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Studies of mercury in water, sediment, and fish in Mississippi: Concentrations, speciation, cycling, and isotopic composition

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STUDIES OF MERCURY IN WATER, SEDIMENT, AND FISH IN MISSISSIPPI:

CONCENTRATIONS, SPECIATION, CYCLING, AND ISOTOPIC COMPOSITION

A Dissertation
presented in partial fulfillment of requirements
for the degree of Doctor of Philosophy
in the Department of Chemistry & Biochemistry
The University of Mississippi

by

GARRY BROWN, JR.

AUGUST 2013

ABSTRACT

The goal of this project is to better understand the biogeochemical cycle of mercury (Hg) in Mississippi to aid those managing the resources and developing related policy. To that end, Hg levels were determined in a total of 202 fish from Enid, Sardis and Grenada Lakes; Enid and Grenada have fish consumption advisories due to Hg. In addition, total-Hg and methylmercury (MeHg) levels were determined in the inflow and outflows from Enid and Sardis Lakes and in bottom sediment and suspended solids in Enid Lake. Potential sources of Hg to Enid Lake were investigated, including precipitation and runoff from urban, agricultural, and wetland/forest areas, which flow into the Yocona River. A range of water quality parameters was measured to determine the primary factors controlling the distribution and transport of Hg species in the watershed.

Concentrations in the fish generally increased with fish size (age) and trophic position. The suggested consumption limit for bass and crappie, commonly targeted by fishers, are <5 meals per month for adults and <12 meals per year for children. Wetland areas were determined to be hotspots for MeHg in the watershed with relatively high concentrations in water and fish, but runoff from agricultural areas likely provides the largest input of Hg to Enid Lake by transport of particle-bound-Hg. Wet deposition accounts for an estimated 33 kg and 15 kg of Hg deposited annually via rainfall to the Little Tallahatchie (Sardis) and Yocona River (Enid) watersheds, respectively. Mercury fluxes for Enid Lake based on concentrations in the inflow and outflow, and water-flow measurements are reported by season and during a storm event.

The feasibility of using remote sensing (satellite imagery) to model the distribution of total suspended solids and Hg following a rainstorm event will be shown. Finally, results from the first study of total-Hg, MeHg, and Hg isotopic composition in sediment from a cold seep from the northern Gulf of Mexico is discussed. Hg levels in the sediment from the cold seep were similar to background sites, and cold seeps are likely not significant sources of MeHg to Gulf waters.

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INTRODUCTION AND ORGANIZATION OF THE DISSERTATION

Humans are exposed to methylmercury (MeHg) mostly through consumption of biota from freshwater and marine systems, yet fundamental questions remain about sources of MeHg to aquatic biota, and the cycling and transport of Hg in watershdeds. MeHg is a neurotoxin that accumulates in tissues and biomagnifies up the aquatic food chain. Research investigating the sources, speciation, and cycling of Hg in the environment, and the risks associated with consumption of fish and shellfish require an interdisciplinary approach, involving:

- Field Work. Collecting representative samples of water, sediment, and biota from the environment is challenging on a number of levels and requires following careful protocols to minimize contamination, preserve the native forms (speciation) of Hg in the sample prior to analyses, and, in the case of biota, to assure proper animal handling and use protocols are being followed. In addition, obtaining accurate ancillary water quality data is critical to assess the factors controlling the transport and fate of Hg. Sampling may also require, as was the case here, collaboration with wildlife biologists and researchers experienced with electrofishing and deep marine sampling.
- Analytical Chemistry. Levels of Hg species in natural waters are exceedingly low and obtaining reliable data requires state-of-the art instrumentation with low detection limits, method development, and attention to quality assurance and analytical figures-of-merit.

- Toxicology. Interpretation of the hazards associated with consumption of different fish species from several waterbodies under different scenarios (children, adult, pregnant females) requires an understanding of toxicology and risk assessment.
- Modeling. Models can serve to shed light on the transport and fate of Hg in watersheds, but models need empirical measurements for testing and refining of the model.

In the present work, six projects focused on two areas, the northern Gulf of Mexico and north Mississippi lakes (Enid, Sardis and Grenada), are described. The motivation and objectives for each project are best described in separate chapters. To some extent the chapters can be thought of as separate papers, one of which has already been submitted for publication. It should be noted that each project utilized sophisticated Hg instrumentation based on cold vapor atomic fluorescence and/or atomic absorption spectrometry, and each required varying levels of method development.

The first chapter provides background on the biogeochemical cycle of Hg, arguably the most complex among the elements. It also introduces the theory and fundamentals of the instruments and analytical techniques used throughout this work. The remaining six chapters have their own abstract, introduction, objectives, materials and methods, results and discussion, and conclusions.

In chapter two, Hg concentrations, speciation, and isotopic composition were determined in sediment collected from a cold seep complex in the northern Gulf of Mexico (nGoM). A cold seep is an area where hydrocarbons diffuse from the seafloor and support a diverse community of chemosynthetic organisms. [Unlike hydrothermal vents, cold seeps do not involve super-heated water.] Although cold seep sediments possess a thriving microbial community capable of methylating Hg and an additional (geologic) source of Hg, little is known about Hg at these deep marine sites. Sediment from three different areas within the Woolsey Mound cold seep complex and from two background sites off the coast of Louisiana was collected, in collaboration with the Mississippi Mineral Resources Institute (MMRI). The primary purpose was to assess whether cold seeps are sources of MeHg to Gulf waters. This chapter presents the first study, as far as we know, of Hg species and isotopic composition in sediment from a cold seep from the nGoM.

Chapter three is a study of the current levels of mercury in fish from Enid, Sardis, and Grenada lakes in north Mississippi. These lakes serve as flood control reservoirs and provide popular recreational activities for the public, including fishing. Two of these lakes (Enid and Grenada) have fish consumption advisories first issued in the 1990's. In this study, Hg was determined in a total of 202 fish mostly from species that are routinely targeted and consumed by fishermen (crappie, bass, and catfish). The data was compared to earlier studies and a risk assessment was conducted.

Chapter four examines a major source of mercury to the Enid and Sardis Lake watersheds: atmospheric deposition. Rainwater was collected on several occasions and levels of mercury species (total-Hg and MeHg) were determined. The purpose was to quantify the amount of mercury deposited to the Enid and Sardis Lake watersheds through wet deposition using concentrations and annual rainfall totals to the region.

Chapter five investigates the dynamics of Hg species in the watershed, focusing on runoff from urban, agriculture, and forest/wetland areas. Runoff serves to mobilize and transport Hg accumulated during dry periods into streams, rivers, and lakes, where it contributes to the load already present in the waterbody. The Yocona River was also sampled periodically to observe temporal changes during the rainstorm events. Samples were analyzed for total-Hg and MeHg, and, for select samples, total suspended solids, total organic carbon, dissolved organic carbon, and particle bound mercury. The data provides insight into the factors controlling the distribution and transport of mercury species and other elements in the system.

Chapter six presents results for a study of Hg in the inflow and outflow of Enid and Sardis lakes during the summer, fall, winter, and spring between 2011 and 2013. The purpose was to evaluate seasonal trends and begin to develop a mercury budget (mass balance) for the impaired waterbodies.

Chapter seven presents preliminary work coupling Hg and total suspended solids measurements with remote sensing (satellite reflectivity imagery) to model mercury transport and dynamics in Enid Lake. Soils in the watershed are highly erodible, resulting in a large amount of sediment discharged into the lake. This provides a mechanism for infusion of pollutants, which are often associated with sediment particles. Moreover, suspended solids and contaminated sediments can greatly affect water quality and aquatic life. Because of costs and other factors, it is not always possible to include extensive field work and laboratory measurements in studies. It is therefore important to develop models which can minimize future work and provide insight into Hg sources and dynamics in watersheds.

Finally, Chapter 8 covers research that is incomplete but far enough along to document so that others may wish to pursue. It includes measurements of Hg and MeHg in the nGOM waters, focusing on the hypoxia zone that develops in the mouth of the Mississippi each summer. The interest is that methylation may be enhanced in the zone where there is depleted oxygen levels and a source of carbon in the water column. Indeed, these are some of the first measurements of MeHg in these areas post Deep Horizon oil spill. Another study that is discussed involves the capture and tagging of fish from a wetland. During this process fish were measured and a muscle plug was removed using a biopsy punch. The fish were then released and the muscle tissue was analyzed for Hg. The goal was to repeat the process at a later date to study the Hg dynamics in the same fish as it grows and ages, however we have not been successful retrieving a tagged fish.

1. INTRODUCTION AND BACKGROUND

1.1 BACKGROUND ON THE BIOGEOCHEMICAL CYCLE OF MERCURY

Mercury is a global health concern due to its toxicity, bioconcentration up the aquatic food chain, and global dispersion through atmospheric pathways. Mercury has maybe the most complex biogeochemical cycling of the elements and is considered a high priority pollutant by the US EPA (US EPA 2005). It exists in several chemical forms (species) in the environment primarily as elemental mercury (Hg^0), inorganic ionic mercury (Hg^{+2}), and organometallic mono-methyl-mercury (MeHg). The physical and chemical properties of these species influence their behavior in the environment. Mercury is dispersed globally via atmospheric pathways from natural sources, such as volcanic emission and biomass burning, and anthropogenic means, such as industrial combustion and mining activities (US EPA 2005). Elemental mercury vapor, which comprises ~99% of mercury in the atmosphere, is slowly converted to Hg^{2+} (primarily through photochemical oxidation mechanisms) (Li et al. 2008). Atmospheric mercury enters into aquatic and terrestrial ecosystems through wet and dry deposition (Hg^{2+} being much more soluble than Hg^0). Once deposited in the water bodies, Hg^{2+} can be converted to MeHg via biotic means (primarily sulfate reducing bacteria) (MDEQ 2002) and abiotic means (chemical reactions) (Clarkson et al. 1991). MeHg is a neurological toxin and soluble in water (Aschner 2007). MeHg, often as the uncharged chloride complex, is readily assimilated into phytoplankton at the base of the aquatic food chain and can reach high levels in top predators ($>1\ \mu g\ g^{-1}$) (MDEQ 2002). Consequently, MeHg bioconcentrates up the aquatic food chain. Because MeHg is almost completely absorbed in the gastrointestinal tract and transported throughout the body, consumption of contaminated fish can lead to severe poisoning in humans and wildlife (MDEQ 2002; Aschner 2007).

1.1.1 Forms of mercury in the environment

The forms of mercury in the environment include elemental (Hg^0), inorganic (Hg^{+1}, Hg^{2+} as salts or complexed with ligands), particulate-bound mercury (P-Hg), and organo-mercury (primarily CH_3Hg^+) (US EPA 2009). Atmospheric deposition is believed to be the major source to many aquatic and terrestrial ecosystems (MDEQ 2002).

Elemental mercury. Elemental mercury (Hg^0) is the dominant form of mercury in the atmosphere (Landing 1995) Hg^0 is a volatile gas that is emitted into the atmosphere both naturally by microbial demethylation or volcanic activity, and anthropogenically by refinery emissions or disposal of mercury-containing products. Hg^0 is slowly oxidized to Hg^{2+} in the atmosphere through photochemical means (Munthe 1992). Chemical reactions within water droplets in the atmosphere (e.g. fog, clouds) can also oxidize Hg^0 to Hg^{2+} (Munthe 1992). The life span of Hg^0 in the atmosphere ranges from one to two years; whereas the life span of Hg^{2+} in the atmosphere is measured in days (MDEQ 2002; ECPD 2009) Atmospheric mercury is deposited to aquatic and terrestrial systems through wet and dry depositions. Hg^0 is not particularly soluble in water and less toxic than organo-mercury compounds.

Inorganic mercury. Inorganic (ionic) mercury is more soluble then Hg^0 in water. Once deposited in water bodies, Hg^{2+} is converted to organic mercury via sulfate reducing bacteria. Most inorganic mercury compounds are white powders or crystals, except for mercuric sulfide (also known as cinnabar), which is red (ATSDR 2009).

Particulate-mercury. Particulate-bound mercury (P-Hg) is mercury bound to particulate matter in the atmosphere (e.g. dust) or natural waters (ECPD 2009). The residence time of P-Hg in the atmosphere depends on the particle size and meteorological conditions but in general is relatively short and P-Hg will fall near its source of emission. Atmospheric P-Hg is eventually deposited to the terrestrial and aquatic systems via wet and dry deposition. A relevant review suggests that P-Hg plays a significant role in the cycling of mercury in natural waters (Keeler et al 1995).

Organo-mercury. As stated earlier, MeHg is a neurological toxin and soluble in water (Aschner 2007) MeHg, often as the uncharged chloride complex, is readily assimilated into phytoplankton at the base of the aquatic food chain and can reach high levels in top predators (MDEQ 2002). Additional details on MeHg are provided in sections below.

1.1.2 Methylation and demethylation of inorganic mercury in aquatic environs

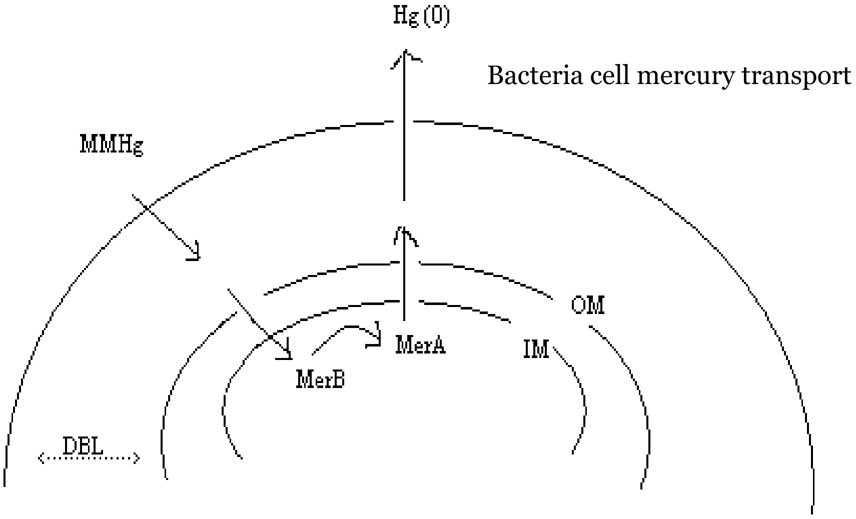
A variety of microorganisms, such as sulfate-dependent bacteria, are involved in the conversion of Hg^{2+} to MeHg. The bacteria and the conditions that favor methylation occur under anaerobic or oxygen poor conditions that are often found in wetlands, river sediments, and on occasion in open waters. Methylation is favored in low pH (acidic) environments with high concentrations of organic matter. For example, acid rain has been shown to increase biomethylation as more MeHg is formed under acidic conditions (Raloff et al 1991).

Exstorm, et. al. (2008) found that the corrinoid-containing protein (methyltransferase) in the bacteria *D. desulfuricans* LS was responsible for the methylation of ionic mercury to MeHg (Ekstrom et al 2008). These proteins are composed of a cobalt-containing center, which are synthesized by sulfate reducing bacteria (SRB). The study performed an experiment in which the corrinoid-containing enzyme was limited in complete oxidizing SRB. A decrease in mercury methylation was seen in the bacteria cultures after limiting this enzyme illustrating the importance of corrinoid-containing protein in mercury methylation (Ekstrom et al 2008).

Vitamin B-12 may also play a role in methylation of Hg in sulfate reducing bacteria (Ridley et al 2007). Ridley, et. al. illustrates Vitamin B-12's role in mercury methylation by three key mechanisms which involves a methyl group transferring via radical reactions and carbonium ions. Mercuric acetate and methylcobalamin (Vitamin B-12) was allowed to react by transferring the carbanion methyl group to yield methylmercury and aquocobalamin.

The mechanism of conversion from MeHg back to Hg^0 occurs through the enzymes mercuric reductase A and B (MerA, MerB) (Kritee 2008 & 2009). The conversion of MeHg to Hg^0 is a two-step process in which both occur in the cytosol of the bacteria cell (Figure 1). MeHg is first transported across the diffusion boundary layer of the bacteria cell attached to a thiol group. Uptake of the organic compound occurs through the inner membrane space to the cytosol where MerB converts CH_3Hg^+ to Hg^{2+}. MerA converts Hg^{2+} to elemental mercury. Hg^0 is diffused out the cell into the aquatic medium and ultimately back to the atmosphere fueling the biogeochemical cycling of mercury.

$$CH3Hg^{+} \xrightarrow{MerB} Hg^{2+} \xrightarrow{MerA} Hg^{0}$$



Kritee et al. (2009) Geochemica et Cosmochimica Acta 1285-1296

Figure 1 Transport and demethylation of methylmercury Transport and demethylation of methylmercury within bacterial cells. MMHg=mono-methyl-mercury; DBL=diffusion boundary layer; IM=inner membrane; OM=outer membrane.

(Adapted from Kritee et al. 2009)

1.1.3 Bioconcentration of mercury up the aquatic food chain

Mercury is among the most highly bioconcentrated trace metals in the human food chain (Waite et al 2002) Once Hg is methylated, it is transported to aquatic forms via ingestion or absorption. Plants and small organisms such as plankton incorporate Hg via passive surface absorption or through food intake. The only route of Hg exposure to autotrophic organisms is through passive absorption. The relatively small amount of Hg that results from these species from a period of passive absorption is not detrimental to the organism (ECDP 2009). Heterotrophic organisms may be exposed to dangerous concentrations via a second route. MeHg biomagnifies through the food chain as predators eat other organisms and absorb the contaminants that their food sources contained. Because MeHg has a long half-life in organisms (months), predator fish, such as Gar and Bass, are continually increasing their concentration of mercury. Over time, an individual who consumes plants or prey contaminated with MeHg will acquire levels greater than in either of its habitat or its food. As a result, top predators contain greater burdens of Hg than the fish they consume (MDEQ 2002; ECDP 2009).

1.1.4 Effects of mercury on the nervous system

Nearly 100 percent of MeHg ingested is absorbed and transported through the blood stream (ASTDR 2009). Once in the blood stream, through the aid of the thiol transporter glutamate, it can enter the brain and affect astrocytes. Astrocytes are the most numerous non-neuronal cell types in the central nervous system. They make up ~50% of human brain volume. Astrocytes perform several functions that are essential for normal neuronal activity, including glutamate uptake (80% of synaptic glutamate), glutamate release, K^+ and H^+ buffering, and water transport (Aschner 2007) According to Aschner, organic mercury causes swelling of astrocytes, reactive oxygen species increase in nerve cells, and efflux of glutathione outside astrocyte

(Aschner 2007). In the cell, MeHg induces astrocytic swelling triggering glutamate release, and inhibits uptake of cystine and cysteine, reducing astrocytic ability to synthesize glutathione. Glutathione is an important intracellular antioxidant in astrocytes.

1.1.5 Mercury concerns in Mississippi

Mercury has emerged as a serious public health concern in the state of Mississippi. A fish consumption advisory was issued for the Enid Reservoir in May 1995 and Yocona River in September 1996 by the MS Department of Health due to high levels of Hg in fish tissue sampled in the waters (MDEQ 2002). Sardis Lake also has relatively high levels of mercury in its fish but MS department of Health has yet to issue a fish consumption advisory. The origin of Hg in these water bodies is unclear but may include atmospheric deposition, geological formations that leach Hg into the watershed, and historic land use practices. Local and regional environmental conditions also have a dramatic impact on the transport and fate of Hg species in a given area. The presence of wetlands, for example, plays a critical role in the cycling of Hg in watersheds and has been shown to be sources of MeHg to ecosystems.

Within Northwest Mississippi there exists a large number of wetlands that were formed during the middle of the last century as a result of dams constructed for retention basins and flood control. Yet, there is very little information on these constructed wetlands with respect to their influence on total-Hg and MeHg dynamics in these Hg-impaired watersheds. It should be noted that wetlands offer many positive benefits to the environment, including habitat for wildlife and natural filtering of water impurities.

Mercury is of concern in another region of the state as well. The impact of the Deep Water Horizon Oil Spill in the Gulf of Mexico and seasonal hypoxia events in the waters around the mouth of the Mississippi River is unknown. Oil introduced into the ecosystem from the spill may alter MeHg levels due to: proliferation of hydrocarbon-degrading- and possibly methylating microorganisms; changes in dissolved oxygen (redox conditions) as a result of increased microbial activity; higher levels of dissolved organic carbon which is known to affect Hg bioavailability; and the sheer amount of Hg introduced into the ecosystem from the oil itself.

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1.2 Instrument fundamentals and theory

The research presented in this dissertation used a variety of instruments to determine mercury (Hg) and other analytes in water, sediment, and fish tissue. Concentrations of Hg in the environment range from parts-per-quadrillion (measured in air and seawater) to parts-per-million or higher (measured in fish tissue and Hg ores). Thus, whether an instrument is suitable to accurately measure Hg in a sample is dictated, in part, by its detection limit.

This chapter outlines the theory and fundamentals of the state-of-the-art instruments utilized in this research, including the Tekran 2600 Ultra-Trace Mercury Analysis System, the Tekran 2700 Automated MeHg Analyzer, the Shimadzu 5000 Total Organic Carbon Analyzer, the Costech Elemental Combustion System, and a YSI field multi-meter. It also includes a short discussion of the quality assurance measures taken and statistical approaches used.

For Hg instrumentation, the design and detection capability has evolved since the early 1900's. The first Hg instrumentation cited in the literature was by Thomas T. Woodson, who credited Dr. C. W. Hewlett for developing the first application for optically measuring Hg using resonance scattering at the 253.7 nm spectral line in a phototube (Woodson 1934). Woodson was credited in 1939 with developing an instrument, which detects Hg vapor in mixtures of air or flue gas utilized in power plants for Hg boilers and Hg vapor. This instrument was called the General Electric Vapor Detector, which contained components still utilized in modern Hg instruments such as the UV-lamp and phototube.

In 1972 Kopp et al. (1972) reported a method for determining Hg in aqueous solutions using the cold vapor technique. The detection limit was between 0.2-10 µg Hg/L. William Fitzgerald (1979) reported the determination of sub-nanogram levels of Hg using a dual stage amalgamation with gold which traps Hg in the elemental phase (Hg^0). This work, along with Nicolas Bloom's work (1988) determining volatile Hg species at picogram levels using a gas chromatography (GC) coupled to a cold-vapor atomic fluorescence detector, was the blue-print for modern day Hg instrumental designs. These methods now serve as United States Environmental Protection Agency (US EPA) protocols designed to measure low levels of Hg in water to determine whether it meets water quality criteria.

1.2.1 Cold Vapor Atomic Fluorescence Spectrometry

1.2.1.1 Tekran® 2600 Total Mercury Analyzer

Cold Vapor Atomic Fluorescence Spectrometry (CVAFS) is extremely sensitive and has lowest detection limit (< 0.1 pg) of all the analytical techniques commonly employed for the determination of Hg. In the study, we determined total-Hg in aqueous solutions and digests following US EPA Method 1631 "Mercury in Water by oxidation, purge and trap, and CVAFS" with the Tekran 2600 (Toronto, Canada). Briefly, in this method bromine monochloride (BrCl) is added to the aqueous samples to convert (oxidize) all Hg species to Hg^{2+} and preserve the sample. Just prior to analysis, the sample is reduced with hydroxylamine hydrochloride ($NH_2OH\cdot HCl$) to destroy free halogens which can damage the gold amalgamation traps. Then a reducing agent, typically stannous chloride ($SnCl_2$), is added to convert Hg^{+2} to Hg^0. The Hg^0 is purged from the solution using a gas-liquid separator with argon gas as the carrier. The vapor stream is carried to a soda lime trap where it is dried. The Hg^0 passes through the water trap and is sequentially concentrated on two gold cartridges, being thermally desorbed from each gold trap before being carried into the cell of a CVAFS for detection. In the spectrometer, Hg^0 flows through a quartz fluorescence cell. A pulsed mercury discharge lamp (positioned at a 90° angle from the detector) excites the Hg^0 atoms in the cell to fluoresce. A photomultiplier tube measures the fluorescence and the read-out occurs on a computer.

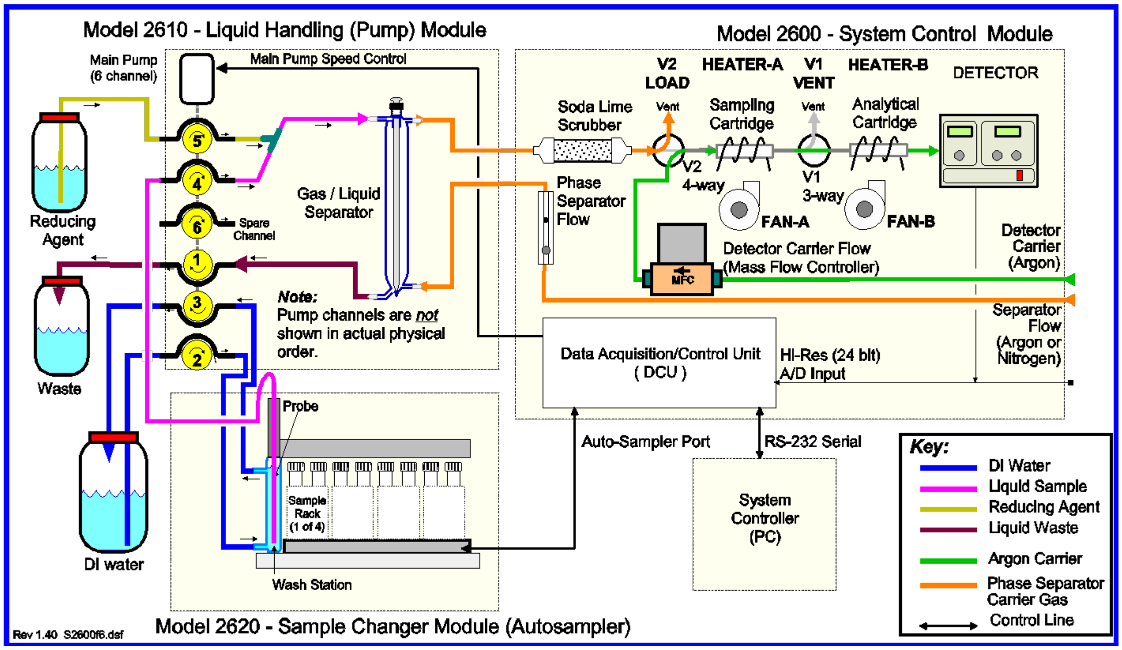

Figure 2 Schematic of Tekran 2600 CVAFS Hg Analysis System

(Used with permission by Tekran®)

1.2.1.2 Tekran® 2700 Methylmercury Analyzer

To determine MeHg in aqueous solutions and digests of sediments and fish tissue, we followed US EPA Method 1630 “Methylmercury in water by distillation, aqueous ethylation, purge and trap, and CVAFS” using the Tekran® 2700 Methyl Mercury Analyzer. A schematic of the instrument is given in Figure 3. Briefly, in this method approximately 30-mL sample aliquot (preserved to 0.4% using HCl) is placed in an I-Chem® glass vial with ~ 2.7 ml of 9% (m/v) citrate buffer. Then, 30 µL of a 1% (m/v) solution of sodium tetraethyl borate is added to the vial and it is quickly capped. Volatile Hg species are formed (methyl-ethyl-mercury for $MeHg^+$ and diethylmercury for Hg^{+2}). The ethylated forms are then separated from the solution by in-vial purging using argon onto a Tenax® carbon trap. The in-vial purging occurs via the autosampler's needle, which is designed to purge the sample with argon and contains a port, which carry the purged Hg species to the carbon trap. After pre-concentration on the carbon trap, the species are thermally desorbed and carried into a GC where they are separated. The volatile species are then passed through a pyrolytic decomposition column, which converts organo-Hg forms to Hg^0, and further into the cell of a CVAFS for detection. The combination of low background (the detector is 90° to the Hg lamp excitation source) and high sensitivity (photomultiplier detection) allows for extremely low detection limits (< 0.02 ng/L), which is required for the low-levels of MeHg found in the environment. We employed a modified version of US EPA Method 1630 developed by Kerry Tate at the Florida Department of Environmental Protection; the method avoids sample distillation and uses propyl-Hg as a surrogate to determine Hg loss and recovery.

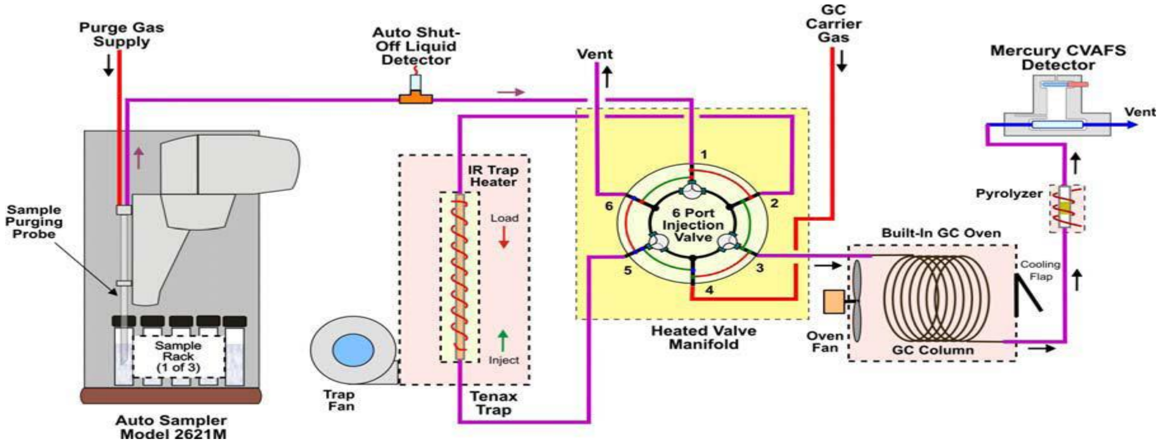

Figure 3 Schematic of the Tekran 2700

(Used with permission by Tekran®)

1.2.2 Thermal decomposition-atomic absorption spectrometry

Thermal Decomposition Atomic Absorption Spectroscopy (TD-AAS) is an analytical method capable of directly determining the concentration of Hg in a sample without any sample preparation. To determine total-Hg in sediments and fish tissue we followed US EPA Method 7473 "Mercury in solids and solutions by thermal decomposition, amalgamation, and atomic absorption spectrophotometry" using the Direct Mercury Analyzer (DMA-80; Milestone Inc., Monroe, CT). A schematic of the instrument is given in Figure 4. Briefly, in this method samples are weighed to the nearest 0.1 mg in a nickel or quartz boat (quartz for solutions containing acid). The boats are automatically introduced into the instrument's combustion tube via the autosampler. The instrument self-seals and oxygen (carrier gas) begins flowing over the sample at a rate of ~ 200 mL min^{-1}. The sample combusts as the temperature is raised to ~550 °C for a pre-specified period. Gaseous combustion products are carried through a Mn_3O_4/CaO-based catalyst (kept at 750 °C), where: oxidation is completed; different chemical forms (species) of Hg in the sample are converted to elemental Hg vapor; and sulfur oxides, nitrogen oxides, and halogens, which can interfere with the analysis, are trapped. Hg^0 and other decomposition products are carried to a tube containing gold-coated sand. There, Hg^0 is selectively trapped (forms an amalgam with gold) while other products are flushed out of the system. Later in the cycle, the trap is rapidly heated to ~700 °C and Hg^0 vapor is carried in a pulse through the single beam spectrophotometer. With sequential flow through two measurement cells with different path lengths, the instrument can be calibrated over two ranges (typically 0–35 and 35– 800 ng of Hg). The Hg concentration is then calculated by the software based on the absorbance measured at 253.7 nm and the weight of the sample. The detection limit for the DMA-80 can reach levels as low as 0.001 ng of Hg.

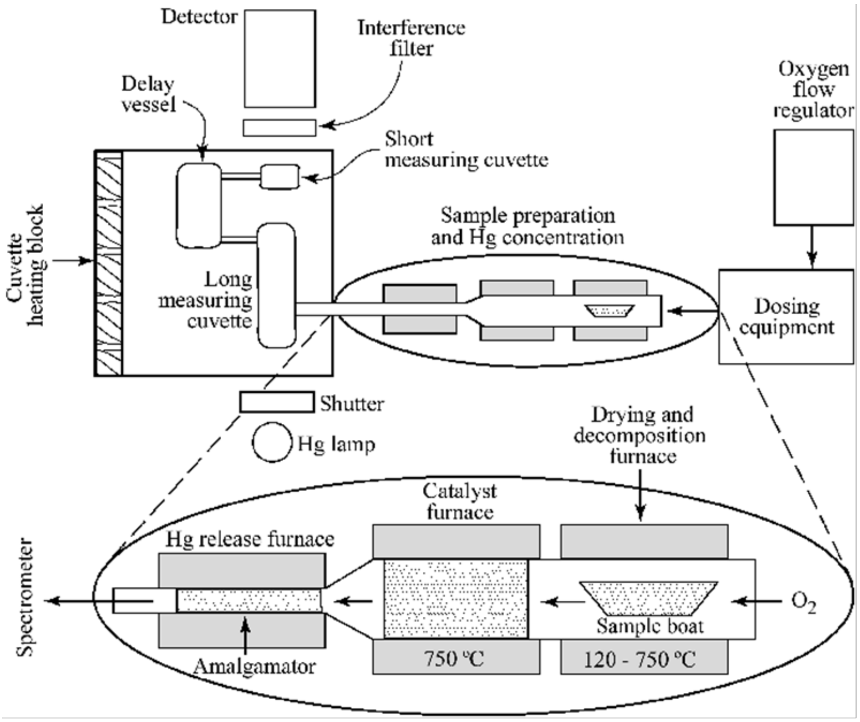

Figure 4 Schematic of a Combustion-CVAAS Hg System

1.2.3 Total Organic Carbon and dissolved organic carbon in water

Total organic carbon (TOC) and dissolved organic carbon (DOC) are important parameters to measure when studying Hg in the environment because mercury species form complexes with organic matter (e.g., humic and fulvic acids). These complexes serve to increase the mobility of mercury in water and soil (Lyons et al 2006).

TOC and DOC were measured in water samples following US EPA Method 415.3 using the Shimadzu 5000 Total Organic Carbon Analyzer. The analysis is carried out by first acidifying the DOC (filtered) and TOC (unfiltered) water samples to 0.4% using HCl. The samples are then sparged using oxygen gas to remove the inorganic carbon. The inorganic-carbon free water is then injected onto a combustion column which is packed with platinum-coated alumina beads that are held at a temperature of ~680°C. The organic carbon compounds are combusted and converted to CO_2, and subsequently detected by a non-dispersive infrared detector. Dissolved nitrogen in the sample is also combusted and converted to NO. The NO is mixed with ozone chemiluminesces for detection by a photomultiplier (Dickson et al. 2007).

1.2.4 Total organic carbon in sediment

Total Organic Carbon in sediment was determined using an Elemental Combustion System (Costech Analytical Technologies Inc. Valencia, CA). Sediment was treated with HCl to remove inorganic carbon. Samples were then weighed into tin capsules, which are placed into an automatic sample holder. The samples are dropped into a combustion chamber and react with oxygen and at temperatures of 1700-1800°C. This converts the carbon to CO2, which is carried to a GC with a thermal conductivity detector. The detector generates a signal that is proportional to the amount of element in the sample.

1.2.5 Field Measurements

An YSI Multi-parameter meter (Yellow Springs, OH) was used in the field to measure a range of water quality parameters including pH, conductivity, oxidative reducing potential (ORP), temperature, and dissolved oxygen (DO). The pH was determined because the methylation of Hg is enhanced in environments with low pH (Liang, 2013). DO and ORP was determined because methylation of mercury occurs primarily via sulfate-reducing bacteria that thrive in reduced and anaerobic conditions, and reduced oxygen levels can stimulate microbial populations to increase biomethylation (Clarkson 1991). Chloride (Cl^-) was measured because the dissolved inorganic Hg species tends to form aqueous Cl^- complexes at neutral and acidic pH in waterbodies. Conductivity measures the level of dissolved ions in water. The meter was calibrated for all parameters prior to recording in-field measurements.

1.3 Quality assurance and quality control and statistical analyses

1.3.1 QA/QC

Each of the methods described above has its own quality assurance measures, the details of which can be found in the method. This section provides a summary of the QA/QC used in the study. When feasible each analytical batch included replicates to assess precision, a reference material to monitor accuracy and bias, blanks to evaluate contamination and determine detection and quantitation limits. A new calibration curve was run each analytical day. QA/QC samples included a calibration check standard, a method blank, standard reference material, and a sample duplicate (or triplicate).

Precision describes the reproducibility of results, that is, the agreement between numerical values for two or more replicate measurements, or measurements that have been made in exactly the same way (Robinson 2005). Precision was determined by analyzing sample replicates and determining if replicates fall within the suggested range for the specific testing protocol. For triplicates the relative standard deviation was determined. For duplicates, precision was determined using the relative percent.

Accuracy describes the correctness of an experimental result expressed as the closeness of the measurement to the true or accepted value and was determined by the evaluation of spike recoveries as well as certified reference materials (CRMs). CRMs used in this study include NIST-1640 and ORMS-4 (for total-Hg in natural water) and DORM-3 (fish muscle reference material). As previously noted, for MeHg determinations, a propyl-Hg surrogate was spiked into each sample for determining recoveries.

1.3.2 Statistical analyses

Statistical analyses were performed using Excel, JMP or Statplus Statistical Software. To assess the data, make comparisons between seasons and locations, and find correlations between variables, various statistical tests were employed, including ANOVA, T-tests, and Pearson's Correlations. Differences that were considered significant was $p<0.05$. ANOVA is a parametric test in which differences in conditions are determined between sets within the data, which are under investigation (Drăghi 2003). Data was analyzed using ANOVA with location and season as treatment factors. T-tests were used to determine the differences between means divided by an estimate of the standard deviation, and Pearson's Correlation was used to determining the relationship between two variables with correlation values between (+) 1 and (-).

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CHAPTER TWO

2. MERCURY CONCENTRATIONS, SPECIATION, AND ISOTOPIC COMPOSITION IN SEDIMENT FROM A COLD SEEP IN THE NORTHERN GULF OF MEXICO

Brown G.; Sleeper K.; Johnson M.; Blum J.; Cizdziel J. *Marine Pollution Bulletin* 2013, in review.

2.1 ABSTRACT

The northern Gulf of Mexico (nGoM) has numerous cold seeps, areas where hydrocarbons diffuse from the seafloor and support a diverse community of chemosynthetic organisms. Although cold seep sediments possess a thriving microbial community capable of methylating mercury (Hg) and an additional (geologic) source of Hg, little is known about Hg at these deep marine sites. We present here the first study of total-Hg, monomethylmercury (MMHg), and mercury isotopic composition in sediment from a cold seep from the nGoM. Sediment from three different areas within the Woolsey Mound cold seep complex and from two background sites off the coast of Louisiana was analyzed. Total-Hg averaged 50 ng/g (n=28), ranged from 31 to 67 ng/g, and decreased with depth (0 to 15 cm). MMHg averaged 0.91 ng/g (n=18), and ranged from 0.2 to 1.9 ng/g. There was no significant difference for total-Hg or MMHg between cold seep and background sites ($p>0.05$). δ^{202}Hg ranged from -0.5 to -0.8 ‰ and becomes more negative with depth ($r=0.989$). Mass independent fractionation as represented by Δ^{199}Hg was small but consistently positive ranging from 0.04 to 0.12 ‰; there was no difference between cold seeps (Δ^{199}Hg = +0.09 ± 0.03; n=7, 1SD) and background sites (Δ^{199}Hg = +0.07 ± 0.02; n=5, 1SD). Overall, our study suggests that releases of hydrocarbons at the seafloor do not significantly alter Hg levels in the sediments compared to background sites, and that cold seeps are likely not significant sources of MMHg to nGoM waters.

2.2 INTRODUCTION

Mercury (Hg) is a persistent, mobile and highly toxic heavy metal pollutant with a complex biogeochemistry that varies with its chemical form (speciation) and redox state (Mason 1994; Fitzgerald and Lamborg 2004). It exists in various inorganic and organic forms in the environment, most notably elemental Hg (Hg^0), oxidized divalent/ionic Hg (Hg^{2+}: which is typically complexed with various ligands and has a strong affinity for sulfides and organic matter), organometallic Hg (of which monomethylmercury (MMHg) is of particular interest), and particle-bound-Hg (PBM), which may include the previous forms. MMHg is a neurotoxin that readily biomagnifies up the aquatic food chain (e.g., Porcella 1994; Clarkson 1990). Because MMHg is almost completely absorbed in the gastrointestinal tract and transported throughout the body, consumption of contaminated fish can lead to severe poisoning in both humans and wildlife (Wren 1986; Clarkson 2002).

The main source of MMHg in the oceanic environment is believed to be in-situ production by natural processes (Fitzgerald et. al. 2007). Mercury can become methylated by a number of biotic and abiotic means, with biological methylation likely dominating in the environment (Fitzgerald et. al. 2007; Mills et. al. 2003; Nikolaus et. al. 2003). A variety of organisms are capable, under certain conditions, of transforming Hg^{2+} to MMHg. Among these, sulfate reducing and Fe-reducing bacteria (SRB) are believed to be the most important (Fitzgerald 2004). The main source of MMHg in open ocean water is not clear but likely involves, to some extent, methylation by microbes in low oxygen microenvironments within particulate organic matter during remineralization processes (Wang et al. 2012; Sunderland 2009). In addition, submarine hydrothermal vents, where both elevated concentrations of Hg and SRB have been found, have been hypothesized as a deep marine source of MMHg (Kraepiel et al.

2003; Lamborg et al. 2006). However, Hg is apparently quickly sequestered near the vents and deep waters near the vents are not typically elevated in Hg so they are likely not a significant source of MMHg to the oceans (Lamborg et al. 2006; Fitzgerald et al. 2007). In contrast, little is known about mercury at cold seeps; as far as we are aware there have been no published studies of Hg at these sites.

The northern Gulf of Mexico (nGoM) is a diverse marine system that supports a vital fishery along the southern coast of the United States. The nGoM has numerous cold seeps, areas at the bottom of the water column where fluids containing hydrocarbons (crude oil and natural gas) and other constituents are released from geologic structures below (Sassen et. al. 2004; Lapham et. al. 2008). These seeps mostly occur along faults and fissures created by sedimentary, salt-driven tectonics (Lapham et. al. 2008; Kennicutt et. al. 1988). As upwelling fluids reach the sediment-water interface, entrained gases may become crystallized into gas hydrates or escape through bubble plumes or mud volcanoes (Dimitrov 2003; Milkov 2004). It is well known that cold seeps, where there are high rates of sulfate reduction and anaerobic hydrocarbon oxidation, support a diverse community of chemosynthetic organisms and microbial populations (e.g., Brooks et. al. 1987; Fisher et. al. 2000; Lanoil et. al. 2001; Lloyd et. al. 2006). The seeps are also responsible for carbonate reefs and chimneys, which represent the degradation and complete mineralization of hydrocarbons in a natural system (Roberts and Aharon 1994).

Cold seeps have been an active area of new discovery and research since the 1980's (Paull et al. 1984); however, little is known about the biogeochemical cycling of Hg at cold seeps. Of particular interest is whether they serve as significant sources of MMHg to Gulf waters and its ecosystem. Given that cold seeps have a thriving microbial community capable of methylating Hg, and have an additional geologic source of Hg (Hg is present in the gases and fluids continually evolved from cold seeps), it is plausible that significant amounts of MMHg may be produced at these sites. Indeed, the chemical environment in cold seeps include areas with low redox potentials and the presence of hydrocarbons, conditions that are ideal to facilitate the growth of microbial organisms that can methylate Hg such as SRB and anaerobic methanotrophic archaea (Stokke et. al. 2012; Levin 2005). The source of the Hg in the petroleum system is not well known but general hypotheses include: 1) Hg liberated in the earth's crust driven by heat and pressure that migrates as a vapor to the traps in which oil and gas accumulate (Wilhelm et. al. 2001), and 2) Hg that is co-deposited with the original organic matter making up the petroleum.

Typically, elemental Hg is the dominant species detected in natural gas, gas condensates and crude oil but dimethyl-Hg and mercuric halides may contribute trace amounts to the total Hg concentration (Wilhelm et. al. 2001). Most crude oils processed in the U.S. have relatively low Hg levels (<10 ng/g) (Wilhelm et. al. 2007); in the Gulf of Mexico, the average concentration of total-Hg in crude oils was 2.1 ng/g from seven different oil streams (Shafawi et al. 1999). Elemental Hg concentrations in natural gas are highly variable but have been reported to range from 1 to 200 ng/g (Ryzhov et. al. 2003). About 20 to 30 tons of Hg is recovered from natural gas by the European Union every year (UNEP 2011). Thus, natural seepage of the petroleum system at cold seeps could provide a significant source of elemental Hg to the deep-water Hg budget.

Recently, mass dependent fractionation (MDF) of the seven stable isotopes of Hg has been demonstrated for geological processes (e.g., Smith et al. 2005) and biological processes (e.g., Kritee et al. 2007). In addition, mass independent fractionation (MIF) has been shown to occur for photochemical processes (e.g., Bergquist and Blum 2007). As a result, Hg isotopic fingerprints have been used as a diagnostic tool to discriminate among Hg sources (e.g. Biswas et al. 2008). For cold seeps, the Hg isotopic composition may differ between cold seep and background sediments due, in part, to: 1) a different proportion of Hg sources (geologic "from below" and deposition "from above"), 2) different levels of biological activity (and associated biotic transformations of Hg), and 3) varying redox conditions and sequestration of Hg as cinnabar (HgS_{solid}).

The main objective of the present study was to determine if there are differences in mercury (total-Hg, MMHg, and Hg isotopic composition) between cold seep and background (control) sites to assess whether cold seeps are significant sources of Hg and MMHg to the nGoM deep marine environment. Here we report results for sediment from three different locations from the Woolsey Mound cold seep complex and from two background areas off the coast of Louisiana.

2.3 MATERIALS AND METHODS

2.3.1 Site Descriptions

Sediments were obtained from 5 different locations in the nGoM off the Louisiana coast southeast of Grand Isle, LA, U.S.A. (Fig. 5). Background sites were designated as Hg-1 and Hg-2, and cold seep sites as Hg-3, Hg-4, and Hg-5. Table 1 provides GPS coordinates and depths for each sampling site. The average water depth was 868 meters. The samples stem from one of the most studied cold seeps in the GoM, Woolsey Mound in Mississippi Canyon 118. The site serves as a seafloor observatory for the long-term study of gas hydrates. Seismic surveys of the sub-bottom geology have been conducted and near-surface accumulation and seepage of the petroleum hydrocarbon system are well known (Simonetti et. al. 2013, and references therein). Extensive coring programs have been conducted for lithologic logging and many have been subsampled for geochemical and microbial analysis (Bowles et al. 2011; Lapham et al. 2008). The rates and distribution of sulfate reduction and methane flux have been mapped and detailed habitat maps provide information on the distribution and locations of various chemosynthetic organisms. In addition, Woolsey Mound is also located relatively close to the Deepwater Horizon/Macondo Well incident, approximately 10 nautical miles to the north-northwest.

