
4°C in a Sorvall high-speed centrifuge. The liquid phase was then decanted into sterile, plastic vials covered in aluminum foil and stored at 4°C for up to 2 weeks prior to analysis.

**Hg Analyses.** The samples were analyzed for total Hg and CH$_3$Hg$^+$ by cold vapor atomic fluorescence spectrometry (CVAFS) according to EPA Methods 1631e (USEPA, 2002) and 1630 (USEPA, 2001), respectively, at The University of Utah. In brief, the water samples were filtered through a 0.45 μm capsule filter and acidified with 2 mL L$^{-1}$ of 9 M H$_2$SO$_4$. Following acidification, all the Hg species in solution were first oxidized to Hg(II) with BrCl and then sequentially reduced first with NH$_2$OH to remove free halogens and then with stannous chloride to convert Hg(II) to volatile Hg(0) that was then purged from solution with argon, concentrated on a gold trap, then released from the trap with a gas stream and analyzed by CVAFS (USEPA, 2002). In this method total mercury includes all BrCl-oxidizable mercury forms and species. According to the EPA “this includes, but is not limited to, Hg(II), Hg(0), strongly organo-complexed Hg(II) compounds, adsorbed particulate Hg, and several tested covalently bound organo-mercurials (e.g., CH$_3$HgCl, (CH$_3$)$_2$Hg, and C$_6$H$_5$HgOOCCH$_3$)” (USEPA, 2002). It may or may not include the recovery of Hg bound in microbial cells, which may require UV photoxidation to be released.

To determine dissolved CH$_3$Hg$^+$, the samples were filtered through a 0.45 μm capsule filter, acidified with 2 mL L$^{-1}$ of 9 M H$_2$SO$_4$, and then concentrated by distillation at 125°C under N$_2$ flow. The samples were then adjusted to pH 4.9 with an acetate buffer and ethylated by adding sodium tetraethyl borate (NaBEt$_4$) to form methylethyl mercury (MeEtHg). The MeEtHg was separated from solution by purging onto a graphitic carbon trap (Carbotrap®) and then thermally releasing the MeEtHg in an inert gas stream. A pyrolytic decomposition column was
used to convert the organo-Hg to elemental Hg(0), which was then detected by CVAFS. In this method, CH$_3$Hg$^+$ includes all acid-distillable Hg that reacts with NaBEt$_4$ to yield MeEtHg. According to the EPA, this “includes but is not limited to, CH$_3$Hg$^+$, strongly organo-complexed CH$_3$Hg compounds, adsorbed particulate CH$_3$Hg, and CH$_3$Hg bound in microorganisms (USEPA, 2001).

Results and Discussion

The amount of Hg in each microcosm system relative to the amount of initially added Hg over time can be seen in Figure 6. Although the error bars are large and differences between the three treatments not significant, clear trends can be seen in the data. First, it is apparent that in each treatment a large amount of Hg was lost from solution within the first 48 hours. After that Hg concentrations in the three treatments remained relatively constant. The greatest loss (> 80%) was observed in the neg control microcosm that only contained GSL and saline water, but no sediment, and the smallest

Figure 6: Total microcosm Hg relative to initially added Hg over time. Error bars represent one standard deviation of the mean of triplicate measurements.
loss occurred in the microcosms treated with molybdate to inhibit the activity of sulfur reducing bacteria (~40%). Approximately 70% of the total Hg addition was lost in the control microcosm in the first 48 hr. There are two possibilities for the losses. The first is that Hg volatilized when the microcosms were opened to remove the water sample for centrifugation prior to measurement.

The second possibility is that some of the Hg sorbed to the sediments in the + Mo and control microcosms. Since less Hg was lost in the microcosms containing sediment, that suggests that most of the Hg was lost to volatilization and that the sediment helped to retain Hg due to sorption. Furthermore, since more Hg was retained in the + Mo system than the control, that suggests that sulfate reducing bacteria are active in the GSL and that they may contribute to losses of Hg from the lake. The microcosms also illustrate the complexity in the biogeochemical cycling of Hg in the lake, because slight increases in solution Hg concentrations starting after one week of incubation in both sediment-containing microcosms suggests that the sediments that initially helped retain Hg in the system, began releasing Hg into solution, demonstrating their potential to be a secondary source of Hg depending on environmental conditions as has been reported by other researchers (Benoit et al., 1999; Covelli et al., 1999; Ullrich et al., 2001).