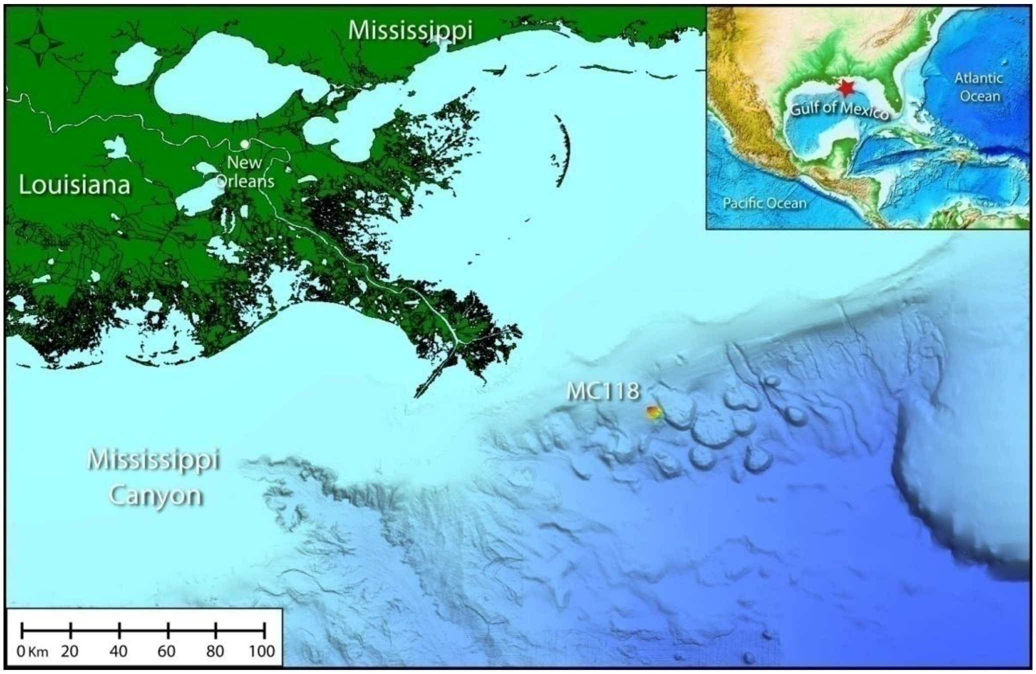

Figure 5 Map showing the general sampling location (MC118) off the Louisiana Coast in the Gulf of Mexico.

Table 1 Coordinates and depth of sampling locations

Table 1. Coordinates and depth of sampling locations

Sample ID	Site	Latitude (N)	Longitude (W)	Depth (m)
Hg-1	Background	28° 52.56853	88° 30.08669	835
Hg-2		28° 52.49157	88° 29.24710	855
Hg-3	Cold Seep	28° 51.49723	88° 29.60435	882
Hg-4		28° 51.43669	88° 29.60297	882
Hg-5		28° 51.23368	88° 29.50391	887

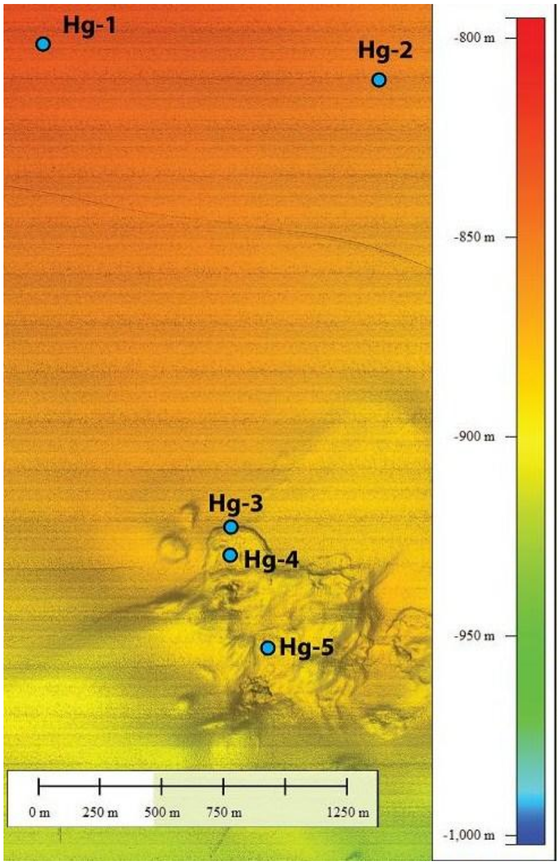

Figure 6 Map showing the specific sampling locations and their relative depths. Hg-1 and Hg-2 are background sites, Hg-3, -4 and -5 represent cold seep sites.

2.3.2 Core collection and handling

Samples were collected by the Mississippi Mineral Resources Institute (MMRI) using a box corer. MMRI has extensive experience studying and sampling Woolsey Mound. The samples contained macro- and micro-organisms that confirm that they were from the cold seep. On board the ship, two punch cores were collected from each box core using acid washed polycarbonate tubes. Cores for sites 1, 2 and 3 were 15 cm in length, cores 4 and 5 were 23 cm in length. The tubes were labeled (including direction), placed into a plastic bag, followed by an outer non-UV penetrable amber seal tight bag. Cores were stored upright in a freezer on the ship and delivered to the University of Mississippi on dry ice within 72 hours. Cores were thawed in a laminar flow clean bench and divided using a titanium knife. Cores 1, 2 and 3 were divided into three 5 cm intervals, and cores 4 and 5 were divided into four equal intervals of about 5.75 cm in length. The sections were transferred into pre-cleaned 250 mL wide-mouth amber glass jars with PTFE-lined caps (I-Chem; VWR Scientific), homogenized using a Teflon coated spoon, and stored in a freezer until analysis. Samples were allowed to air dry in a laminar flow clean bench prior to analysis.

2.3.3 Analytical methods

Because the aim of this study was simply to compare mercury speciation and isotopic composition between cold seep and background sites, redox measurements, high resolution depth profiles, and additional ancillary geochemical data were not carried out in this investigation. We note that the site has been geochemically characterized in a number of other studies (e.g. Lapham et al. 2008; Lutken et al. 2011).

2.3.3.1 Determination of total organic carbon (TOC)

Organic carbon (%) was determined using an Elemental Combustion System (Costech Analytical Technologies Inc., Valencia, CA). Before analysis, sediment samples were treated with 6 M hydrochloric acid to drive off inorganic carbon (US EPA 2002). About 5 mg of the treated sample was placed in tin capsules and combusted in the instrument. Methionine was used to calibrate the instrument and as a calibration check. Recoveries ranged between 89-110%.

2.3.3.2 Determination of Total-Hg

Total-Hg was determined using a DMA-80 (Milestone Inc., Shelton, CT, USA) following US EPA Method 7473 (US EPA 2007). The analyzer is based on release of Hg upon thermal decomposition of samples, isolation of Hg by amalgamation with gold, and detection using atomic absorption spectrometry. The Hg concentration is based on the absorbance measured at 253.7 nm and the weight of the sample. Here, about 0.25 g of sediment was weighed to the nearest 0.1 mg in Ni boats. Reference materials including MESS-3 (sediment) and NIST SRM 1573a (tomato leaf) were used to calibrate the instrument and as calibration check every 10 samples; results were within 15% of the certified values. During each run a subset of samples were analyzed in duplicate. The relative percent difference was <10%. Blanks were run every 10 samples to assure that Hg was not being carried over between samples. The amount of Hg for the blanks was negligible and corresponded to a concentration of ~0.20 ng/g using the typical weight of an analyzed sample. The method detection limit for the analysis was estimated at 0.1 ng/g.

2.3.3.3 Determination of Methyl-Hg

MMHg was measured using an automated analyzer (Tekran 2700; Tekran Inc. Toronto, Canada) following digestion of sediment using a procedure developed by the Florida Department of Environmental Quality (Tate 2012). Briefly, approximately 1 gram samples were digested with 5 ml of 25% KOH in methanol in 50-ml Teflon tubes. The samples were heated to 90°C for 1 hour using a hot-block. The digest was diluted to 50-ml with Purge and Trap grade methanol. 100-µL of the diluted digest was placed in a 100 ml volumetric flask along with 9-ml of a 9% (m/v) citrate buffer and diluted to the mark using methanol. A 30-mL aliquot of the solution was then transferred into an I-Chem® glass vial and 20 μL of a 1% (m/v) solution of sodium tetraethyl borate was added and the vial quickly capped. Volatile ethylated-Hg species were purged from solution onto a Tenax® carbon trap with ultrapure argon gas. The Hg species were then thermally desorbed and carried into a gas chromatograph. The separated volatile species were then passed through a pyrolytic decomposition tube and the elemental Hg vapor was carried into a cell for cold vapor atomic fluorescence detection.

For quality control, propyl-Hg was added to each sample prior to digestion as a surrogate to gauge losses during sample preparation; recoveries ranged between 70-120%. A calibration curve was constructed using a methylmercury (II) chloride (Sigma-Aldrich®) standard and r^2 values of 0.999 were achieved. A lab fortified blank (LFB) and method blank was run along with the sediment samples. The method blank consisted of 0.10 g of methionine added to 5.0 mL of 25% KOH/MeOH and 1.0 gram of Ottawa sand in a 50 ml acid washed digestion tube and was digested in the same manner as the sediment samples. LFB was prepared in the same manner, except without the sand, and the solution was spiked with MMHgCl standard to attain a final solution concentration of 4.0 ng/L after dilution. The recovery of the LFB was between 70% and 120%.

2.3.3.4 Determination of Hg isotopic composition

High precision Hg isotope ratios were measured at the University of Michigan using methodology described in detail elsewhere (Blum and Bergquist, 2007; Gehrke et al., 2009; Blum et al. 2011). In short, Hg^0 was released from the sediment by combusting the samples in a furnace at 750°C. The gases were passed through a second furnace held at 1000°C before being bubbled through a 1% $KMnO_4$ solution where Hg was trapped as Hg^{+2}. Sample recoveries were monitored and procedural blanks determined. Hg isotopic compositions were determined using a Nu Instruments MC-ICP-MS. Instrumental mass-bias was corrected using a Tl internal standard (NIST 997) and sample-standard bracketing using NIST 3133 solution at the same concentration and in the same matrix. Mass-dependent isotope concentrations are reported as described by Blum and Bergquist (2007) as δ^{202}Hg in permil (‰) relative to the NIST 3133 Hg standard following the equation:

$$\delta^{202}\mathrm{Hg} = 1000^{*}\{[(^{202}\mathrm{Hg}/^{198}\mathrm{Hg})_{\mathrm{sample}}]/[(^{202}\mathrm{Hg}/\,^{198}\mathrm{Hg})_{3133}]-1\}$$

Mass-independent Hg isotope fractionation (MIF) is reported as Δ^{199}Hg and Δ^{201}Hg in permil (‰), and is defined as the difference between measured δ^{199}Hg and δ^{201}Hg values and the predicted MDF values according the equations reported by Blum and Bergquist (2007):

$$\Delta^{199}\mathrm{Hg} = \delta^{199}\mathrm{Hg}_{\mathrm{measured}} - (\delta^{202}\mathrm{Hg}_{\mathrm{measured}} * 0.252)$$

$$\Delta^{201}\mathrm{Hg} = \delta^{201}\mathrm{Hg}_{\mathrm{measured}} - (\delta^{202}\mathrm{Hg}_{\mathrm{measured}} * 0.752)$$

Analytical uncertainty was determined using replicate analyses of UM-Almaden. For the analytical session in which these Gulf of Mexico samples were measured, UM-Almaden δ^{202}Hg = -0.53 ± 0.06, Δ^{199}Hg = -0.04 ± 0.06 and Δ^{201}Hg = -0.03 ± 0.06 (permil ± 2SD, n = 5). External reproducibility of the sample preparation process was assessed using the sediment reference material MESS-3 (National Research Council of Canada, Beaufort Sea marine sediment). Process recovery of Hg for this analysis of MESS-3 = 97%, and isotope results are δ^{202}Hg = -1.93, Δ^{199}Hg = 0.04 and Δ^{201}Hg = 0.00, which are in close agreement with long-term results (δ^{202}Hg = -1.88 ± 0.14, Δ^{199}Hg = 0.01 ± 0.05 and Δ^{201}Hg = -0.04 ± 0.05 (permil +/- 2SD, n = 32). The uncertainties associated with the long-term isotopic results for MESS-3 are a good estimate of external reproducibility associated with these Gulf of Mexico samples.

2.4 RESULTS AND DISCUSSION

2.4.1 Total Organic Carbon

Sources of organic matter at the deep water sites in this study may include surface phytoplankton productivity, chemosynthetic community organic matter from local biomass, and petroleum derived from seep fluids (Lapham et al. 2008). Whereas the sediments are exposed to all these sources, the heterogeneity is believed to be dominated by the upward flux of methane, hydrocarbon and petroleum (Lapham et al. 2008). In the present study, sediment organic carbon averaged 1.5% ± 1.1 (1SD, n=31), ranged from 0.3% to 6.5%, and decreased slightly with depth (Table 2). Excluding one background sample that contained an anomalously high level of organic carbon (6.5%), there was little difference between background (1.57% ± 0.56) and cold seeps (1.37% ± 0.45). The sediments at all the deep marine sites in this study will contain reduced muds with decaying organisms from the water column. In addition, cold seep sediments may contain petroleum hydrocarbons (at ppm levels or higher) delivered to the seafloor from a deeper petroleum system. It should be noted that at some cold seeps hydrocarbon fluids migrate through the sediment fast enough escape through bubbles or mud volcanoes (e.g., Lapham 2008). Our TOC values are toward the upper end of that reported by Lapham et al. (2008) who studied microbial activity in surficial sediments at MC118 cold seep field. The values are also slightly higher than those reported by Delaune et al. (2008) and within the range reported by Trefry et al. (2007) for offshore drilling sites along the Louisiana coast. Drilling platforms result in considerable detrital fallout of organic matter due to the high population of fish at these sites. Methylation in sediment by sulfate reducing bacteria (SRB) is supported by organic carbon, which can act as a substrate for SRB metabolism (Liu, et al 2009). Levels of MMHg and total-Hg have been correlated with organic carbon in sediments from the nGoM (Trefry et al. 2006, Delaune et al. 2008). We also observe this trend though weaker than those reported by Trefry and Delaune (Fig. 7).

Table 2 Summary statistics for deep marine sediments from the Northern Gulf of Mexico.

Table 2. Summary statistics for organic carbon, total-Hg and MeHg in deep marine sediments from the northern Gulf of Mexico

Location	Depth (cm)	Organic Carbon (%)			Total-Hg (ng/g)					MeHg (ng/g)					
		n	mean	1SD	n	mean	1SD	min	max	n	mean	1SD	min	max	% of Total
Background	0-5	4	3.1	2.3	4	59.5	5.6	53.4	66.9	3	0.64	0.15	0.52	0.80	1.1
	5-10	2	1.4	26%	2	50.3	3.9	46.1	54.4						
	10-15	2	1.1	5.2%	4	46.4	3.4	42.9	50.6	3	0.83	0.73	0.20	1.63	1.8
	All	8	2.2	1.8	10	52.7	7.4	42.9	66.9	6	0.73	0.48	0.20	1.63	1.4
Cold Seep	0-5	5	1.5	0.5	6	57.8	3.9	53.1	63.2	6	1.12	0.63	0.22	1.92	1.9
	5-10	11	1.3	0.5	6	50.1	6.4	42.2	55.7						
	bottom*	7	1.2	0.3	6	35.4	3.9	31.2	40.8	6	0.86	0.52	0.42	1.78	2.4
	All	23	1.3	0.5	18	48.8	9.9	31.2	63.2	12	0.99	0.57	0.22	1.92	2.0
All		31	1.5	1.1	28	50.2	9.2	31.2	66.9	18	0.91	0.54	0.20	1.92	1.8

Concentrations are on a dry-weight basis. MeHg was not measured in the intermediate depths. *Includes interval 10-15 cm from site Hg-3 and 18-23 cm from sites Hg-4 and Hg-5.

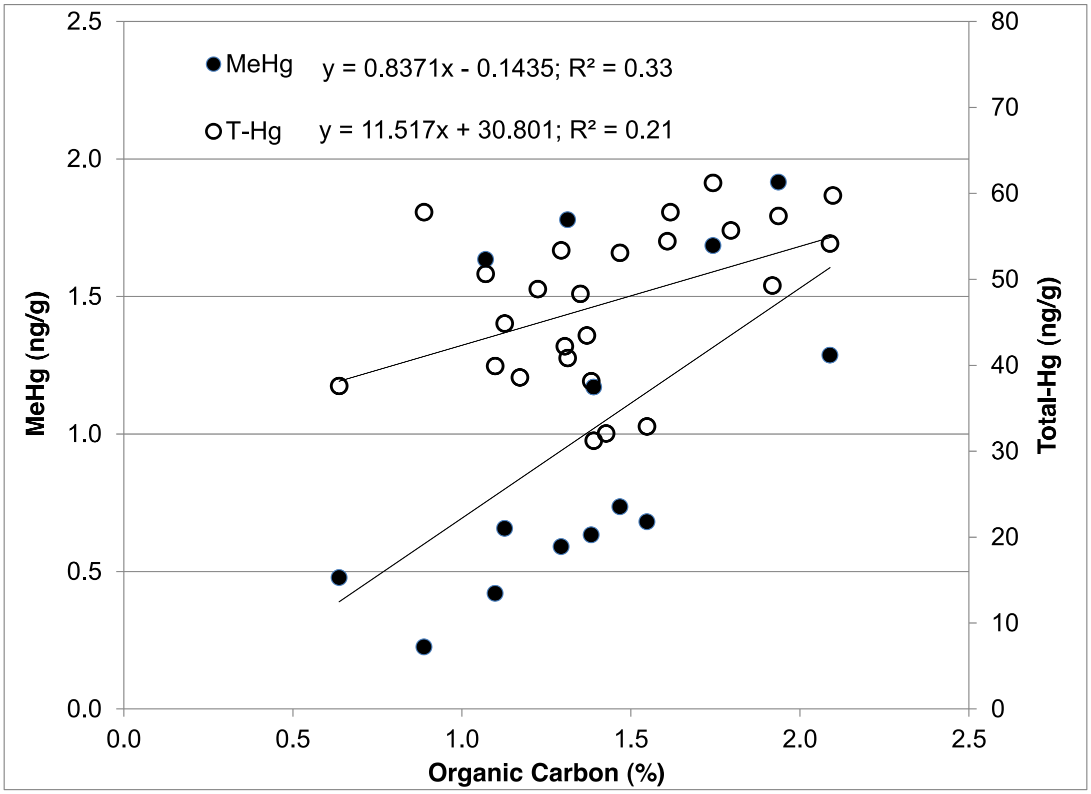

Figure 7 Concentrations of MMHg and total-Hg versus organic carbon for deep marine sediments from MC118 in the northern Gulf of Mexico. Anomalous data point at 6.5% carbon not included for clarity of other data.

2.4.2 Total-Hg and MMHg

Total-Hg averaged 50.2 ± 9.2 ng/g and ranged from 31.2 to 66.9 ng/g (Table 2). There was no significant difference between cold seep and background sites; background averaged 52.7 ± 7.4 ng/g and cold seeps averaged 48.8 ± 9.9 ng/g. Our results are similar, albeit slightly lower than, the 40 to 80 ng/g reported for background sites in a study of total-Hg in sediment near GoM oilrig platforms (Trefry et al. 2007) and suggests that cold seeps are not significant sources of Hg to these deep marine sediments (Table 3). Hg in sediment near oilrig drilling sites can be much higher due to the presence of barite ($BaSO_4$), which is a component of spent drilling mud that contains relatively high levels of Hg; regulations set the upper limit of Hg in barite to 1 µg/g (Trefry et al. 2007).

Table 3 Total-Hg and MeHg in marine sediments from the northern Gulf of Mexico (other studies)

Table 3. Total-Hg and MeHg in marine sediments from the northern Gulf of Mexico (other studies)

Location Name	Coordinates	Depth (m)	Total-Hg (ng/g)	MeHg (ng/g)	Reference
MP299	29°15.43' 88°46.38'	60	61 ± 6	0.42±0.20	Trefry et. al 2007 ("far field" samples)
EI346	28°09.83' 91°22.14'	92	47 ± 3	0.30±0.10	
EB 963	28°00.65' 90°07.47'	540	71±3	0.65±0.24	
GC 112	27°51.32' 90°44.09'	534	79±8	0.87±0.20	
MC 496	28°27.03' 89°22.44'	556	71±5	0.51±0.18	
ST 53	28°51.484' 90°27.551'	19	31.88±7.8	0.41±0.05	DeLaune et al. 2008
C6B	28°52.169' 90°28.040'	19	27.01±7.22	0.46±0.13	
C4	28°57.019 90°31.783	13	37.79±2.49	0.31±0.03	Meihuey 2006
C6B	28°52.230' 90°28.045’	19	41.06±2.41	0.47±0.06	
C8	28°47.312 90°16.668	23	11.02±1.77	0.12±0.02	

Total-Hg decreased with sediment depth (Fig. 8). For background sites, top of the cores (0-5 cm) averaged 59.5 ± 5.6 ng/g while the bottom portion (10-15 cm) averaged 46.4 ± 3.4 ng/g. For cold seep sites, top of the cores averaged 57.8 ± 3.9 ng/g while the bottom portion averaged 35.4 ± 3.9 ng/g. Thus, even when taking into account changes in depth there appears to be no difference in Hg between cold seep and background sites. Moreover, the similar trend lines for Hg with depth suggest similar deposition rates of Hg to these locations.

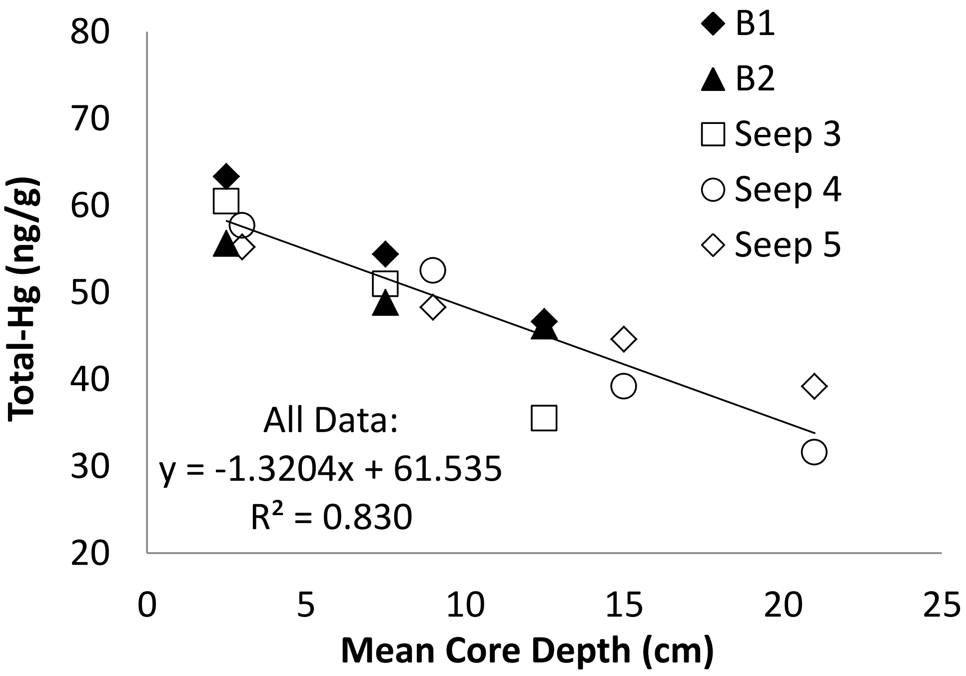

Figure 8 Total-Hg versus core depth for sediments from background sites (B1, B2) and cold seep sites (Seep 3, 4, 5) collected off the coast of Louisiana in the Gulf of Mexico. R^2 values for individual cores B1, B2, Seep 3, Seep 4 and Seep 5 were 0.998, 0.942, 0.981, 0.975 and 0.987, respectively.

MMHg averaged 0.91 ± 0.54 ng/g and ranged from 0.20 to 1.92 ng/g (Table 2). Mean concentrations at the cold seep sites were slightly greater than the background sites (0.99 ± 0.57 ng/g versus 0.73 ± 0.48 ng/g) but the difference was not significant. While there are no other reports of MMHg in cold seep sediment, several studies report MMHg in surficial sediment in the northern GoM. Trefey et al. (2007) found 0.44 ± 0.27 ng/g (range 0.11 to 1.0 ng/g, n=39), and Delaune et al. (2008) found 0.46 ± 0.13 (range 0.32 to 0.58 ng/g, n=3), at "background" sites away from offshore drilling rigs. MMHg levels were found to be slightly greater near offshore drilling sites (Delaune et al. 2008). In another study, MMHg was found to range from 0.12 to 0.47 ng/g in sediment collected from a hypoxic zone off the coast of Louisiana (Meihuey 2006). Our results are slightly higher but overlap the range reported in these studies. The percent of total-Hg in the methylated form was also higher in the cold seep sediments (2.4%) versus background sediments (1.4%). We found no consistent trend for MMHg with depth however as noted earlier we did not divide our core to obtain a high-resolution depth profile.

Overall, the results suggest that cold seep sediment may have slightly higher levels of MMHg than background sites, but the variation in the data does not allow us to definitively draw that conclusion. Even if there is an increase in methylation rate at cold seeps, it appears the difference is small and would not constitute a major source of MMHg to the deep marine environment. However, a more detailed study (beyond the scope of this study) examining in-situ methylation rates and redox conditions and measurements of Hg in chemosynthetic organisms from cold seeps would provide a more definitively answer.

2.4.3 Hg isotopic composition

If cold seeps introduce Hg from deep geologic sources then one might expect cold seep sediments to have a different isotopic signature than background areas where the source of mercury is primarily associated with deposition of particulates generated or introduced to the water column. This is because “geologic” Hg would have potentially undergone different fractionation processes in the subsurface and during mobilization and transport. Indeed, different isotopic signatures have been observed for Hg in different coal deposits (Biswas et al., 2008; Lefticariu et al., 2011), reflecting differing atmospheric and geological sources of Hg. However, we find that the isotopic composition between the sites is indistinguishable both in MDF (δ^{202}Hg=-0.66±0.10 for cold seeps and -0.70±0.12 for background sites) and MIF (Δ^{199}Hg =0.07±0.02 for cold seeps and 0.09±0.03 for background sites) (Table 4). Depth profiles revealed Hg has lower δ^{202}Hg near the surface and increases with depth (r^2=0.978 for the core cold seep site 5). The total range of δ^{202}Hg was -0.5 to -0.8- permil (see Fig. 9). This range is similar to the ranges reported for recent deep-water materials off the coast of Portugal (Mil-Homens et al. 2012) and pre-anthropogenic sediments from the Mediterranean (Gehrke et al. 2009). Other GOM sediment samples (from a more coastal setting 5-25 km off the Louisiana coast) have lower δ^{202}Hg and Δ^{199}Hg (Rice et al 2007; Blum 2011) that most likely reflect greater terrestrial and anthropogenic inputs of Hg (see data labeled Coastal in Fig 9).

Table 4 Mercury isotopic composition of sediment from the northern Gulf of Mexico.

Table 4. Mercuy isotopic composition of sediments from the northern Gulf of Mexico. Mercury isotopic compositon are reported in delta (permil) notation. Uncertainties are based on n measurements.

General Location	Core ID	Depth (cm)	n	δ204	1SD	δ202	1SD	δ201	1SD	δ200	1SD	δ199	1SD	Δ204	1SD	Δ201	1SD	Δ200	1SD	Δ199	1SD
Background	1A	5-10	1	-0.89		-0.59		-0.41		-0.26		-0.11		-0.01		0.03		0.04		0.04	
	2A	0-5	2	-0.90	0.01	-0.56	0.00	-0.35	0.01	-0.26	0.01	-0.06	0.05	-0.07	0.01	0.07	0.01	0.02	0.01	0.08	0.05
	2A	5-10	2	-1.09	0.11	-0.73	0.09	-0.46	0.08	-0.33	0.01	-0.10	0.08	0.00	0.03	0.09	0.01	0.03	0.03	0.08	0.06
	2A	10-15	2	-1.23	0.03	-0.79	0.04	-0.60	0.00	-0.37	0.03	-0.16	0.02	-0.05	0.09	0.00	0.03	0.03	0.01	0.04	0.01
	2B	5-10	1	-0.94		-0.63		-0.45		-0.24		-0.07		0.00		0.02		0.07		0.08	
	Mean ± 1SD		n=5	-1.01	0.15	-0.66	0.10	-0.45	0.09	-0.29	0.05	-0.10	0.04	-0.03	0.03	0.04	0.04	0.04	0.02	0.07	0.02
Cold Seep	3A	5-10	1	-1.17		-0.79		-0.55		-0.36		-0.14		0.01		0.04		0.04		0.06	
	3B	5-10	1	-1.13		-0.76		-0.54		-0.34		-0.11		0.00		0.03		0.04		0.09	
	4A	6-12	1	-1.19		-0.79		-0.59		-0.35		-0.11		-0.01		0.00		0.04		0.09	
	5A	0-6	2	-0.87	0.05	-0.54	0.06	-0.38	0.01	-0.23	0.02	-0.03	0.01	-0.07	0.14	0.02	0.05	0.03	0.01	0.10	0.03
	5B	6-12	2	-0.95	0.09	-0.61	0.09	-0.42	0.11	-0.27	0.00	-0.04	0.02	-0.04	0.04	0.03	0.04	0.03	0.04	0.12	0.00
	5A	12-18	2	-1.17	0.05	-0.75	0.04	-0.50	0.03	-0.35	0.03	-0.10	0.01	-0.05	0.01	0.07	0.06	0.03	0.00	0.09	0.00
	5A	18-24	2	-1.21	0.02	-0.83	0.01	-0.57	0.02	-0.38	0.02	-0.17	0.05	0.03	0.00	0.05	0.01	0.04	0.01	0.04	0.05
	Mean ± 1SD		n=7	-1.08	0.16	-0.70	0.12	-0.49	0.09	-0.32	0.06	-0.09	0.06	-0.03	0.04	0.04	0.03	0.04	0.00	0.09	0.03
All	Mean ± 1SD		n=12	-1.06	0.14	-0.70	0.10	-0.49	0.08	-0.31	0.05	-0.10	0.04	-0.02	0.03	0.04	0.03	0.04	0.01	0.08	0.03

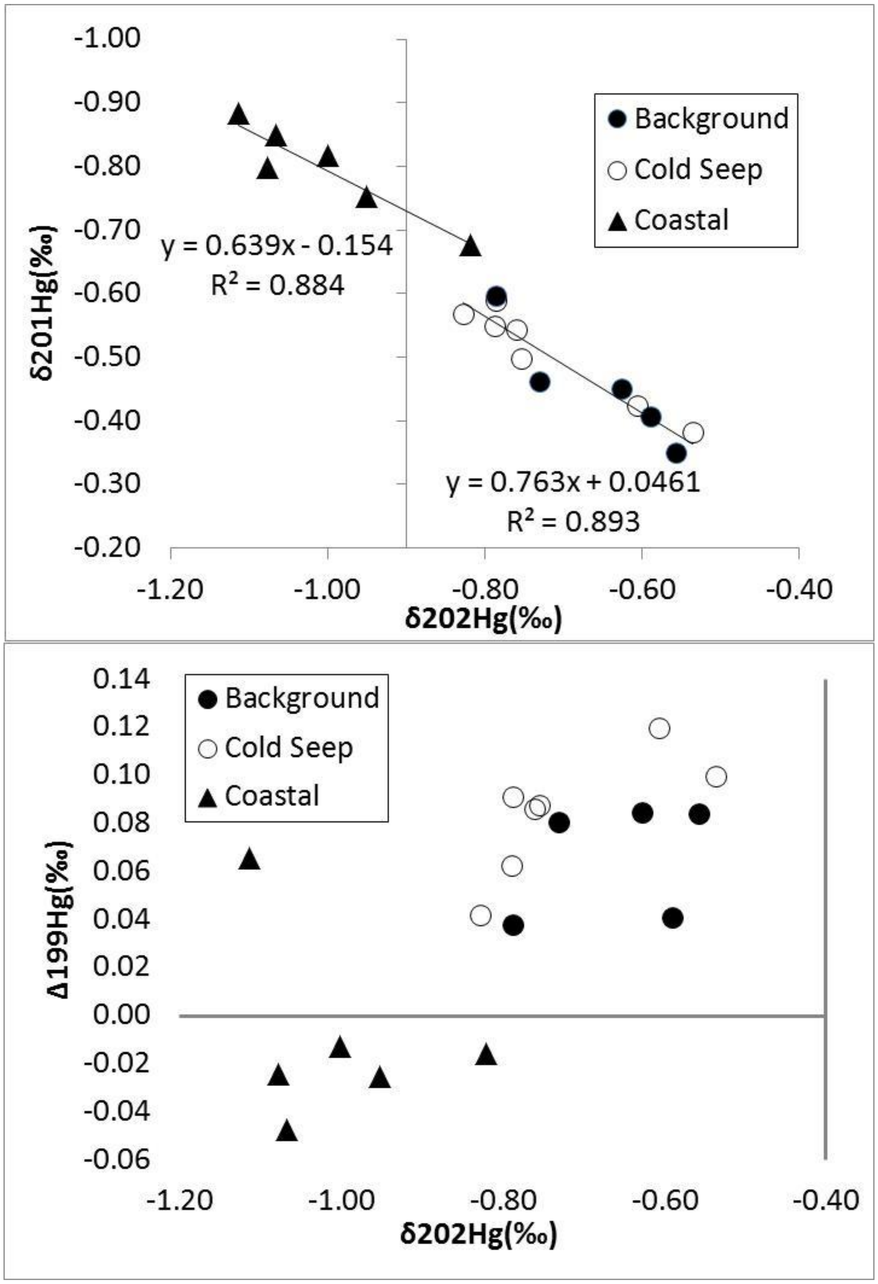

Figure 9 Isotope signature plots for sediments from the northern Gulf of Mexico. Background and cold seep samples are from an average depth of 868 m; coastal samples (Blum, 2011) are from near the mouth of the Mississippi and Atchafalaya rivers (Lat 28.9, Lon -90.5 and Lat 28.7, Lon -91.3) at an average depth of 17.4 m (Rice et al. 2009).

2.5 CONCLUSIONS

This first study of mercury speciation and isotopic composition in sediment at cold seeps in the nGoM suggests that hydrocarbon releases at cold seeps have little influence on total-Hg concentrations in the sediment. This is supported by the similar isotopic composition between cold seep and background sites. Hg derived from geologic sources (from below) and mobilized in fluids released at cold seeps is apparently insufficient to significantly alter the Hg isotopic signature of mercury that has been deposited with sediment particulates (from above). Whereas the average MMHg levels and percentages of total-Hg were greater at cold seeps compared to background sites, the differences were not statistically significant. From this perspective cold seeps do not appear to be major sources of MMHg to nGoM waters. A detailed examination of redox conditions and methylation rates is needed to fully understand the biogeochemical cycling of mercury at cold seeps.

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CHAPTER THREE

3. MERCURY IN FISH FROM ENID, SARDIS AND GRENADA LAKES: CONCENTRATIONS, TRENDS, AND RISK ASSESSMENT

3.1 ABSTRACT

The Mississippi Department of Health issued fish consumption advisories for Enid Lake in 1995 and Grenada Lake in 2001 due to high levels of the mercury (Hg) in fish. In the present study, total mercury (Hg) concentrations were determined in the muscle tissue of a total of 202 fish from Enid, Sardis and Grenada Lakes in North Mississippi by combustion-atomic absorption spectrometry. Species analyzed include those that are routinely targeted and consumed by fisherman: white crappie (WC), largemouth bass (LMB), and catfish, including channel catfish (CC), blue catfish (BC), and flathead catfish (FH). Gizzard shad (GS), a common forage fish lower on the trophic scale, was also analyzed. The purpose was to re-assess Hg levels in fish from these lakes and the human-health risk associated with consumption of edible fillets using current guidelines. In addition, MeHg/Total-Hg ratios were determined in the liver of select fish; this ratio has been used to evaluate the trends in Hg accumulation in fish.

Mercury levels in the fish generally increased with fish size and trophic position. For all lakes combined, FH had the highest mean levels of Hg (1093 ng/g, n = 2), followed by BC (439 ng/g, n = 10), LMB (357 ng/g, n = 54), CC (255 ng/g n =47), WC (187 ng/g, n = 56), and GS (36 ng/g, n = 33). On average fish from Grenada Lake had higher levels of Hg for LMB (469 ng/g), CC (395 ng/g), and GS (70 ng/g), compared to Sardis and Enid Lakes which had similar mean levels for LMB (312 ng/g and 289, respectively). For WC, averages were similar between Enid (214 ng/g) and Grenada (199 ng/g), while Sardis had lower levels (147 ng/g). There were too few FH and BC to make comparisons.

Concentrations in LMB from Enid Lake tended to be lower than reported for fish collected in the early 1990's; there were no data available for the other lakes and species for comparison. This is a promising development but it is unclear whether this is related to recent emission caps for US coal- and oil-fired electric generating units. Interestingly, ratios of MeHg in liver to total-Hg in muscle were <0.5 for all fish analyzed; others have suggested that ratios of <0.5 indicates that the Hg accumulation is decreasing.

A risk assessment indicated that the hazard index (quotient) levels from Enid were greater than one for most fish collected which suggest a hazard health effect will occur at the representative intake value. The suggested consumption limit for adults (on a monthly basis) and children (on a yearly basis), respectively are 4 and 11 meals for both LMB and WC, and slightly more, 6 and 16 meals for CC.

3.2 INTRODUCTION

Mercury (Hg) is among the most highly bioconcentrated trace metals in the human food chain (Waite et al. 2002). Humans are exposed to Hg primarily through consumption of contaminated fish. Mercury from both natural and anthropogenic sources enter aquatic systems through wet and dry deposition, and through inputs from streams and rivers. Mercury is methylated by micro-organisms, particularly sulfate reducing bacteria which thrive in anaerobic conditions often found in sediment. Once methylated, the organo-metallic water-soluble form (MeHg) complexes with various ligands (e.g. Cl^-) in aquatic systems and with thiols in biota. MeHg is assimilated into aquatic species through passive and direct assimilation with a major jump occurring at the base of the aquatic food chain (Bodaly et al. 1997). Small organisms such as plankton incorporate Hg via passive surface absorption or through food intake (Bryan, et al. 1979). The only route of Hg exposure to autotrophic organisms is through passive absorption; the relatively small amount of Hg that results is apparently not detrimental to the organism (ECPD 2013). Heterotrophic organisms are exposed to Hg via a second route, the diet. MeHg biomagnifies through the food chain as predators eat other organisms and absorb the contaminants in their food. Because MeHg has a relatively long half-life (~3.3 years in fish), its concentration increases in fish as they age, particularly for top predators, which have higher metabolic rates and are positioned higher on the food chain (Van Walleghem, et al. 2013).

Humans are at risk when consuming fish fillets with elevated levels of Hg, because >95% of the Hg in fish-muscle tissue is in the MeHg form (Bloom 1992). MeHg is a neurotoxin that can cross the blood-brain barrier in humans; it can also affect astrocytes, non-neuronal cells in the central nervous system (Aschner et al 2007). In the US, the Environmental Protection Agency (EPA) and the Food and Drug Association (FDA), as well as state agencies (Table 5) issue regulations for mercury in water and fish. Although it is recognized that individuals have different risks based on body-weight and age with fetuses and children being more susceptible to the effects of MeHg than adults, single Hg concentration thresholds are still widely referenced and vary by agency. For example, the FDA uses 1 μg/g of Hg in fish tissue (though set for commercial fish), whereas the EPA uses a more restrictive 0.3 μg/g value as screening values for consumption advisories. The Mississippi Department of Environmental Quality (MDEQ) lists the aquatic life criteria for safe levels of Hg in fish as 0.153 ppm, which is lower than both the FDA and EPA criteria.

Table 5 Select regulations for mercury in the United States

Medium	Source	Species	Criterion		Agency*	Notes
Water	Drinking Water	Hg	2 ng/g	Maximum Contaminant Level	US EPA	Safe Drinking Water Act
	Water Quality Criteria	Hg	0.77 ug/L (chronic)	Aquatic Life: Freshwater	US EPA	Clean Water Act
	Aquatic Life Support Criteria	Hg^{2+}	12 ng/L	Aquatic Life: Freshwater	MDEQ	Total maximum Daily Load
	Aquatic Life Support Criteria	Hg^{2+}	25 ng/L	Aquatic Life: Saltwater	MDEQ	Total maximum Daily Load
	Drinking Water	Hg	≤ 0.151 µg/L	Public Water Supply	MDEQ	Total maximum Daily Load
Air	Indoor Air Concentration	Hg^0	25 µg/m^3	Occupational settings where mercury is handled	ATSDR	Method of analysis: real-time air monitoring instrument
	Indoor Residential Occupancy Level	Hg^0	≤ 0.1 µg/m^3	Indoor Air quality after a spill	ATSDR	Level acceptable for occupancy of any structure after a spill
Fish	Muscle	MeHg	0.3 µg/g	Fish Consumption	US EPA	Based on consumption rate of 0.0175 kg/day
	Muscle	Hg	1 µg/g	Fish Consumption	US FDA	Set for mercury in commercial fish
	Whole Body	Hg	0.153 µg/L	Water quality criteria	MDEQ	Total maximum Daily Load

*US EPA- United States Environmental Protection Agency; MDEQ- Mississippi Department of Environmental Quality; ATSDR- Agency for Toxic Substances and Disease Registry; US FDA- United States Food and Drug Administration

In the state of Mississippi, numerous of lakes have been placed on the EPA advisory list of waterbodies impaired with Hg (US EPA 2013). Among these are Enid and Grenada Lakes (MDEQ 2002, 2008). The origins of mercury in these waterbodies are unclear, with no known point sources of Hg; atmospheric deposition and watershed runoff are considered non-point sources.

Several studies have used ratios of MeHg and total-Hg in fish muscle and liver to assess status of Hg in fish (Collin et al 2009; Cizdziel et al 2003; Wagemann et al 2000). For fish with high levels of Hg in fish muscle (> ~0.5 ng/g) Hg in the liver tends to be higher than in the muscle. Collin et al. (2013) investigated methylation and demethylation processes in tilapia after dietary exposure to isotopically labeled Hg and MeHg. The study found that MeHg had faster inter-organ transportation from liver toward muscles, and recorded the percentage of MeHg being lower with an increase in total amount of Hg in the muscle. Another study looked at the ratios of MeHg/Total-Hg in liver tissue, which suggested that greater ratios for higher trophic levels are due to diet intake and the different ways of processing Hg between piscivores and non-piscivores (Cizdziel 2003).

In the present study, total-Hg was determined in muscle tissue for 202 fish from Enid, Sardis, and Grenada Lakes located in North Mississippi. Fish species studied include most of the fish commonly targeted by fishers, including largemouth bass (LMB), channel catfish (CC), white crappie (WC), flathead catfish (FH), and blue catfish (BC). The purpose was to assess Hg levels in fish from these lakes and to determine the human-health risk associated with consumption of edible fillets. In addition, the MeHg/Total-Hg ratios were determined in the liver of select WC, LMB, CC and FH to evaluate trends in Hg accumulation in individual fish.

3.3 MATERIALS AND METHODS

3.3.1 Study Area

Grenada, Sardis, and Enid lakes are located in North Mississippi (Fig. 10). The lakes were constructed in the 1940's and 1950's to control the flood prone regions in the Mississippi delta, which in 1927 suffered a devastating flood (Donald, 2004). Congress enacted the Yazoo Headwater Project in 1936 that lead the way for the construction of reservoirs, levees, and dams in the flooded region. The surface area of Grenada, Sardis, and Enid lakes are 142 km^2, 131.5 km^2, and 84.9 km^2, respectively. Water levels are regulated for flood control and recreational usage with fall and winter months having the lowest levels and spring and summer months having the highest.

3.3.2 Fishing and Sampling

We collected and analyzed fish species that are most commonly targeted and consumed by the fishing public (Table 6). The Mississippi Department of Wildlife, Fisheries, and Parks (MDWFP) collected fish from Grenada and Sardis Lakes and the USDA National Sedimentation Laboratory (NSL) collected fish from Enid Lake. Fish were captured by electro-shocking, a non-lethal survey method in which the fish are temporarily paralyzed. In short, electrodes are used to deliver 3-4 amps. The voltage delivered varies with the conductivity of each lake. The boat serves as the cathode and the anode was mounted to the bow of the boat. The impact of the electrical field spans ~12 ft from the front of the boat to a depth of 6-8 ft. Fish from Grenada Lake were collected near Young's, Grenada, Bryant and Gums Crossing Landing Areas. Fish from Sardis were collected from Hurricane Creek and Teckville Landing areas. Fish from Enid Lake were collected near Cossar State Park and recreational areas.

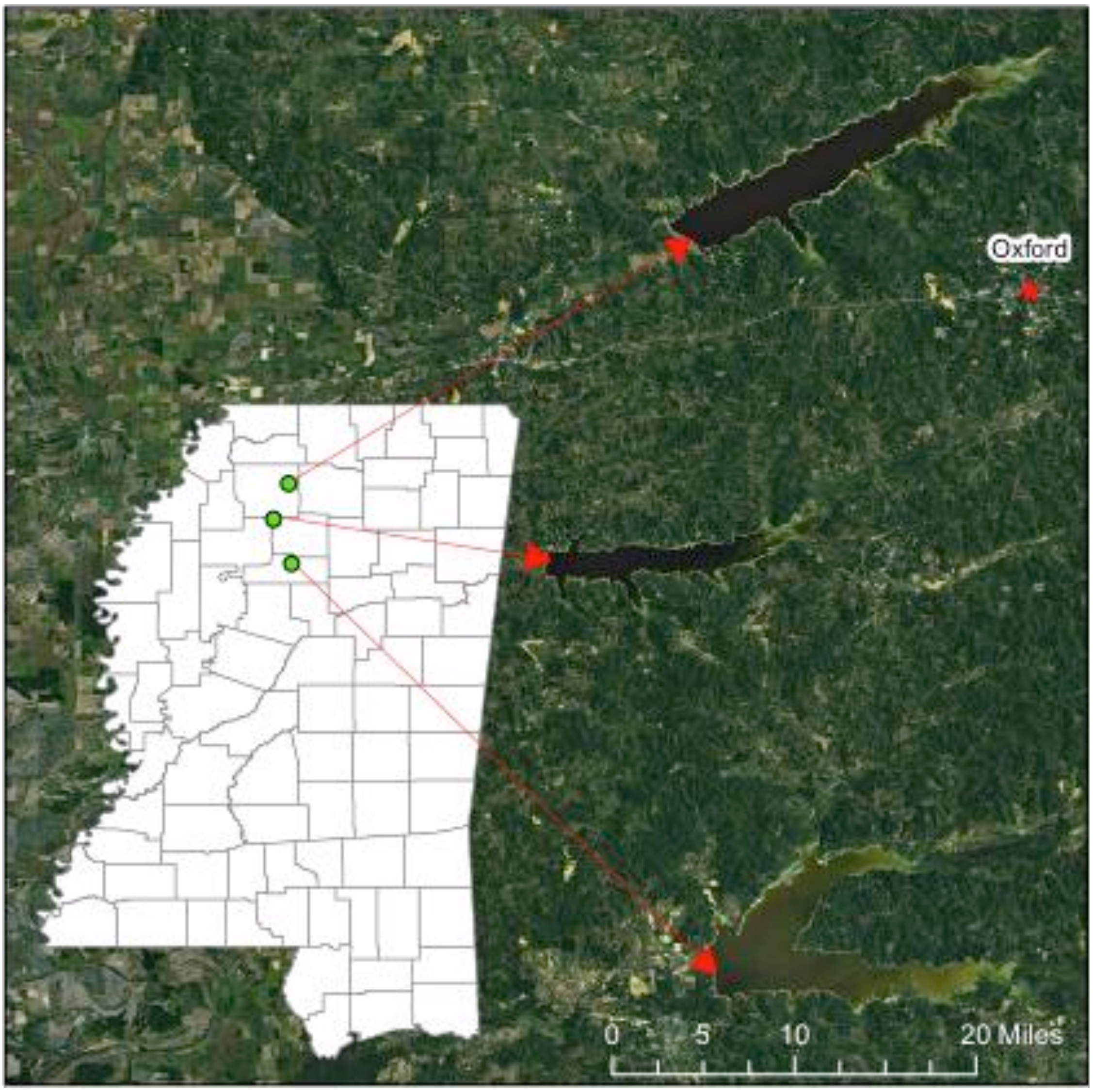

Figure 10 Sardis (top), Enid (center), and Grenada (bottom) lakes in relation to State of Mississippi and the town of Oxford.

Table 6 Fish species and number of fish collected in North Mississippi Lakes.

Species	Sardis	Grenada	Enid
Crappie (*Pomoxis annularis*)	20	20	16
Largemouth Bass (*Micropterus salmoides*)	21	18	15
Channel Catfish (*Ictalurus punctatus*)	18	14	15
Blue Catfish (*Ictalurus furcatus*)	4	6	--
Flathead Catfish (*Pylodictis olivaris*)	0	2	--
Gizzard Shad (*Dorosoma cepedianum*)	21	12	--
TOTAL	84	72	46

Figure 11 Wildlife biologists from the Mississippi Department of Wildlife, Fisheries, and Parks conducting fish surveys using electro-shocking.

Fish were placed in a cooler directly on ice and transported to the University of Mississippi Chemistry laboratory. Fish were processed in the lab by dissecting the muscle, liver and other vital organs (gills, gonad (when present), kidney, heart, sperm eggs to be used in subsequent analysis) using a titanium knife. The samples were transferred to individual vials and plastic bags for storage. All samples were kept frozen until analysis.

3.3.3 Trophic level

The trophic level is the position a fish occupies in the aquatic food chain. It is routinely determined based on fish diet studies (Pauly et al. 1998). MeHg biomagnifies in the aquatic food chain with top predator fish containing a greater accumulation of MeHg in muscle tissue. The trophic levels of species collected in this study are 4.4 ± 0.8 (WC), 3.9 ± 0.6 (CC), 3.8 ± 0.7 (FC), 3.8 ± 0.6 (LMB), 3.4 ± 0.8 (BC), and 2.4 ± 0.21 (GS) (Froese, 2013). Whereas levels of Hg generally follow trophic status, concentrations in individual fish also depend on age and size (weight) of the fish, such that young (small) fish of a high trophic level may have less Hg then old (larger) fish of a lower trophic level.

3.3.4 Determination of Total-Hg

Total-Hg in fish tissue was determined using a DMA-80 (Milestone Inc., Shelton, CT, USA). Following US EPA method 7473. The analyzer is based on release of Hg upon thermal decomposition of samples, isolation of Hg by amalgamation with gold, and detection using atomic absorption spectrometry. Fish samples were allowed to thaw and about 0.2 grams of sample was obtained using a titanium knife or biopsy punch and placed in nickel boats for analysis.

Method detection limits were determined to be 0.01 ng of Hg. The instrument was calibrated using liquid Hg standards (Spex Certiprep). A calibration curve with an r^2 value of 0.999 was obtained. Certified Reference Material DOLT-2 (Dogfish liver Tissue, National Research Council of Canada) was used as a reference check and was run every 10th sample. Recoveries were between 80% and 120%. Duplicate samples were analyzed for species and obtained a relative percent difference of less than 20%.

3.3.5 Determination of MeHg

MeHg was determined in select samples of fish-liver by cold-vapor atomic fluorescence spectrometry (CVAFS) using a Tekran 2700 (Tekran Inc. Toronto, Canada). Prior to analysis, tissue samples were digested following a standard procedure used by the Florida Department of Environmental Quality (Tate 2012). Briefly, approximately 0.5 to 1 gram of tissue (wet weight) was digested with 5 ml of 25% KOH in methanol in 50-ml Teflon tubes. The samples were heated to 90°C for 1 hour using a hot-block. The digest was diluted to 50-ml with Purge and Trap grade methanol. 100-µL of the diluted digest was placed in a 100 ml volumetric flask along with 9-ml of a 9% (m/v) citrate buffer and diluted to the mark using methanol. A 30-mL aliquot of the solution was then transferred into an I-Chem® glass vial and 30 µL of a 1% (m/v) solution of sodium tetraethyl borate was added and the vial quickly capped. Volatile ethylated-Hg species were purged from solution onto a Tenax® carbon trap with ultrapure argon gas. The Hg species were then thermally desorbed and carried into a gas chromatograph. The separated volatile species were then passed through a pyrolytic decomposition tube and the elemental Hg vapor was carried into a cell for measurement by CVAFS.

For quality control, propyl-Hg was added to each sample prior to digestion as a surrogate to gauge losses during sample preparation; recoveries ranged between 77-115%. A calibration curve was constructed using a methylmercury (II) chloride (Sigma-Aldrich®) standard and r^2 values of 0.99 were achieved. A lab-fortified blank (LFB) was run along with the sediment samples. The LFB consisted of 0.10 g of methionine added to 5.0 mL of 25% KOH and was spiked with MeHgCl standard to attain a final solution concentration of 4.0 ng/L after dilution. This was performed in a 50 ml acid washed digestion tube and digested in the same manner as the fish liver samples. The recovery of the LFB was 80%. DOLT-2 was also digested in the same manner as the liver samples and the recovery was between 101-107% from the certified reference value. Samples run in duplicates obtain RPD less then 15% and samples run in triplicates obtained relative standard deviations (RSD) of less than 10%.

3.3.6 Assessing the risk of consuming fish from the lakes

To estimate exposure to MeHg through consumption of contaminated fish, we used the most recent US EPA guidelines for assessment of Hg risk to populations with high consumption of self-caught freshwater fish (US EPA 2011). Intake Rate (IR) was calculated as follows:

$$\text{Intake Rate } (\mu g/kg/d) = \frac{FTC * MCF * FCR * CAF}{BW}$$

Where: FTC is the concentration of total-Hg in fish-muscle measured in μg/g; MCF is the mercury conversion factor (0.95), which is the fraction of Hg in fish tissue that is MeHg; FCR is the fish consumption rate (173 g/day); CAF is the cooking adjustment factor (1.5 unitless); BW is the body weight measured (60 kg for adults and 14.5 kg for children).

The intake value is then used to calculate a Hazard Quotient (HQ), which is a ratio of an individual's exposure level over a time period to a MeHg reference dose (RfD) (0.1 μg/kg-day); when the hazard quotient reach is >1, there is a potential for toxicity (US EPA 2011). This followed by a consumption limit calculation which estimates the amount of fish that a normal size adult and child could consume each month without adverse effects (Huggett et al 2001; US EPA 2004);

$$\mathrm{MCL} = \frac{\mathrm{RfD} * \mathrm{BW}}{C_m} \times \frac{30.44 \text{ d/mo}}{\mathrm{IR}}$$

Where: MCL is the monthly consumption limit, C_m is the concentration of Hg in the fish-muscle tissue, and the other abbreviations have the same meaning as above. Note: we did not include fish <12” because these are below size limit restrictions for WC and are generally put back into the lake by fishers.

3.3.7 Statistical Data Analysis

Correlation, analysis of variance (ANOVA) and analysis of covariance (ANCOVA) were carried out using JMP 5.0 software. Hg concentration was log-transformed to reduce heterogeneity in variances among lakes to satisfy the assumptions of ANOVA/ANCOVA. For crappie and channel catfish, ANOVA was used as neither length nor weight were correlated with Hg concentration. For LMB, both weight and length were positively correlated with Hg concentration. Thus, ANCOVA was employed using weight as a covariate; length and weight were highly correlated with each other and therefore only weight was included in the ANCOVA to avoid multi-co-linearity effects. Adding the covariate does affect the interpretation of the analysis in this, as indicated by the differences between the least square means and the unadjusted means. Least square means were used as they account for the covariate. In the case of the ANOVA’s for crappie and channel catfish, the least square means and means are the same because no covariate was included. Finally, Tukey's tests were used to provide pairwise means differences among lakes.

3.4 RESULTS

3.4.1 Fish-muscle Hg

The average and median Hg concentration for each species and each lake is shown in Table 7. Individual fish data is presented in appendix 3.1. Each individual lake results are presented below. The data are discussed and interpreted in Section 4.0 (Discussion). Statistical output from the software is presented in the appendix 3.2.

3.4.2 Grenada Lake

A total of 72 fish were collected from Grenada Lake including 20 WC, 18 LMB, 14 CC, 6 BC, 2 FH and 12 GS. The two largest fish, FC with lengths of 88 cm and 74.5 cm, had the highest Hg concentrations at 1461 and 725 ng/g, respectively. Mercury levels in LMB averaged 469 ng/g and ranged between 125 ng/g and 1066 ng/g. Blue catfish had an average concentration of 431 ng/g and ranged between 343 ng/g to 547 ng/g. Channel catfish had concentration averages of 395 ng/g with a range of 200 ng/g to 665 ng/g and WC had concentration averages of 199 ng/g and ranged between 99 ng/g to 383 ng/g. Gizzard shad had the lowest concentration of Hg with an average concentration of 70 ng/g and with a range between 42 ng/g to 149 ng/g.

Table 7 Average concentrations (wet weight ng/g), standard deviations (1SD), ranges, and medians of Hg in fish tissue collected in North MS Lakes

	Grenada						Sardis					Enid			All Lakes Combined				
Mercury (ng/g)	Crappie	LMB	CC	GS	BC	Flathead	Crappie	LMB	CC	GS	BC	Crappie	LMB	CC	Crappie	LMB	CC	GS	BC
Average	199	469	395	70	431	1093	147	312	222	38	448	214	289	147	187	357	255	36	439
1SD	77	287	125	40	80	520	35	182	89	21	287	41	214	56	35	98	127	23	12
min	99	125	200	42	343	725	109	51	142	8	290	120	108	69	147	289	147	38	431
max	383	1066	666	149	547	1461	237	723	432	73	878	285	954	272	214	469	395	70	448
median	180	377	381	55	424	1093	138	274	190	41	311	215	196	142	199	312	222	54	439

3.4.3 Sardis Lake

A total of 84 fish were collected from Sardis Lake, which included 21 GS, 20 WC, 21 LMB, 18 CC, and 4 BC. Result showed the greatest amount of total-Hg in the BC with an average concentration of 448 ng/g and range between 290 ng/g to 878 ng/g. The average Hg concentrations in LMB were 792 ng/g and ranged between 102 ng/g to 1810 ng/g. Channel catfish concentrations averaged 222 ng/g with a range of 142 ng/g to 432 ng/g and WC concentrations averaged 147 ng/g and ranged between 109 ng/g to 237 ng/g. Gizzard shad contained the least amount of Hg in fish tissue with an average concentration of 38 ng/g and with a range between 8 ng/g to 73 ng/g.

3.4.4 Enid Lake

A total of 46 fish were collected from Enid Lake, which included 16 WC, 15 LMB, and 15 CC. The average Hg concentrations in largemouth bass were 289 ng/g and ranged between 108 ng/g to 954 ng/g. CC and WC concentrations averaged 147 ng/g and 214 ng/g with a range of 69 ng/g to 272 ng/g and 120 ng/g to 285 ng/g, respectively.

3.4.5 Liver-to-Muscle Ratios

The ratio of total-Hg in the liver to total-Hg in the muscle varied among species with Enid Lake CC (2.28), and Grenada Lake FC (0.85) having the highest ratios. The lowest ratio of total-Hg in the liver to total-Hg in the muscle was seen in Sardis Lake's WC (0.29) and LMB (0.38) and Enid Lake LMB (0.57). The LMB total-Hg liver-to-muscle ratio is within the range of what has been reported in the literature (Goldstien, 1996; Cizdziel, 2003). The ratios of MeHg in liver to total-Hg in liver were less than 0.43 for all fish collected with Enid Lake's CC having the lowest ratio at 0.09 and LMB having the highest ratio (0.43).

Table 8 Average concentrations (ng/g, wet weight) and concentration ratios for select fish from North Mississippi Lakes

			Concentrations			Concentration Ratios		
Lake Location	Fish ID	n	T-Hg in Liver	T-Hg in Muscle	MeHg in Liver	T-Hg Liver-to-Muscle	MeHg Liver-to-Muscle	Liver MeHg-to-T-Hg
Grenada	Flat Head Catfish	1	0.65	1.09	0.2	0.59	0.19	0.31
Sardis	White Crappie	2	0.04	0.16	0.01	0.29	0.1	0.3
	Largemouth Bass	2	0.22	0.58	0.08	0.38	0.13	0.34
Enid	Largemouth Bass	3	0.4	0.57	0.2	0.57	0.26	0.43
	Channel Catfish	3	0.34	0.15	0.03	2.28	0.2	0.09

Trophic levels play a significant role in the levels of Hg in liver to muscle ratio (Cizdziel et al. 2003; Allen-Gil et al. 1997; Lasorsa et al. 1995). Results showed that total-Hg in liver to total-Hg in muscle were varied among species. All the fish liver analyzed for total-Hg and MeHg are piscivores and have close trophic levels. Therefore, no trophic level assumption could be deduced from the data collected for the samples analyzed.

The lower ratio corresponding to uptake behavior in the current study may be related to the decrease in atmospheric deposition, which was suggested as the reason mercury levels in fish muscle decrease in Enid and Grenada.

The liver acts as a detoxifier and demethylates Hg (Houserova, et al. 2005; Collin, et al 2009). A large percentage of Hg is in the inorganic form in the liver. It is thought that the conversion of inorganic Hg is a measurement of the fish organ's response to the Hg exposure. It would be ideal to determine the level of MeHg in the fish muscle to make an accurate assessment of the amount of dominant Hg species in both the liver and the muscle.

3.4.6 Enid Lake Human Health Risk Assessments

A risk assessment was calculated on Enid Lake to determine the exposure and hazard index adults and children may face when eating fish contaminated with Hg. The assessment is also a guide, which reports safe levels for consumption of fish species contaminated with mercury. The results indicated that adults and, most severely, children would have a higher exposure to a greater amount of Hg when eating LMB. The average index for adults consuming LMB is 1.65 x 10^{-4} mg/kg/d and for children the value was 7.52 x 10^{-4} mg/kg/d (Fig. 19). The average hazard indexes for children were >1 for all fish sampled in Enid Lake and HI for adults were >1 for LMB (Fig. 18). Largemouth bass average hazard index was 1.65 for adults and 7.96 for children. The consumption rate for adults and children consuming both WC and LMB per month were similar, 4.66 meals/month and 3.07 meals/month, respectively (Fig. 17).

The risk assessment is based on average adult weight of 70 kg and an average child weight of 14.5 kg. It is assumed that risk may vary among a population consuming contaminated fish. According to the US EPA Risk Assessment Guidance for Superfund, in human risk assessments the toxicity term should be expressed as a point estimate. A risk assessment, which considers variability and uncertainty, takes into account probability and distribution for random variables. We are certain that different people eat different amounts of fish but we may be uncertain about how much variability there is in the fish intake among the population.

3.5 DISCUSSION

3.5.1 Hg concentrations with size and trophic level

Fish size (length or weight) reflects the age or maturity of the fish, which can correlate with the Hg concentration in its tissues (Pauly 1984). Length is also used as an indicator in determining fishery length limits in state waters (Sackett et al. 2013). In this study, the average length of fish was relatively uniform between lakes for WC, and similar for LMB for Grenada and Sardis (Figure 12). Gizzard shad tended to be smaller in Sardis compared to Grenada, while CC were slightly bigger in Sardis compared to the other lakes.

Regardless of the lake, Hg concentrations tended to increase with both fish length and weight (Figures 13 and 14). This trend was strongest for LMB in Enid ($r=0.94$, $p<0.01$) and Sardis ($r=0.77$, $p<0.01$). This is not surprising considering LMB have relatively high metabolism, growth rates and its diet; as a piscivore (primarily) the species consume relatively high numbers of forage fish (e.g. gizzard shad). Sackett et al. (2013) also reported strong correlations for LMB for increasing Hg concentrations with body length.

Excluding the FH and BC, which were few in numbers and relatively large in size, Hg levels generally increased with trophic level, with GS the lowest and LMB the highest. For all lakes combined, FH had the highest mean levels of Hg (1093 ng/g, n = 2), followed by BC (439 ng/g, n = 10), LMB (357 ng/g, n = 54), CC (255 ng/g n =47), WC (187 ng/g, n = 56), and GS (36 ng/g, n = 33).

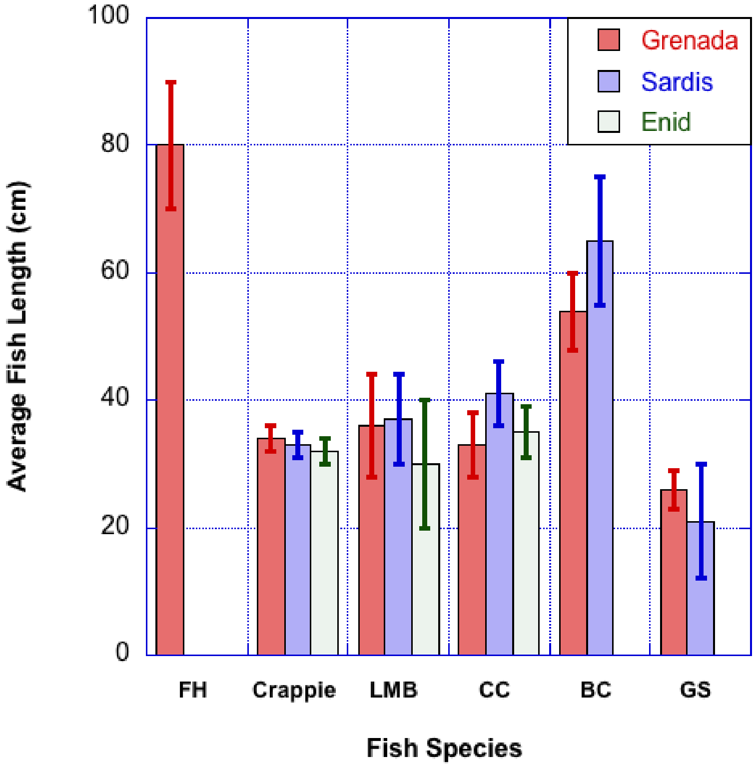

Figure 12 Average length of fish species collected from Grenada, Sardis, and Enid Lakes located in North Mississippi, USA. FH = Flathead Catfish, Crappie = White Crappie, LMB = Largemouth Bass, CC = Channel Catfish, BC = Blue Channel Catfish, GS = Gizzard shad. (Error bars represent ±1SD)

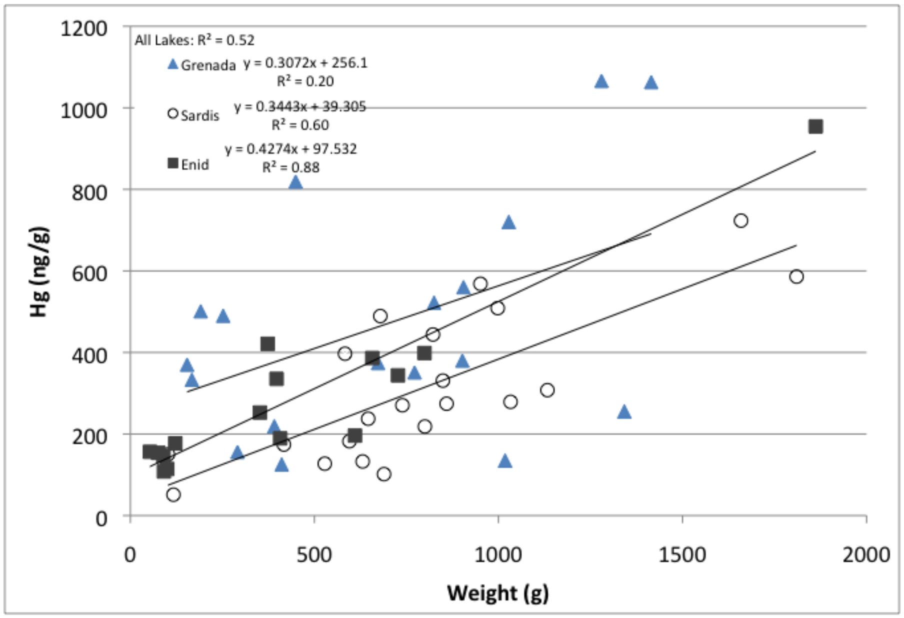

Figure 13 Total-Hg in fish-muscle vs. weight for Largemouth Bass collected from Grenada, Sardis, and Enid Lakes.

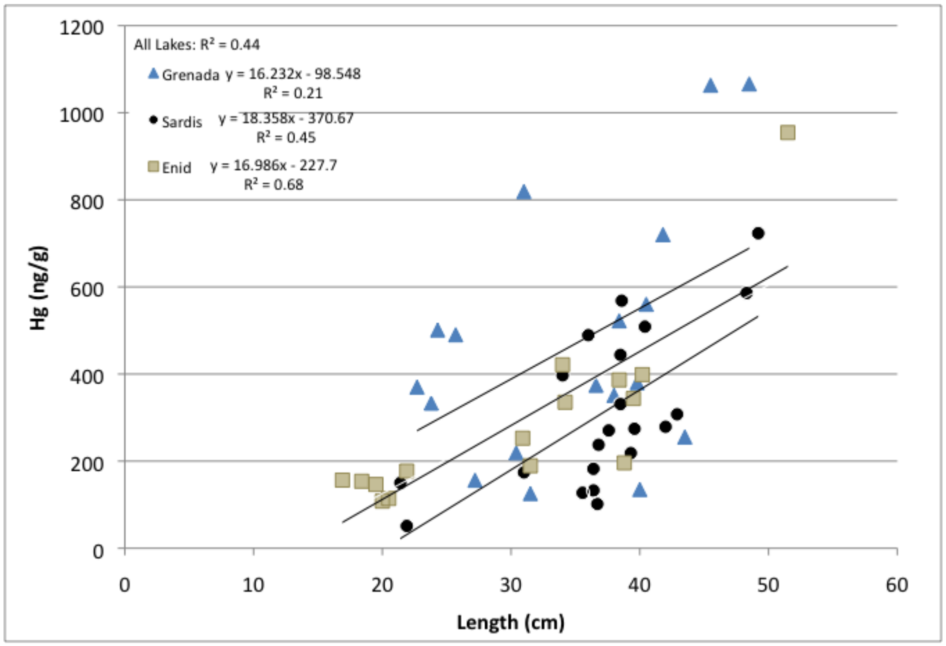

Figure 14 Total-Hg in fish-muscle vs. length for Largemouth Bass collected from Grenada, Sardis, and Enid Lakes.

3.5.2 Comparison of fish-Hg between lakes

Average total-Hg concentrations between the lakes and between species are compared in Figure 15. A statistical comparison using least square means is given in Table 9. For LMB, WC, and CC, Grenada had the highest levels, and the difference was statistically significant compared to Sardis for LMB and WC, and Enid and Sardis for CC. On the other hand, Sardis tended to have the lowest levels. Enid was intermediate. The reason for the seemingly higher levels in Grenada are unclear but may be related to water chemistry and water-soil interactions as the geology between sites differs. It is noteworthy that MeHg is known to be elevated in lakes with low pH. On average Grenada lake seems to have a slightly lower pH (neutral to slightly acidic) whereas Enid and Sardis are closer to neutral or slightly above.

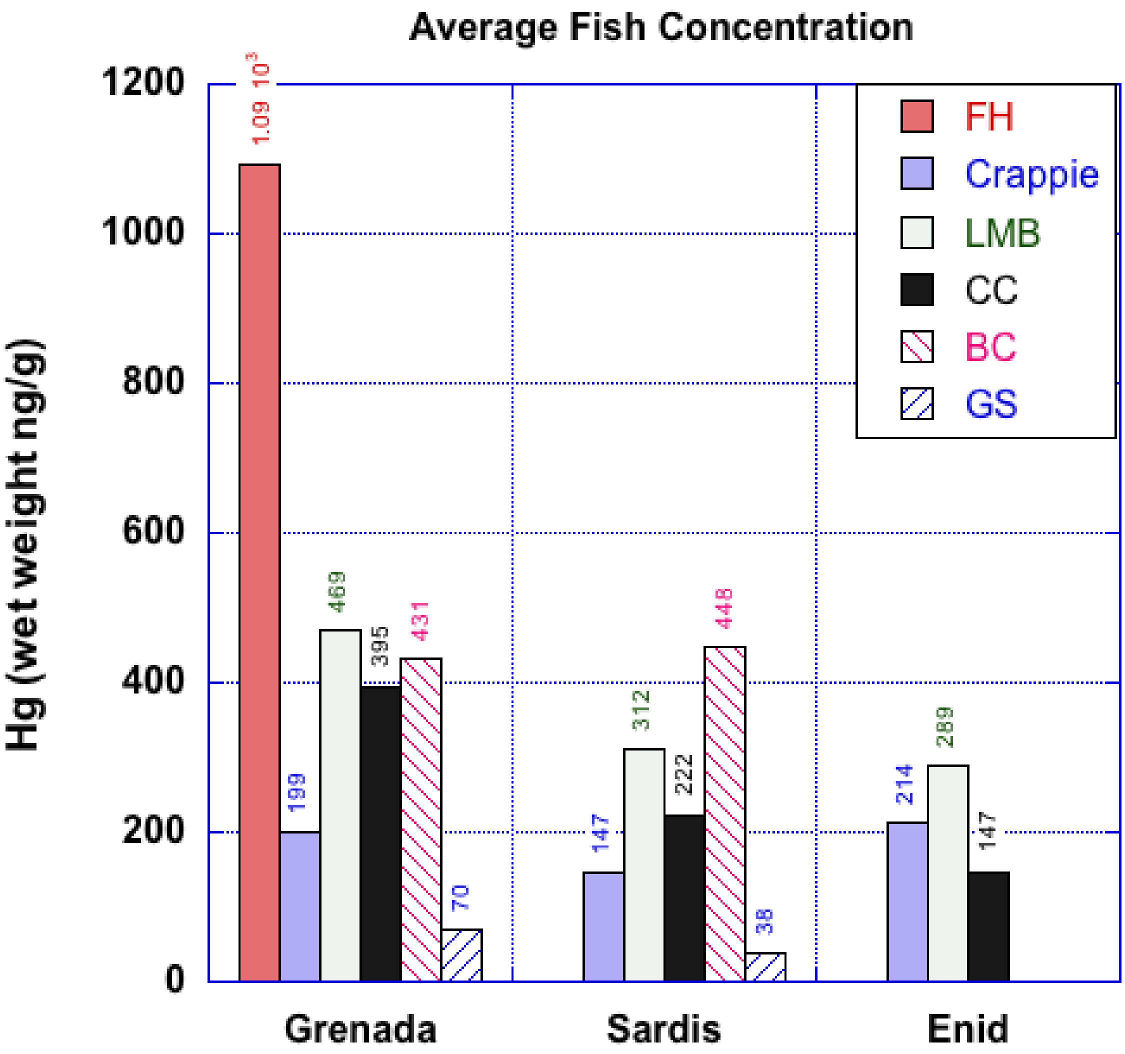

Figure 15 Average Total-Hg (ng/g) in fish-muscle collected from Grenada, Sardis, and Enid Lakes located in North Mississippi, USA. Numbers above bars represent average concentration values. FH = Flathead Catfish, Crappie = White Crappie, LMB = Largemouth Bass, CC = Channel Catfish, BC = Blue Channel Catfish, GS = Gizzard shad.

Table 9 Least square means (ng/g) and differences for fish-Hg between north Mississippi lakes. Levels not connected by the same letter are significantly different ($p<0.05$).

Species	Data / Test	Grenada	Enid	Sardis
Largemouth Bass	Least-squares:	379	295	229
	Significance:	A	A, B	B
Crappie	Least-squares:	210	186	143
	Significance:	A	A	B
Channel Catfish	Least-squares:	377	209	138
	Significance:	A	B	C

3.5.3 Decreasing levels of mercury compared to earlier decades

Mercury levels in LMB from the current study are lower compared to the MDEQ fish sampling conducted in 1992-1994 and 2009 (Figure 16). Data also show a decline in Enid and Grenada WC, CC, and FH. The major non-point source of Hg contamination in watersheds is proposed to be atmospheric Hg (Fitzgerald et al 1998). It is evident by the current data collected in our study that the input of Hg into the river has declined since last analyzed. A number of factors may be attributed to this decline. One obvious factor includes the difference in quality assurance techniques performed in the early nineties compare to the new millennium. EPA Methods such as 7473, 1631, and 245.7 enacted prior to the 1992-1994 results was due to the Clean Water Act in 1999, which was a means for providing reliable measurements at the lowest EPA ambient water quality criteria for mercury under the National Toxics Rule and in the Great Lakes and Tribes (40 CFR 132.6).

Water conditions must also be taken into account for each sampling time period. Runoff entering into the water containing Hg-bearing organic matter and soils treated with pesticides with Hg composed in them can also contribute Hg inputs to a river (Brinkman and Rasmusseen 2012). Although Hg is no longer used in farming products, according to the US EPA in the United States mercuric chloride was used as a pesticide in the early 90's (US, EPA 1994).

Another factor may be an actual decline of atmospheric Hg in the Mid-South region. Monson, B. A et al (2011) correlated declines in Hg concentrations of largemouth bass with regional declines in Hg deposition in the Great Lakes region. The National Atmospheric Deposition Program-Mercury Deposition Network database was accessed to determine the historical Hg deposition amounts in the Mid-South region between the years of 1999-2011. No evidence of a decline in the region was observed for the years of 1999-2011. The amount of deposition to the region was consistently on average between 7 ng/L to 13 ng/L (MDN accessed June 2013). More information is needed regarding deposition rates in the early 90's in the Mid-South region during the time of sample collection to support or refute this claim. A US EPA report "Regulatory Impact Analysis for the Final Mercury and Air Toxics Standards" stated that anthropogenic mercury in the US is on a decline since 1990. The Hg emissions from electric generating units declined from 59 tons to 29 tons in 2010 and Hg emissions from non-electric generating units declined from 205 tons in 1999 to 52 tons in 2005.

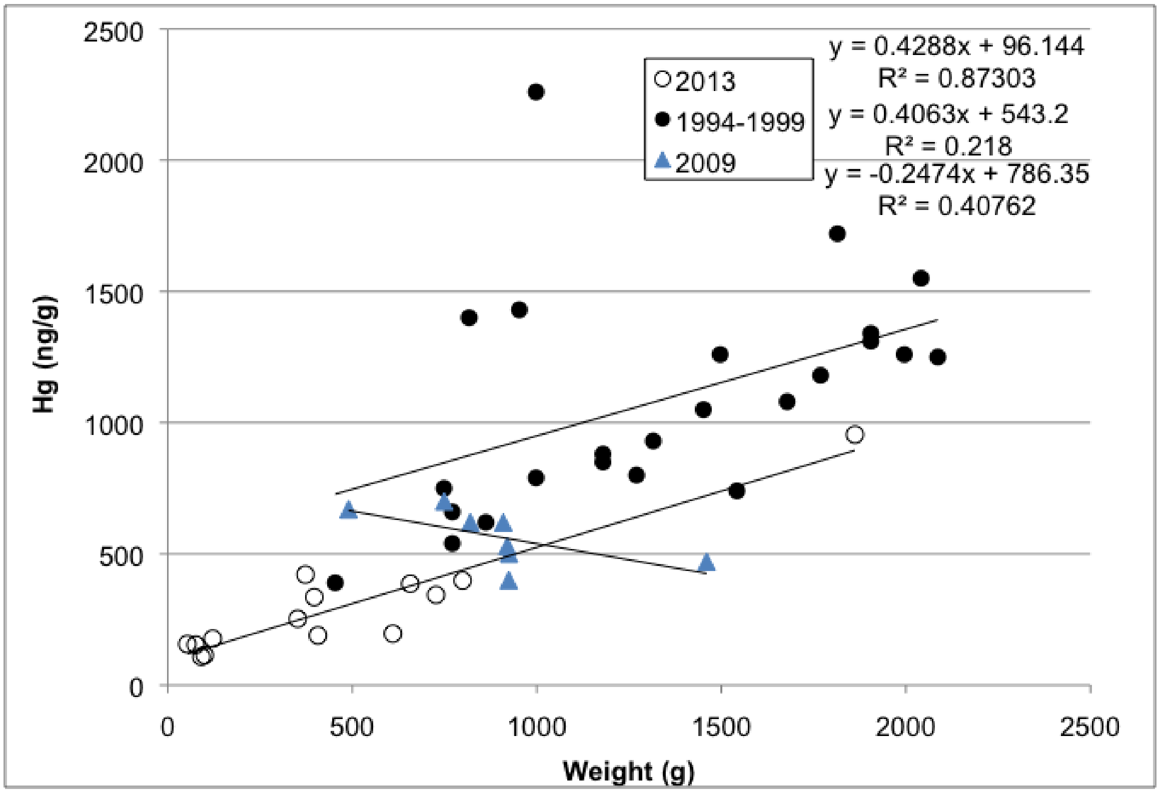

Figure 16 Total-Hg vs. weight for largemouth from Enid Lake over the last two decades. Data from 1992-1994 and 2009 was used by permission from the Mississippi Department of Environmental Quality.

3.5.4 Liver-to-muscle concentration ratios

For liver-to-muscle ratios, during a fish exposure to a contaminated water system for a long time, MeHg accumulates in the liver at a greater rate before being metabolized to the muscle (Helle, et al 1976). For short-term exposures, Hg will decrease at a slower rate in the muscle than in the liver. MeHg in liver to total-Hg in muscle, which has ratios greater than 1 follows the latter explanation, describing fish exposure in Hg contaminated water systems. All species had MeHg in liver to total Hg in muscle ratios less than 0.5, with Sardis Lake's white crappie (0.10) and largemouth bass (0.13) having the lowest ratios. Ratios of 0.5 indicate that Hg is being lost from fish.

3.5.5 Risk assessment

A risk assessment was calculated for Enid Lake using the current findings of Hg concentration in fish species. This assessment was modeled from a similar assessment for Enid Lake conducted by Huggett et al (2000). Huggett et al (2000) reported that almost all mean Hg concentrations in largemouth bass, gar, and crappie exceed the FDA action level of 1.0 ppm. The assessment stated that the Hazard Indexes were >1 for adults and children. The source of the Hg was hypothesized to be atmospheric deposition to the lake.

In our study, we found Hg concentration in fish below the EPA action level. The Hazard Indexes for most fish were > 1 for both adults and children. The magnitude for fish consumption rate levels between adults and children were high, with children having a smaller consumption rate. When the Mississippi Department of Health issued a fish consumption advisor on Enid and Grenada Lakes, the length of fish measured with high levels of Hg in fish tissue were 69 cm and above.

The US EPA Office of Air Quality Planning and Standards and Environmental Impacts Health Division conducted a risk assessment to simulate the projected hazard index in the year of 2016. The assessment factored in data from 3,141 watersheds, which is only about 4% of U.S. watersheds but includes the east coast of the US where the majority of coal-fired plants are located. The assessment associated high levels in fish tissue to methylation in the water column and indirectly to atmospheric deposition. The assessment predicts a significant reduction in Hg emissions from U.S. coal- and oil-fired electric generating units since 2005, when Hg emissions were estimated to be 52.9 tons. In addition, a few additional reductions are expected to occur without additional regulations to reduce mercury emissions. These reductions may be accomplished by regulations and federal enforcement actions that achieve Hg reductions as a co-benefit of controls for nitrogen monoxide and sulfur dioxide emissions. The projected year of 2016 was used in the simulation because 2016 is the year when regulations under Section 112 of the Clean Air Act would need to be implemented for electric generating units Hg emissions by federal officials.

A study in Lake Superior showed a decline in fish Hg concentrations, which was consistent with regional declines in Hg deposition in the region (Drevnick et al 2007). Levels are lower in fish tissue since last determined in the early 90's large-scale study. It is possible that the lower level of Hg determined in fish collected in Enid Lake is due to a decrease in atmospheric Hg to the lake, as what is currently being measured in watersheds of other studies.

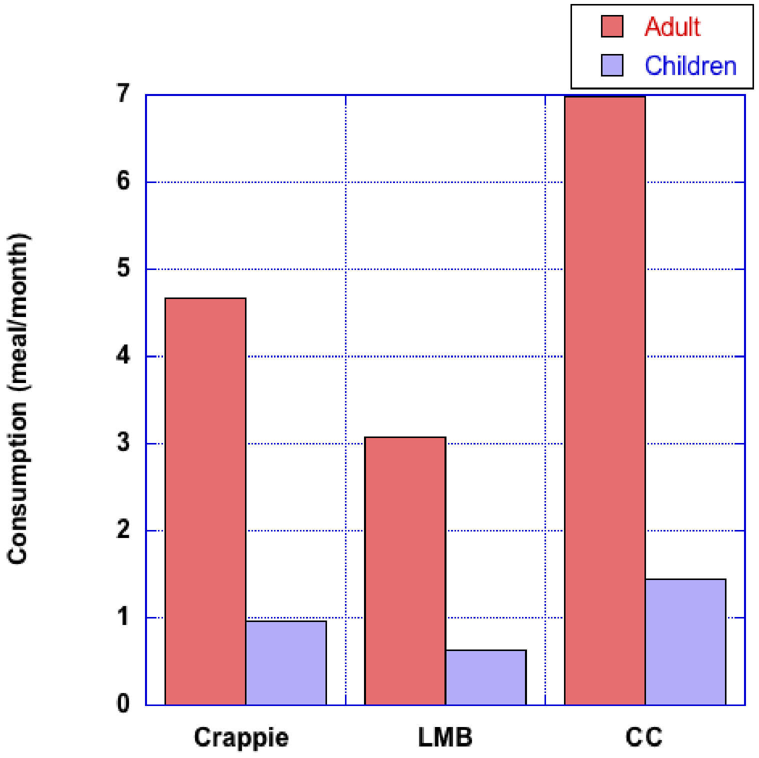

Figure 17 Suggested consumption limits for white crappie, largemouth bass, and channel catfish from Enid Lake for adults and children.

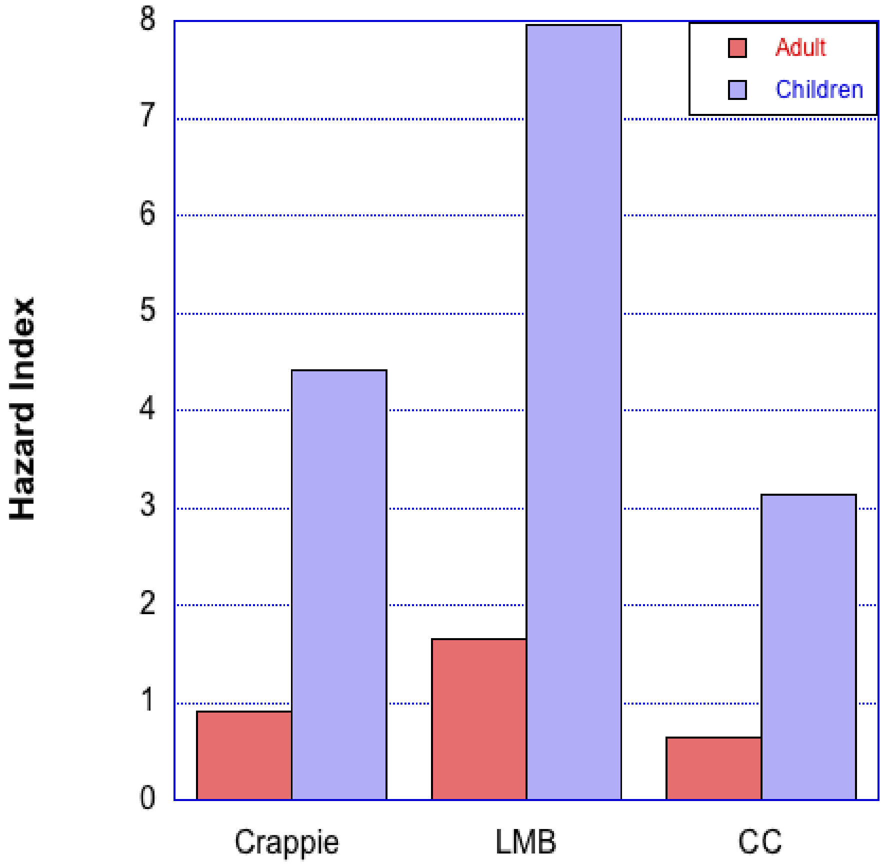

Figure 18 Hazard Index (HI) for adults and children consuming white crappie, largemouth bass, and channel catfish from Enid Lake. The HI is calculated by dividing the intake by the reference dose for methylmercury (1.0 x 10-4 mg/kg-d). HI values greater than one suggest a hazard health effect will occur at the representative intake value.

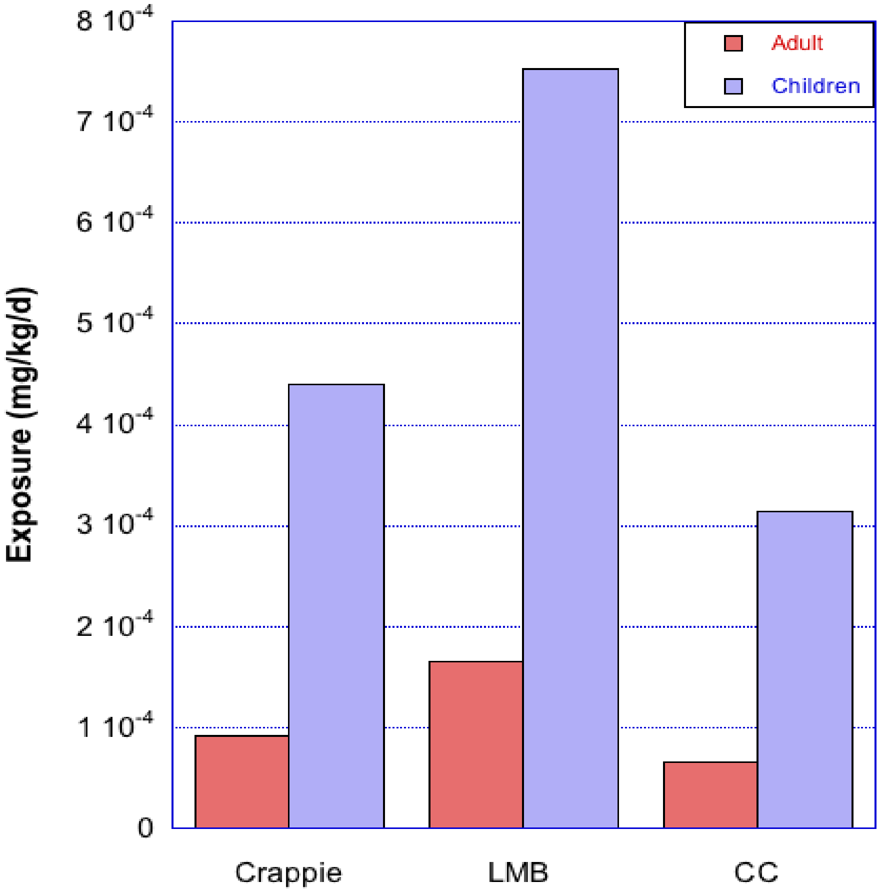

Figure 19 Exposure associated with adults and children consumption (intake) of fish collected in Enid Lake for white crappie, largemouth bass, and channel catfish.

Table 10 Mean hazard indexes and consumption limits by Lake and fish species. HI in red suggest a hazard health effect will occur at the representative intake value

Lake	Species	Mean Hazard Index		Mean Consumption Limit (meals/month)	
		Adult	Child	Adult	Child
Grenada	Crappie	0.85	4.11	5	1
	Bass	1.56	7.55	3	0.5
	Catfish	1.81	8.75	2	0.5
Enid	Crappie	0.91	4.41	4	1
	Bass	1.65	7.96	3	0.5
	Catfish	0.65	3.14	7	1
Sardis	Crappie	0.63	3.02	6	1
	Bass	1.42	6.88	3	0.5
	Catfish	0.95	4.58	4	1

Catfish = Channel Catfish. MCL conservatively rounded down.

3.6 CONCLUSIONS

The purpose of this study was to re-assess total-Hg levels in fish muscle tissue of fish types most commonly captured in Grenada, Enid, and Sardis Lakes, and to calculate a human health risk assessment for muscle tissue from Enid. The re-assessment showed that Grenada and Enid Lake's Hg concentration levels are lower in the fish muscle of largemouth bass and other fish sampled compared to studies conducted in 1992-1994 and 2009. Mercury levels generally increased with fish size and trophic level. If the earlier data is reliable then it is possible that the decline in Hg levels may be attributed to a decrease in atmospheric deposition given that there are no major point sources of Hg in the watershed. The ratio of total-Hg in the liver to total-Hg in the muscle varied among species. The MeHg in liver to total-Hg in muscle ratios for all fish analyzed were less than 0.5, suggesting that Hg is being "lost" from the fish. A risk assessment indicated that the hazard index (quotient) levels from Enid were greater than one for most fish collected which suggest a hazard health effect will occur at the representative intake value. The suggested consumption of largemouth bass for adults is <4 meals per month and <8 meals per year for children. Based on these results Grenada, Sardis, and Enid Lakes do not present a severe consumption risk of Hg contamination. The public should, however, limit the consumption of larger fish, which tend to have greater concentrations of Hg in fillets. Fish sampling surveys should be conducted about every 10 years to re-assess the current levels of Hg in fish.