In Figure 7 the concentrations of CH$_3$Hg$^+$ relative to the amount of total Hg in the microcosm solutions for the three treatments are plotted over time. Although the values are very low relative to those that have been reported for the south arm of the GSL (Figure 4; Naftz et al., 2008; Wurtsbaugh & Jones 2012), they are within observed ranges for marine systems. In seawater values greater than 1% are considered anomalous due presumably to competition with Cl (Ullrich et al., 2001).
As with the total Hg values, there are no differences in CH$_3$Hg$^+$ between the three treatments. However, the trends are clear. First, in the neg control systems, CH$_3$Hg$^+/\text{THg}$ changes minimally over the course of the two week experiment indicating that little methylation occurs in the water column. In the control microcosms, CH$_3$Hg$^+/\text{THg}$ increases from ~0.4% to 0.6% over two weeks, indicating first that the microbes are active in the microcosms and CH$_3$Hg$^+$ is being generated. In the +Mo microcosms, however, CH$_3$Hg$^+/\text{THg}$ has significantly decreased by the end of the experiment. Shutting down up to 95% of sulfate reducing bacteria activity results in a clear difference relative to the control, clearly indicating that in the hypersaline deep brine layer of the GSL sulfate reducing bacteria are actively producing CH$_3$Hg$^+$. This finding contrasts with suggestions that microbial methylation of Hg is minimal in saline waters (Olson & Cooper, 1974; Blum & Bartha, 1980; Compeau & Bartha, 1983, 1984, 1987). It also supports the finding of high CH$_3$Hg$^+$ concentrations in the GSL. (Naftz et al., 2008).

Figure 7: Changes in the CH$_3$Hg$^+/\text{THg}$ ratio in the microcosm solution phases plotted over time. The error bars represent 12% standard error.

Electrical Conductivity (EC), DOM, DOC, dissolved total S, sulfate, and Cl concentrations, salinity as well as redox potentials are system properties that were not measured,
but would have added strength to this small microcosm study and helped to interpret the results. It would also have been interesting to analyze sediment mineralogy, specifically for metal sulfides, and iron, aluminum and manganese oxides.

Two other system properties that have not been measure before but could have a large impact if present are MeI and MeSn, therefore it would have been nice to have measured their concentrations in solution. Initial conditions of the microcosm also should have been measured so there would be a time 0 reading of Hg and CH$_3$Hg$^+$, as well as having more time points to plot. Although, any solutions added after the microcosm were anaerobically sealed were prepared under aerobic conditions and purged with N$_2$(g), they may have introduced oxygen into the microcosms and affected the speciation of Hg and CH$_3$Hg$^+$.

The final collection of water from the microcosms is the step at which most of the Hg (g) was lost through volatilization so the total of what was added to the vials was not seen when measured. In the future volatile Hg evolving in the traps would be captured and quantified so that a more accurate mass balance could be performed. Finally, it would have been interesting to analyze microbes in the sediments and sediment pore water for the presence of the two genes (hgcA and hgcB) that have been found to be involved Hg methylation (Sonke et al., 2013).

While I chose to set the microcosms up with a concentration of total Hg equal to measured levels of CH$_3$Hg$^+$, a future study might consider spiking samples with levels of total Hg to the actual observed levels of total Hg. Following and measuring sulfate reduction would also have been an important step in linking the CH$_3$Hg$^+$ to sulfate reduction in GSL, this could be accomplished by using a radiolabeled $^{35}$sulfate and liquid scintillation counter or by doing a gene expression analysis such as a PhyloChip to determine which microbes are the most metabolically
active in each sample and a GeoChip to check for the presence of sulfate reduction and mercury related genes.

**SUMMARY/CONCLUSION**

The biogeochemistry of Hg in saline aqueous environments is highly dynamic and complex. Its behavior in the GSL is further complicated by the unusual conditions of high elevation, high solar radiation, and extremely high salinity. Although there is much that could be improved with the microcosm study, both the experimental results and the literature review suggest that both biotic and abiotic processes contribute to Hg methylation in the lake. Although the presence of MeSn and MeI in the GSL has not been investigated, based on the environmental history of the region they probably occur in the lake and could contribute to Hg methylation. At the very least it would be good to determine background levels of MeI before proposed biofuel production involving high levels algae that produce MeI as a by-product are introduced into the lake. I also recommend testing for microbes other than sulfate reducing bacteria that are able to methylate Hg such as iron reducing bacteria and other microbes containing the hgcAB cluster genomes.
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