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APPENDIX 3.1: Fish-Muscle Hg and Risk Assessment Values

GRENADA LAKE: individual fish muscle Hg concentration (ng/g wet wt.) and risk assessment values.

Species	Fish ID	Length (cm)	Weight (g)	Muscle (ng/g)	Adult Intake (mg/kg/d)= (CF*IR*EF*ED)/(BW*AT)	Adults Hazard Index= Intake (mg/kg/d)/ Reference dose of MeHg (1.0E-4)	Children Intake (mg/kg/d) = (CF*IR*EF*ED)/(BW*AT)	Children Hazard Index= Intake (mg/kg/d)/ Reference dose of MeHg (1.0E-4)	Adults Monthly Consumption Limits = (RfD* BW/Cm)X(30.44d/mo/IR)	Children Monthly Consumption Limits = (RfD* BW/Cm)X (30.44d/mo/IR)
Blue Catfish (*Ictalurus furcatus*)	BC1	44.5	699	352	1.50E-04	1.50	7.25E-04	7.25	2.7	0.6
	BC2	43.2	574	419	1.79E-04	1.79	8.63E-04	8.63	2.3	0.5
	BC3	54.5	1641	497	2.12E-04	2.12	1.02E-03	10.23	1.9	0.4
	*BC1	58.1	1428	547	2.33E-04	2.33	1.13E-03	11.26	1.8	0.4
	*BC2	56.6	1421	429	1.83E-04	1.83	8.83E-04	8.83	2.2	0.5
	*BC3	47.8	800	343	1.46E-04	1.46	7.06E-04	7.06	2.8	0.6
Average		51	1094	431	1.84E-04	1.84	8.88E-04	8.88	2.3	0.5
1SD		6	454	80	3.41E-05	0.34	1.65E-04	1.65	0.4	0.1
min		43	574	343	1.46E-04	1.46	7.06E-04	7.06	1.8	0.4
max		58	1641	547	2.33E-04	2.33	1.13E-03	11.26	2.8	0.6
median		51	1111	424	1.81E-04	1.81	8.73E-04	8.73	2.3	0.5
Flathead catfish	FC1	74.5	ND	1461	6.23E-04	6.23	3.01E-03	30.08	0.7	0.1
	FC2	88	ND	725	3.09E-04	3.09	1.49E-03	14.93	1.3	0.3
Average		81	ND	1093	4.66E-04	4.66	2.25E-03	22.50	1.0	0.2
1SD		10	ND	520	2.22E-04	2.22	1.07E-03	10.71	0.5	0.1
min		75	ND	725	3.09E-04	3.09	1.49E-03	14.93	0.7	0.1
max		88	ND	1461	6.23E-04	6.23	3.01E-03	30.08	1.3	0.3
median		81	ND	1093	4.66E-04	4.66	2.25E-03	22.50	1.0	0.2
Threadfin shad (*Dorosoma petenense*) ACTUALLY	BF1	15	24	20	ND	ND	ND	ND	ND	ND
	BF2	14	19	52	ND	ND	ND	ND	ND	ND
	BF3	13.5	17	41	ND	ND	ND	ND	ND	ND
	BF4	10.5	11	45	ND	ND	ND	ND	ND	ND
	BF5	9.5	6	35	ND	ND	ND	ND	ND	ND
Average		13	15	39	ND	ND	ND	ND	ND	ND
1SD		2	7	12	ND	ND	ND	ND	ND	ND
min		10	6	20	ND	ND	ND	ND	ND	ND
max		15	24	52	ND	ND	ND	ND	ND	ND
median		14	17	41	ND	ND	ND	ND	ND	ND
Gizzard shad (*Dorosoma cepedianum*)	GS1	28.5	182	47	ND	ND	ND	ND	ND	ND
	GS2	24.0	131	149	ND	ND	ND	ND	ND	ND
	GS3	29.6	256	49	ND	ND	ND	ND	ND	ND
	GS4	29.6	216	60	ND	ND	ND	ND	ND	ND
	GS5	26.6	164	ND	ND	ND	ND	ND	ND	ND
	GS6	24.3	132	72	ND	ND	ND	ND	ND	ND
	GS7	31.5	258	42	ND	ND	ND	ND	ND	ND
Average		28	191	70	ND	ND	ND	ND	ND	ND
1SD		3	54	40	ND	ND	ND	ND	ND	ND
min		24	131	42	ND	ND	ND	ND	ND	ND
max		32	258	149	ND	ND	ND	ND	ND	ND
median		29	182	55	ND	ND	ND	ND	ND	ND

GRENADA LAKE: individual fish muscle Hg concentration (ng/g wet wt.) and risk assessment values.

Species	Fish ID	Length (cm)	Weight (g)	Muscle (ng/g)	Adult Intake (mg/kg/d)= (CF*IR*EF*ED)/(BW*AT)	Adults Hazard Index= Intake (mg/kg/d)/ Reference dose of MeHg (1.0E-4)	Children Intake (mg/kg/d) = (CF*IR*EF*ED)/(BW*AT)	Children Hazard Index= Intake (mg/kg/d)/ Reference dose of MeHg (1.0E-4)	Adults Monthly Consumption Limits = (RfD* BW/Cm)X(30.44d/mo/IR)	Children Monthly Consumption Limits = (RfD* BW/Cm)X (30.44d/mo/IR)
Crappie (*Pomoxis*)	Cr1	ND	704	333	1.42E-04	1.42	6.86E-04	6.86	2.88	0.60
	Cr2	35.6	725	251	1.07E-04	1.07	5.17E-04	5.17	3.82	0.79
	Cr3	38.6	883	194	8.27E-05	0.83	3.99E-04	3.99	4.95	1.02
	Cr4	31.0	456	176	7.51E-05	0.75	3.62E-04	3.62	5.45	1.13
	Cr5	35.5	784	152	6.48E-05	0.65	3.13E-04	3.13	6.31	1.31
	Cr6	34.3	563	135	5.76E-05	0.58	2.78E-04	2.78	7.11	1.47
	Cr7	32.5	535	183	7.80E-05	0.78	3.77E-04	3.77	5.24	1.09
	Cr8	36.5	853	190	8.10E-05	0.81	3.91E-04	3.91	5.05	1.05
	Cr9	37.0	706	229	9.77E-05	0.98	4.71E-04	4.71	4.19	0.87
	Cr10	35.5	695	383	1.63E-04	1.63	7.89E-04	7.89	2.51	0.52
	Cr11	35.5	730	275	1.17E-04	1.17	5.66E-04	5.66	3.49	0.72
	Cr12	37.3	887	115	4.90E-05	0.49	2.37E-04	2.37	8.35	1.73
	Cr13	31.5	472	139	5.93E-05	0.59	2.86E-04	2.86	6.91	1.43
	Cr14	32.3	415	163	6.95E-05	0.70	3.36E-04	3.36	5.89	1.22
	Cr15	35.2	786	143	6.10E-05	0.61	2.94E-04	2.94	6.71	1.39
	Cr16	35.0	689	153	6.52E-05	0.65	3.15E-04	3.15	6.27	1.30
	Cr17	34.2	651	99	4.22E-05	0.42	2.04E-04	2.04	9.70	2.01
	Cr18	33.5	534	323	1.38E-04	1.38	6.65E-04	6.65	2.97	0.62
	Cr19	30.5	392	150	6.40E-05	0.64	3.09E-04	3.09	6.40	1.33
	Cr20	32.0	508	192	8.19E-05	0.82	3.95E-04	3.95	5.00	1.04
Average		34	648	199	8.48E-05	0.85	4.09E-04	4.09	5.5	1.1
1SD		2	155	77	3.29E-05	0.33	1.59E-04	1.59	1.9	0.4
min		31	392	99	4.22E-05	0.42	2.04E-04	2.04	2.5	0.5
max		39	887	383	1.63E-04	1.63	7.89E-04	7.89	9.7	2.0
median		35	692	180	7.65E-05	0.77	3.70E-04	3.70	5.3	1.1
Largemouth bass (Micropterus salmoides)	LMB1	31.5	411	125	5.35E-05	0.53	2.58E-04	2.58	7.7	1.6
	LMB2	43.5	1342	255	1.09E-04	1.09	5.26E-04	5.26	3.8	0.8
	LMB3	40	1018	135	5.75E-05	0.57	2.78E-04	2.78	7.1	1.5
	*LMB1	38	772	351	1.50E-04	1.50	7.23E-04	7.23	2.7	0.6
	*LMB2	40.5	905	560	2.39E-04	2.39	1.15E-03	11.53	1.7	0.4
	*LMB3	41.8	1028	720	3.07E-04	3.07	1.48E-03	14.82	1.3	0.3
	*LMB4	36.6	673	374	1.59E-04	1.59	7.70E-04	7.70	2.6	0.5
	*LMB5	39.8	902	380	1.62E-04	1.62	7.82E-04	7.82	2.5	0.5
	*LMB6	38.4	825	522	2.23E-04	2.23	1.07E-03	10.75	1.8	0.4
	*LMB7	45.5	1415	1063	4.53E-04	4.53	2.19E-03	21.88	0.9	0.2
	*LMB8	30.4	391	219	9.34E-05	0.93	4.51E-04	4.51	4.4	0.9
	*LMB9	24.3	191	501	ND	ND	ND	ND	ND	ND
	*LMB10	27.2	291	156	ND	ND	ND	ND	ND	ND
	*LMB11	22.7	154	370	ND	ND	ND	ND	ND	ND
	*LMB12	23.8	167	333	ND	ND	ND	ND	ND	ND
	*LMB13	25.7	252	490	ND	ND	ND	ND	ND	ND
	*LMB14	31	449	819	1.16E-05	0.12	5.62E-05	0.56	1.2	0.2
	*LMB15	48.5	1280	1066	1.52E-05	0.15	7.32E-05	0.73	0.9	0.2
Average		35	693	469	1.56E-04	1.56	7.55E-04	7.55	3.0	0.6
1SD		8	422	287	1.26E-04	1.26	6.07E-04	6.07	2.2	0.5
min		23	154	125	1.16E-05	0.12	5.62E-05	0.56	0.9	0.2
max		49	1415	1066	4.53E-04	4.53	2.19E-03	21.88	7.7	1.6
median		37	723	377	1.50E-04	1.50	7.23E-04	7.23	2.5	0.5
Channel catfish (*Ictalurus punctatus*)	CC1	39.8	510	666	2.84E-04	2.84	1.37E-03	13.71	1.4	0.3
	CC2	36	309	552	2.35E-04	2.35	1.14E-03	11.36	1.7	0.4
	CC3	35	269	311	1.33E-04	1.33	6.40E-04	6.40	3.1	0.6
	CC4	34.6	310	456	1.94E-04	1.94	9.39E-04	9.39	2.1	0.4
	CC5	31.6	237	393	1.68E-04	1.68	8.09E-04	8.09	2.4	0.5
	CC6	40	433	312	1.33E-04	1.33	6.42E-04	6.42	3.1	0.6
	CC7	36.6	265	513	2.19E-04	2.19	1.06E-03	10.56	1.9	0.4
	CC8	34.8	263	345	1.47E-04	1.47	7.10E-04	7.10	2.8	0.6
	CC9	28	143	200	ND	ND	ND	ND	ND	ND
	CC10	28.8	154	369	ND	ND	ND	ND	ND	ND
	CC11	22	64	282	ND	ND	ND	ND	ND	ND
	*CC1	36.5	319	440	1.88E-04	1.88	9.06E-04	9.06	2.2	0.5
	*CC2	32.5	200	261	1.11E-04	1.11	5.37E-04	5.37	3.7	0.8
	*CC3	27.9	159	423	ND	ND	ND	ND	ND	ND
Average		33	260	395	1.81E-04	1.81	8.75E-04	8.75	2.4	0.5
1SD		5	117	125	5.38E-05	0.54	2.60E-04	2.60	0.7	0.1
min		22	64	200	1.11E-04	1.11	5.37E-04	5.37	1.4	0.3
max		40	510	666	2.84E-04	2.84	1.37E-03	13.71	3.7	0.8
median		35	264	381	1.78E-04	1.78	8.57E-04	8.57	2.3	0.5

SARDIS LAKE

Species	Fish ID	Length (cm)	Weight (g)	Muscle (ng/g)	Adult Intake (mg/kg/d)= (CF*IR*EF*ED) /(BW*AT)	Adults Hazard Index= Intake (mg/kg/d)/Reference dose of MeHg (1.0E-4)	Children Intake (mg/kg/d)= (CF*IR*EF*ED)/(BW*AT)	Children Hazard Index= Intake (mg/kg/d)/Reference dose of MeHg (1.0E-4)	Adults Monthly Consumption Limits = (RfD* BW/Cm)X(30.44d/mo/IR)	Children Monthly Consumption Limits = (RfD* BW/Cm)X(30.44d/mo/IR)
Crappie (Pomoxis)	CF1	35.0	749	128	5.46E-05	0.55	2.63E-04	2.63	7.5	1.6
	CF2	30.5	380	119	5.08E-05	0.51	2.45E-04	2.45	8.1	1.7
	CF3	32.4	499	182	7.75E-05	0.78	3.74E-04	3.74	5.3	1.1
	CF4	36.0	734	122	5.18E-05	0.52	2.50E-04	2.50	7.9	1.6
	CF5	32.6	498	110	4.69E-05	0.47	2.27E-04	2.27	8.7	1.8
	CF6	31.0	509	142	6.04E-05	0.60	2.91E-04	2.91	6.8	1.4
	CF7	34.2	561	150	6.39E-05	0.64	3.08E-04	3.08	6.4	1.3
	CF8	31.0	471	139	5.92E-05	0.59	2.86E-04	2.86	6.9	1.4
	CF9	32.6	612	149	6.33E-05	0.63	3.06E-04	3.06	6.5	1.3
	CF10	35.4	630	166	7.06E-05	0.71	3.41E-04	3.41	5.8	1.2
	CF11	33.0	570	136	5.82E-05	0.58	2.81E-04	2.81	7.0	1.5
	CF12	30.5	474	136	5.78E-05	0.58	2.79E-04	2.79	7.1	1.5
	CF13	32.5	525	237	1.01E-04	1.01	4.88E-04	4.88	4.0	0.8
	CF14	33.0	556	109	4.64E-05	0.46	2.24E-04	2.24	8.8	1.8
	CF15	32.5	395	113	4.82E-05	0.48	2.33E-04	2.33	8.5	1.8
	CF16	31.6	498	231	9.83E-05	0.98	4.75E-04	4.75	4.2	0.9
	CF17	35.6	742	134	5.70E-05	0.57	2.75E-04	2.75	7.2	1.5
	CF18	34.6	608	143	6.11E-05	0.61	2.95E-04	2.95	6.7	1.4
	CF19	34.0	648	158	6.75E-05	0.67	3.26E-04	3.26	6.1	1.3
	CF20	33.5	569	136	5.82E-05	0.58	2.81E-04	2.81	7.0	1.5
Average		33	561	147	6.26E-05	0.63	3.02E-04	3.02	6.8	1.4
1SD		2	104	35	1.49E-05	0.15	7.20E-05	0.72	1	0
min		31	380	109	4.64E-05	0.46	2.24E-04	2.24	4	1
max		36	749	237	1.01E-04	1.01	4.88E-04	4.88	9	2
median		33	559	138	5.87E-05	0.59	2.83E-04	2.83	7	1
Largemouth bass (*Micropterus salmoides*)	LMB1	49.2	1659	723	3.08E-04	3.08	1.49E-03	14.89	1.3	0.3
	LMB2	35.6	528	127	5.43E-05	0.54	2.62E-04	2.62	7.5	1.6
	LMB3	39.6	859	274	1.17E-04	1.17	5.65E-04	5.65	3.5	0.7
	LMB4	21.4	102	150	ND	ND	ND	ND	ND	ND
	LMB5	36.4	631	133	5.66E-05	0.57	2.73E-04	2.73	7.2	1.5
	LMB6	38.5	849	331	1.41E-04	1.41	6.81E-04	6.81	2.9	0.6
	LMB7	36.0	679	489	2.09E-04	2.09	1.01E-03	10.07	2.0	0.4
	LMB8	37.6	739	271	1.15E-04	1.15	5.57E-04	5.57	3.5	0.7
	LMB9	38.5	822	444	1.89E-04	1.89	9.14E-04	9.14	2.2	0.4
	LMB10	31.0	417	174	7.42E-05	0.74	3.58E-04	3.58	5.5	1.1
	LMB11	34.0	583	397	1.69E-04	1.69	8.17E-04	8.17	2.4	0.5
	LMB12	48.3	1810	586	2.50E-04	2.50	1.21E-03	12.06	1.6	0.3
	LMB13	38.6	951	568	2.42E-04	2.42	1.17E-03	11.70	1.7	0.3
	LMB14	40.4	998	509	2.17E-04	2.17	1.05E-03	10.47	1.9	0.4
	LMB15	36.4	595	182	7.77E-05	0.78	3.75E-04	3.75	5.3	1.1
	LMB16	42.9	1133	308	1.31E-04	1.31	6.33E-04	6.33	3.1	0.6
	LMB17	42.0	1033	279	1.19E-04	1.19	5.74E-04	5.74	3.4	0.7
	LMB18	39.3	800	219	9.32E-05	0.93	4.50E-04	4.50	4.4	0.9
	LMB19	36.8	646	238	1.01E-04	1.01	4.89E-04	4.89	4.0	0.8
	LMB20	36.7	689	102	4.33E-05	0.43	2.09E-04	2.09	9.4	2.0
	LMB21	21.9	117	51	ND	ND	ND	ND	ND	ND
Average		37	792	312	1.43E-04	1.43	6.88E-04	6.88	3.8	0.8
1SD		7	408	182	7.53E-05	0.75	3.63E-04	3.63	2	0
min		21	102	51	4.33E-05	0.43	2.09E-04	2.09	1	0
max		49	1810	723	3.08E-04	3.08	1.49E-03	14.89	9	2
median		38	739	274	1.19E-04	1.19	5.74E-04	5.74	3	1
Channel catfish (*Ictalurus punctatus*)	CC1	36.5	307	152	6.46E-05	0.65	3.12E-04	3.12	6.3	1.3
	CC2	37.0	340	198	8.42E-05	0.84	4.07E-04	4.07	4.9	1.0
	CC3	44.5	476	316	1.35E-04	1.35	6.50E-04	6.50	3.0	0.6
	CC4	42.5	581	248	1.06E-04	1.06	5.12E-04	5.12	3.9	0.8
	CC5	41.0	430	156	6.65E-05	0.67	3.21E-04	3.21	6.2	1.3
	CC6	49.0	761	162	6.92E-05	0.69	3.34E-04	3.34	5.9	1.2
	CC7	47.6	719	199	8.47E-05	0.85	4.09E-04	4.09	4.8	1.0
	CC8	48.0	919	194	8.26E-05	0.83	3.99E-04	3.99	5.0	1.0
	CC9	42.0	543	334	1.42E-04	1.42	6.88E-04	6.88	2.9	0.6
	CC10	31.0	217	405	1.73E-04	1.73	8.34E-04	8.34	2.4	0.5
	CC11	46.5	737	142	6.04E-05	0.60	2.92E-04	2.92	6.8	1.4
	CC12	40.3	466	184	7.85E-05	0.79	3.79E-04	3.79	5.2	1.1
	CC13	43.5	664	432	1.84E-04	1.84	8.90E-04	8.90	2.2	0.5
	CC14	41.0	522	178	7.59E-05	0.76	3.66E-04	3.66	5.4	1.1
	CC15	40.0	452	187	7.96E-05	0.80	3.84E-04	3.84	5.1	1.1
	CC16	38.0	520	163	6.95E-05	0.69	3.35E-04	3.35	5.9	1.2
	CC17	40.5	475	145	6.18E-05	0.62	2.98E-04	2.98	6.6	1.4
	CC18	38.7	437	209	8.89E-05	0.89	4.29E-04	4.29	4.6	1.0
Average		42	531	222	9.48E-05	0.95	4.58E-04	4.58	4.84	1.00
1SD		5	175	89	3.81E-05	0.38	1.84E-04	1.84	1	0
min		31	217	142	6.04E-05	0.60	2.92E-04	2.92	2	0
max		49	919	432	1.84E-04	1.84	8.90E-04	8.90	7	1
median		41	498	190	8.11E-05	0.81	3.92E-04	3.92	5	1

SARDIS LAKE: individual fish muscle Hg concentration (ng/g wet wt.) and risk assessment values.

Species	Fish ID	Length (cm)	Weight (g)	Muscle (ng/g)	Adult Intake (mg/kg/d)= (CF*IR*EF*ED) /(BW*AT)	Adults Hazard Index= Intake (mg/kg/d)/Reference dose of MeHg (1.0E-4)	Children Intake (mg/kg/d)= (CF*IR*EF*ED)/(BW*AT)	Children Hazard Index= Intake (mg/kg/d)/Reference dose of MeHg (1.0E-4)	Adults Monthly Consumption Limits = (RfD* BW/Cm)X(30.44d/mo/IR)	Children Monthly Consumption Limits = (RfD* BW/Cm)X(30.44d/mo/IR)
Gizzard shad (*Dorosoma cepedianum*)	GS1	26.5	140	45	ND	ND	ND		ND	ND
	GS2	26.5	151	73	ND	ND	ND		ND	ND
	GS3	26.0	120	72	ND	ND	ND	ND	ND	ND
	GS4	27.5	88	59	ND	ND	ND	ND	ND	ND
	GS5	12.5	12	ND	ND	ND	ND	ND	ND	ND
	GS6	10.0	8	ND	ND	ND	ND	ND	ND	ND
	GS7	9.5	7	ND	ND	ND	ND	ND	ND	ND
	GS8	31.2	225	44	1.87E-05	0.19	9.05E-05	0.90	21.8	4.5
	GS9	27.5	184	35	ND	ND	ND	ND	ND	ND
	GS10	35.0	378	49	ND	ND	ND	ND	ND	ND
	GS11	25.6	126	63	ND	ND	ND	ND	ND	ND
	GS12	24.4	128	45	ND	ND	ND	ND	ND	ND
	GS13	29.0	203	29	ND	ND	ND	ND	ND	ND
	GS14	27.2	107	38	ND	ND	ND	ND	ND	ND
	GS15	26.9	137	40	ND	ND	ND	ND	ND	ND
	GS16	10.5	7	10	ND	ND	ND	ND	ND	ND
	GS17	10.9	8	42	ND	ND	ND	ND	ND	ND
	GS18	9.9	5	9	ND	ND	ND	ND	ND	ND
	GS19	8.7	4	10	ND	ND	ND	ND	ND	ND
	GS20	9.8	6	10	ND	ND	ND	ND	ND	ND
	GS21	8.7	5	8	ND	ND	ND	ND	ND	ND
Average		20	98	38	ND	ND	ND	ND	ND	ND
1SD		9	99	21	ND	ND	ND	ND	ND	ND
min		9	4	8	ND	ND	ND	ND	ND	ND
max		35	378	73	ND	ND	ND	ND	ND	ND
median		26	107	41	ND	ND	ND	ND	ND	ND
Blue Catfish (Ictalurus furcatus)	BC1	72.6	ND	298	1.27E-04	1.27	6.14E-04	6.14	3.2	0.7
	BC2	78.2	ND	878	3.75E-04	3.75	1.81E-03	18.08	1.1	0.2
	BC3	64	2590	290	1.24E-04	1.24	5.96E-04	5.96	3.3	0.7
	BC4	55	1431	324	1.38E-04	1.38	6.68E-04	6.68	3.0	0.6
Average		67.5	2011	448	1.91E-04	1.91	9.22E-04	9.22	2.6	0.5
1SD		10.1	820	287	1.23E-04	1.23	5.92E-04	5.92	1.0	0.2
min		55.0	1431	290	1.24E-04	1.24	5.96E-04	5.96	1.1	0.2
max		78.2	2590	878	3.75E-04	3.75	1.81E-03	18.08	3.3	0.7
median		68.3	2011	311	1.33E-04	1.33	6.41E-04	6.41	3.1	0.6

ENID LAKE										
Species	**Fish ID**	**Length (cm)**	**Weight (g)**	**Muscle (ng/g)**	**Adult Intake (mg/kg/d)= (CF*IR*EF*ED)/(BW*AT)**	**Adults Hazard Index= Intake (mg/kg/d)/Reference dose of MeHg (1.0E-4)**	**Children Intake (mg/kg/d)= (CF*IR*EF*ED)/(BW*AT)**	**Children Hazard Index= Intake (mg/kg/d)/Reference dose of MeHg (1.0E-4)**	**Adults Monthly Consumption Limits = (RfD* BW/Cm)X(30.44d/mo/IR)**	**Children Monthly Consumption Limits = (RfD* BW/Cm)X(30.44d/mo/IR)**
Crappie (Pomoxis)	Cr1	32	415	183.0	7.80E-05	0.78	3.77E-04	3.77	5.25	1.09
	Cr2	31	445	224.8	9.59E-05	0.96	4.63E-04	4.63	4.27	0.88
	Cr3	34	479	206.1	8.79E-05	0.88	4.24E-04	4.24	4.66	0.96
	Cr4	33	509	247.0	1.05E-04	1.05	5.09E-04	5.09	3.89	0.80
	Cr5	33	452	190.7	8.13E-05	0.81	3.93E-04	3.93	5.03	1.04
	Cr6	30	391	260.2	1.11E-04	1.11	5.36E-04	5.36	3.69	0.76
	Cr7	30	384	180.4	7.69E-05	0.77	3.71E-04	3.71	5.32	1.10
	Cr8	34	506	257.6	1.10E-04	1.10	5.30E-04	5.30	3.73	0.77
	Cr9	34	527	217.9	9.29E-05	0.93	4.49E-04	4.49	4.41	0.91
	Cr10	33	484	212.5	9.06E-05	0.91	4.37E-04	4.37	4.52	0.94
	Cr11	30	385	120.4	5.14E-05	0.51	2.48E-04	2.48	7.97	1.65
	Cr12	33	469	246.5	1.05E-04	1.05	5.07E-04	5.07	3.89	0.81
	Cr13	31	399	171.5	7.31E-05	0.73	3.53E-04	3.53	5.60	1.16
	Cr14	35	458	222.1	9.47E-05	0.95	4.57E-04	4.57	4.32	0.90
	Cr15	31	421	284.6	1.21E-04	1.21	5.86E-04	5.86	3.37	0.70
	Cr16	30	320	205.1	8.75E-05	0.87	4.22E-04	4.22	4.68	0.97
Average		32	440	214.4	9.14E-05	0.91	4.41E-04	4.41	4.66	0.97
1SD		2	56	40.6	1.73E-05	0.17	8.35E-05	0.84	1.09	0.23
min		30	320	120.4	5.14E-05	0.51	2.48E-04	2.48	3.37	0.70
max		35	527	284.6	1.21E-04	1.21	5.86E-04	5.86	7.97	1.65
median		32	449	215.2	9.18E-05	0.92	4.43E-04	4.43	4.46	0.92
Largemouth bass (*Micropterus salmoides*)	LMB1	19.5	90	146.4	ND	ND	ND	ND	ND	ND
	LMB2	31.5	407	189.3	8.07E-05	0.81	3.90E-04	3.90	5.07	1.05
	LMB3	51.5	1862	954.4	4.07E-04	4.07	1.96E-03	19.65	1.01	0.21
	LMB4	38.8	610	196.0	8.36E-05	0.84	4.04E-04	4.04	4.90	1.01
	LMB5	34	373	421.2	1.80E-04	1.80	8.67E-04	8.67	2.28	0.47
	LMB6	20.5	100	114.3	ND	ND	ND	ND	ND	ND
	LMB7	34.2	397	335.2	1.43E-04	1.43	6.90E-04	6.90	2.86	0.59
	LMB8	30.9	352	252.6	1.08E-04	1.08	5.20E-04	5.20	3.80	0.79
	LMB9	21.9	122	177.0	ND	ND	3.64E-04	ND	ND	ND
	LMB10	39.5	727	343.8	1.47E-04	1.47	7.08E-04	7.08	2.79	0.58
	LMB11	20	91	108.1	ND	ND	ND	ND	ND	ND
	LMB12	16.9	53	156.4	ND	ND	ND	ND	ND	ND
	LMB13	38.4	657	386.4	1.65E-04	1.65	7.96E-04	7.96	2.48	0.51
	LMB14	40.2	799	398.6	1.70E-04	1.70	8.21E-04	8.21	2.41	0.50
	LMB15	18.4	76	153.8	ND	ND	ND	ND	ND	ND
Average		30	448	288.9	1.65E-04	1.65	7.52E-04	7.96	3.07	0.64
1SD		10	468	213.6	9.79E-05	0.98	4.66E-04	4.72	1.31	0.27
min		17	53	108.1	8.07E-05	0.81	3.64E-04	3.90	1.01	0.21
max		52	1862	954.4	4.07E-04	4.07	1.96E-03	19.65	5.07	1.05
median		32	373	196.0	1.47E-04	1.47	6.99E-04	7.08	2.79	0.58
Channel catfish (*Ictalurus punctatus*)	CC1	34.3	284	141.6	6.04E-05	0.60	2.91E-04	2.91	6.78	1.40
	CC2	31	223	84.2	3.59E-05	0.36	1.73E-04	1.73	11.40	2.36
	CC3	34.6	378	271.6	1.16E-04	1.16	5.59E-04	5.59	3.53	0.73
	CC4	40.1	671	157.0	6.70E-05	0.67	3.23E-04	3.23	6.11	1.27
	CC5	38.3	490	134.7	5.75E-05	0.57	2.77E-04	2.77	7.12	1.48
	CC6	31.4	249	108.5	4.63E-05	0.46	2.23E-04	2.23	8.85	1.83
	CC7	34.5	361	150.9	6.43E-05	0.64	3.11E-04	3.11	6.36	1.32
	CC8	36.8	318	104.9	4.47E-05	0.45	2.16E-04	2.16	9.15	1.90
	CC9	33.4	378	217.7	9.29E-05	0.93	4.48E-04	4.48	4.41	0.91
	CC10	37.5	507	106.2	4.53E-05	0.45	2.19E-04	2.19	9.04	1.87
	CC11	37.9	467	110.0	4.69E-05	0.47	2.26E-04	2.26	8.72	1.81
	CC12	41.2	576	151.5	6.46E-05	0.65	3.12E-04	3.12	6.33	1.31
	CC13	33.1	307	172.3	7.35E-05	0.73	3.55E-04	3.55	5.57	1.15
	CC14	34.5	349	221.0	9.43E-05	0.94	4.55E-04	4.55	4.34	0.90
	CC15	27.8	182	69.1	ND	ND	ND	ND	ND	ND
Average		35	383	146.8	6.50E-05	0.65	3.14E-04	3.14	6.98	1.45
1SD		4	136	55.6	2.27E-05	0.23	1.10E-04	1.10	2.22	0.46
min		28	182	69.1	3.59E-05	0.36	1.73E-04	1.73	3.53	0.73
max		41	671	271.6	1.16E-04	1.16	5.59E-04	5.59	11.40	2.36
median		35	361	141.6	6.24E-05	0.62	3.01E-04	3.01	6.57	1.36

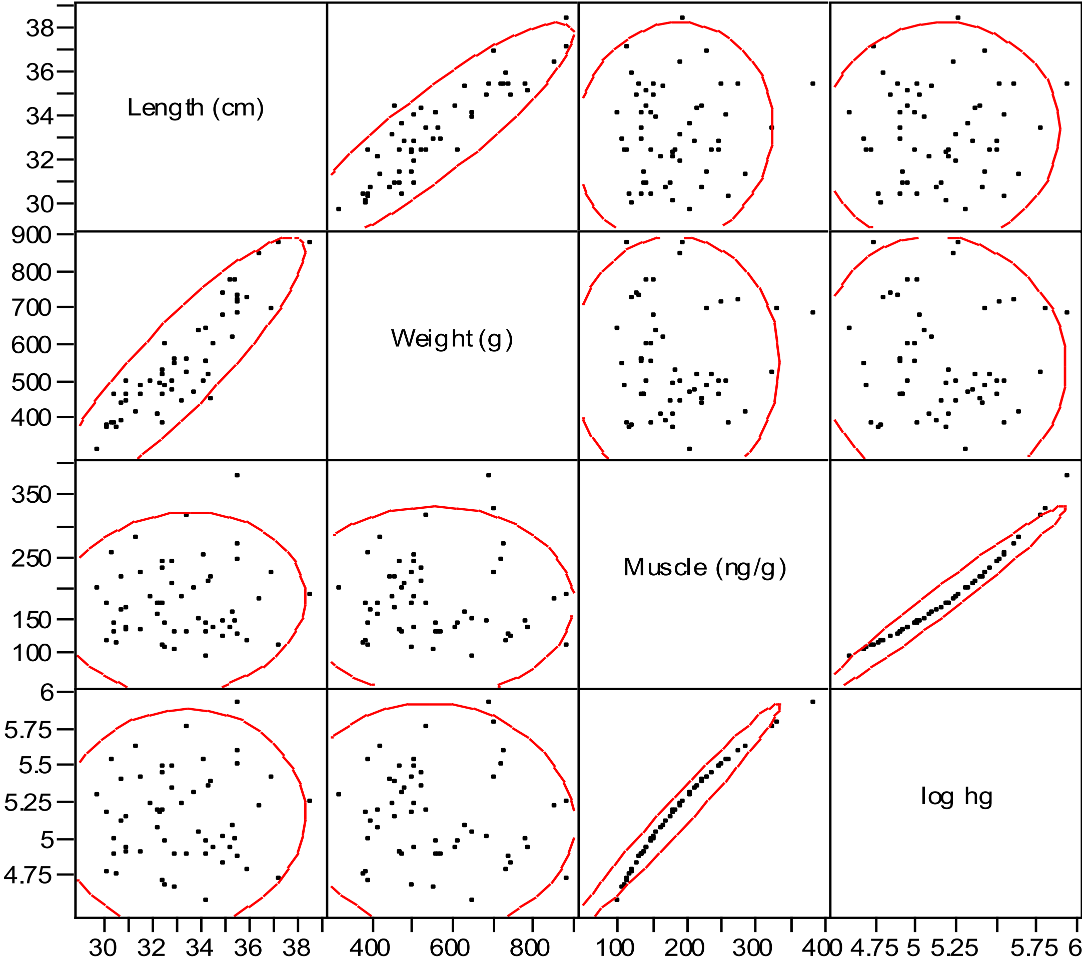
APPENDIX 3.2: Statistical Analysis Output

Correlations

Crappie
Multivariate Correlations

	Length (cm)	Weight (g)	Muscle (ng/g)	log hg
Length (cm)	1.0000	0.8942	0.0649	0.0419
Weight (g)	0.8942	1.0000	-0.0606	-0.0902
Muscle (ng/g)	0.0649	-0.0606	1.0000	0.9832
log hg	0.0419	-0.0902	0.9832	1.0000

Scatterplot Matrix



Pairwise Correlations

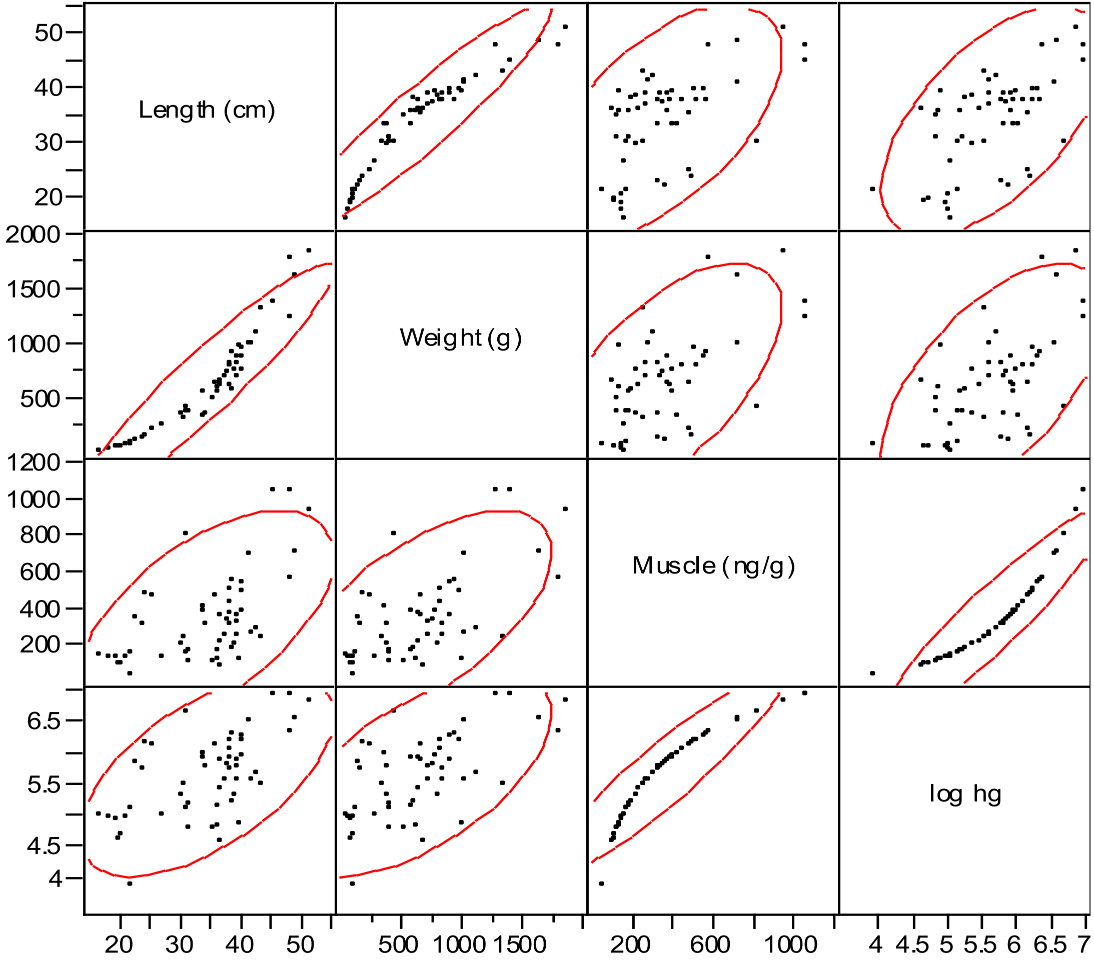
Variable	by Variable	Correlation	Count	Signif Prob	Plot Corr
Weight (g)	Length (cm)	0.8942	55	0.0000	▨
Muscle (ng/g)	Length (cm)	0.0649	55	0.6379	▨
Muscle (ng/g)	Weight (g)	-0.0104	56	0.9391	▨
log hg	Length (cm)	0.0419	55	0.7613	▨
log hg	Weight (g)	-0.0467	56	0.7324	▨
log hg	Muscle (ng/g)	0.9833	56	0.0000	▨

Largemouth Bass

Multivariate Correlations

	Length (cm)	Weight (g)	Muscle (ng/g)	log hg
Length (cm)	1.0000	0.9391	0.5721	0.5913
Weight (g)	0.9391	1.0000	0.6277	0.6050
Muscle (ng/g)	0.5721	0.6277	1.0000	0.9301
log hg	0.5913	0.6050	0.9301	1.0000

Scatterplot Matrix



Pairwise Correlations

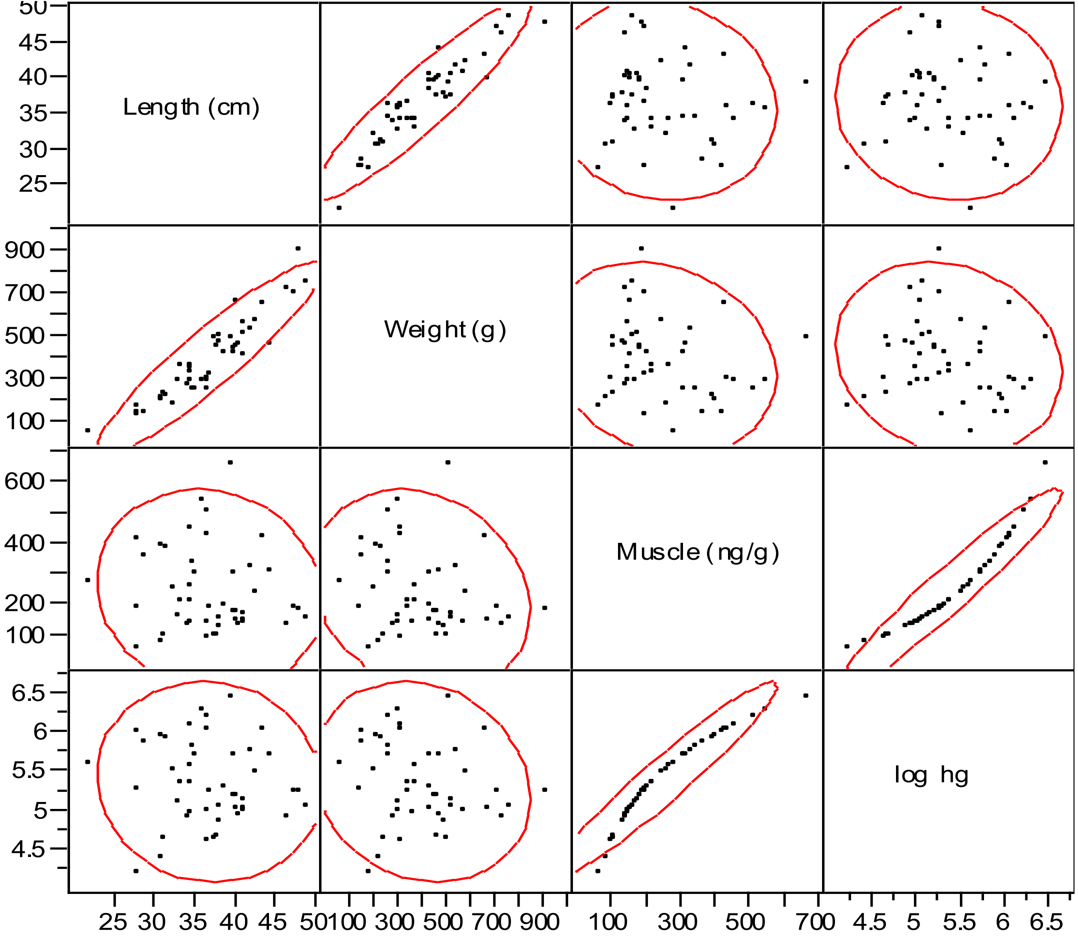
Variable	by Variable	Correlation	Count	Signif Prob	Plot Corr
Weight (g)	Length (cm)	0.9391	54	0.0000	▨
Muscle (ng/g)	Length (cm)	0.5721	54	0.0000	▨
Muscle (ng/g)	Weight (g)	0.6277	54	0.0000	▨
log hg	Length (cm)	0.5913	54	0.0000	▨
log hg	Weight (g)	0.6050	54	0.0000	▨
log hg	Muscle (ng/g)	0.9301	54	0.0000	▨

Channel Catfish

Multivariate Correlations

	Length (cm)	Weight (g)	Muscle (ng/g)	log hg
Length (cm)	1.0000	0.9263	-0.1009	-0.0582
Weight (g)	0.9263	1.0000	-0.1906	-0.1534
Muscle (ng/g)	-0.1009	-0.1906	1.0000	0.9611
log hg	-0.0582	-0.1534	0.9611	1.0000

Scatterplot Matrix



Pairwise Correlations

Variable	by Variable	Correlation	Count	Signif Prob	Plot Corr
Weight (g)	Length (cm)	0.9263	47	0.0000	▨
Muscle (ng/g)	Length (cm)	-0.1009	47	0.4999	▨
Muscle (ng/g)	Weight (g)	-0.1906	47	0.1994	▨
log hg	Muscle (ng/g)	0.9611	47	0.0000	▨

Crappie

Response log hg – no covariate
Whole Model
Actual by Predicted Plot

Summary of Fit

RSquare	0.26087
RSquare Adj	0.232979
Root Mean Square Error	0.274409
Mean of Response	5.16925
Observations (or Sum Wgts)	56

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	1.4085645	0.704282	9.3530
Error	53	3.9909154	0.075300	Prob > F
C. Total	55	5.3994799		0.0003

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	5.1812349	0.036873	140.52	<.0001
lake[Enid]	0.1677842	0.054114	3.10	0.0031
lake[Grenada]	0.0469387	0.051133	0.92	0.3628

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
lake	2	2	1.4085645	9.3530	0.0003

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
Enid	5.3490191	0.06860225	5.34902
Grenada	5.2281736	0.06135971	5.22817
Sardis	4.9665120	0.06135971	4.96651

LSMeans Differences Tukey HSD

Alpha=0.050 Q=2.41127LSMean[i] By LSMean[j]

Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	Enid	Grenada	Sardis
Enid	0 0 0 0	0.12085 0.09204 -0.1011 0.34278	0.38251 0.09204 0.16057 0.60444
Grenada	-0.1208 0.09204 -0.3428 0.10109	0 0 0 0	0.26166 0.08678 0.05242 0.4709
Sardis	-0.3825 0.09204 -0.6044 -0.1606	-0.2617 0.08678 -0.4709 -0.0524	0 0 0 0

Level			Least Sq Mean
Enid	A		5.3490191
Grenada	A		5.2281736
Sardis		B	4.9665120

Levels not connected by same letter are significantly different

Largemouth Bass

Response log hg – using weight as a covariate
Whole Model
Actual by Predicted Plot

Summary of Fit

RSquare	0.470763
RSquare Adj	0.439008
Root Mean Square Error	0.496118
Mean of Response	5.672638
Observations (or Sum Wgts)	54

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	3	10.946928	3.64898	14.8252
Error	50	12.306674	0.24613	Prob > F
C. Total	53	23.253602		<.0001

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	5.0540825	0.124423	40.62	<.0001
Weight (g)	0.0009537	0.000162	5.90	<.0001
lake[Enid]	0.0008921	0.105463	0.01	0.9933
lake[Grenada]	0.2514776	0.096249	2.61	0.0118

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Weight (g)	1	1	8.5732226	34.8316	<.0001
lake	2	2	2.4358458	4.9482	0.0109

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
Enid	5.6875997	0.13275164	5.48196
Grenada	5.9381853	0.11703129	5.96602
Sardis	5.4343379	0.11025067	5.55737

LSMeans Differences Tukey HSD

Alpha=0.050   Q=2.41542LSMean[i] By LSMean[j]

Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	Enid	Grenada	Sardis
Enid	0 0 0 0	-0.2506 0.1779 -0.6803 0.17912	0.25326 0.17672 -0.1736 0.68012
Grenada	0.25059 0.1779 -0.1791 0.68029	0 0 0 0	0.50385 0.16017 0.11697 0.89073
Sardis	-0.2533 0.17672 -0.6801 0.1736	-0.5038 0.16017 -0.8907 -0.117	0 0 0 0

Level			Least Sq Mean
Grenada	A		5.9381853
Enid	A	B	5.6875997
Sardis		B	5.4343379

Levels not connected by same letter are significantly different

Channel Catfish
Response log hg – no covariate

Summary of Fit

RSquare	0.580316
RSquare Adj	0.56124
Root Mean Square Error	0.348486
Mean of Response	5.383726
Observations (or Sum Wgts)	47

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	7.388667	3.69433	30.4204
Error	44	5.343473	0.12144	Prob > F
C. Total	46	12.732140		<.0001

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	5.398688	0.051118	105.61	<.0001
lake[Enid]	-0.474725	0.072882	-6.51	<.0001
lake[Grenada]	0.5318438	0.074193	7.17	<.0001

Effect Tests

Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
lake	2	2	7.3886667	30.4204	<.0001

Least Squares Means Table

Level	Least Sq Mean	Std Error	Mean
Enid	4.9239634	0.08997873	4.92396
Grenada	5.9305318	0.09313683	5.93053
Sardis	5.3415689	0.08213896	5.34157

LSMeans Differences Tukey HSD

Alpha=0.050 Q=2.42548LSMean[i] By LSMean[j]

Mean[i]-Mean[j] Std Err Dif Lower CL Dif Upper CL Dif	Enid	Grenada	Sardis
Enid	0	-1.0066	-0.4176
	0	0.1295	0.12183
	0	-1.3207	-0.7131
	0	-0.6925	-0.1221
Grenada	1.00657	0	0.58896
	0.1295	0	0.12418
	0.69246	0	0.28776
	1.32067	0	0.89017
Sardis	0.41761	-0.589	0
	0.12183	0.12418	0
	0.1221	-0.8902	0
	0.71311	-0.2878	0

Level				Least Sq Mean
Grenada	A			5.9305318
Sardis		B		5.3415689
Enid			C	4.9239634

Levels not connected by same letter are significantly different

CHAPTER FOUR

4. MERCURY SPECIES IN RAINWATER COLLECTED IN OXFORD, MISSISSIPPI: ESTIMATING WET DEPOSITION TO THE NORTH MISSISSIPPI REGION

4.1 ABSTRACT

Mercury is a toxic environmental pollutant that is dispersed globally through the atmosphere. Mercury is deposited to terrestrial and aquatic systems through wet and dry deposition. Indeed atmospheric deposition is considered a major non-point source that contributes significant Hg loads to watersheds. The purpose of this study was to evaluate levels of mercury species in the rainwater and to quantify the amount of mercury deposited to the Enid and Sardis Lake watersheds in north Mississippi through rainfall. Enid Lake, in the Yocona River watershed, is considered impaired with a fish consumption advisory due to elevated levels of Hg in fish tissue. Total-Hg and MeHg concentrations were determined in rainwater collected in Oxford, Mississippi on several occasions in 2012. Total-Hg averaged 5.1 ng/L and ranged from 3.4 to 7.2 ng/L. This is below that modeled by the Mercury Deposition Network (MDN) in 2011, the most recent year data was available; note that there is very limited data for Hg in this region and the model interpolates data from other states. Levels were above EPA criterion (3.5 ng/L) for lake water. The vast majority of the Hg in the rainwater was inorganic Hg^{+2}; MeHg averaged <3% of the total-Hg. An estimated 33 kg and 15 kg of Hg are deposited annually via rainfall to the Little Tallahatchie (Sardis) and Yocona River (Enid) watersheds, respectively.

4.2 INTRODUCTION

Atmospheric mercury enters into aquatic and terrestrial ecosystems through wet (precipitation) and dry deposition. Atmospheric deposition contributes significantly to Hg loading to watersheds and, in some cases, is the only major source of Hg (Ryaboshapko et al. 2002). The dominant form of Hg in the atmosphere is Hg^0 with a life-span in the atmosphere ranging from one to two years (Li et al. 2008). Divalent mercury (Hg^{2+}) is the more soluble form of Hg and has a life span in the atmosphere measured in days (ECPD, 2009). Once deposited in waterbodies, Hg^{2+} can be converted to the toxic organic form, methylmercury (MeHg), primarily through sulfate reducing bacteria in anoxic sediment.

Mercury in precipitation is widespread, and some measured levels in the United States, exceed US EPA safe level for lakes and rivers. The agency set a national human health and water quality criterion for Hg in rivers and lakes at 7.9 ng/L and 3.5 ng/L, respectively. In one study it found that rain in Louisiana and New York exceeded the EPA's safe level 96% of the time contributing to contamination in the state's waterbodies (National Wildlife Federation, 2003).

Mississippi is a state with abundant water resources and a thriving commercial and recreational fishing industry. It is also a state with four coal-fired power plants and is in proximity to numerous other coal-burning power facilities, which are recognized as a significant source of Hg to the atmosphere and local environs. Coal is expected to remain the nation's primary source of energy and several new coal-fired power plants are scheduled to come online near Mississippi. Identifying the fate and transport of Hg is critical to improving water quality and ecosystem health (Fulkerson and Nnadi 2007).

A fish-consumption advisory was issued for Enid Reservoir, located in North Mississippi, by the Mississippi Department of Health due to high levels of Hg in fish tissue sampled in the waters. The origin of Hg in this waterbody is unclear but may include atmospheric deposition.

The purpose of this study was to evaluate levels of Hg species in the rainwater and to quantify the amount of mercury deposited to the Enid and Sardis Lake watersheds through wet deposition. Mercury species (total-Hg and MeHg) were analyzed in rain samples collected on three different occasions in Feburary and March 8, 2012, on the campus of the University of Mississippi (Oxford, MS. USA). Wet deposition was estimated using the average concentration and the average annual rainfall to the region. In addition, Hg concentrations in precipitation are compared to the level defined as safe by the EPA for Hg in lakes and streams.

4.3 METHODS

The average annual precipitation rate for the state of Mississippi is 59.6 inches (South ERCC, 2003). Rainwater was collected outside the Department of Chemistry and Biochemistry at the University of Mississippi along Science Row Street (latitude 34° 21' 57" N and longitude 89° 31' 31" W) using acid washed 2 liter polycarbonate bottles (Nalgene®) with a 11-in diameter low-density polyethylene funnel (LDPE). The setup was secured on a ring stand and placed on the site for collection (Figure 20). Approximately 10 ml of rainwater was used to clean out the bottles before collection. Once collected, half of the rainwater samples were filtered using a 0.45-micron quartz fiber filter. Both filtered and unfiltered samples were preserved to 0.4% acidity using 12N HCl. The samples were analyzed separately for total-Hg and MeHg.

Figure 20 Rainwater collection apparatus (acid-washed LDPE funnels and 2 L polycarbonate bottle) outside Coulter Hall on the University of Mississippi campus.

4.3.1 Total Mercury Analysis

For total-Hg, 50 mL samples were analyzed using a 2600 Mercury Analyzer (Tekran®, Toronto, CA) following EPA method 1631 “Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry” (CVAFS) (as described earlier). In short, bromine monochloride (BrCl) is used as an oxidizing agent to digest Hg species in the samples. The oxidizing agent is added to the filtered and unfiltered samples for digestion. The sample is reduced with hydroxylamine hydrochloride ($NH_2OH \cdot HCl$) to destroy free halogens. Stannous chloride ($SnCl_2$) reduces Hg^{2+} to Hg^0 for subsequent detection by a CVAFS photomultiplier tube.

4.3.2 Methyl Mercury Analysis

Samples were analyzed using a 2700 MeHg Analyzer (Tekran®, Toronto, CA) following a slightly modified version of EPA Method 1630, “Direct ethylation using $NaBEt_4$, purge and trap, and Cold Vapor Atomic Fluorescence Spectrometry” (as described earlier). In short, 30-mL sample aliquots are placed in an I-Chem® amber glass vial with a citrate acid buffer and ethylated in the vial by the addition of $NaBEt_4$; volatile mercury species are formed (methyl-ethyl-mercury for $MeHg^+$ and diethylmercury for Hg^{+2}). The ethylated forms are then separated from the solution by in-vial purging with high purity argon gas onto a Tenax carbon trap. After pre-concentration the trapped species are thermally desorbed and carried into a GC where the species are separated. The volatile species are then passed through a pyrolytic decomposition column, which converts organo-Hg forms to Hg^0, and further into the cell of a CVAFS for detection. Each sample was spiked with 1 ng/L propyl-Hg before ethylation to serve as a surrogate to measure analytical recoveries.

4.3.3 Quality Assurance

For total-Hg, standards 0.5 ng/L, 1 ng/L, 2.5 ng/L, 5 ng/L, and 10 ng/L. The r^2 value for the calibration was > 0.99, and the recovery values for all calibration standards were between 80-122%. The analytical procedure for total-Hg was modified towards the end of the experimental run as a result of insufficient $SnCl_2$ thereby showing a higher percent recovery of rainwater unfiltered III and Ongoing Precision and Recovery (OPR). A standard of 1 parts per trillion of MeHg was used for OPR1.

For MeHg, a propyl-Hg surrogate was included. Recovery of the surrogate was between 70-130% of its expected value. MeHg and propyl-Hg standards were prepared in the same flask at concentration values of 0.02 ng/L, 0.06 ng/L, 0.2 ng/L, 0.4 ng/L, 1 ng/L, and 2 ng/L. The r^2 value for the MeHg and propyl-Hg calibrations was > 0.99. The recovery values for the calibration standards ranged between 80-120%. Ongoing precision and recovery (OPR) was analyzed for MeHg. The recoveries for the OPR were between 80-100%.

4.4 RESULTS AND DISCUSSION

4.4.1 Mercury levels in precipitation

Mercury and MeHg were determined in rain collected on three separate occasions: 2/4/12, 2/13/12, and 3/8/12. Concentrations of total-Hg averaged 5.1 ng/L and ranged from 3.4 to 7.2 ng/L (Table 11). These levels are lower compared to the levels recorded at a station located near the Mississippi Gulf, which averaged 10.6 ng/L from 2003 to 2011. Our data is also slightly lower than the 10 to 14 ng/L estimated by the Mercury Deposition Network (MDN) for the most recent data available (2011) (see figure 22). Note that the MDN has very limited data for mercury in our region, and uses data from other states to interpolate deposition here. The 2011 model also estimates a wet deposition rate in the state of Mississippi to be between 14.0 to 18 $\mu g/m^2$ (figure 22). The vast majority of the Hg in the rainwater was inorganic Hg^{+2}; MeHg averaged <3% of the total-Hg. For MeHg averaged <3% of the total-Hg; the vast majority of the mercury in the rainwater is inorganic Hg^{+2}.

Table 11 Concentrations (ng/L) of mercury species in rain from Oxford, MS*

Date	**Total-Hg**				
	n	Mean	precision	min	max
2/14/12	2	4.7	29%	4	5.4
2/13/12	2	3.5	3%	3.4	3.5
3/8/12	3	7.1	0.04 (SD)	7.1	7.2
Total	7	5.1		3.4	7.2
Date	**MeHg**				
	n	Mean	precision	min	max
2/14/12	2	0.11	17%	0.1	0.12
3/8/12	3	0.16	0.01(SD)	0.15	0.16
Total	5	0.14		0.1	0.16

*filtered through 0.45 μm; MeHg not determined for 2/13/12

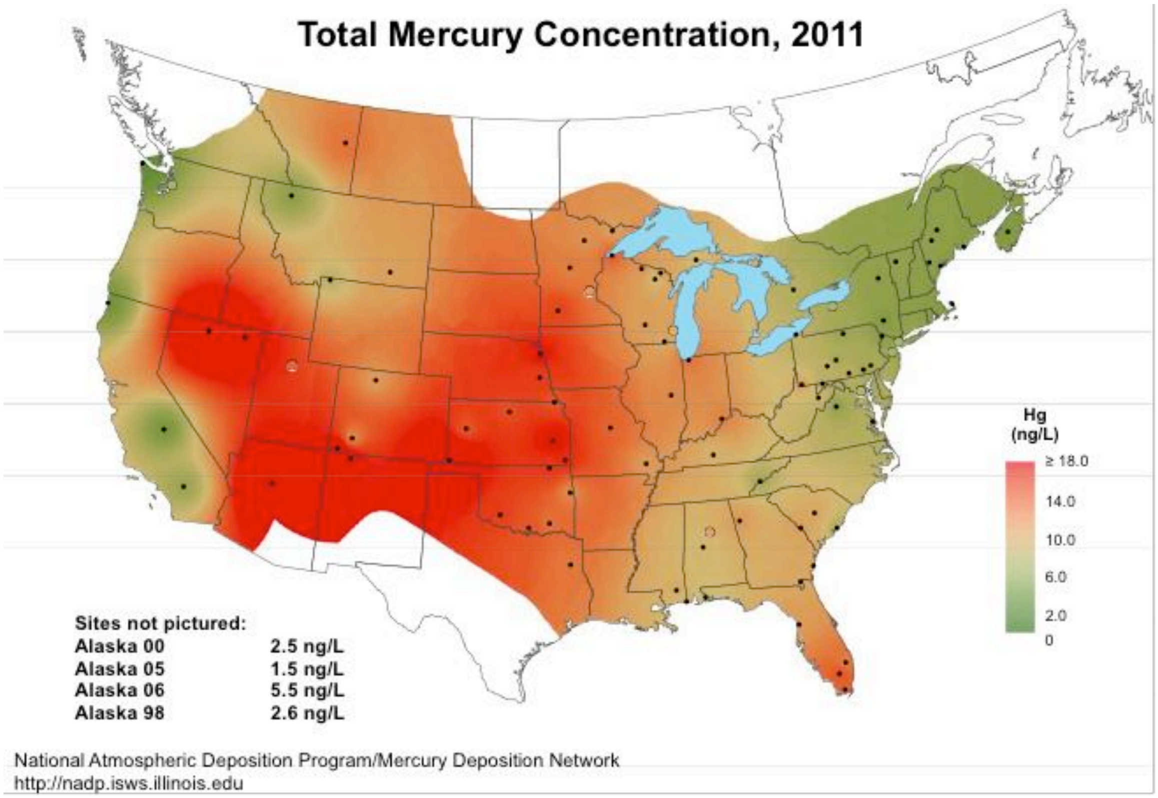
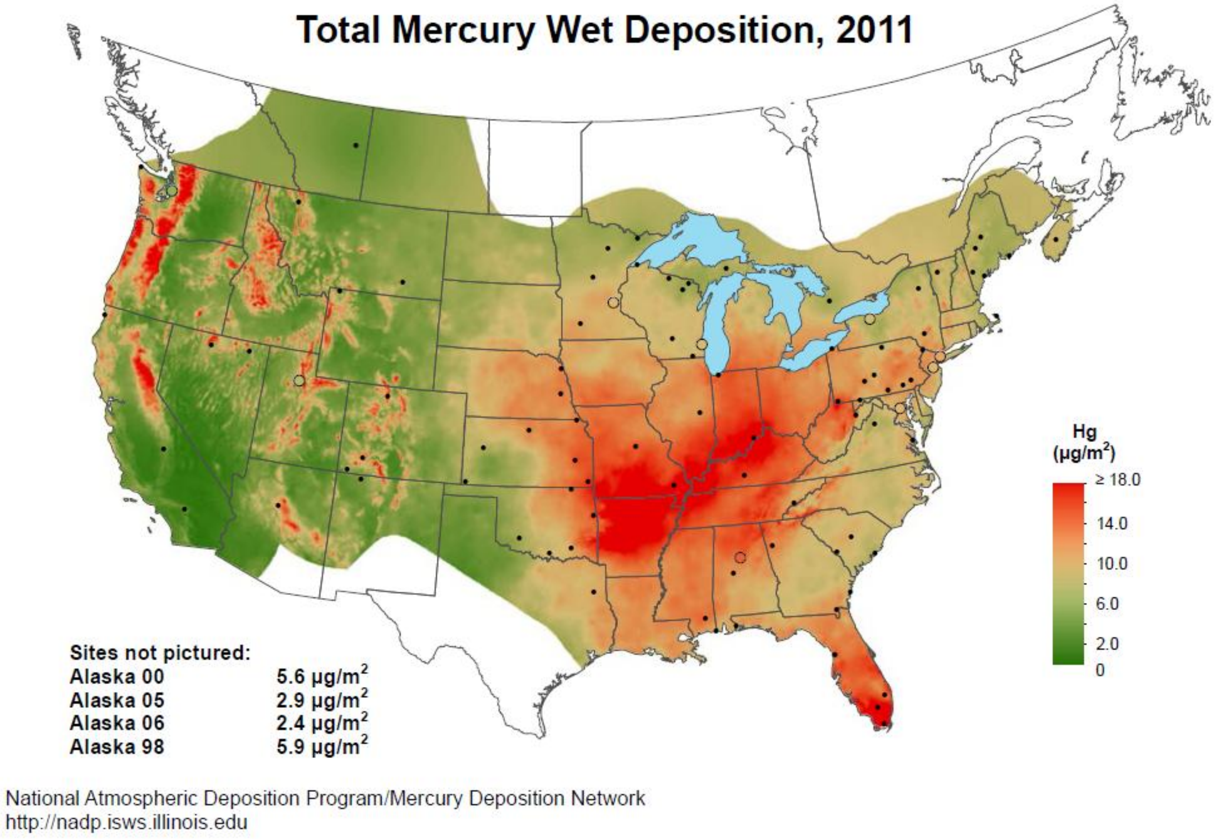


Figure 21 National Atmospheric Deposition Program/Mercury Deposition Network map illustrating total mercury concentrations in the United States and Canada during 2011.



National Atmospheric Deposition Program/Mercury Deposition Network
http://nadp.isws.illinois.edu

Figure 22 National Atmospheric Deposition Program/Mercury Deposition Network map illustrating total mercury wet deposition in the United States and Canada during 2011.

4.4.2 Changes in Hg concentrations and speciation during a precipitation event

Concentrations of total-Hg and MeHg were measured in rain collected during the start and end of a rainfall event in Oxford, MS on 8 of March 2012. The results for total-Hg are presented in Figure 23. As expected, levels were higher in unfiltered samples; Hg^{+2} is surface reactive and tends to bind to aerosols and fine particulate matter in water. Reflecting a “wash-out” of the atmosphere, levels of total-Hg were higher during the start (mid-day) of the event (7.13 ± 0.04 ng/L, filtered; 9.72 ± 0.085 ng/L, unfiltered), whereas near the end of the event (evening) levels decreased (4.18 ± 0.03 ng/L, filtered; 5.35 ng/L unfiltered). These levels were also at or above the EPA human health standard for Hg in lakes (3.5 ng/L) indicated by the red line on the graph.

Concentrations for MeHg were exceedingly low and <3% of the total-Hg levels. At the start of the event, average MeHg levels were 0.150 ng/L for filtered and 0.152 ± 0.008 ng/L for unfiltered. Levels at the end of the rain were similar, 0.17 ng/L for filtered and 0.152 ± 0.003 ng/L for unfiltered. Given the precision of the analyses, there were no differences discernible between the start and end of the event (Figure 24). One likely source of MeHg in the air, and subsequently in rain, is fine dust particles from dried soil and sediment that become an airborne. There may also be some in-situ production via a reaction between labile Hg^{+2} complexes and an unknown methylating agent, possibly acetate or similar molecules (Hammerschmidt et al. 2007).

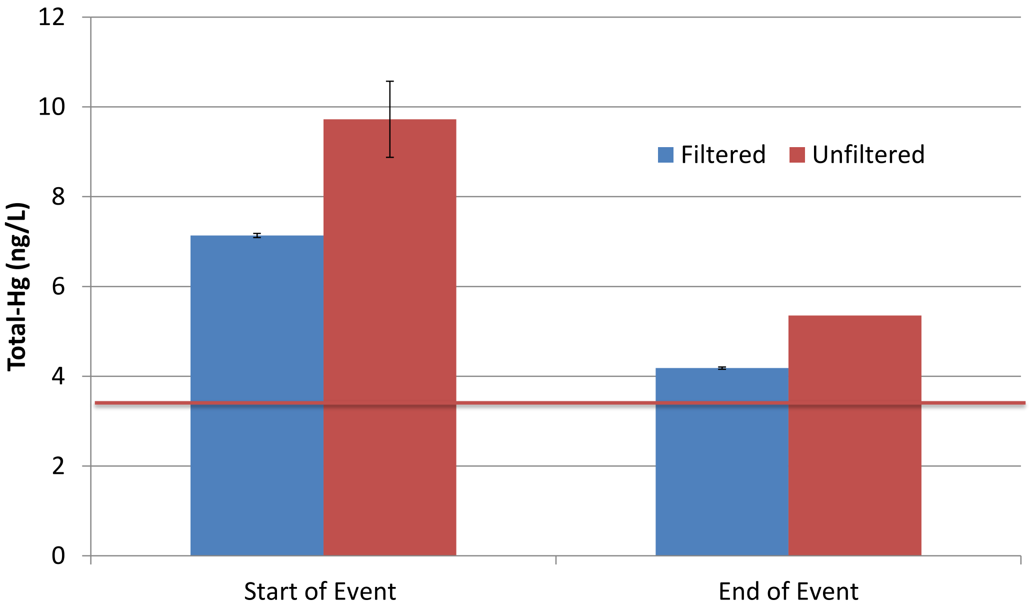


Figure 23 Average total-Hg concentrations in rainwater (March 8, 2012). The red line represents the EPA criterion for human health for lake water (3.5ng/L).

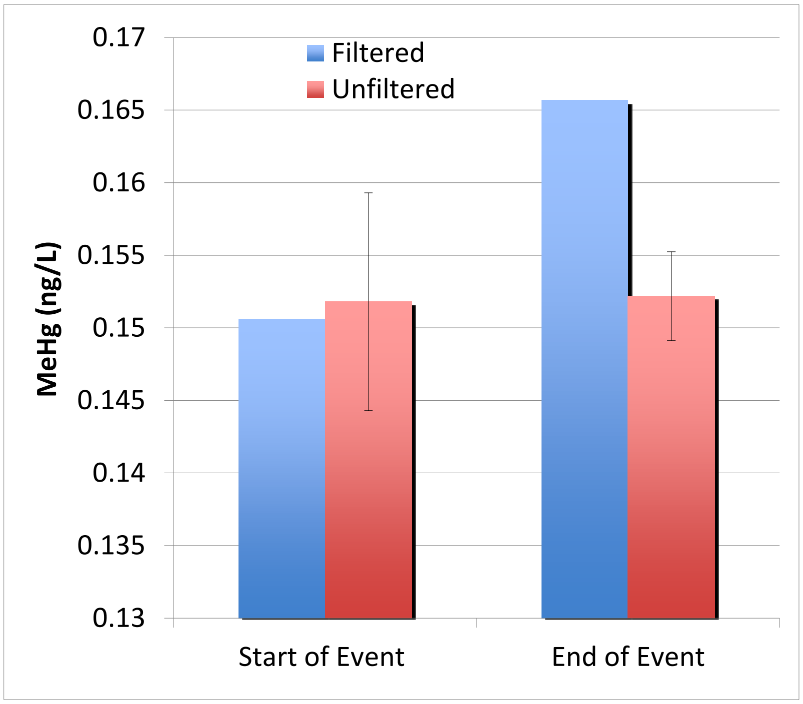


Figure 24 Average MeHg concentration in rainwater (March 8, 2012).

4.4.3 Mercury loading to watershed via wet deposition

Mercury loadings to the Yocona (Enid Lake) and Little Tallahatchie (Sardis Lake) watersheds were estimated based on the mean mercury concentration in the rain, the average annual precipitation to the region, and the watershed area, and direct Hg species deposition directly to Sardis and Enid Lake was determined by:

$$\text{Hg Wet Deposition Rate } (\mu g\cdot m^{-2}\cdot y^{-1}) = C_{Hg} \times I / 1000$$

Where C_{Hg} = concentration of Hg in precipitation ($\mu g\cdot m^{-3}$) and I = precipitation intensity ($mm\cdot y^{-1}$). The total-Hg and MeHg loadings to the Yocona watershed, through wet deposition, were 15kg and 0.39 kg, respectively and the direct Total-Hg and MeHg deposition directly to Enid Lake was 0.66 and 0.028, respectively (Table 12). The Grenada Lake watershed was not included in these analyses because the rain events measured were considered too distant from the site where the rain event occurred.

One of the factors that impacts levels of Hg in rainfall is the nature of the storm. Large convective summer thunderstorms routinely occurring in the south reach high levels in the atmosphere and scavenge a pool of ionic mercury in the upper troposphere (Zhang et al 2012; Selin et al 2008; Guentzel 2001). Thus, the maximum in Hg wet deposition in the southeast is sometimes not associated with local sources (Driscoll et al 2013). Moreover, summer storms may contribute more Hg to the watershed based on these higher levels. Thus, our deposition estimates may actually be somewhat low since we used data for rainfall events in the winter/spring.

Table 12 Deposition of Hg species to the Little Tallahatchie and Yocona Watersheds and direct calculations to Sardis and Enid Lakes.

Watershed	Depostion to watershed			Lake	Deposition directly to lake		
	Area (km^2)	Total-Hg (kg)	MeHg (kg)		Area (km^2)	Total-Hg (kg)	MeHg (kg)
Little Tallahatchie	4328	33	0.86	Sardis	131.5	0.99	0.031
Yocona	1942	15	0.39	Enid	85	0.66	0.028

4.5 CONCLUSIONS

Concentrations of total-Hg and MeHg were measured in rain collected for three separate rainfall events in Oxford, MS. Wet deposition both directly and indirectly (through runoff, which has elevated Hg levels; see next chapter) is a significant source of Hg to Enid and Sardis lakes. An estimated 33 kg and 15 kg of Hg are deposited annually via rainfall to the Little Tallahatchie (Sardis) and Yocona River (Enid) watersheds, respectively and 0.99 and 0.66 deposited directly to the Lakes, respectively. The vast majority of the Hg in the rainwater was inorganic Hg^{+2}; MeHg averaged <3% of the total-Hg.

Acknowledgements

The MeHg instrument was obtained by a grant from the US EPA (#CD-95450510-0). Special thanks to Ife Olayemi for help with measurements of mercury in the rainfall.

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CHAPTER FIVE

5. MERCURY SPECIATION AND TRANSPORT IN RUNOFF FROM URBAN, AGRICULTURAL, AND FOREST/WETLAND AREAS IN THE YOCONA RIVER WATERSHED DURING RAIN STORM EVENTS

5.1 ABSTRACT

To investigate the sources and transport of mercury (Hg) to an impaired waterbody it is necessary to understand the dynamics of Hg species in the watershed. Mercury is mobilized in runoff and contributes to the load already present in the waterbody. Watersheds with diverse land use areas and periods of dry weather followed by storm events add complexity to understanding the sources of Hg in headwaters. In the present study, samples of runoff were collected from urban, agriculture, and forest/wetland areas in the Yocona River watershed; the Yocona River feeds into Enid Lake, and both waterbodies have fish consumption advisories due to elevated levels of Hg. The Yocona River was also sampled during storm events to observe temporal changes in mercury species as the river flow changed. Samples were analyzed for total-Hg (T-Hg), methyl-Hg (MeHg), and, for select samples, total suspended solids (TSS), total organic carbon (TOC), dissolved organic carbon (DOC), and particle bound mercury (PBM).

As the river rose to near peak levels during the storm, concentrations of T-Hg increased for both filtered (<0.45 μm) and unfiltered fractions (from about 3 ng/L to as high as 24 ng/L). In a subsequent rain event, the following day, concentrations were lower (3 ng/L filtered; 8 ng/L unfiltered), possibly because much of the readily mobilized Hg was flushed from the system the prior day. MeHg concentrations were also elevated during higher flows but decreased as the water returned to baseline flow. The fraction of MeHg in the unfiltered water decreased during the storm event, not surprisingly suggesting that Hg^{+2} species are more readily accumulated and washed from the landscape. Mercury concentrations were moderately correlated with DOC and TOC, presumably due to its affinity with organic matter.

Because the soils in the area are highly erodible and can be mobilized in runoff, soils were assayed for Hg levels. Forest soil and wetland sediment contained higher concentrations (63.4 ± 3.6 ng/g and 38.9 ± 1.8 ng/g, respectively) than agriculture soils (18.9 ± 1.9 ng/g). Loss-on-ignition (an estimate of organic matter) correlated with Hg levels in sample from forest (16.0% ± 1.2) followed by wetland (13.3% ± 3.6) and agriculture (11.9% ± 1.5).

For the runoff, concentrations and speciation varied between categories. Average Hg concentrations were highest in the urban runoff (~26 ng/L), followed by forest/wetland (~18 ng/L), and agriculture (~15 ng/L). Once again forest/wetland had the greatest DOC levels. The proportion of Hg associated with particles also varied, with over 60% for agriculture, about 50% for urban runoff, and 20% for forest/wetland. Considering that ~40% of the land in the watershed is agriculture and that agricultural fields have a greater potential for erosion, agricultural runoff likely contributes the most Hg to Enid Lake, followed by forest/wetland, and urban areas.

5.2 INTRODUCTION

Mercury (Hg) is a global health concern due to its toxicity, bioconcentration up the aquatic food chain, and global dispersion through atmospheric pathways (US EPA 2005). MeHg is a water-soluble neurotoxin and readily assimilated into phytoplankton (often as the uncharged chloride complex) at the base of the aquatic food chain. Because it is only slowly eliminated from organisms, it can accumulate in fish tissue and reach high levels (>1 μg/g) in predators, especially piscivores. Thus, concentrations in fish generally increase with fish size (age), and consumption of contaminated fish can lead to toxicity in humans and wildlife (Hong Seok et al. 2013; US FDA 2013, Aschner et al. 2007).

In the state of Mississippi, Hg has emerged as a serious public health concern. A fish consumption advisory was issued for the Enid Reservoir in May 1995 and Yocona River in September 1996 by the MS Department of Health due to high levels of Hg in fish tissue sampled in the waters. The origin of Hg in the Enid Reservoir is unclear but may include atmospheric deposition, geological formations that leach Hg into the watershed, and historic land use practices.

During a rainstorm event Hg is deposited into waterbodies directly through wet deposition and indirectly through runoff. Runoff is commonly categorized by the land use, with urban, agriculture and forest/wetland areas dominating most watersheds. During dry periods Hg accumulates on surfaces naturally through dry deposition and by other means. For example, runoff from impervious pavement in urban areas entrains a variety of pollutants, including Hg, that have been deposited on the pavement from vehicle emissions, oil drippings, and other sources; runoff from agricultural fields may contain pesticides and fertilizers; and runoff from forest and wetlands may contain relatively high levels of dissolved organic matter (DOC) in the form of humic and fulvic acids that can complex metals, including Hg, and transport them to streams and lakes.

Measuring Hg species in runoff, streams, and rivers during rain events provides a means to evaluate the sources and estimate the load of Hg species entering an impaired lake. The approach has been used, for example, to evaluate the impact of agriculture and urbanization (Lyons et al. 2006), determine fluxes of Hg species into and out of Lake Champlin (Stanley and Chalmers 2012), determine critical factors in implementing river clean-up projects (Ismail and Salim 2013), and examine Hg species transport through an urban watershed (Mason and Sullivan 1998). In each of these studies, Hg speciation and concentration varied with land cover. Lyons et al. (2006) reported higher concentrations of dissolved Hg (2.6 ng/L) in urbanized regions compared to an agriculture region (0.52 ng/L) in Scioto River Valley in Ohio, USA. Zuo et al. (2013) reported Hg in urban runoff to be primarily associated/bound with particles (particle bound mercury (PBM)). Runoff from urbanized areas has been reported high and other studies attribute the higher concentrations to Hg^{2+} wash from impervious surfaces during rain events (Brinkmann and Rasmussen 2012; Mason and Sullivan 1998). Brinkman and Rasmusseen studied the fate of Hg in agricultural fields; they found that irrigation and subsequent drainage as the primary source of Hg to nearby rivers. Mercury in agriculture runoff is also believed to be primarily in the particulate form (Caron et al. 2008). In contrast, wetland and forest runoff contributes a greater percentage of Hg in the dissolved form, likely due to higher levels of DOC (Hurley et al 1998).

In this study, the concentration of total-Hg (T-Hg) and methyl-Hg (MeHg) was determined in runoff (both filtered and unfiltered) from urban, agricultural, and forest/wetland areas in the Yocona River Watershed located in Lafayette County in north Mississippi, USA.

The purpose was to investigate the sources and dynamic of Hg in a watershed that contains two waterbodies (Enid Lake and the Yocona River) which have fish consumption advisories, and determine if any particular area serves as a MeHg "hotspot" in the watershed. Grab samples from each land use category were collected and composited. The Yocona River was also sampled during the storm events. Particle bound mercury (PBM) was also determined. Sediment samples were collected to determine the total-Hg and %LOI in the land areas. Precipitation was collected and measured but is discussed in a separate chapter (Brown, Chapter 4). Ancillary measurements included total suspended solids (TSS), total organic carbon (TOC), dissolved organic carbon (DOC). The data allowed calculation of the partition coefficient (Log K_d).

Our hypotheses were that: 1) forest/wetland runoff would contain the highest concentrations and percentages of MeHg due to conditions that favor methylation (e.g., anoxic sediments and sulfate reducing bacteria) 2) urban runoff from parking lots would contain relatively high levels of inorganic mercury (Hg^{2+}), particularly in the earlier stages of the rain event, but that levels will decrease as the impervious surface becomes washed; and 3) Hg in agricultural runoff will be primarily present as particle bound mercury.

5.3 METHODS

5.3.1 Study Area

The study area is located in the Yocona River Watershed in Lafayette County in northern Mississippi (Table 13 and Figure 25). The Yocona River watershed is 1943 km^2, consisting of 39% agriculture, 38% forest, 17% wetland, and 2% urban (MDEQ 2002). The Yocona River was sampled opportunistically during a heavy storm event at Highway 7 near Oxford, Mississippi.

Table 13 Sampling locations for runoff and the Yocona River

Type	Latitude (N)	Longitude (W)
Agriculture runoff (1)	34°16'09.17"	89°31'18.29"
Agriculture runoff (2)	34°21'07.17"	89°38'56.84"
Urban runoff (1)	34°21'41.03"	89°33'53.48"
Urban runoff (2)	34°21'45.38"	89°31'54.86"
Wetland/Forest (1)	34°21'23.59"	89°33'08.89"
Wetland/Forest (2)	34°22'19.25"	89°36'07.73"
Yocona River	34°16'23.65"	89°31'17.64"

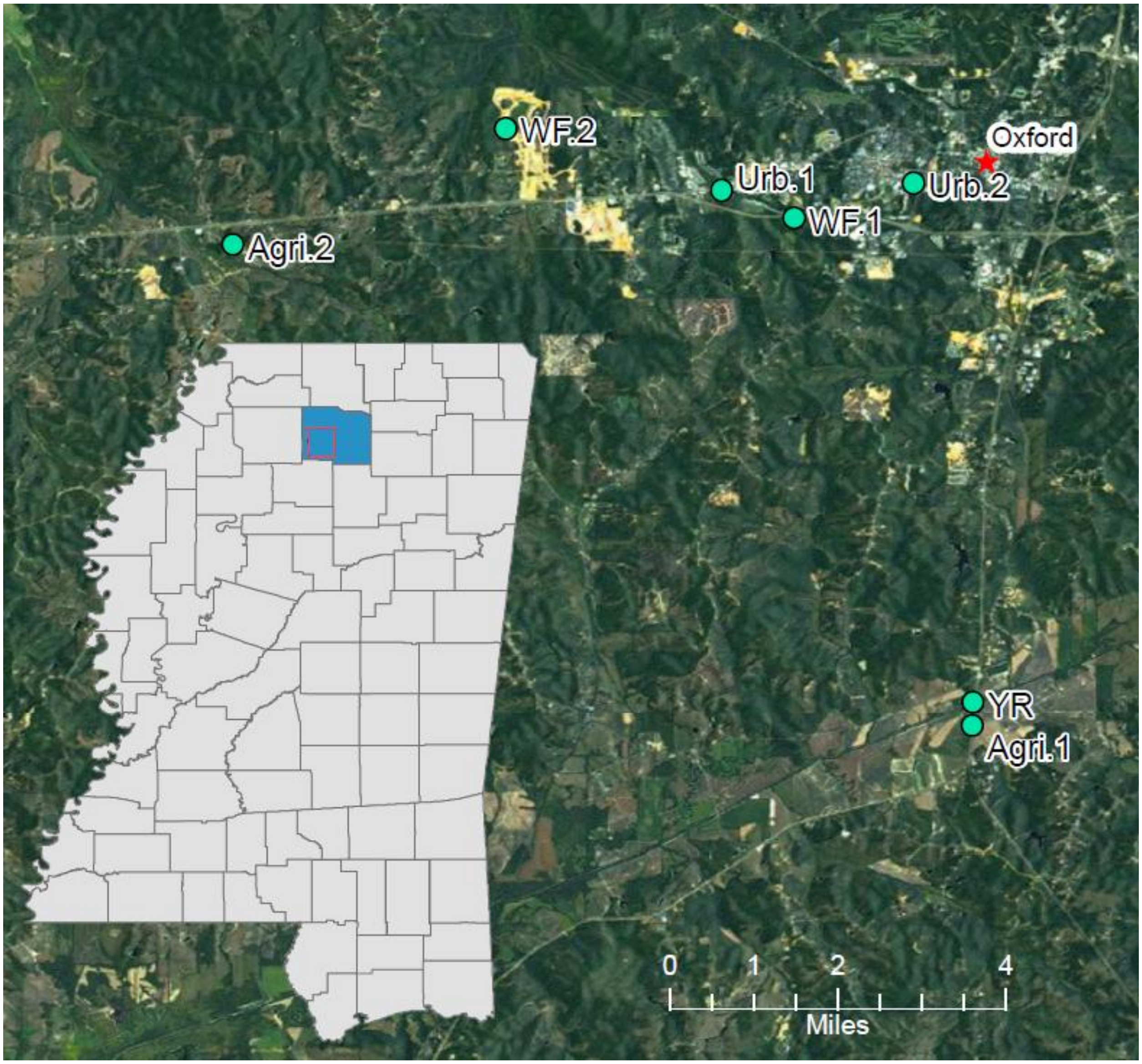


Figure 25 Map of study area showing location of Lafayette County (blue) in Mississippi and aerial view (right). Areas sampled include Wetland/Forest (WF), Agriculture (Agri), Urban (Urb), and the Yocona River (YR).

5.3.2 Sample Collection

Yocona River. The Yocona River was sampled on August 16 and 17, 2012 prior to a rainstorm event, at the beginning of the event, and at two points thereafter, and during rain events on December 5 and 28, 2012 (Fig. 26). The August sampling occurred after an extended dry period. The prior month had only 3 days with any significant precipitation (>2 cm); the rest of the days were either rain free or had trace levels. A USGS hydrologic (sonar) stream gauge was use to determine the water level (and discharge flow) of the river during each sampling event (Fig. 26). Streams and rivers are generally considered relatively well mixed during high flows (Colman and Breault 2000).

Runoff. Composite samples of runoff from wetland/forest, agriculture, and urban land areas were collected during a storm event on December 5, 2012 and from wetland/forest and agricultural areas during a storm event on December 28, 2012. Two different sites within each land category were collected (~ 1 L per site) and composited into acid washed 2 liter polycarbonate bottles (Nalgene®). Precipitation totals on these days were 0.97 cm and 3.15 cm, respectively. Once collected, samples were transported to the lab and filtered using a pre-heated (500°C, 4 hours) and weighed 0.45-micron quartz fiber filter. Both filtered and unfiltered samples were preserved to 0.4% acidity using 12N HCl. The samples were analyzed separately for total-Hg, MeHg, DOC, TOC, and TSS. Urban runoff was collected only on the December 5, 2012 sampling.

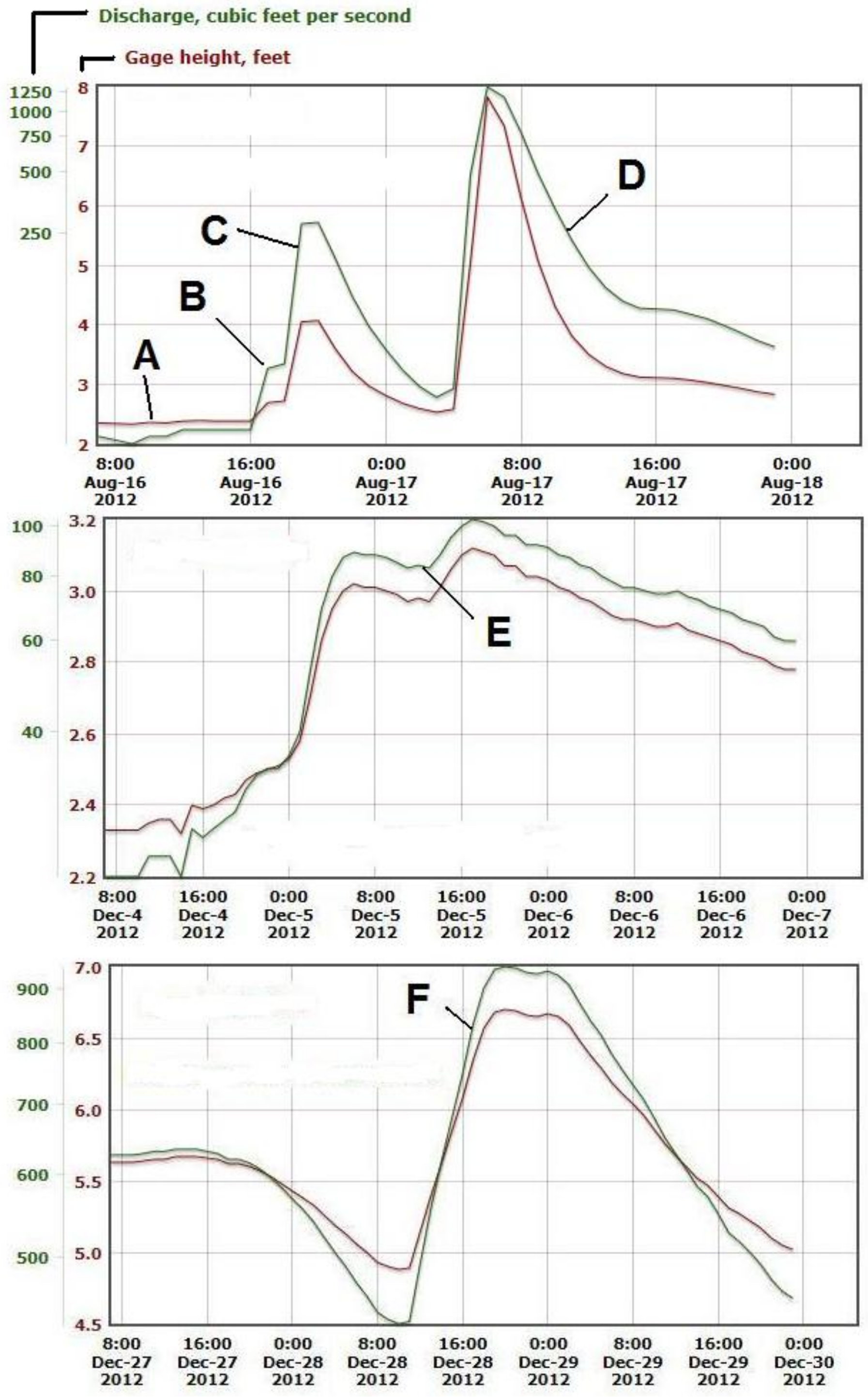


Figure 26 USGS stream gauge (#0727400) data showing the relative height and discharge of the Yocona River measured at Highway 7 near Oxford, MS. Samples were collected at the times indicated.

5.3.3 Total-Hg analysis

Total-Hg was determined by EPA Method 1631using a Tekran 2600 Cold Vapor Atomic Fluorescence Spectrometer (CVAFS) Mercury Analyzer (2600 Tekran; Tekran Inc. Toronto, Canada) for all samples (US EPA 2002). Briefly, all Hg in the sample is oxidized to Hg^{2+} with B 300 µL of BrCl. Prior to analysis, the sample is reduced with hydroxylamine hydrochloride ($NH_2OH{\cdot}HCl$) to destroy free halogens. Stannous chloride ($SnCl_2$) reduces Hg^{2+} to Hg^0. The Hg^0 is separated from the solution using a gas-liquid separator. The Hg is sequentially concentrated on two gold cartridges and is thermally desorbed from each gold trap into argon gas stream that carriers the Hg into the cell of a CVAFS for detection. A calibration curve was generated using a NIST-traceable Hg standard solution (Spex Certiprep; Metuchen, NJ, USA) diluted with 5% (v/v) HNO_3 prepared from deionized water obtained from a Milli-Q system (Millipore, Bedford, MA, USA). The curve generated a R^2 value of 0.99 and results were within 1% of the certified value. Samples for each land category was spiked using the Hg standard and results were between 85-106%. Each sample was run in triplicate analysis; the standard deviations for each sample were within ±1 S.D. with a relative standard deviation between 1-13%.

5.3.4 MeHg analysis

A Methyl Mercury Analyzer (2700 Tekran; Tekran Inc. Toronto, Canada) was used to determine MeHg in runoff samples. A modified version of EPA method of 1630 was used for analysis. The procedure is carried out without sample distillation and uses a propyl-Hg surrogate to quantify MeHg recovery (Tate 2010). A 30-mL sample aliquot was placed in an I-Chem® glass vial with a citrate buffer and ethylated in the vial by the addition of sodium tetraethyl borate ($NaBEt_4$); volatile mercury species are formed (methyl-ethyl-mercury for $MeHg^+$ and diethylmercury for Hg^{+2}). The ethylated forms are then separated from the solution by purging with argon onto a Tenax carbon trap. The system is designed for in-vial purging as a way for faster analysis and to eliminate memory issues occurring during analysis. After preconcentration the trapped species are thermally desorbed and carried into a gas chromatograph where the species are separated. The volatile species are then passed through a pyrolytic decomposition column, which converts organo-Hg forms to Hg^0, and further into the cell of a CVAFS for detection. Propyl-Hg was added to each sample as a surrogate to gauge losses during sample preparation using the Methylmercury Analyzer; recoveries ranged between 70-113%. A calibration curve was constructed using a methylmercury (II) chloride in H_2O (MeHgCl; Alfa Aesar®, Ward Hill, MA. USA) standard and R^2 values of 0.99 were achieved. The method detection limit for the analysis was 0.02 ng/L. Results for spiked samples using the MeHgCl standard were between 70-87%. Each sample was run in triplicate analysis; the standard deviations for each sample were within ±1 S.D. with a relative standard deviation between 3-11%.

5.3.5 Particle Bound-Hg and Total Suspended Solids analysis

Particle bound Hg was determined for total-Hg by combustion using Cold Vapor Atomic Absorption Spectrometer (CVAAS) (DMA-80; Milestone Inc., Shelton, CT, USA). After water samples were filtered through a pre-weighed 0.45µm quartz wool filter, the filters were dried under a laminar flow hood, placed in Ziploc bags, and stored at approximately -20 ºF in a deep freezer until analysis. Filters were heated at 300°C prior to filtering runoff samples to assure the filters was free from Hg. The analyzer is based on release of Hg upon thermal decomposition of samples, isolation of Hg by amalgamation with gold, and detection using atomic absorption spectrometry. The Hg concentration is based on the absorbance measured at 253.7 nm and the weight of the sample. Reference materials including MESS-3 (sediment) and Joaquin Soil were used to calibrate the instrument and as calibration check for every 10 samples; recoveries were between 98 to 102 % of the certified value. Blank filters were run every 10 samples to assure that Hg was not being carried over between samples. The amount of Hg for the blanks was negligible. The method detection limit for the analysis was estimated at 0.2 ng/g.

Total Suspended Solids was determined by passing approximately 160 ml to 500 ml of sample through a 0.45µm quartz wool filter that was combusted prior to filtering (Burford et al 2012). The filter was allowed to dry at room temperature under a laminar flow hood and reweighed to determine the TSS concentration using the following formula:

TSS (mg/L)= ((Residual + Filter (mg)) - Filter (mg)) / sample filtered (mL)) * 1000 (mg/L)

The formula used in determining TSS concentration was based on the U.S. Environmental Protection Agency method 160.2 (US EPA method 160.2).

5.3.6 Total Organic Carbon (TOC) and Dissolved Organic Carbon (DOC) analysis

Runoff samples were determined for TOC and DOC using a Shimadzu Total Organic Carbon 5000 (SSM-5000A; Shimadzu Scientific Instruments USA). Organic carbon was determined by filtering the runoff samples through 0.45µm quartz wool and acidifying the filtered and unfiltered water samples to 0.4% using concentrated HCl. The samples were then sparged using oxygen gas to remove the inorganic carbon. The inorganic free water sample was then injected onto a combustion column which is packed with platinum-coated alumina beads that is held at a temperature of 680°C. The non-purgeable organic carbon compounds were combusted and converted to CO_2, and subsequently detected by a non-dispersive infrared detector. The non-purgeable dissolved nitrogen in the sample was also combusted and converted to NO. The NO is mixed with ozone chemiluminesces for detection by a photomultiplier (Dickson et al. 2007). The Shimadzu 5000 was calibrated following US EPA method 415.3 (US EPA Method 415.3). Calibration for the instrument was prepared from an organic carbon primary dilution standard using a solution made from potassium hydrogen phthalate (KHP) in deionized water obtained from a Milli-Q system (Millipore, Bedford, MA, USA) with r2 values reported as 0.999. The RSD values for the sample replicates was reported as ≤ 6%.

5.3.7 Partition Coefficient (Log K_d)

The soluble form of mercury, Hg^{2+} forms complexes with organic matter in the water column. These complexes are important in the mobility of Hg^{2+} in water and soil (Lyons et al 2006). In the dissolved phase, Hg can also bind to colloids, matter microscopic in size that can pass through filters of 0.45 microns. The partition coefficient can estimate the binding affinity of Hg to colloids. Calculated as log of K_d, partition coefficient is a measure of the speciated particulate Hg concentration divided by the filtered concentration of Hg in the water at equilibrium expressed in units of L/Kg. (Mason et al 1998; Lyons et al 2006):

$$\text{Log } K_d = \frac{\text{Particulate Hg (ng/Kg)}}{\text{Dissolved Hg (ng/L)}}$$

The average value for the partition coefficient for suspended Hg divisible with dissolved Hg in pore water at equilibrium is reported as 5.3 L/Kg with a range between 4.2 and 6.9 L/Kg (Allison et al 2005). A decrease in log K_d, as TSS increases, can be explained by Hg bound to colloids passing through the micro filters. This process increases during high particulate load such as load occurring during a storm event (Mason et al 1998).

5.3.8 Sediment Analysis

Total-Hg in sediment was determined using a DMA-80 (Milestone Inc., Shelton, CT, USA) following US EPA Method 7473 (US EPA 2007). The analyzer is based on release of Hg upon thermal decomposition of samples, isolation of Hg by amalgamation with gold, and detection using atomic absorption spectrometry. Here, about 0.2 g of sediment was weighed to the nearest 0.1 mg in Ni boats. The Certified Reference material MESS-3 (sediment) was used to calibrate the instrument and as calibration check every 10 samples; results were within 20% of the certified values. Relative percent differences among samples run in triplicate were <10%. Organic matter was estimated by calculating the loss-on-ignition (LOI) using the DMA for combustion purposes, and was determined by weighing the boats before and after combustion. Whereas a factor can be used to convert organic matter to organic carbon, it depends on the type of organic matter present, which can vary from site to site (EPA 2002).

5.4 RESULTS

Table 14 shows the levels of total-Hg, MeHg, TSS, PBM, DOC, TOC, and the percent of Hg associated with particles in the Yocona River and headwaters (runoff) during rainstorm events in the Yocona River watershed. Table 15 gives a Pearson's correlation matrix for Hg species and water quality parameters.

5.4.1 Soils and sediments

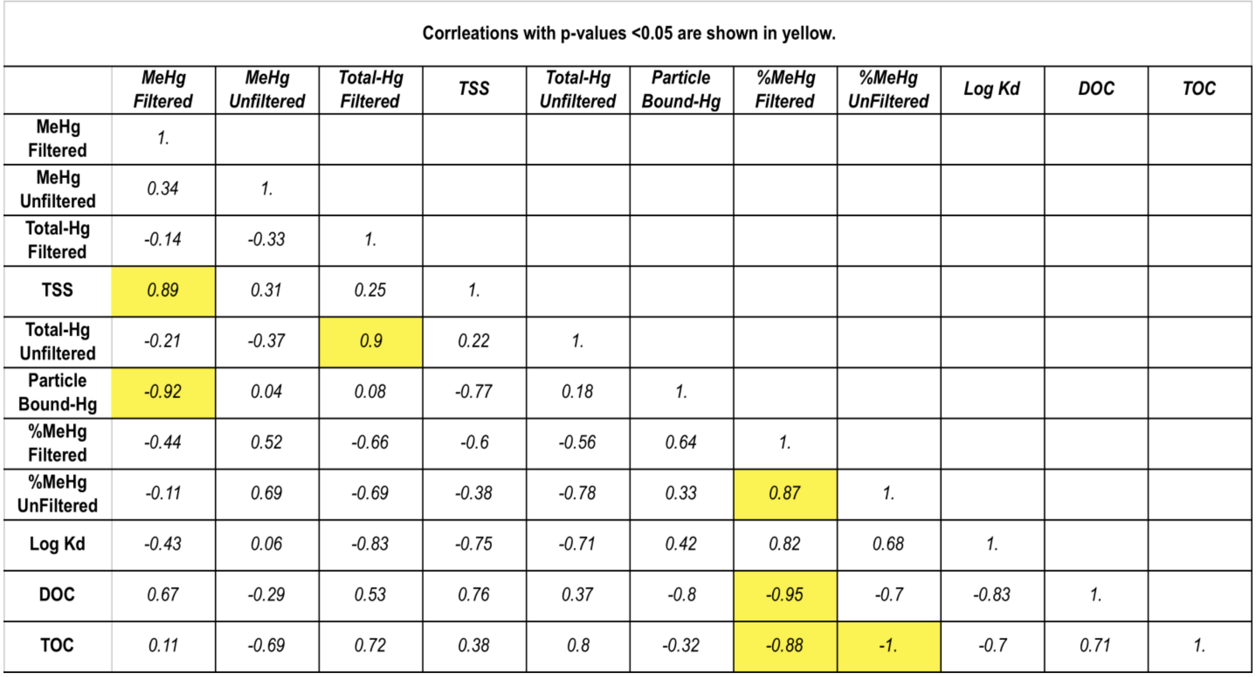
Because the soils in the area are highly erodible and can be mobilized in runoff, soils were determined for Hg. Forest soil and wetland sediment contained higher total-Hg concentrations (63.4 ± 3.6 ng/g and 38.9 ± 1.8 ng/g, respectively) than agriculture soils (18.9 ± 1.9 ng/g). Loss-on-ignition (an estimate of organic matter) correlated with Hg levels with forest (16.0% ± 1.2) followed by wetland (13.3% ± 3.6) and agriculture (11.9% ± 1.5)

Table 14 Concentrations of Total-Hg, MeHg, Total Suspended Solids (TSS), Particle Bound Mercury (PBM), Total Organic Carbon (TOC), and Dissloved Organic Carbon (DOC) in the Yocona River and headwaters during rainstorm events in the Yocona River watershed

Sample	Date	Description (see figure 2)		Gauge Height (ft)	Discharge (ft^3/sec)	Filtered (<0.45 μm)			Unfiltered			TSS (mg/L)	PBM (ng/g)	% of Hg associated with particles >0.45 μm	DOC (mg/L)	TOC (mg/L)	Log K_D
						Total-Hg (ng/L)	MeHg (ng/L)	MeHg (% of total)	Total-Hg (ng/L)	MeHg (ng/L)	MeHg (% of total)						
Yocona River	16-Aug-12	Base flow	A	2.7	25	2.6**	0.21 ± 0.01	6	2.6 ± 0.1	0.20 ± 0.02	15	7.5	NES	~0	13.0 ± 0.2	14.6*	-
		Start of Event	B	3.1	45	4.2**	0.38 ± 0.01	1.3	4.2*	0.63 ± 0.01	3.3	173	88.6	~0	14.2**	14.2 ± 0.4	4.1
		Peak of first event	C	4.2	279	21.6 ± 0.1	0.29 ± 0.04	11.3	24 ± 1	0.77 ± 0.03	6.6	159	217 ± 71	~10%	_	17.6 ± 0.4	4.0
	17-Aug-12	Decline of 2nd event	D	4.1	331	3.3 ± 0.4	0.37 ± 0.01	7.2	8 ± 1	0.51 ± 0.01	7.6	188	93 ± 34	~59%	15.8*	16.6*	4.4
	5-Dec-12	After rise	E	3.1	83	0.7 ± 0.2	0.15*	66.2	1.3 ± 0.3	0.17*	0.7	5.6	122.9	~60%	13.03*	14.58*	5.3
	28-Dec-12	Major event	F	6.6	820	12.7 ± 0.1	0.18*	1.4	22 ± 6	0.30 ± 0.01	1.3	208.5	88.3	~42%	--	--	3.8
Urban	5-Dec-12	Parking lots		NA		13.2 ± 0.6	0.14 ± 0.01	1.0	25.7 ± 0.3	0.12 ± 0.01	1.0	46.4	315	~49%	11.3**	11.3*	4.4
Forest / Wetland	5-Dec-12	Small stream exiting a forested area and originating from a wetland area		NA		12.4 ± 0.8	0.19 ± 0.01	1.5	15.2 ± 0.2	0.20 ± 0.02	1.5	62.4	172	~21%	15.13*	18.7 ± 0.7	4.1
	28-Dec-12					16 ± 1	0.09*	0.5	20 ± 1	0.13 ± 0.01	0.6	42	103.4	~20%	--	--	3.8
Agriculture	5-Dec-12	Runoff from cotton and soybean fields		NA		1.0 ± 0.1	0.67 ± 0.02	67.0	2.8 ± 0.4	0.74 ± 0.03	26.2	7	200 ± 36	~64%	7.0 ± 0.4	10.25*	5.3
	28-Dec-12					7.8 ± 0.1	0.11 ± 0.01	1.4	27 ± 5	0.16 ± 0.01	0.6	253.8	67 ± 9	~71%	--	--	3.9

NA = Not Applicable; NES = not enough solids; ± 1 standard deviation; *duplicate measurements (relative percent difference ≤ 15%); **used unfiltered or total values because filtered or dissolved value was above but within uncertainty of unfiltered or total value.

Table 15 Pearson's correlation matrix for water parameters measured in all Runoff collected in Land areas in the Yocona River Watershed.



Corrleations with p-values <0.05 are shown in yellow.

	MeHg Filtered	MeHg Unfiltered	Total-Hg Filtered	TSS	Total-Hg Unfiltered	Particle Bound-Hg	%MeHg Filtered	%MeHg UnFiltered	Log Kd	DOC	TOC
MeHg Filtered	1.										
MeHg Unfiltered	0.34	1.									
Total-Hg Filtered	-0.14	-0.33	1.								
TSS	0.89	0.31	0.25	1.							
Total-Hg Unfiltered	-0.21	-0.37	0.9	0.22	1.						
Particle Bound-Hg	-0.92	0.04	0.08	-0.77	0.18	1.					
%MeHg Filtered	-0.44	0.52	-0.66	-0.6	-0.56	0.64	1.				
%MeHg UnFiltered	-0.11	0.69	-0.69	-0.38	-0.78	0.33	0.87	1.			
Log Kd	-0.43	0.06	-0.83	-0.75	-0.71	0.42	0.82	0.68	1.		
DOC	0.67	-0.29	0.53	0.76	0.37	-0.8	-0.95	-0.7	-0.83	1.	
TOC	0.11	-0.69	0.72	0.38	0.8	-0.32	-0.88	-1.	-0.7	0.71	1.

5.4.1 Yocona River

Total-Hg concentrations for sampling in the Yocona River during the start of the storm event for filtered and unfiltered samples were ~2.6 ng/L and 4.2 ng/L, respectively. Concentrations for both filtered and unfiltered total-Hg samples increase to 21.6 ng/L and 24 ng/L, respectively, and decreased as the flow decreased to baseline levels (2.9 ng/L and 2.6 ng/L, respectively). The percentage of Hg associated with particles increased as the river flows increased. MeHg levels during the start of the storm event for filtered and unfiltered were 0.4 ng/L and 0.6 ng/L, respectively. MeHg levels also increased with river flow, and decreased as the water fell to base flow. TSS was high (and relatively constant) during the storm event compared to base flow levels. DOC and TOC increased slightly as the river levels increased and decreased as levels returned to base flow. An anomalously high data point for DOC (804.6

mg/L) determined for the first peak (C in figure 2) was statistically omitted; the value was much higher than the TOC and the vial may have been contaminated. The partition coefficient (K_d) was relatively constant and averaged 4.2 ± 0.2.

5.4.2 Runoff by land category

The concentration of total-Hg and MeHg varied between urban, agriculture, and forest/wetland land areas (Table 14). Total-Hg concentrations for both filtered and unfiltered samples were highest in urban runoff (13.2 ± 0.6 ng/L and 25.7 ± 0.3 ng/L, respectively) followed by forest/wetland (~15 ng/L and ~18 ng/L) and agriculture (~5 ng/L and ~15 ng/L). Concentrations of MeHg ranged from about 0.1 ng/L to about 0.8 ng/L, with no clear differences between runoff categories. PBM, on the other hand, was highest in agriculture, with about 70% of the Hg associated with particles, followed by urban runoff (~50%), and forest/wetland (~20%). Concentrations of Hg in the particulate matter itself were high, especially in the urban runoff (314.9 ng/g). TSS was highest in the agricultural runoff (~250 mg/L). Forest/wetland runoff tended to have higher DOC and TOC levels. The Log K_d (sorption coefficient) values were relatively uniform between runoff types averaging 4.3 ± 0.5; there may have been too few samples (precision) to tease out differences in K_d that may exist.

Grab samples (river edge sampling) were collected form the Yocona River during storm event. Total-Hg levels for samples from the December 28 event (river discharge ~820 ft^3/sec) were higher (12.7 ± 1 ng/L and 22.2 ± 6 ng/L, filtered and unfiltered, respectively) than the December 5 event (~85 ft^3/sec; 0.7 ± 0.02 ng/L and 1.3 ± 0.3 ng/L). DOC and TOC averaged 13.0 mg/L and 14.6 mg/L, respectively, for the December 5th event; they were not determined for December 28 sampling event.

5.5 DISCUSSION

5.5.1 Mercury in Soil and Sediment

Methylation in sediment, by sulfate reducing bacteria, can be facilitated by organic carbon, which can act as a substrate for sulfate reducing bacteria metabolism (Liu et al 2009). Sediment and soil samples were collected from agriculture, forest, and wetland areas to compare the amount of Hg and organic carbon present between land areas and to gauge the contribution sediment and soil will have in the process of erosion and runoff into the Yocona River. There was a strong correlation between total-Hg concentrations and LOI, which can be explained because Hg has a high affinity to organic matter (Fig. 27). Total-Hg has also been correlated with TOC in sediments (Trefry et al. 2006, Delaune, et al. 2008 Liu, et al. 2009). The strong affinity of Hg to organic matter is primarily associated with coordination at the reduced sulfur sites within the organic matter (Ravichandran, 2004).

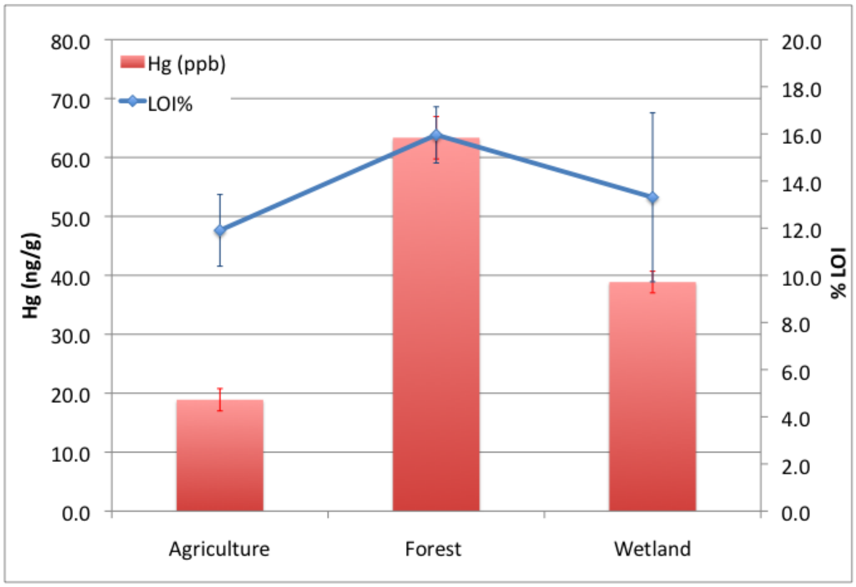


Figure 27 Total-Hg and percent loss-on-ignition (organic matter) of sediment and soil samples for agriculture, forest, and wetland collected in the Yocona River Watershed.

5.5.2 Levels of Mercury Species in the Yocona River during a Storm Event

The Yocona River was sampled periodically during a rain storm event and after the river returned to base flow. For the August 16-17 event, the water level had two peaks (Fig. 28). Total-Hg levels were at their greatest during the first peak of the storm event (designated as C), and decreased in concentration thereafter (Fig. 29). This may reflect a "washing out" effect, where the easily mobilized Hg "pulses" through the system with the initial high flows. DOC also seemed to follow this trend; unfortunately the data point at time C was anomalously high (much higher than the TOC) and was removed (Fig. 28). As discussed earlier, Hg has an affinity to organic matter and the organic matter load increases in the river as a result of runoff inputs to the river; thus it is plausible that the increased Hg load is at least in part due to it being complexed to the organic matter.

As the river rose the percentage of Hg associated with particulate matter increased from about 10% to about 60%. The relationship between total-Hg and PBM in river systems is important. Indeed, interactions of Hg with particulate matter have been suggested as the major factor driving the distribution in Hg in rivers (Mason and Sullivan 1998). Concentrations of total-Hg and PBM were highly correlated during the storm event (Fig. 29)

The partition coefficient is a function of binding affinity and is strongly affected by level of organic matter in the particles. Log K_d values for our study remained relatively constant throughout the storm event (averaging 4.3 ± 0.5); the values reflect the strong bonding Hg has with particulate matter, especially particulate matter containing organic matter. The range of K_d in our study is similar to that reported in rivers and estuaries collected during high particulate loads in water columns (Mason et al 1998; Lyons et al 1997; Hurley et al 1995; Benoit et al 1998). In the present study, there was a moderate correlation (r=-0.75) between TSS and Log K_D.

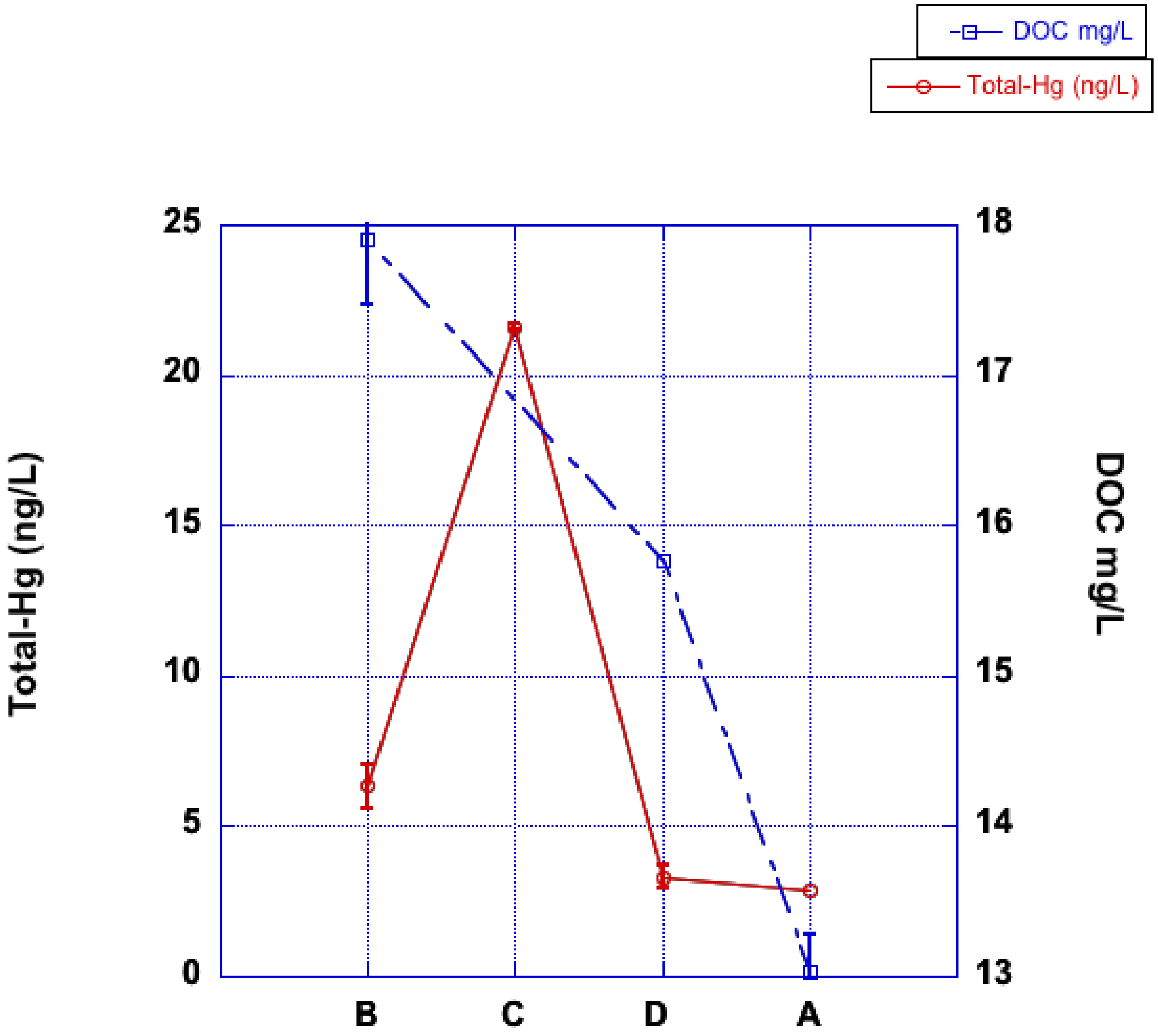


Figure 28 Total-Hg concentrations and dissolved organic carbon in the Yocona River during a storm event on August 16-18, 2012. B is the start of the storm event; C is near a peak in river flow; D is from a subsequent rain event the next day; A is at the base flow of the river.

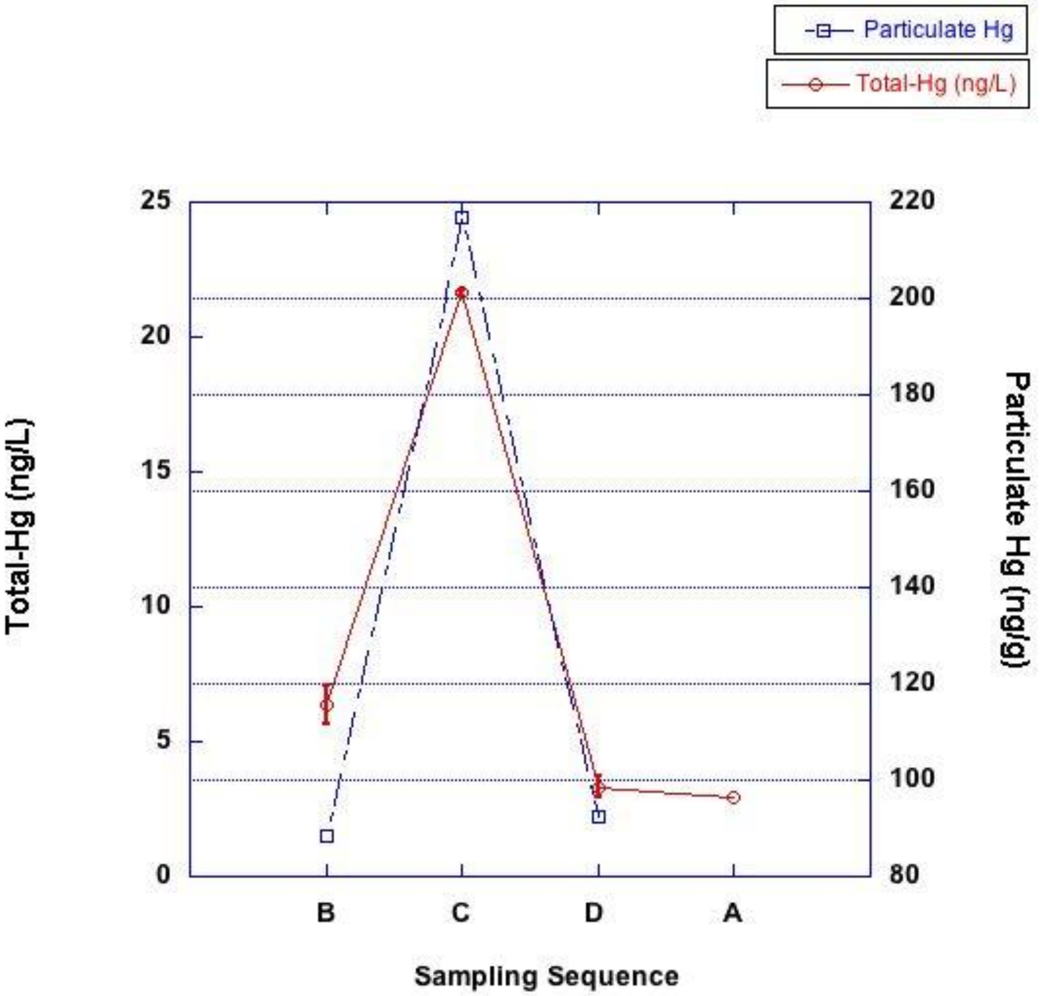


Figure 29 Dissolved total-Hg and Particulate-Hg determined in the Yocona River on August 16-18, 2012. B is the start of the storm event; C is near a peak in river flow; D is from a subsequent rain event the next day; A is at the base flow of the river.

MeHg concentrations did not follow a similar trend compared to total-Hg as the water level fluctuated. Levels for MeHg were highest at the start of the rain event, decreased during the first flow peak, increased during the second flow peak, and declined to base flow levels after the storm event. The initial high levels of MeHg, during the start of the rain event, may be due to MeHg from surrounding land areas becoming mobile and entering into the river. Levels of MeHg declined during base flow occur after washout as the system returns to steady-state. The second spike of MeHg may be due to newly induced release from areas in the watershed, such as overflows of wetlands/ponds that only occur during very intense rains with high flows. MeHg is considerably more mobile than inorganic-Hg (Mason and Sullivan 1998). The relationship for the MeHg and DOC for our data is presented in Figure 30.

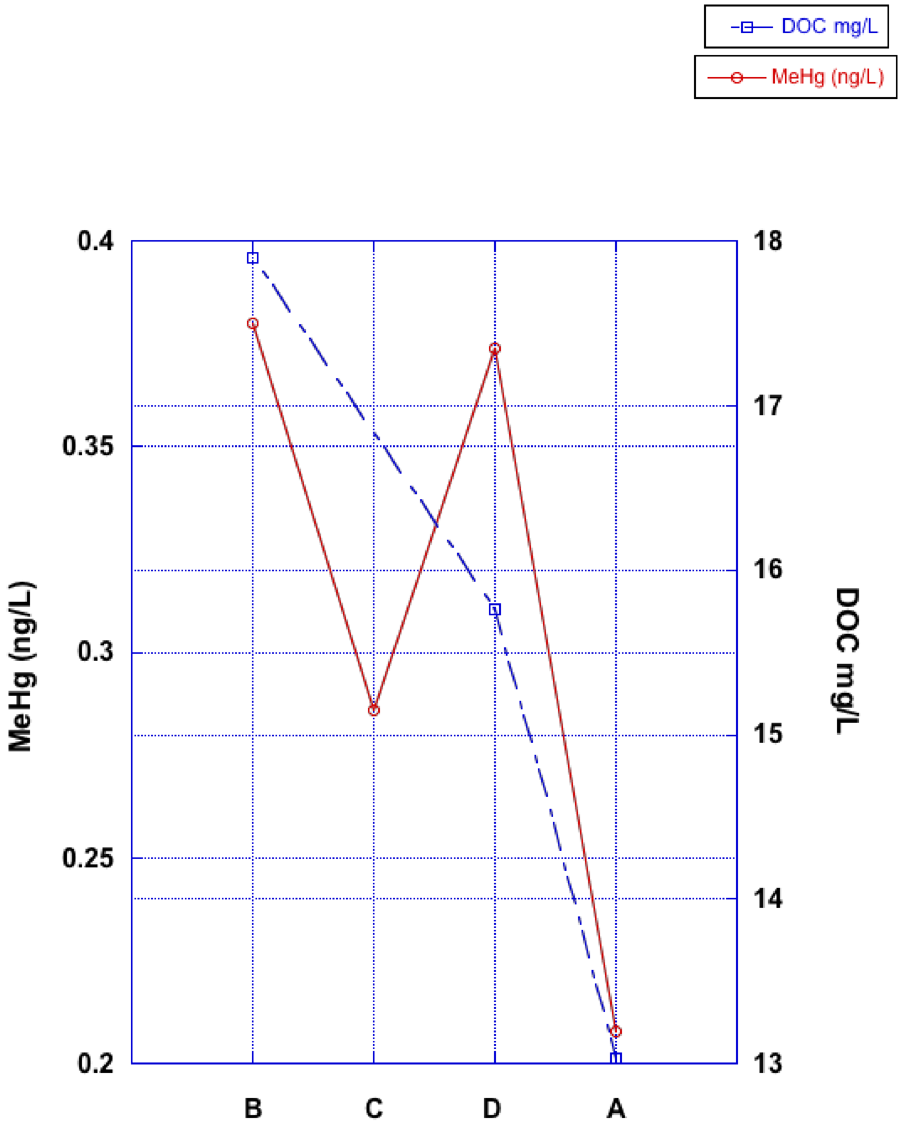


Figure 30 MeHg and dissolved organic carbon in the Yocona River on August 16-18, 2012. B is the start of the storm event; C is near a peak in river flow; D is from a subsequent rain event the next day; A is at the base flow of the river.

5.5.3 Urban Runoff

In an urban watershed high loads of Hg are transported during storm events (Mason and Sullivan 1998). In our study, urban runoff had higher concentrations of total-Hg in both filtered and unfiltered samples compared to forest/wetland and agriculture land categories. Runoff from urban parking lots may have higher levels of inorganic Hg, which has deposited on the impervious pavement via wet or dry deposition and from vehicles. This Hg may be readily transported with the runoff as the surface becomes washed, especially in the earlier stages of the rain event where there may be a high concentration of Hg deposited on the surface (Fulkerson et al. 2007). PBM-concentrations were also high in the urban runoff (Fig. 31). Sources of this particulate matter on urban surfaces are manifold but include atmospheric deposition and dust. The combination of particulate and Hg sources contributes to the high Hg load when the surface becomes washed during a storm event. Lyons et al. (1997) reported that in urbanized areas P-Hg was the dominant form of mercury.

Results for MeHg indicated urban runoff contained the least amount in both filtered and unfiltered samples compared to forest/wetland and agriculture land categories (Fig. 32). Low levels in urban runoff compared to other “natural” areas (wetland/forest/agriculture) is not surprising given that methylation is enhanced in environments that foster the growth of sulfate reducing, such as sediment and/or anoxic waters. MeHg typically complexes with inorganic metals, such as chlorides, to form mercuric salts in water systems (Hem, J. 1970). Therefore DOC is less likely to be a factor in MeHg mobility or contributing source in an urban area. In our study, MeHg was inversely correlated with DOC (r^2=-0.96; p=0.04). We found relatively high DOC levels in urban runoff compared to other land categories (Fig. 32). This was surprising but consistent with the literature (Gardner and Carey 2004).

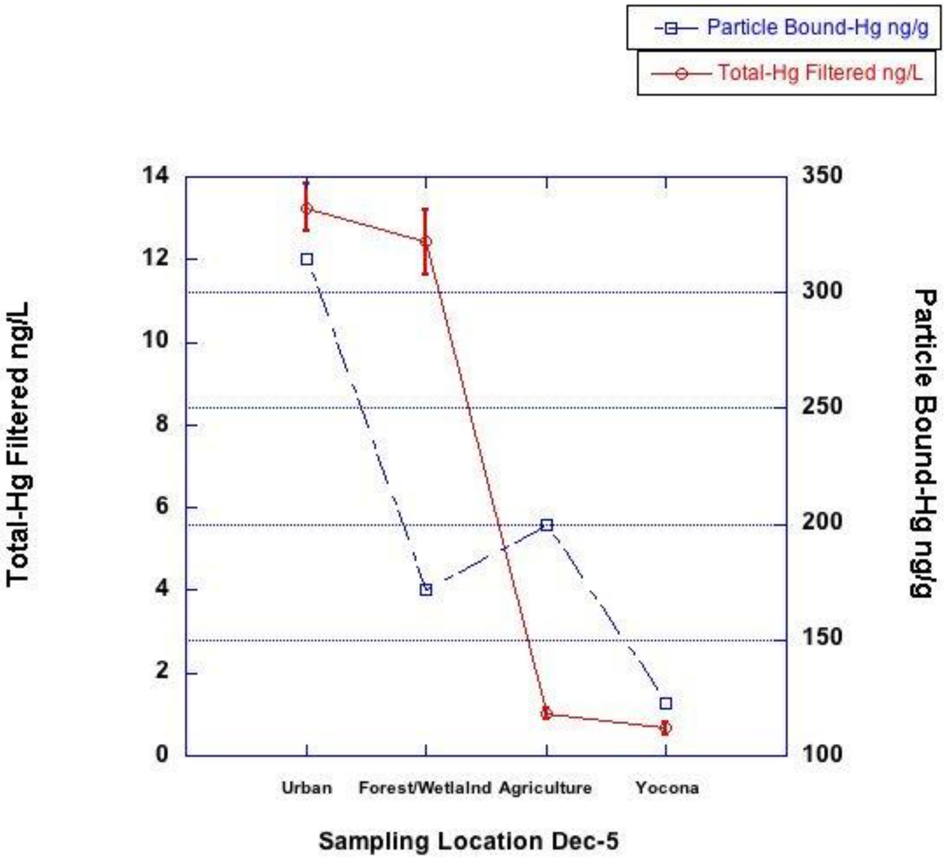


Figure 31 Dissolved Total-Hg and Particulate-Hg in runoff and the Yocona River collected on December 5 and 28, 2012.

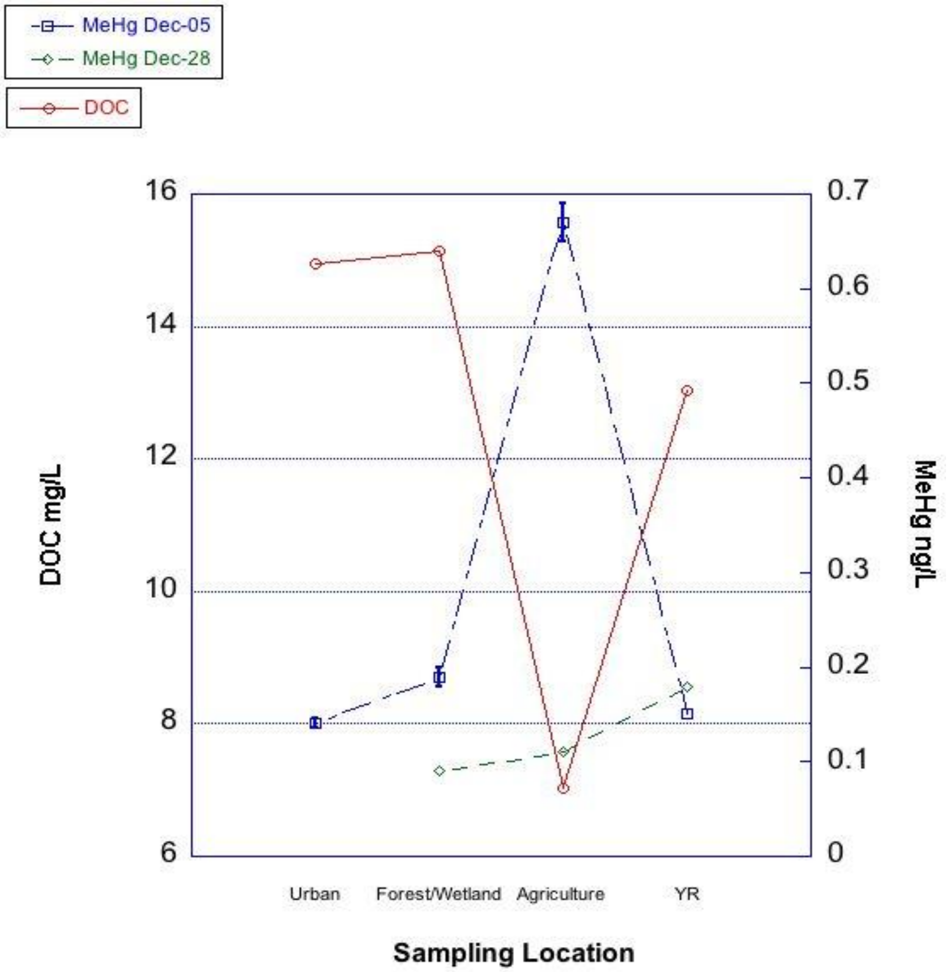


Figure 32 Dissolved organic carbon and dissolved MeHg in runoff and the Yocona River on December 5 and 28, 2012.

5.5.4 Agriculture Runoff

Agriculture is the dominant land area in the Yocona River watershed. It contributes 39% of the landmass and runoff from this land area contributes a significant proportion of the Hg load to the river. Brinkman and Rasmusseen (2012) showed that agriculture farming and irrigation play a role in the transport of silt carrying drainage water that carries a high load of Hg to surrounding water bodies. Balogh et al. (1998) reported that soil inputs, from the surrounding agriculture area dominate Hg loadings to the Minnesota River. Other studies have reported the dominant form of mercury species in agriculture as particle bound mercury (Hurley et al. 1995; Balogh et al. 2000; Caron et al. 2008).

In the present study, about 70% of the Hg in the agricultural runoff was associated with particles >0.45µm. Concentrations of Hg in the particulate matter for agricultural runoff was ~60 ng/g and ~200 ng/g for the two rain events, which is higher than the soil itself. This may because the soil was measured on a bulk basis, whereas the runoff likely contains a higher fraction of the fines which have higher Hg levels in part due to surface area. Concentrations of dissolved MeHg were highest in agriculture runoff for both sampling events, and the runoff also had a higher percentage of MeHg to total-Hg. The source of the MeHg is not clear and needs further study, but it is worth noting that MeHg, mercuric chloride and arsenic were all used as a pesticides and biocides in historical farming practices (UNEP, 2002; US EPA 1994).

5.5.5 Forest/Wetland Runoff Event Sampling

In a study of rivers in Michigan Rivers, it was shown that watersheds dominated by forest and wetlands contribute more dissolved mercury to the rivers (Hurley et al. 1998). In the present work, Forest/Wetland runoff had relatively high total-Hg concentrations and DOC levels (Fig. 33). TSS was also high and correlated with total-Hg levels (Fig. 34). These relationships have been seen in other studies (Shanley et al. 2012, Riscassi et al. 2011, Wall et al., 2005) and are due to Hg being complexed to suspended solids in the water column.

We hypothesized that MeHg levels would be higher in the forest/wetland category due to conditions which promote methylation, particularly in wetlands, such as anoxic sediment and the presence of sulfate reducing bacteria. Results, however, showed no significant difference with the other sampled land areas. Timing is important, and we may have missed the initial flush of the system; testing this hypothesis would require multiple samples to attain a better temporal picture of the changes from the individual runoff areas.

The Log K_d was inversely correlated ($p=0.03$) to TSS in the runoff collected on December 5, 2012 for all land areas (Fig. 35). DOC and MeHg had a negative correlation for runoff collected on December 5, 2012. This may be due to a competitive inhibition from an increased load of DOC, which could sequester MeHg from the water system and influence Hg bioavailability (Benoit, J. M., et al. 2003).

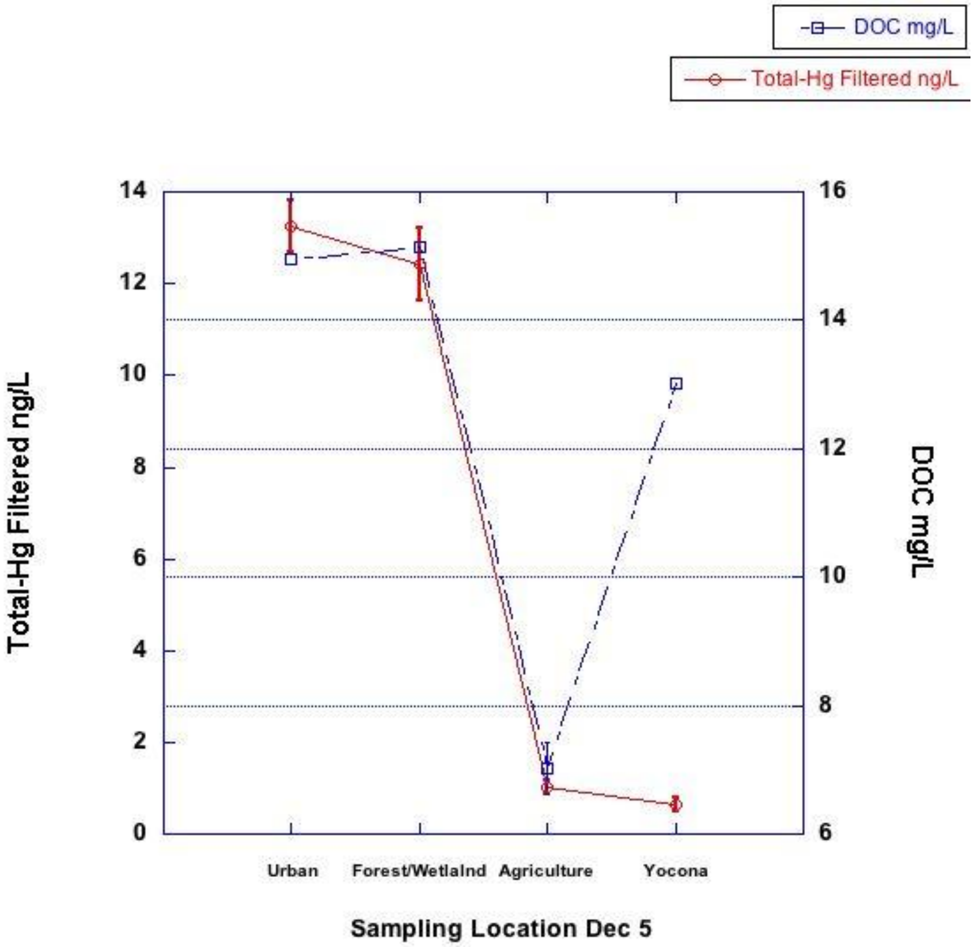


Figure 33 Dissolved organic carbon vs. Dissolved Total-Hg determined in runoff and the Yocona River on December 5, 2012.

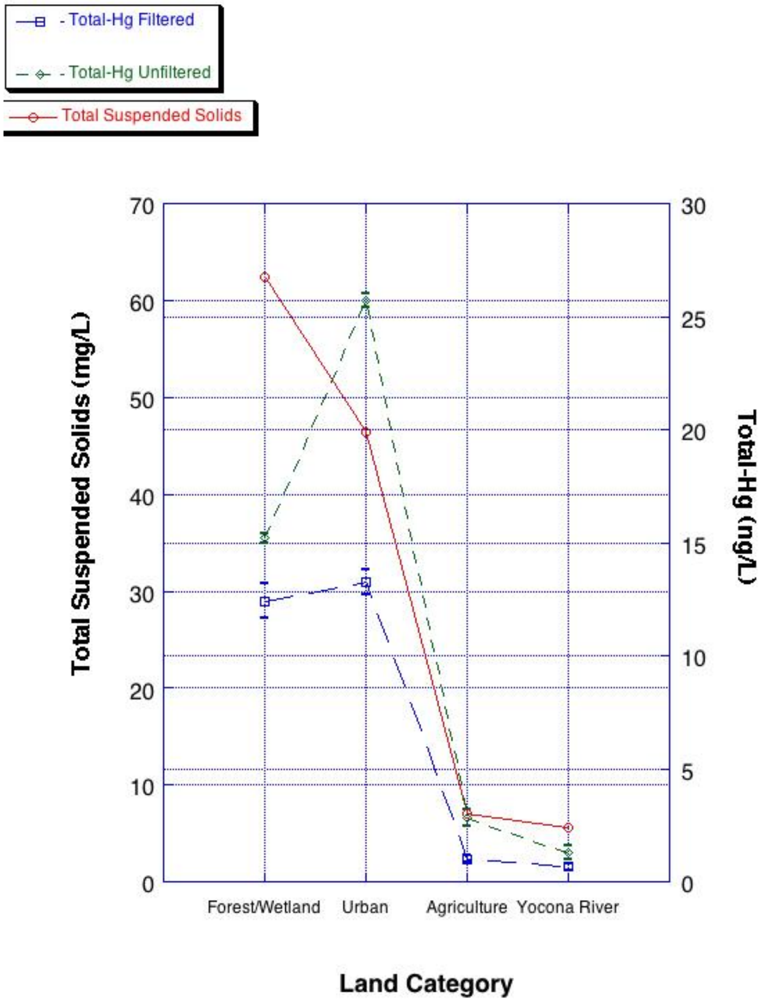


Figure 34 Total-Hg vs. Total Suspended Solids in runoff and the Yocona River collected on December 5, 2012.

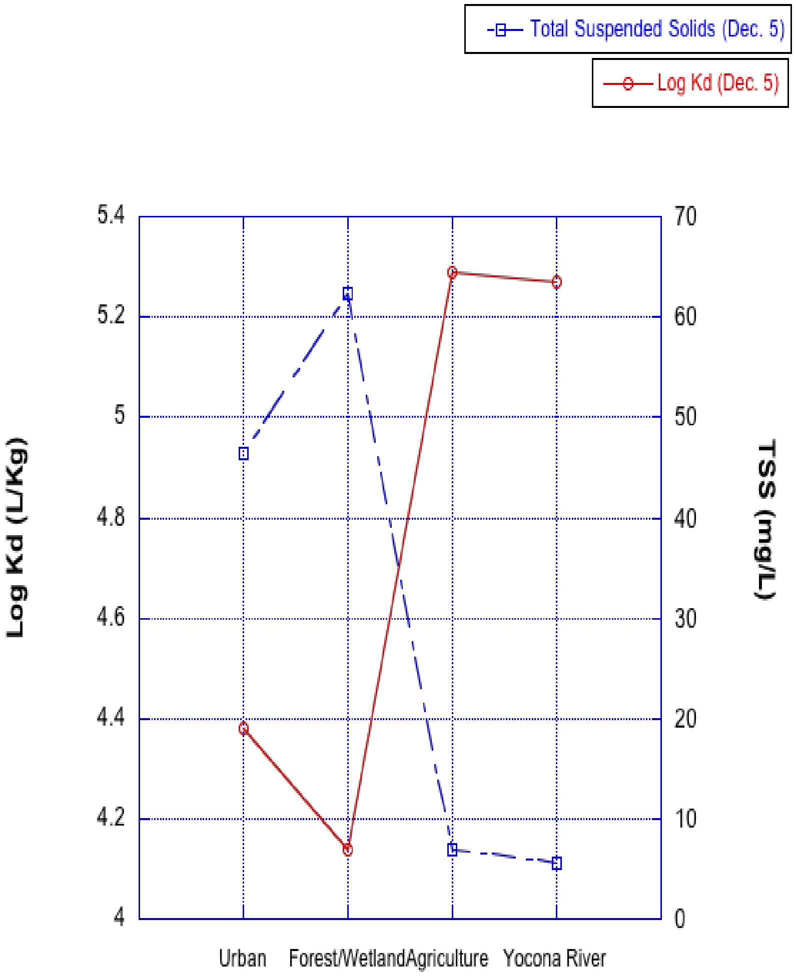


Figure 35. Partition coefficient vs. total suspended solids collected in composite runoff samples on December 5, 2012 in the Yocona River Watershed (Oxford, MS. USA)

5.6 CONCLUSIONS

A large proportion of the Hg entering Enid Lake through the Yocona River occurs during heavy storm events, when Hg is mobilized and carried by runoff in the watershed. Opportunistic sampling of the Yocona River during storm events show that Hg levels are generally greatest at peak flows and decline afterward. This is attributed to total-Hg and MeHg binding affinity with organic matter and suspended solids within the water. Urban runoff had the highest concentrations of total-Hg but given the low percentage of land that is paved in the watershed its contribution to the Yocona River and Enid Lake is relatively small. Agricultural runoff, on the other hand, likely contributes the majority of the Hg load. DOC was higher in forest/wetland runoff which may increase the transport and bioavailability of Hg. Particulates served as a major transport medium in the system. Overall, this study shows the complexity of Hg cycling in a watershed with multiple land categories and the importance of characterizing the inputs of Hg from each.

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CHAPTER SIX

6. SEASONAL TRENDS AND FLUXES OF MERCURY SPECIES IN ENID AND SARDIS LAKES, AND ASSOCIATED WETLANDS

6.1 ABSTRACT

Seasonal effects on the concentrations and fluxes of mercury (Hg) species were studied in Enid and Sardis Lakes, the former having a fish-consumption advisory for Hg, and in headwater wetlands within the Yocona River watershed. Water samples were collected from the inflows and outflows of the lakes during each season between 2011 and 2013. Water quality parameters including oxidizing reducing potential (ORP), dissolved organic carbon (DOC), pH, chloride, conductivity, and temperature were determined in-situ.

Total-Hg concentrations in the outflow of both Enid and Sardis Lakes were highest in the winter and lowest in the summer and fall. For example, levels in Enid Lake in the winter were 7.92 ± 0.16 ng/L (filtered) and 8.41 ± 1.17 ng/L (unfiltered), whereas the summer was 1.25 ± 0.07 ng/L (filtered) and 1.91 ± 0.21 ng/L (unfiltered). The increase of total-Hg in the winter likely stems from the increased load from suspended solids, known to bind Hg, during frequent precipitation events in the winter and spring; whereas the summer and fall tend to have longer periods between storms allowing the lake to settle.

In contrast, MeHg levels averaged around ~0.2 ng/L in the lakes and about double (~0.4 ng/L) in the wetlands, but spiked during the summer months, particularly for the wetlands where levels reached as high as 1.3 ng/L. During the summer the percent of Hg in the MeHg form was ~25% in the lake outflow and >50% in the wetland. This seasonal trend is due to higher methylation rates in the summer resulting from higher temperatures, microbial activity, and more anoxic conditions. MeHg in the wetlands was negatively correlated with ORP (r=-0.67; p=0.09)

and the percent of Hg in the methylated form increased with decreasing ORP; this correlation was weaker when data from the lakes were included (r=-0.29).

Mercury flux was estimated for Enid Lake using Hg concentrations in Yocona River (inflow) and in water collected below the dam (outflow), and discharge measurements based on USGS stream gauge and Army Corps of Engineer flow-release data. Data were collected during stable periods (no precipitation events in the prior few days) during each season, and during a summer storm event. In the winter, the net flux of total-Hg concentration was the most negative (~30 g/day being lost from the lake) primarily due to management of lake levels; there was a high discharge rate as the lake level is lowered to accommodate spring rains. In the summer, the net flux of MeHg was substantially more negative than fall and spring (with more exiting the lake than entering) despite having similar magnitude of water inflow and outflow. This is assumed to be from build-up of MeHg in the lake due to higher methylation and evaporation rates. During the storm event the flux of Hg in the lake became the most positive, suggesting that storm events contribute a significant portion of Hg to the lakes. Sources and losses of Hg not accounted for include direct wet and dry deposition to the water surface, volatilization of Hg to the atmosphere, and sequestration of Hg as sulfide complexes in sediments. Overall, the information obtained from this study provided insight into the factors controlling the seasonal distribution of mercury in the studied reservoirs. An estimated Hg mass balance of Enid Lake was calculated to determine annual fluxes to the river.

6.2 INTRODUCTION

Humans have detrimental health effects when exposed to methylmercury (MeHg). Biological areas affected include neurological pathways, kidney, liver and lungs (Yu et al. 2011). Humans, particularly infants, are susceptible to MeHg exposure because Hg and MeHg have the ability to cross the blood-brain and placental barriers (Aschner and Aschner 1990).

Identifying the fate and transport of Hg in waterbodies is critical to improving water quality and ecosystem health (Fulkerson and Nnadi 2006). Mercury levels in natural surface waters can vary by season. MeHg concentrations tend to increase in the summer months due to higher methylation rates resulting from higher temperatures, microbial activity, and more anoxic conditions (Pai et al. 1997). A number of factors, including pH, organic matter, and suspended solids, play a critical role in Hg speciation, mobility, and bioavailability (Pai et. Al. 1997).

A fish-consumption advisory was issued for the Enid Reservoir in 1995 and Yocona River in 1996 by the Mississippi Department of Health due to high levels of Hg in fish tissue. The purpose of this study was to 1) investigate temporal trends and seasonal affects for Hg species in Enid Lake, Sardis Lake, and wetland areas and 2) to use Hg concentrations and flows for the inflow and outflow of the Lakes to construct a preliminary Hg (mass balance) budget.

6.3 MATERIALS AND METHODS

6.3.1 Study Area

The study area is located in northern Mississippi and includes the Yocona River, which supplies Enid Lake, the Little Tallahatchie River, which supplies Sardis Lake (Fig. 36). In addition, samples were collected from wetlands near the University of Mississippi campus (Fig. 37). Enid and Sardis Reservoirs are man-made reservoirs built in the 1940's by the Army Corps of Engineers to provide flood control in the northern region of Mississippi Yazoo River Basin (see Chapter 3). The Yocona and Little Tallahatchie Rivers are inflows of Enid Lake and Sardis Lakes, respectively. Within this region, two wetland areas (also constructed in the 1940's) were targeted for intensive study. The wetlands are located in the Little Tallahatchie watershed and serve as a natural backwater storage area.

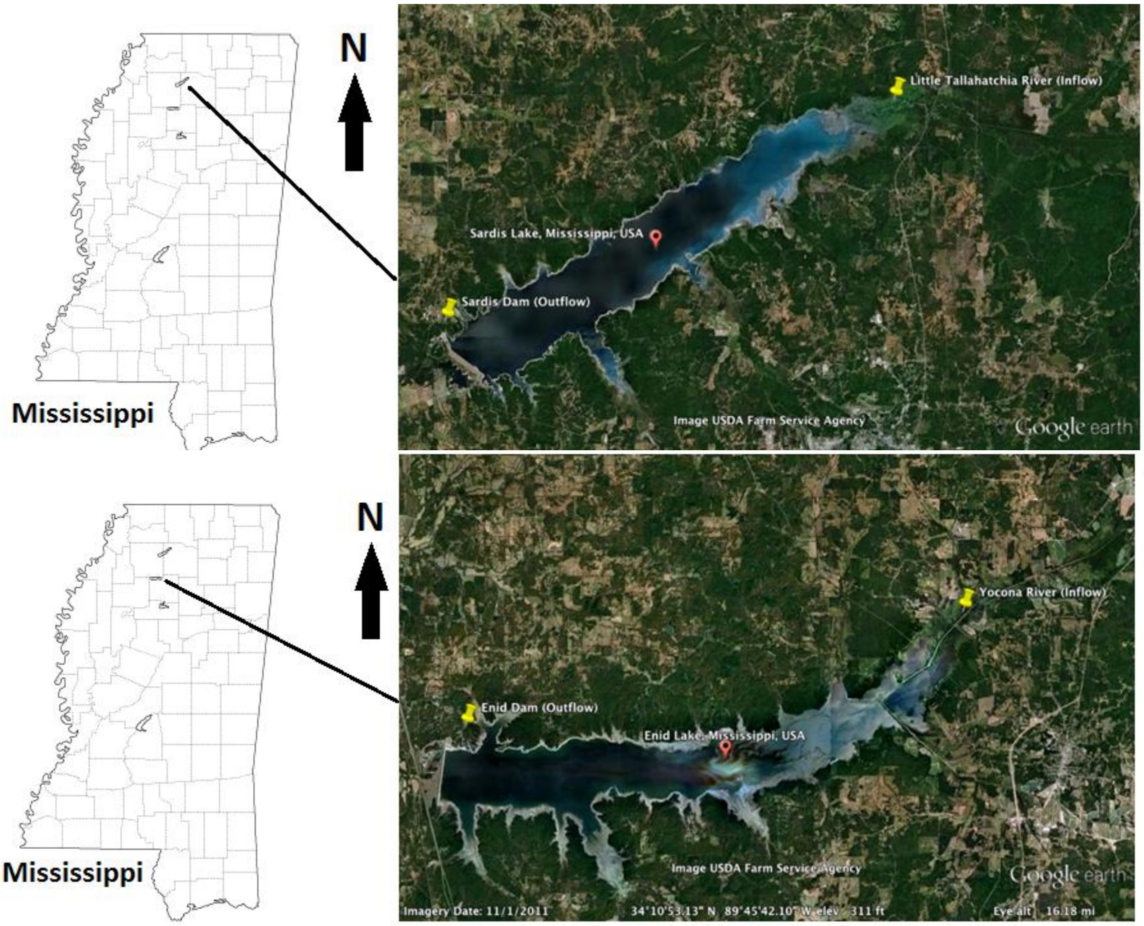


Figure 36 Sample locations for the inflows and outflows of Sardis (top) and Enid (bottom) lakes.

Figure 37. Map showing the location of the wetlands (left) and a photo of the wetland (right).

6.3.2 Sample Collection, Preservation, and Field Measurements

Water samples were collected on a seasonal basis over the course of nearly two years from the inflows and outflows of both Enid and Sardis Lakes, and from wetlands near the University of Mississippi campus (see study area). Samples were collected, preserved, and analyzed following standard US EPA procedures (US EPA Methods 1630 and 1631). Samples were collected from the shoreline by two individuals using the "clean-hands, dirty hands" technique (US EPA Method 1669) or using a 12 foot Grab Sampler collection device. Samples were placed directly into acid washed fluoropolymer bottles or amber glass borosilicate bottle specifically for MeHg analysis. The bottles were then doubly bagged in polyethylene and placed into a clean cooler containing ice. The samples were transported to the laboratory and stored in the fridge at about 4°C until processed. Water samples were filtered and preserved within 48 hours of collection, using a glass fiber filter with pore size of 0.45 µm, and preserved to 0.4% using 12 N HCl. Samples were in the refrigerator until analysis.

An YSI meter was used in the field to obtain a list of water quality parameters such as the pH, conductivity, oxidative reducing potential (ORP), temperature, and dissolved oxygen (DO). The meter was calibrated in the lab the same day prior to fieldwork.

6.3.3 Sample Analysis and Quality Assurance

Mercury species concentrations were determined in water samples using the Direct Mercury Analyzer (Particulate-Hg), Tekran® 2600 Mercury Analyzer (Total-Hg), and Tekran® 2700 Methyl Mercury Analyzer (MeHg) (Brown Chapter 1). Samples were general run in triplicate analysis and duplicate analysis (RPD < 20%). Certified Reference Materials (CRMs) used in this study include NIST-1640 and ORMS-4 (for total-Hg in natural water) and DORM-3 (fish muscle reference material). For MeHg determinations, a propyl-Hg surrogate was spiked into each sample for determining recoveries. Recoveries for all CRMs were between 70% to 120%.

6.3.4 Flux Calculations

A mercury budget for Enid Lake was determined by quantitatively examining the primary input to the lake (the Yocona River) and output (below the dam) of mercury species to determine the overall net flux. This is a first approximation because there are sources and transformations not accounted for (e.g., inputs from other streams, direct deposition to the water surface, leaching, losses through volatilization) and because these are snapshots in time. Nevertheless, the river flows and lake levels are expected to be the dominant factors which can lead to a better understanding of Hg transport and cycling in the system.

Water was collected from the inflow of Enid Lake in the Yocona River (near US Highway 7). The USGS hydrologic (sonar) stream gauge was used to determine water discharge flow at the sample collection site. Similarly, water was collected from the outflow of the lake below the dams. Outflow rates were obtained from the Army Corp of Engineers stream gauge. Together the data was used to estimate tributary mass discharges (loadings) from sample concentration data of Hg species and continuous flow records. The formula used to calculate net loadings of Hg species is based on the Mississippi Department of Environmental Quality Total Maximum Daily Load and was (MDEQ 2002):

$$\text{Load} = \text{Concentration} * \text{Flow}$$

Where: Load is measured in grams per day (g/day) and Flow was measured in cubic feet per second (cfs). A unit conversion factor of 2.45 was also used for the load calculations.

6.4 RESULTS AND DISCUSSION

This section focuses on seasonal trends for Enid Lake, Sardis Lake and wetlands, and on fluxes of Hg species in Enid Lake. The appendices contain additional data and information collected for the samples.

6.4.1 Seasonal Trends

Concentrations of total-Hg and MeHg in Enid and Sardis Lakes (as measured in the lake's outflow) varied by season (Figs. 38 and 39). For Enid Lake, total-Hg concentrations were highest during the winter, for both filtered (7.92 ± 0.16 ng/L) and unfiltered (8.41 ± 1.167 ng/L), and lowest in the summer, for both filtered (1.25 ng/L) and unfiltered (1.91 ± 0.21 ng/L). For Sardis Lake, total-Hg concentrations were also highest during the winter, for both filtered (5.18 ± 0.15 ng/L) and unfiltered (5.28 ± 0.50 ng/L), and lowest in the summer (0.73 ± 0.11 ng/L) for filtered, and fall (1.01 ± 0.23 ng/L) for unfiltered.

The higher levels of total-Hg in the water in the winter and spring likely stems from an increased load of suspended solids, known to bind Hg, during the relatively frequent precipitation events in the winter and spring; whereas the summer and fall tend to have longer periods between storms allowing the lake to settle more (Fig. 40). Another possible factor contributing to the lower levels in the summer months is increased losses to the atmosphere due to greater photo-reduction of Hg species to the more volatile and less soluble Hg^0 form as solar fluxes increase. Jong-Sung et al. (2008) also found total-Hg concentrations in water to be higher in the winter (3.3 ± 0.1 ng/L) compared to the summer (2.2 ± 0.4 ng/L) for the Juam Reservoir in Korea. They attributed the differences to be related to elevated levels of dissolved gaseous mercury (DGM) and associated volatilization in the summer.

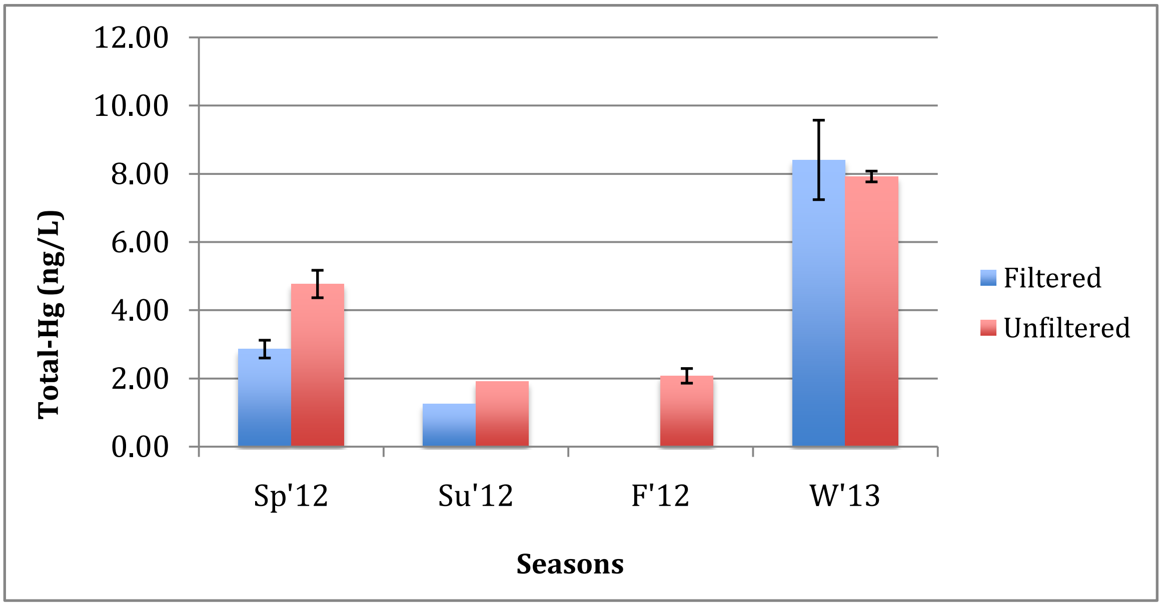


Figure 38. Total-Hg (ng/L) concentrations by season for filtered and unfiltered samples collected in the outlet from Enid Lake.

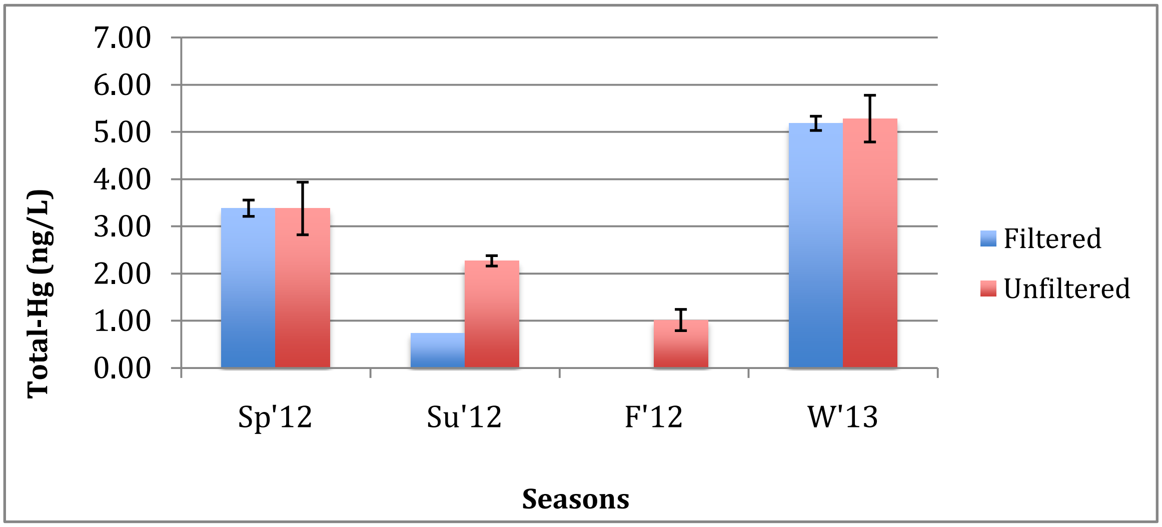


Figure 39 Total-Hg (ng/L) concentrations by season for filtered and unfiltered samples collected in the outlet from Sardis Lake.

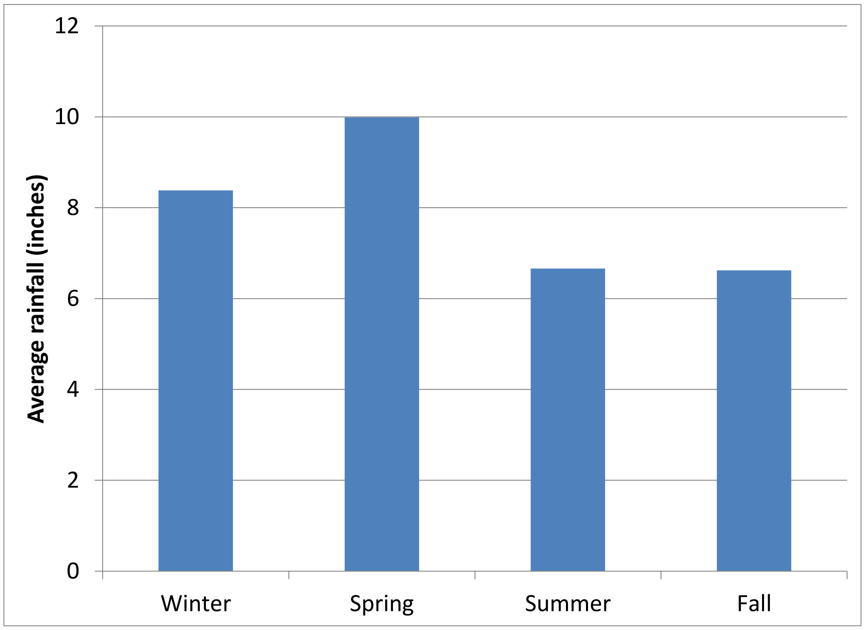


Figure 40 Average rainfall in Oxford, Mississippi by season from 2008-2013.

MeHg concentrations varied by season and location but were highest in the summer for Enid Lake and Pond 2, where unfiltered concentrations were as high as 0.7 ng/L and 1.3 ng/L, respectively. For Enid Lake, MeHg concentrations were 0.74 ng/L (filtered) and 0.79 ng/L (unfiltered) (Fig. 41). For Sardis Lake, MeHg concentrations were somewhat lower, but also peaked in the spring and summer, with summer levels being slightly higher (0.18 ± 0.01 ng/L, filtered) and (0.21 ng/L, unfiltered). The percent of Hg in the methylated form was higher during the summer in both Enid (60% for filtered and 41% for unfiltered) and Sardis Lakes (25.3% for filtered and 9% for unfiltered). The percentages of MeHg of total-Hg in the wetlands were also highest in the summer, with >70% of the Hg being in the methylated form (Fig. 42). It is well known methylation rates increase in the summer months due to higher temperatures, increasing anoxic conditions, and greater microbial activity (Gilmour et al 1992; King et al 2002; Muhaya et al. 1997). Others have shown a net flux of MeHg from the sediments to the water column (Sellers et al 1997).

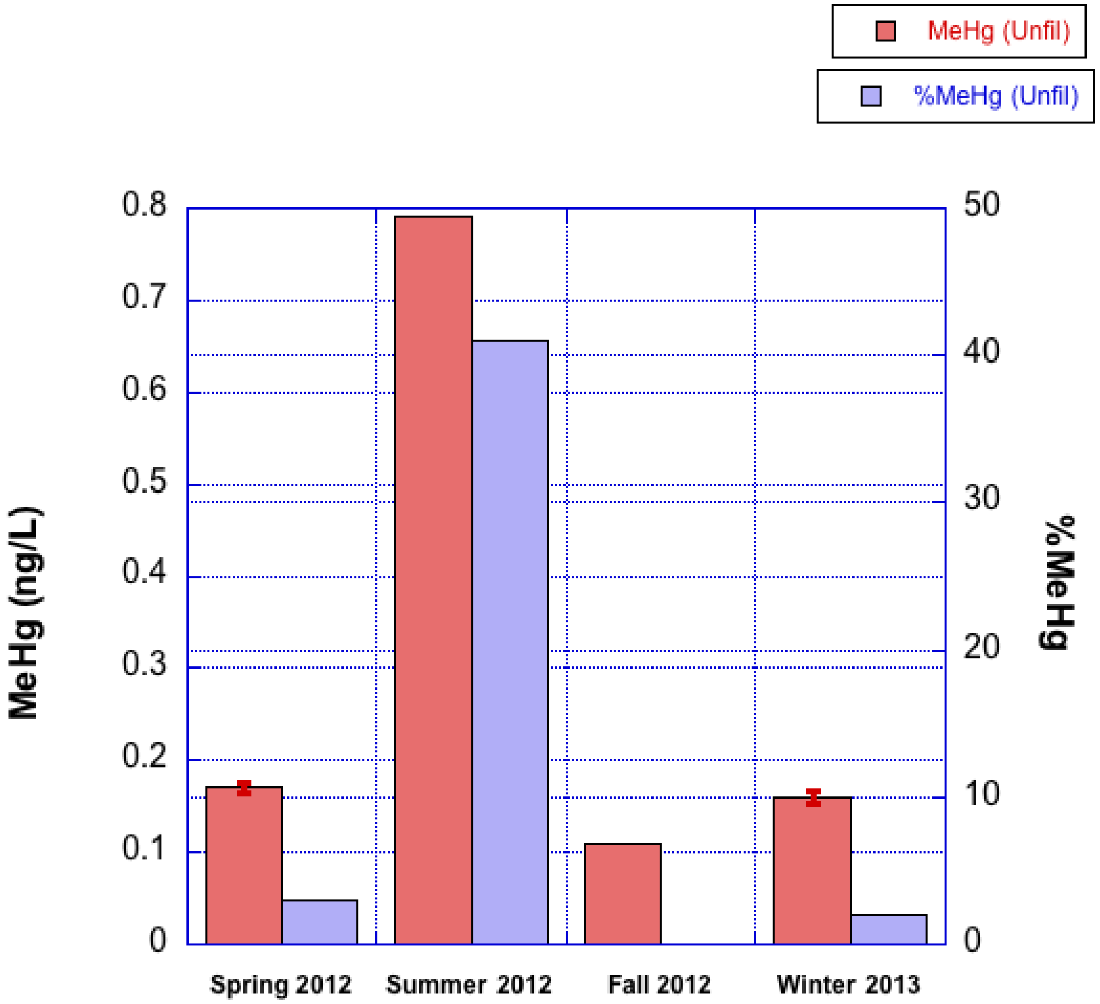


Figure 41 MeHg concentrations and percentage of Hg in the MeHg form in water from the outlet of Enid Lake. Percent MeHg is not shown for fall 2012 because total-Hg was below the instrumental detection limit (0.2 ng/L).

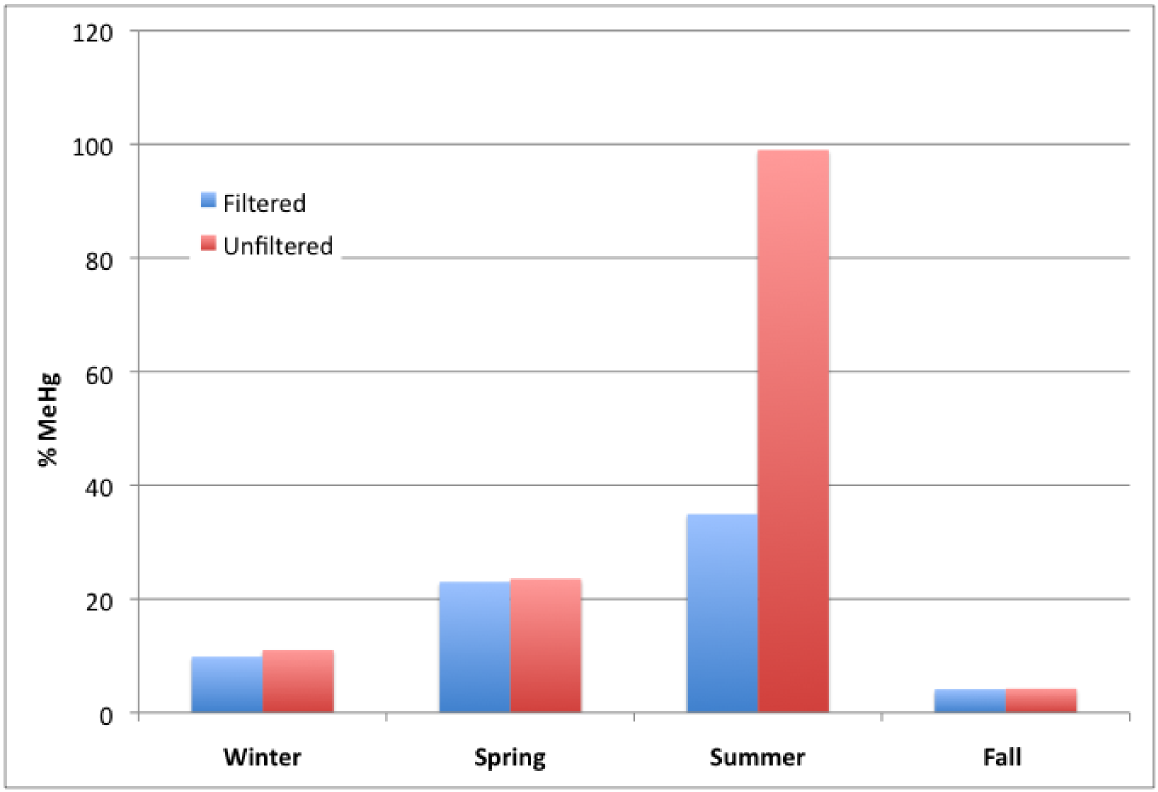


Figure 42 Percent of Hg in the methylated form in a wetland in the Little Tallahatchie watershed by season.

Particulate bound mercury (PBM) concentrations were highest in the summer for both Enid (428 ng/g) and Sardis (282 ng/g) Lakes, and lowest in the fall (103 ng/g, Enid) and (129 Sardis). Whereas different concentrations of PBM may reflect different sources, the samples were collected during stable periods when suspended solids were relatively low and very little mass of suspended material was collected and the variability in the data was high. Thus, these results should be viewed with caution. It is noted, however, that an increased Hg loading rate during spring melts and summer/fall storm events in tributaries associated with Lake Michigan were associated with particulate loading from sediment resuspension and/or erosion processes (Hurley et al. 1998). This is further discussed in section 6.4.2.

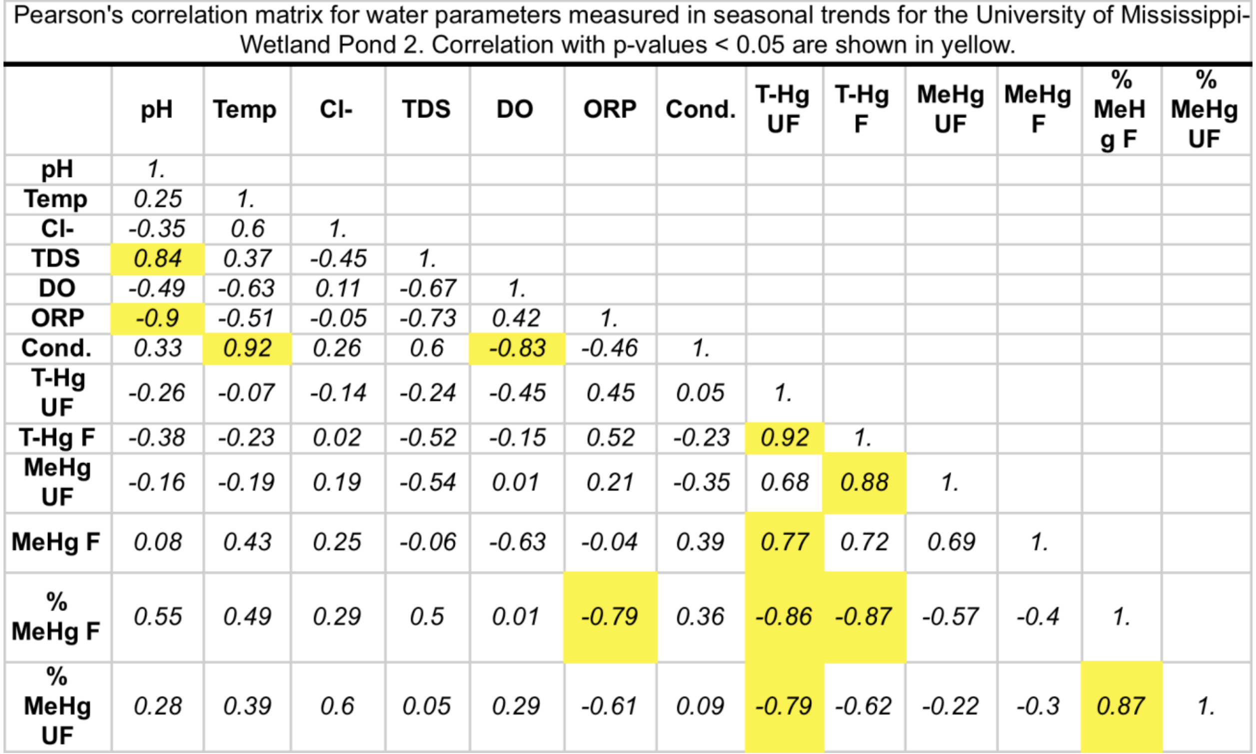
A Pearson's correlations matrix revealed the relationship between Hg and ancillary data (Tables 16 and 17). ORP levels were lowest during the summer months (-663 mV, pond I) and (-516 mV, pond 2) and correlated (negatively) with MeHg levels ($r=-0.67$, $p=0.09$). There was negative correlation between the percent of MeHg of total-Hg (in filtered water) and ORP ($r =-0.79$; pond 2). As noted lower DO and redox conditions favor methylation. MeHg concentrations were also correlated with chloride in the water ($r = 0.76$, $p<0.05$; pond 1, filtered); it is not uncommon to find Hg species complex with chloride and dissolved solids in water.

Table 16 Pearson’s correlation matrix for mercury and water quality parameters measured in Enid and Sardis Lakes and associated wetlands.

Correlations with p-values <0.05 are shown in yellow. F = Filtered (0.45µm); UF = unfiltered.

	pH	Temp	Cl-	TDS	DO	ORP	Cond.	T-Hg UF	T-Hg F	MeHg UF	MeHg F	% MeHg F	% MeHg UF	PBM	Log Kd
pH	1.														
Temp	0.83	1.													
Cl-	0.08	0.32	1.												
TDS	0.08	0.02	-0.09	1.											
DO	-0.61	-0.49	0.4	-0.59	1.										
ORP	0.15	0.06	-0.7	0.45	-0.67	1.									
Cond.	0.38	0.38	0.07	0.93	-0.7	0.45	1.								
T-Hg UF	-0.38	-0.68	-0.15	-0.03	0.25	-0.33	-0.27	1.							
T-Hg F	-0.3	-0.7	-0.16	-0.03	0.28	-0.29	-0.27	0.9	1.						
MeHg UF	-0.04	0.19	0.14	-0.32	0.11	-0.29	-0.25	-0.04	-0.19	1.					
MeHg F	0.08	0.28	0.09	-0.16	-0.01	-0.17	-0.07	-0.16	-0.28	0.96	1.				
% MeHg F	0.12	0.48	0.17	-0.41	0.13	-0.04	-0.21	-0.66	-0.69	0.7	0.74	1.			
% MeHg UF	-0.12	0.23	0.03	-0.39	0.22	0.17	-0.27	-0.64	-0.63	0.53	0.55	0.91	1.		
PBM	0.29	0.52	0.85	0.14	0.07	-0.29	0.37	-0.34	-0.33	0.09	0.1	0.19	0.1	1.	
Log Kd	0.55	0.48	0.49	0.21	-0.3	-0.47	0.36	0.13	0.08	0.12	0.13	-0.21	-0.56	0.46	1.

Table 17 Pearson's correlation matrix for water parameters measured in seasonal trends for the University of Mississippi- Wetland Pond 2. Correlation with p-values < 0.05 are shown in yellow.



Pearson's correlation matrix for water parameters measured in seasonal trends for the University of Mississippi- Wetland Pond 2. Correlation with p-values < 0.05 are shown in yellow.

	pH	Temp	Cl-	TDS	DO	ORP	Cond.	T-Hg UF	T-Hg F	MeHg UF	MeHg F	% MeHg F	% MeHg UF
pH	1.												
Temp	0.25	1.											
Cl-	-0.35	0.6	1.										
TDS	0.84	0.37	-0.45	1.									
DO	-0.49	-0.63	0.11	-0.67	1.								
ORP	-0.9	-0.51	-0.05	-0.73	0.42	1.							
Cond.	0.33	0.92	0.26	0.6	-0.83	-0.46	1.						
T-Hg UF	-0.26	-0.07	-0.14	-0.24	-0.45	0.45	0.05	1.					
T-Hg F	-0.38	-0.23	0.02	-0.52	-0.15	0.52	-0.23	0.92	1.				
MeHg UF	-0.16	-0.19	0.19	-0.54	0.01	0.21	-0.35	0.68	0.88	1.			
MeHg F	0.08	0.43	0.25	-0.06	-0.63	-0.04	0.39	0.77	0.72	0.69	1.		
% MeHg F	0.55	0.49	0.29	0.5	0.01	-0.79	0.36	-0.86	-0.87	-0.57	-0.4	1.	
% MeHg UF	0.28	0.39	0.6	0.05	0.29	-0.61	0.09	-0.79	-0.62	-0.22	-0.3	0.87	1.

6.4.2 Mercury Flux Measurements for Enid Lake

Water flows (cubic feet per second) and concentration of total-Hg and MeHg in the inflow and outflow of Enid and Sardis Lakes by season and during a storm event is given in Table 18. Based on these measurements the net flux of Hg species was calculated and is also given in Table 18. The seasonal data was obtained during periods of stable weather (no rain during the prior few days). As noted, this is a first approximation because there are sources and transformations not accounted for (e.g., inputs from other streams, direct deposition to the water surface, leaching, losses through volatilization) and because these are snapshots in time.

Nevertheless, because the river flows and lake levels are expected to be the dominant factors this work has led to a better understanding of Hg transport and cycling in the system.

For three of the four seasonal measurements there was little difference between inflows and outflows for both water flows and Hg concentrations, and thus the net flux was near zero, except for winter when ~30 g of Hg was being lost per day (Fig. 43). The winter negative net flux is a result of water level management of the lake by the Army Corps of Engineers. The lake discharge levels are higher in the fall and winter months increasing from 50 cfs (summer base flow discharge level) to 1200 cfs in the fall, and 2200 cfs in the winter. These high discharge rates are to lower the lake level to accommodate spring rains, serving as a flood control for the Yazoo River Basin. Of course the lake can't always have a negative flux and serve as a "source" of mercury. So when is the flux positive (inputs > outputs)? Storm events, as have been discussed in previous chapters, clearly represent major inputs to the lake by introducing suspended solids that have Hg-bound to them (Fig. 44).

For MeHg, the data followed the same trend, except that the net flux in the summer was substantially more negative. This is likely related to a build-up of MeHg in the lake due to higher methylation and evaporation rates. Sellers et al. (2001) measured external inputs of MeHg in a lake water column during summer months and reported that the lake accumulated MeHg several times greater than all its inputs, which demonstrated that in-lake MeHg production makes an important contribution to the mass of MeHg in the water column during the summer months.

Table 18 Water flows, concentrations, and fluxes of total-Hg (top) and MeHg (bottom) for Enid Lake*

Date	Description	Water Flows (ft^3/s)		Hg (ng/L)		Net Flux (g/day)
		Inflow	Outflow	Inflow	Outflow	
4/13/12	Spring 2012	60	50	4.7	4.77	0.11
6/27/12	Summer 2012	9	50	1.6	1.91	-0.20
12/8/12	Fall 2012	30	50	1.29	2.07	-0.16
2/1/13	Winter 2013	556	2200	11.45	7.92	-27.09
8/16/12	Storm Event (Su' 12)	279	50	24.6	1.91	16.58

Date	Description	Water Flows (ft^3/s)		MeHg (ng/L)		Net Flux (g/day)
		Inflow	Outflow	Inflow	Outflow	
4/13/12	Spring 2012	60	50	0.26	0.17	0.02
6/27/12	Summer 2012	9	50	0.25	0.79	-0.09
12/8/12	Fall 2012	30	50	0.17	0.11	-0.001
2/1/13	Winter 2013	556	2200	0.45	0.17	-0.28
8/16/12	Storm Event (Su' 12)	279	50	0.53	0.1	0.43

*Unfiltered samples

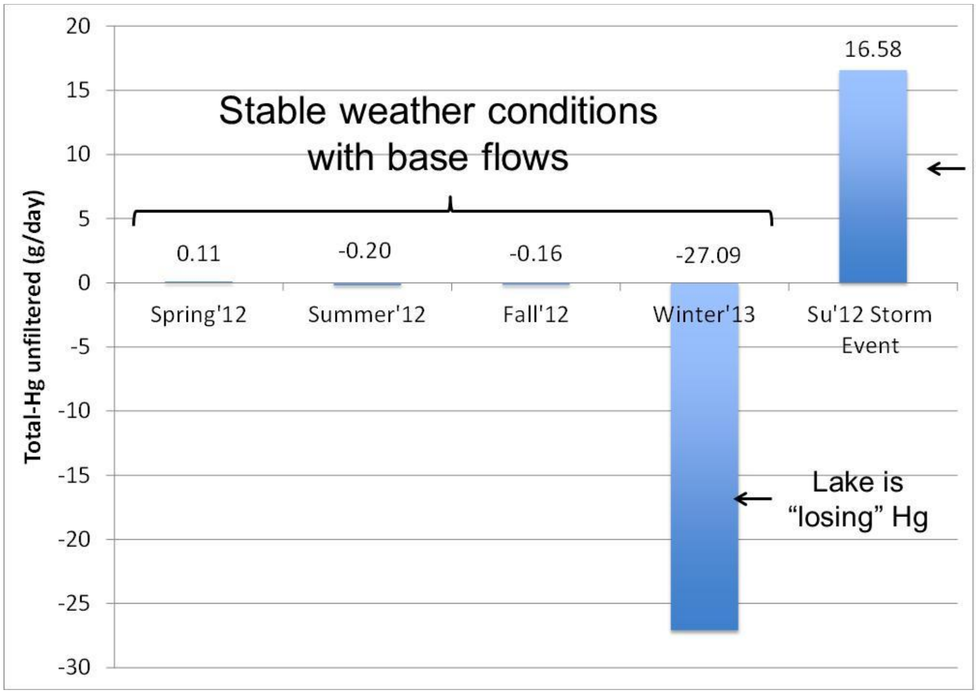


Figure 43 Net flux (g/day) for total-Hg (unfiltered) for Enid Lake by season (during stable weather periods) and with a storm event.

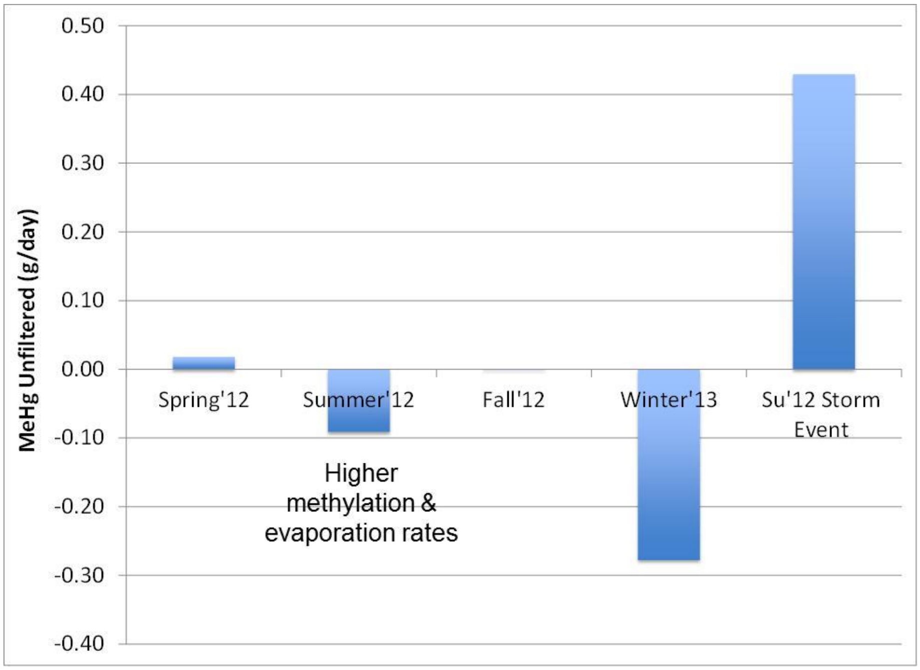


Figure 44 Net flux (g/day) for MeHg (unfiltered) for Enid Lake by season (during stable weather periods) and with a storm event.

The data garnered for this dissertation is the start of a mass balance Hg budget for Enid Lake (Fig. 45). Eventually all known inputs, outputs and storage terms will be used to estimate the range of values for Hg loading into Enid Lake. Inputs include atmospheric deposition (both wet and dry), tributaries, and groundwater. Outputs include efflux (volatilization) to the atmosphere, outflow via the lower Yocona River, biota removal, and sediment storage. The figure includes details of the measurement and future work needed to complete the budget.

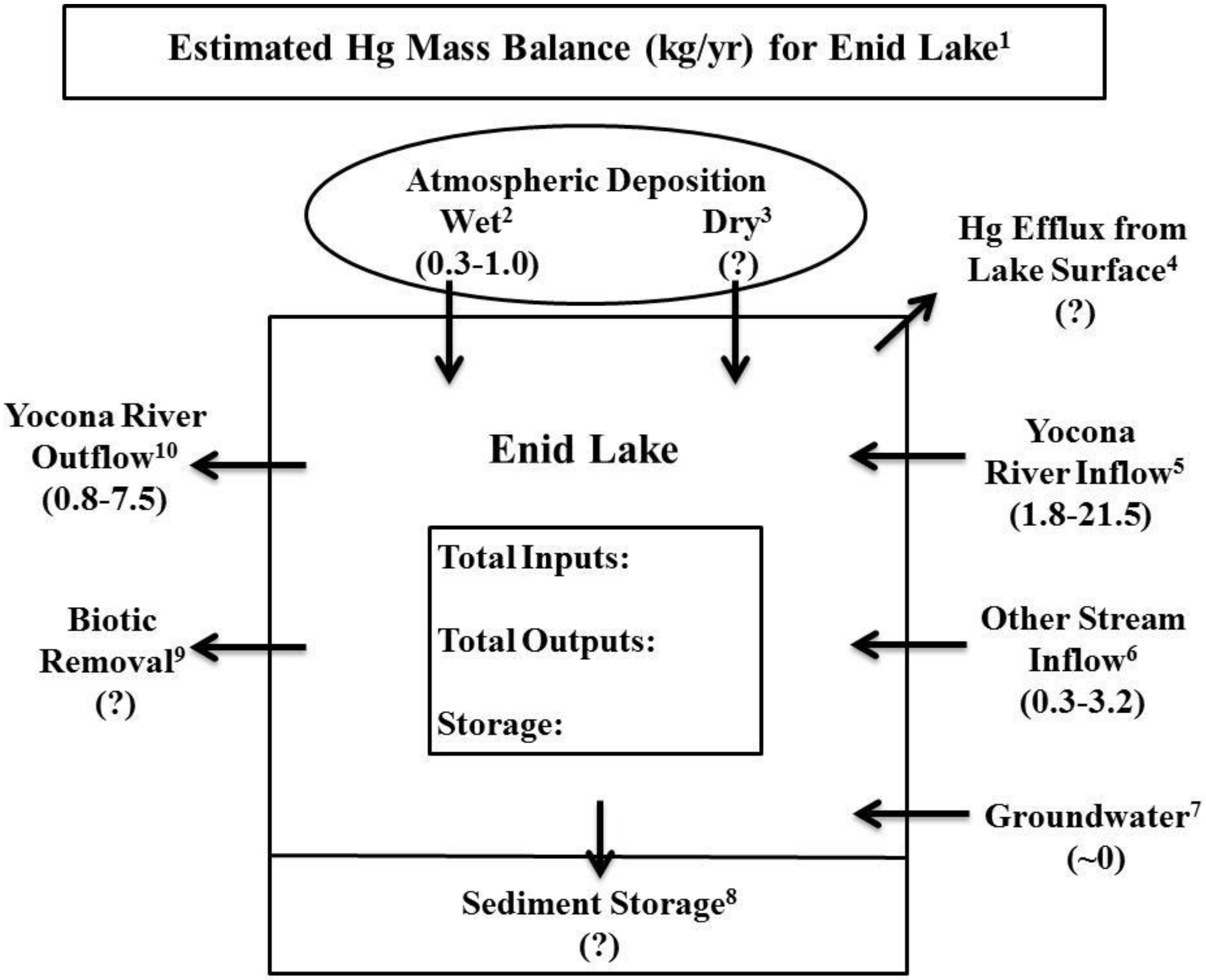


Figure 45 Estimated Hg mass balance for Enid Lake. Inputs and Outputs designated with a superscript are described below.

1. Question marks indicate that the input or output has not been determined or that not enough data is available to make a good estimate yet. The approaches to obtain the inputs and outputs are described summarized individually below. Future plans are to determine ranges and uncertainties for these initial estimates, and to complete the mass balance.
2. Wet deposition was calculated based on the mean Hg concentration in the rain, the average annual precipitation to the region, and the watershed area. Wet Deposition Rate (WDR) ($\mu g \cdot m^{-2} \cdot y^{-1}$) = C_{Hg} x I / 1000; where: C_{Hg}: conc. of Hg in precip. ($\mu g \cdot m^{-3}$), and I: precipitation intensity ($mm \cdot y^{-1}$). The amount deposited annually = WDR * lake area. Deposition to the entire watershed was estimated at 15 kg, but only deposition directly to the lake is considered here; a portion of the remainder may expected to enter the lake via runoff through the Yocona River and other streams. The range was obtained by using a factor of 0.5 (mean 0.66 kg/yr) to address dry and wet years. Note: Concentrations in rain were determined on 3 separate occasions and averaged; however only rain in the winter was sampled. Future work will examine levels during other seasons; the summer, in particular, may have higher levels as convective thunderstorm reach high into the atmosphere and tap a pool of accumulating gaseous oxidized mercury (e.g. Lyman and Jaffe 2012).
3. Dry deposition will be estimated using regional published data sources as well as local sources: Regional data from the National Atmospheric Deposition Program (NADP) Mercury Deposition Network (MDN); Local data from our own research group using a Tekran ambient air speciation system. For the speciation data, deposition will be estimated using an inferential model (e.g., Fang et al. 2001) with a range of deposition velocities (Zhang et al. 2011).
4. Hg volatilization (efflux) from the air-water interface can be a significant source of Hg loss from lakes (Fitzgerald et al. 1991). A number of factors contribute to the rate of efflux, including temperature, concentrations (in both air and water) and wind. Efflux will be measured using a flux chamber following the recent approaches reported in the literature (e.g., Miller et al. 2011; Suchanek et. al 2009; Poissant et al. 2004).
5. Yocona River is the primary source of water entering Enid Lake. Water was collected Yocona River (near US Highway 7) during base flows, storm events, and by season. The

USGS hydrologic (sonar) stream gauge was used to determine water discharge flow at the time of sample collection. Annual mean flows (based on 1952-2012 data) together with average Hg concentrations for peak flow and base flows were used to estimate the maximum and minimum of annual Hg discharge to the lake: Load = Concentration * Flow * 2.54 *365 / 1000; where: concentration is in µg/L, flow is in cubic feet per second, 2.54 is a unit conversion factor, 365 is days/yr, and 1000 is to convert to kg.

6. About 15% of the water entering the lake enters through streams other than the Yocona River based on Mississippi Department of Environmental Quality land use and watershed/catchment maps.
7. Groundwater contribution is expected to be small because the volume of groundwater entering the lake is a small fraction compared to river inputs and because the concentration of Hg in groundwater (collected in the watershed) is low and near detection limits (~0.2 ng/L).
8. Storage in bed sediments will be estimated based on Hg concentrations in surficial sediments. Only four sediment samples have been collected (using an Ekman dredge); greater coverage is needed to extrapolate Hg concentrations from the sediment to the entire lake (using an inverse-weighted interpolation algorithm). Sedimentation rates, which will vary depending on proximity to the river, will need to be revisited to calculate annual mean loading.
9. Hg incorporated into fish and other organisms is removed from the lake by sport fishers as well as by wildlife. An estimate of this loss will be made using a creel survey data and Hg concentration in fishes (recently determined).
10. The output to the Yocona River (below the dam) was estimated as described above (note 5). Concentrations were determined in the outflow at peak and base flows. The mean discharge used was 1000 cfs (Army Corp of Engineer data for 1962-2010).

6.5 CONCLUSIONS

Seasonal trends and fluxes of mercury species in the inflow and outflow of Enid and Sardis Lakes and wetland areas were investigated. In the winter, the net flux of total-Hg concentration was the most negative but can be attributed due to management of lake levels. In the summer, the net flux of MeHg was substantially more negative than fall and spring, likely from in-situ production of MeHg due to conditions that favor methylation. During the storm event the flux of Hg in the lake became the most positive, suggesting that storm events contribute a significant portion of the Hg entering the lake. Wetlands contained high percentages for MeHg of total-Hg in the summer months, presumably due to increased microbial methylation rates. This study has shown that monitoring inflow and outflows in reservoirs for Hg species by season can shed light on the sources and cycling of Hg in the system.

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APPENDIX 6.1: Enid Inflow average Hg concentrations and ancillary parameters.

Enid Inflow (Yocona River)															
Seasons	**pH**	**T (°C)**	**Cl-**	**TDS (mg/L)**	**DO (mg/L)**	**ORP (mV)**	**Cond. (µs/cm)**	**T-Hg UF (ppt)**	**T-Hg F (ppt)**	**MeHg UF (ppt)**	**MeHg F (ppt)**	**% MeHg Dissolved in Total-Hg Dissolved**	**% MeHg in Total-Hg**	**P-Hg ng/g**	**Log K_d**
Spring 2012	6.78	17.4	0.1	65	2.57	236.97	85.33	4.70 ± 0.14	3.82 ± 0.22	0.26*	0.23 ± 0.07	6	6	410.38	5.0
Summer 2012	7.23	28.13	10.13	60.23	7.97	-396.83	98.27	1.6*	1.08*	0.25*	0.22 ± 0.01	20	15	1502.13	6.1
Fall 2012	7.15	16.5	NA	87.1	8.0	195.3	112.5	1.30 ± 0.29	0.66 ± 0.15	0.17*	0.15	23	13	376.03	5.8
Winter 2013	6.37	8.6	1.62	39.00	10.27	-375.93	41.57	11.45 ± 0.59	7.14 ± 0.58	0.45 ± 0.017	0.36 ± 0.005	5	4	201.64	4.5
Avg.	6.88	17.66	3.95	62.83	7.21	-85.12	84.43	4.76	3.17	0.28	0.24	13.55	9.48	622.55	5.3
1SD	0.39	8.02	5.41	19.73	3.27	348.39	30.66	4.72	2.99	0.12	0.08	9.37	5.63	593.47	0.8
MIN	6.37	8.60	0.10	39.00	2.57	-396.83	41.57	1.29	0.66	0.17	0.15	4.98	3.92	201.64	4.5
MAX	7.23	28.13	10.13	87.10	10.27	236.97	112.53	11.45	7.14	0.45	0.36	23.11	15.45	1502.13	6.1

*Relative Percent Difference < 9%

APPENDIX 6.2: Enid Outflow average Hg concentrations and ancillary parameters.

Enid River (Outflow)															
Seasons	**pH**	**T (°C)**	**Cl-**	**TDS (mg/L)**	**DO (mg/L)**	**ORP (mV)**	**Cond. (µs/cm)**	**T-Hg UF (ppt)**	**T-Hg F (ppt)**	**MeHg UF (ppt)**	**MeHg F (ppt)**	**% MeHg Dissolved in Total-Hg Dissolved**	**% MeHg in Total-Hg**	**P-Hg ng/g**	**Log K_d**
Spring 2012	7.53	21.53	0	42.9	1.13	230.47	61.37	4.77 ± 0.40	2.86 ± 0.26	0.17 ± 0.006	0.12 ± 0.007	4	3	149.06	4.7
Summer 2012	7.27	27.13	3.6	29.25	6.3	-394.6	47.3	1.91*	1.25*	0.79*	0.74*	60	41	428.30	5.5
Fall 2012	6.96	14.5	NA	44.2	9.1	105.7	54.1	2.07 ± 0.21	< DL	0.11*	0.08*	NA	5	103.33	ND
Winter 2013	6.82	10.3	3.19	34.45	10.67	-413.27	38.30	7.92 ± 0.16	8.41 ± 1.2	0.16 ± 0.007	0.11 ± 0.012	1	2	170.33	4.3
Avg.	7.14	18.37	2.27	37.70	6.81	-117.93	50.27	4.17	4.17	0.31	0.26	21.61	13.02	212.75	4.9
1SD	0.32	7.46	1.98	7.10	4.19	334.25	9.83	2.82	3.76	0.32	0.32	32.88	18.86	146.39	0.6
MIN	6.82	10.30	0.00	29.25	1.13	-413.27	38.30	1.91	1.25	0.11	0.08	1.25	2.08	103.33	4.3
MAX	7.53	27.13	3.63	44.20	10.67	230.47	61.37	7.92	8.41	0.79	0.74	59.54	41.24	428.30	5.5

*Relative Percent Difference < 20%

APPENDIX 6.3: Sardis Inflow average Hg concentrations and ancillary parameters.

Sardis Inflow (Little Tallahatchie River)															
Seasons	**pH**	**T (°C)**	**Cl-**	**TDS (mg/L)**	**DO (mg/L)**	**ORP (mV)**	**Cond. (µs/cm)**	**T-Hg UF (ppt)**	**T-Hg F (ppt)**	**MeHg UF (ppt)**	**MeHg F (ppt)**	**% MeHg Dissolved in Total-Hg Dissolved**	**% MeHg in Total-Hg**	**P-Hg ng/g**	**Log K_d**
Spring 2012	7.10	17.9	0.33	89.7	1.7	236.2	118.77	4.28 ± 0.63	3.03 ± 0.11	0.24 ± 0.01	0.33 ±0.06	10.9	6	149.93	4.7
Summer 2012	7.33	25.5	5.5	29.25	6.87	-401.1	45.4	2.10*	1.00*	0.18 ± 0.01	NA	NA	9	162.57	5.2
Fall 2012	8.53	14.3	NA	66.3	8.53	83.6	81.03	1.38 ± 0.17	0.63 ± 0.29	0.158 ± 0.002	0.13*	21.3	11	153.12	5.4
Winter 2013	5.84	8.2	4.16	55.90	8.68	-420.43	58.43	6.78 ± 0.25	3.84 ± 0.74	0.32 ± 0.01	0.22 ± 0.01	5.6	5	167.99	4.6
Avg.	7.20	16.48	3.33	60.29	6.44	-125.43	75.91	3.64	2.12	0.22	0.23	12.60	7.56	158.40	5.0
1SD	1.10	7.23	2.68	25.06	3.27	335.41	32.14	2.43	1.55	0.07	0.10	7.97	3.05	8.34	0.4
MIN	5.84	8.20	0.33	29.25	1.70	-420.43	45.40	1.38	0.63	0.16	0.13	5.61	4.73	149.93	4.6
MAX	8.53	25.50	5.50	89.70	8.68	236.20	118.77	6.78	3.84	0.32	0.33	21.28	11.41	167.99	5.4

*Relative Percent Difference < 15%

APPENDIX 6.4: Sardis Outflow average Hg concentrations and ancillary parameters.

Sardis Lake (Outflow)

Seasons	pH	T (°C)	Cl-	TDS (mg/L)	DO (mg/L)	ORP (mV)	Cond. (µs/cm)	T-Hg UF (ppt)	T-Hg F (ppt)	MeHg UF (ppt)	MeHg F (ppt)	% MeHg Dissolved in Total-Hg Dissolved	% MeHg in Total-Hg	P-Hg ng/g	Log K_d
Spring 2012	7.19	21.33	0	64.57	1.27	231.67	91.07	3.37 ± 0.56	3.38 ± 0.17	0.19 ± 0.03	0.15 ± 0.01	4.5	6	137.64	4.6
Summer 2012	7.37	29.03	3.3	32.72	6.9	-398.17	53.7	2.26 ± 0.11	0.73*	0.21*	0.18 ± 0.01	25.3	9	281.67	5.6
Fall 2012	7.08	14.0	NA	55.68	8.73	96.63	67.73	1.01 ± 0.23	< DL	0.09*	0.067 ± 0.001	NA	9	129.31	ND
Winter 2013	6.51	8.9	2.18	43.55	11.20	-397.87	46.37	5.28 ± 0.50	5.18 ± 0.15	0.15 ± 0.13	0.13 ± 0.01	2.5	3	166.54	4.5
Avg.	7.04	18.31	1.83	49.13	7.03	-116.93	64.72	2.98	3.10	0.16	0.13	10.77	6.55	178.79	4.9
1SD	0.37	8.79	1.68	13.93	4.22	329.22	19.68	1.81	2.24	0.05	0.05	12.60	2.95	70.42	0.6
MIN	6.51	8.90	0.00	32.72	1.27	-398.17	46.37	1.01	0.73	0.09	0.07	2.53	2.85	129.31	4.5
MAX	7.37	29.03	3.30	64.57	11.20	231.67	91.07	5.28	5.18	0.21	0.18	25.27	9.23	281.67	5.6

*Relative Percent Difference < 13%

APPENDIX 6.5: University of Mississippi- Pond I (UM-Pond I) average Hg concentrations and ancillary parameters.

UM-Pond I

Sampling Seasons	pH	T (°C)	Cl^-	TDS (mg/L)	DO (mg/L)	ORP (mV)	Cond. (µs/cm)	T-Hg UF (ppt)	T-Hg F (ppt)	MeHg UF (ppt)	MeHg F (ppt)	% MeHg Dissolved in Total-Hg Dissolved	% MeHg in Total-Hg	P-Hg ng/g	P-Hg ng
Fall 2011	6.73	14.63	NA	20.15	9.13	319.27	24.83	2.58*	1.3 ± 0.5	0.11*	0.054*	4	4	903	0.51
Winter 2012	7.91	10.8	NA	269.15	9.27	269.15	14.8	4.83*	3.48*	0.53*	0.34*	10	11	NA	NA
Spring 2012	7.23	28.13	10.13	60.23	7.97	-396.83	98.27	2.26*	3.07*	0.53*	0.71 ± 0.05	23	24	NA	NA
**Summer 2012	7.58	32.8	6.57	21.67	5.9	-663.7	38.8	1.03 ± 0.20	1.59 ± 0.13	1.33 ± 0.03	0.55*	35	129	NA	NA
Avg.	7.36	21.59	8.35	92.80	8.07	-118.03	44.17	2.67	2.36	0.63	0.41	17.96	41.89	903	0.51
1SD	0.50	10.54	2.52	119.02	1.56	488.75	37.39	1.58	1.08	0.51	0.28	13.79	58.44	NA	NA
MIN	6.73	10.80	6.57	20.15	5.90	-663.70	14.75	1.03	1.30	0.11	0.05	4.12	4.24	903	0.51
MAX	7.91	32.80	10.13	269.15	9.27	319.27	98.27	4.83	3.48	1.33	0.71	34.92	128.72	903	0.51

**Cl and ORP results obtain In-Lab; *Relative Percent Difference < 15%

APPENDIX 6.6: University of Mississippi- Pond II (UM-Pond II) average Hg concentrations and ancillary parameters.

UM-Pond II

Sampling Seasons	pH	T (°C)	Cl-	TDS (mg/L)	DO (mg/L)	ORP (mV)	Cond. (µs/cm)	T-Hg UF (ppt)	T-Hg F (ppt)	MeHg UF (ppt)	MeHg F (ppt)	% MeHg Dissolved in Total-Hg Dissolved	% MeHg in Total-Hg	P-Hg ng/g	P-Hg ng
Fall 2011	6.17	13.47	0.13	34.45	10.27	321.43	41.43	0.28 ± 0.01	0.12	0.15*	0.05*	44	55	130	0.51
Winter 2012	7.83	10.7	NA	27.3	7.85	180.8	31.3	2.44*	2.56*	0.81*	0.37*	14	33	NA	NA
Spring 2012	7.92	29.33	0.8	47.02	1.13	57.47	78.63	2.67*	1.71*	0.41*	0.43 ± 0.03	25	15	NA	NA
**Summer 2012	8.82	32.3	5.47	37.05	6.6	-516.0	65.3	0.39 ± 0.19	0.45 ± 0.27	0.45*	0.32*	71	113	NA	NA
Avg.	7.68	21.46	2.13	36.45	6.46	10.93	54.17	1.45	1.21	0.45	0.29	38.78	54.12	130	0.51
1SD	1.11	10.95	2.91	8.16	3.87	367.45	21.68	1.28	1.13	0.27	0.17	25.01	42.62	NA	NA
MIN	6.17	10.70	0.13	27.30	1.13	-515.97	31.27	0.28	0.12	0.15	0.05	14.42	15.18	130	0.51
MAX	8.82	32.33	5.47	47.02	10.27	321.43	78.63	2.67	2.56	0.81	0.43	71.38	113.16	130	0.51

**Cl and ORP results obtain In-Lab; *Relative Percent Difference < 10%

CHAPTER SEVEN

7. MERCURY IN WATER, SUSPENDED SOLIDS AND SEDIMENT IN ENID LAKE: PRELIMINARY ASSESSMENT OF MODELING MERCURY TRANSPORT USING REMOTE SENSING

7.1 ABSTRACT

A fish-consumption advisory was issued by Mississippi Department of Health in 1995 for Enid Lake and parts of the Yocona River in north Mississippi due to elevated levels of mercury (Hg) in fish. In the present study, we determined Hg, total suspended solids (TSS), and a wide range of other water quality parameters in water collected at multiple locations in Enid Lake following a heavy rain storm event. In addition sediment was collected on a transect extending from near the dam to near the mouth of the Yocona River, and Hg was measured based on size fraction. The purpose was to determine the current concentrations and distribution of Hg in the lake, and to use these empirical measurements and remote sensing technology to calibrate and validate numerical models designed to simulate the flow of suspended solids and Hg introduced by the Yocona River.

Concentrations of Hg averaged 9.4 ± 3.6 ng/L (1 SD, n=18) and ranged from 4.9 to 16.3 ng/L for unfiltered water; Hg in the dissolved fraction (<0.45 µm) averaged 7.5 ± 3.2 ng/L and ranged from 3.8 to 14.4 ng/L. Mercury concentration was correlated with TSS ($r=0.683$, $p<0.05$). Concentrations in the sediment averaged 65.2 ng/g, and ranged from 40.7 to 89.7 ng/g. The bulk of the sediment (>80%) consisted of particles <125 µm in diameter. Concentrations of Hg in these fines were greater than the larger size fractions, not surprising given that Hg^{+2} is surface reactive. Mercury also has an affinity for organic matter. Indeed levels of Hg and organic matter were higher in the sediment from the more shallow part of the lake near the mouth of the Yocona River compared to the deeper water areas near the dam. Remote sensing data was used

to model the distribution of TSS and Hg associated with suspended solids in Enid Lake following a storm event. Overall, this study shows that suspended sediment particle size, organic matter, and water flow characteristics are important factors controlling the distribution of Hg in the lake, and that modeling suspended solids and Hg transport using spectral data acquired remotely by satellites is not only feasible but a powerful way to provide timely data on the dynamics of Hg in reservoirs. Moreover, the results from this study are directly applicable to other large lake systems in Mississippi and elsewhere.

7.2 INTRODUCTION

Mercury (Hg) is a widely distributed and persistent pollutant in the environment. The chemical forms of Hg in air, water, and sediment include elemental mercury Hg(0), inorganic ionic mercury Hg(II), and the organic form methylmercury (MeHg). When Hg enters the water and soil, microorganisms can transform it into MeHg, which is a toxic form that accumulates in fish, shellfish and other aquatic organisms (Selin et al. 2010). When humans consume contaminated fish, they are exposed to MeHg. The adverse effects of MeHg in humans include neuro-developmental, cardiovascular, and immunological deficits (Karagas et al. 2012; Grandjean et al. 2010).

The Yazoo River Basin, the largest basin in Mississippi, includes four large flood control reservoirs: Arkabutla Lake, Sardis Lake, Enid Lake and Grenada Lake. The lakes provide significant natural and recreational resources. Soils in the region are highly erodible, resulting in a large amount of sediment discharged into these water bodies. Suspended solids can greatly affect water quality and aquatic life by reducing light penetration, damaging fish gills, and providing a mechanism for introducing mercury (Hg) and other pollutants, which are often associated with sediment particles.

Mercury levels in fish in Enid Lake and the Yocona River prompted a fish consumption advisory by Mississippi Department of Health in 1995. To reduce Hg levels, Total Daily Maximum Loads (TMDLs) for Hg were established in 2002 for the lake watershed. Subsequently, the Mississippi Department of Environmental Quality (MDEQ) adopted a criterion of 12 ng/L to protect aquatic life with a margin of safety of 50% (MDEQ 2002). However, the Hg loads due to atmospheric deposition and nonpoint sources were not included in the TMDL computation and the Hg levels measured are now more than a decade old (Huggett et al. 2001). Moreover, the processes of fate and transport of mercury in water bodies were not considered.

Understanding the dynamic processes of contaminated sediment movement and fate and transport of mercury in these large recreation lakes is important to manage the water quality of the lakes and provide useful information for fish consumption advisories and potential risk assessment. The processes of contaminated sediment transport and settling in the lake are particularly critical to lake water quality because of associations between sediment and other pollutants (nutrients, PCBs, mercury, etc.).

Sediment plays an important role in the fate and transport processes of Hg in water bodies. Mercury may adsorb to sediment particles and also desorb from sediment to the water, and the processes of adsorption/desorption have been described elsewhere (Katsenovich et al 2010), with pH, temperature, sediment size, playing an important role. Bed sediment associated Hg can be released gradually into the water column due to diffusion and sediment resuspension (Kuwabara et al 2003). The adsorption/ desorption of Hg by sediment and Hg release from bed sediment are important processes to study the fate of Hg in water bodies. In a water body, mass may exchange between the water column and bed surface through a variety of mechanisms. The most common one is the diffusion flux across the bed surface. In the case of lakes or reservoirs, the flow is slow, so the release is normally through diffusion between the bed sediment and water column. The diffusive flux of Hg from the bottom sediment can be estimated using Fick's first law, which indicates that the flux is directly proportional to the Hg concentration gradient between bed sediment and water column, and the porosity of sediment.

There is considerable interest in applying remote sensing techniques to provide maps of suspended materials and pollutants in lakes because of the high temporal and spatial variability, which renders field sampling methods as inadequate for some studies (Miller and McKee 2004). To that end, researchers have been using a variety of satellite imagery to study, in particular, the dynamics of suspended solids in waterbodies. Moderate-resolution Imaging Spectroradiometer (MODIS) instruments are part of satellites launched in 1999 and 2002. MODIS collect reflected and emitted energy from the earth's surface in 36 spectral bands from 0.4 to 14.4 µm. For suspended sediment, reflectance measured in the red portion (~600-700 nm) of the visible spectrum and the near IR is commonly used (Miller and McKee 2004).

7.3 MATERIALS AND METHODS

7.3.1 Study Site

Enid Lake is the smallest of four large reservoirs constructed in the 1940's and 1950's to control flood prone regions in the Yazoo River Basin in northwest Mississippi (Figure 46). It was impounded by Enid Dam on the Yocona River in Yalobusha County. The surface area is approximately 84.9 km^2, though water levels fluctuate by season, being regulated for flood control and recreational usage. Generally the lowest lake levels occur in the fall and winter and the highest in the spring and summer. The soils in the region are highly erodible, and the erosion rate has been recognized as one of the highest place in the nation (Bennett and Rhoton 2009). The lake is particularly popular for fishing with crappie and largemouth bass being the most targeted and consumed fish. However, the fishing enterprise is impaired by mercury, and a fish consumption advisory was issued by MDEQ in 1995. In order to reduce the mercury level in the lake, mercury Total Maximum Daily Load (TMDL) has been established in the lake watershed (MDEQ 2002).

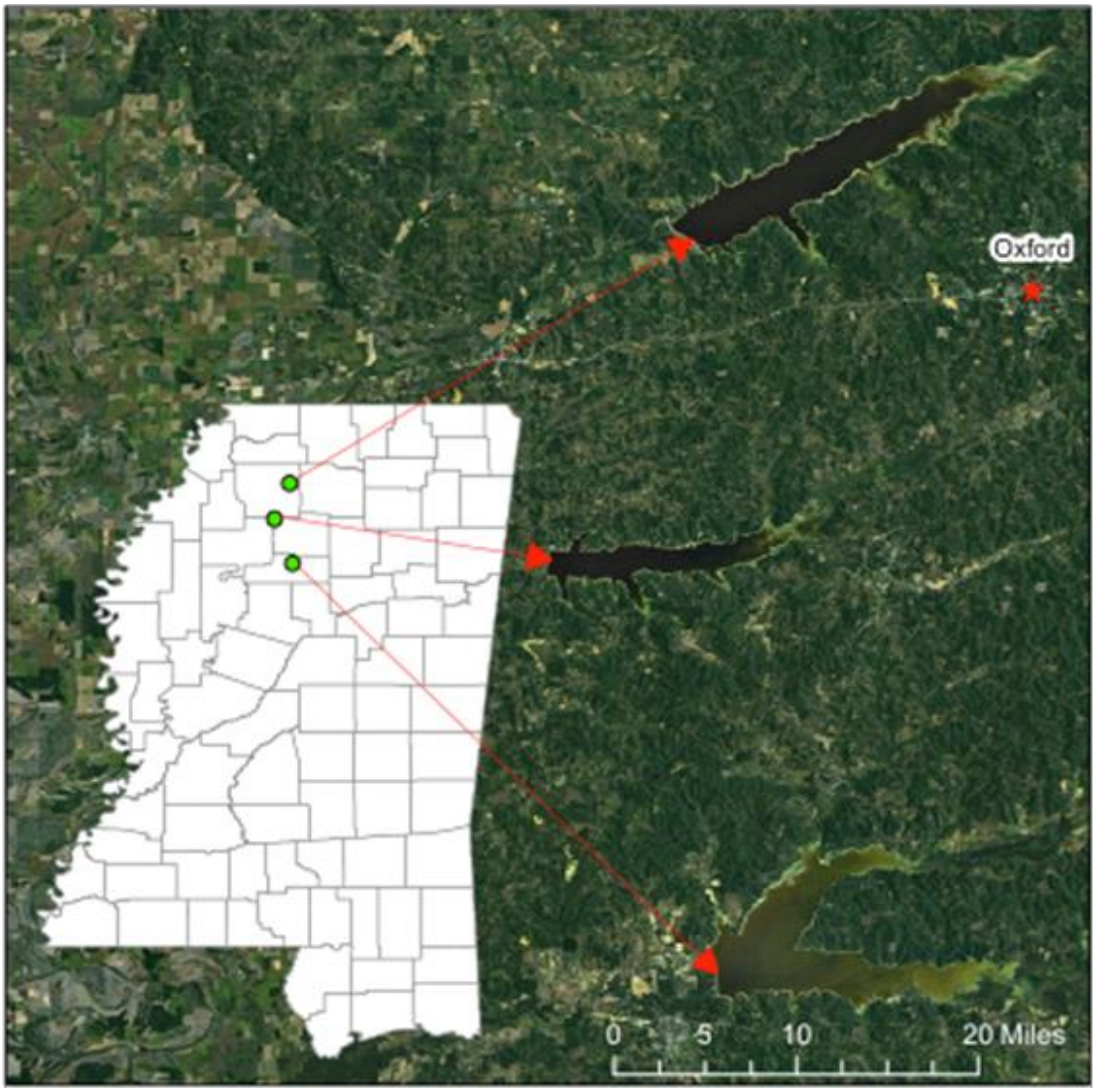

Figure 46 Enid (center), Sardis (top), and Grenada (bottom) lakes in relation to State of Mississippi and the town of Oxford. Arkabutla lake, located to the northwest, is not shown.

7.3.2 Water collection and field measurements

Samples from Enid Lake were collected on March 12, 2013, which followed a heavy rainstorm a few days earlier. The delay was necessary to have a clear day to get the best possible satellite spectral image data, which is discussed further below. The lake was still turbid from inputs of suspended solids associated with high flows from the storm event. The height and flow of the Yocona River, by far the largest contributor of water to the lake, during this period is shown in figure 47. Samples of water were collected into acid-washed Teflon or polycarbonate bottles just below the surface using clean protocols. Samples were obtained from a total of 18 sites distributed throughout the lake (figure 48).

In addition, standard water quality parameters, including pH, chloride, dissolved oxygen (DO), total dissolved solids (TDS), conductivity, and temperature, were collected in the field using a YSI multi-meter. The meter was calibrated the same day in the laboratory prior to fieldwork. In the field, water was collected in a stainless steel bucket and measured as the boat traveled between sampling sites (figure 49).

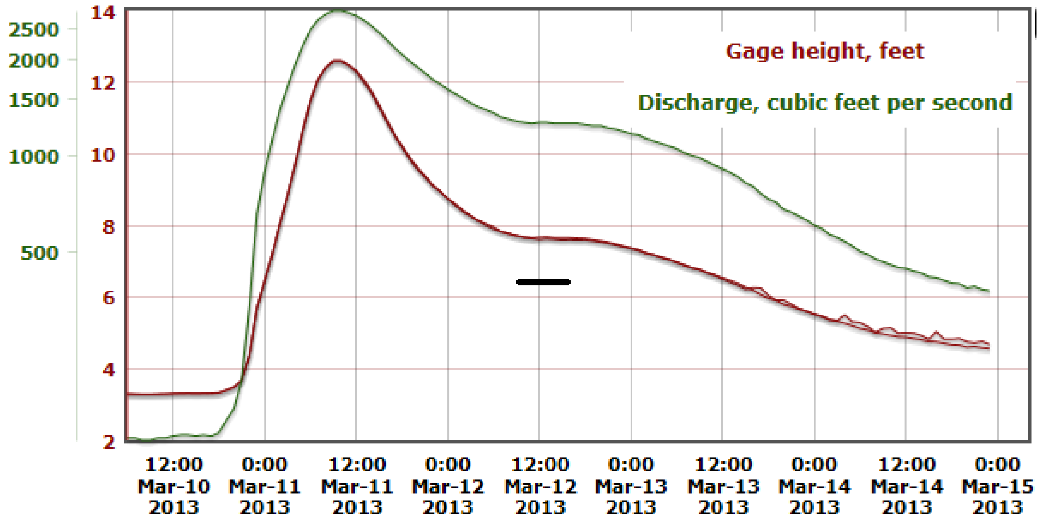

Figure 47 Yocona River flows during the period of sample collection (represented by the black line) on March 12, 2013.

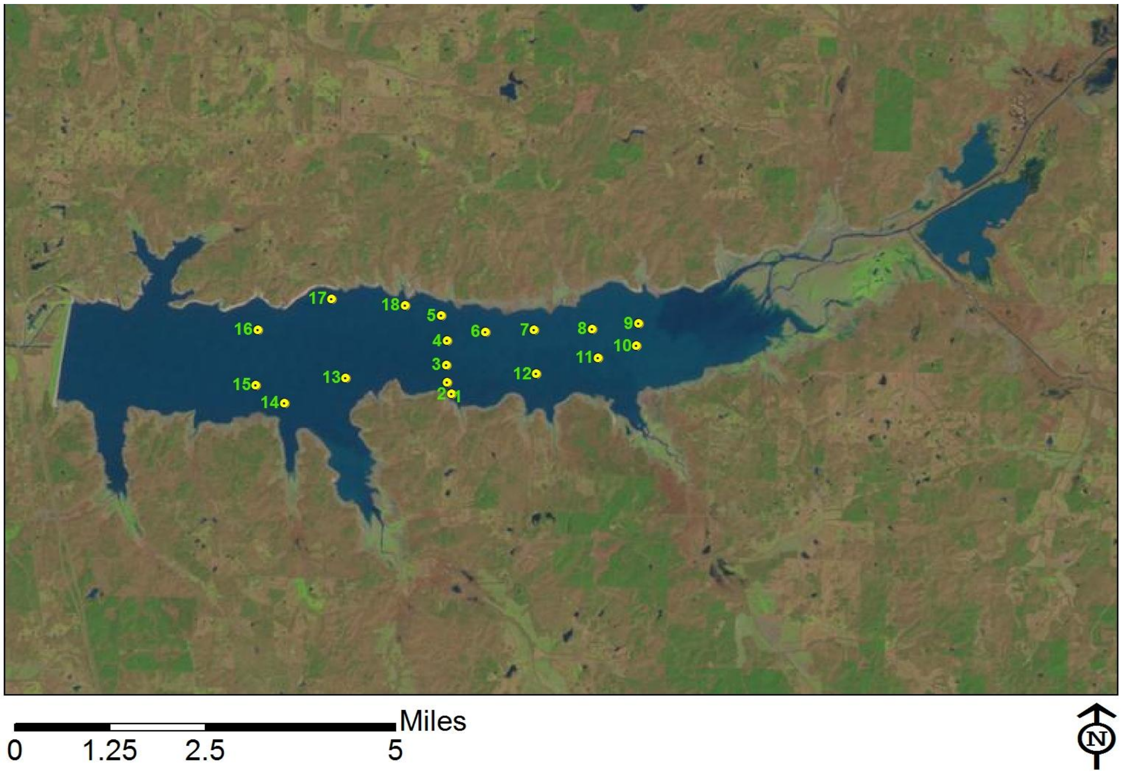

Figure 48 Location of water samples acquired in Enid Lake, Mississippi, on March 12, 2013.

Figure 49 Collecting water quality measurements using the YSI multi-meter while traversing between sampling points on Enid Lake (stainless steel bucket with water not shown). Note turbid water and clear skies for best satellite imagery.

7.3.3 Sediment collection and storage

Bulk surficial sediments were collected from four sites that transect the length of Enid Lake (figure 50). Sediment was collected by the National Sedimentation Laboratory using an Ekman Dredge. Samples were placed into acid-washed glass bottles, put in a cooler on ice, and transported to the lab where they were stored in a fridge until processing.

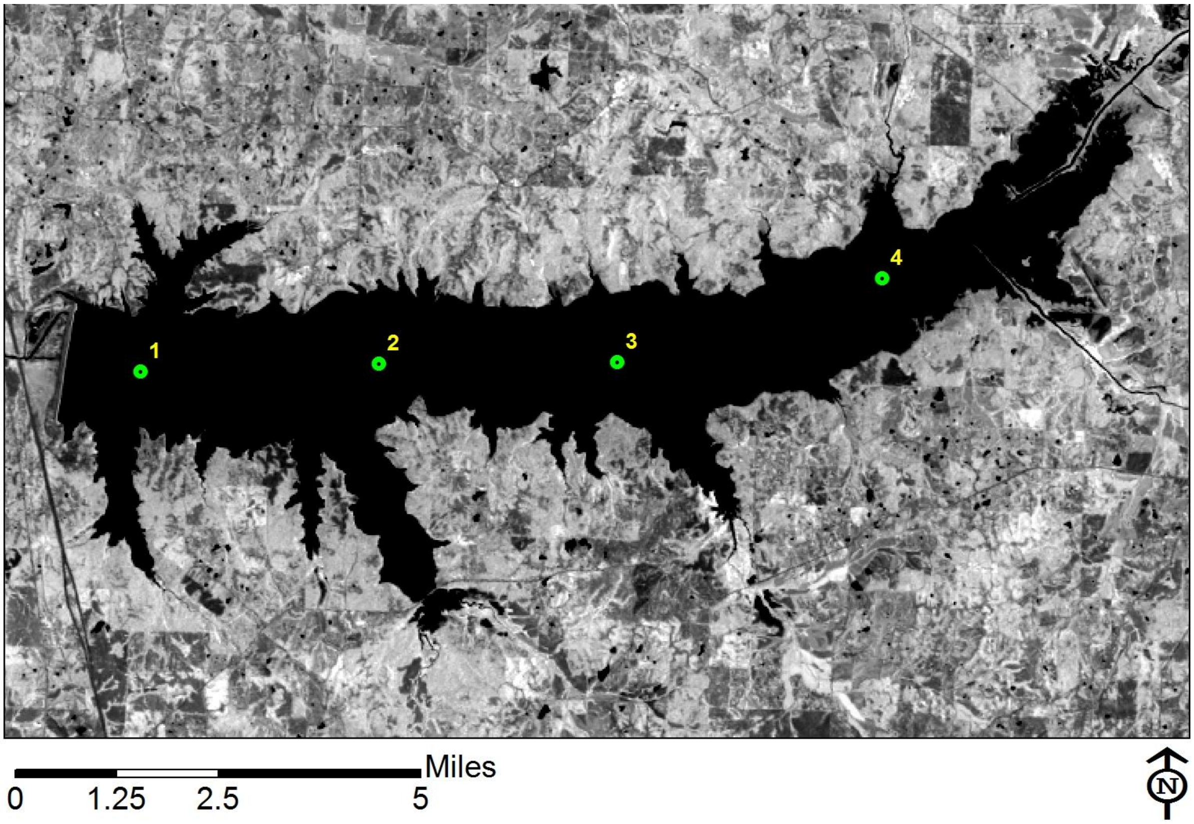

Figure 50 Locations of sediment samples acquired in Enid Lake, Mississippi on May 14, 2013.

7.3.4 Total suspended solids (TSS) and particle bound mercury (PBM) analysis

Total Suspended Solids was determined by passing a known volume of sample (between 150 ml to 500 ml depending on sediment load) through a 0.45 μm quartz wool filter that was combusted prior to filtering to remove any Hg. The filter was allowed to dry at room temperature under a laminar flow hood and reweighed to determine the TSS concentration using the following formula [US EPA Method 160.2]:

TSS (mg/L) = ((Residual + Filter (mg)) - Filter (mg)) / sample filtered (mL)) * 1000 (mg/L)

To determine PBM, the filters were then analyzed by combustion atomic absorption spectrometry using a direct mercury analyzer (DMA-80) following US EPA Method 7473. The instrument was calibrated using a standard solution containing known amounts of Hg. Reference materials including MESS-3 (sediment) and Joaquin Soil were used to as calibration checks every 10 samples; recoveries were between 88 to 115 % of the certified values. Blank filters were run every 10 samples to assure that Hg was not being carried over between samples. The amount of Hg for the blanks was negligible. The method detection limit for the analysis was estimated at 0.2 ng/g. The technique has been thorough discussed in earlier chapters.

7.3.5 Particle size distribution analysis

Sediments were homogenized in their container by stirring with a Teflon-coated spatula. A portion of the sediment was transferred to plastic weighing boats and allowed to air-dry in a clean laminar flow hood. Once dry, the sample was crushed using a clean mortar and pestle and a few grams were set aside for total-Hg analyses. The remaining portion was weighed and placed into a beaker with DI water and sonicated for 1 hour to break up adhering particles. The sample was then wet-sieved through stainless steel meshed screens with openings of 1000 µm, 500 µm, 250 µm, and 125 µm. The screen contents were visually inspected to confirm that there were no clumps; if necessary a spatula was used to further gently break up adhering materials. The screens were then allowed to air-dry and the contents were weighed. Particle size distribution was determined on a weight percent basis. The difference between the initial starting weight and the combined weights of sediment collected on the screens was used for the <125 µm category.

7.3.6 Determination of total-Hg and loss-on-ignition in sediment

Total-Hg was measured in the bulk sediment and in each size fraction using a direct mercury analyzer (DMA-80) based on thermal decomposition, amalgamation, and atomic absorption spectrometry following EPA Method 7473. Quality assurance protocols were the same as discussed earlier. To obtain Hg data for the <125 µm fraction, samples were dry sieved and the material passing through the fine mesh was analyzed. Loss-on-ignition (LOI), which is used as an estimate of organic matter, was determined by weighing the boats before and after combustion.

7.3.7 Remote sensing (satellite imagery) and associated modeling

Modeling was done at the National Center for Computational Hydroscience and Engineering (NCCHE). The Center has developed a new remote sensing based technique using NASA's satellite imagery (Landsat Thematic Mapper) to estimate and map surface suspended sediment concentrations in large lakes and rivers. MODIS NIR reflectivity data on a 250 m x 250 m grid was used for construction of a TSS and Hg distribution maps.

7.4 RESULTS AND DISCUSSION

Data and summary statistics for Hg and water quality are presented in Table 19. There were differences between the sites nearer the dam (e.g., sites 15, 16, 17) and those closer to the Yocona River (e.g., sites 9, 10, 11, 12). The deeper water near the dam tended to be cooler, with higher dissolved oxygen, and slightly higher total dissolved solids and conductivity (Table 19). However, the biggest difference was total suspended solids, which averaged 13.6 ± 1.9 mg/L near the dam and 48.9 ± 5.3 ng/L away from the dam. This likely reflects the deposition (loss) of particles as the water slows as it traverses the lake. This gradient in TSS, which was not apparent visually, was fortunate because it offered an ideal test case for calibrating and validating numerical models of TSS and associated Hg transport (discussed below).

Concentrations of Hg in the water averaged 9.4 ± 3.6 ng/L (1 SD, n=18) and ranged from 4.9 to 16.3 ng/L for unfiltered water. Mercury in the dissolved fraction (<0.45 µm) averaged 7.5 ± 3.2 ng/L and ranged from 3.8 to 14.4 ng/L. A Pearson's correlations coefficient matrix is presented in Table 20. Several variables are highly correlated with each other, such as conductivity and TDS, which is not surprising. Mercury concentration in the water was correlated with TSS ($r=0.68$, $p<0.05$), showing that the Yocona River, the primary source of the suspended material, is a major source of Hg to the lake. Whereas the amount of suspended material influences the Hg concentration in the water, the concentrations also depend on the characteristics and source of the suspended material. Indeed, PBM (ng/g) was negatively correlated with TSS ($r= -0.56$; $p<0.05$, respectively). This likely reflects the distribution of particle sizes in the water, where there is a greater fraction of larger particles (with less surface area) near river inputs (areas with high TSS) compared to near the dam; Hg^{+2} the dominant species of Hg in the water is surface reactive. Of course sedimentation rates are higher near river inputs. The particle size distribution of sediment in the lake is discussed below.

Table 19 Sampling locations and summary statistics for mercury concentrations and water quality data for Enid Lake on March 12, 2013 after a heavy rain event.

Sample Site ID	Location (see figure 3)		Water Quality Data							Mercury Data						Log K_d (L/Kg)
	N	W	T (°C)	Cl- (mg/L)	TDS (mg/L)	DO (mg/L)	ORP (mV)	Cond. (μs/cm)	TSS (mg/L)	Total-Hg (ng/L) Unfiltered	Total-Hg (ng/L) Filtered	PBM (ng/g)	PBM (pg/L)	PBM (ng/g)	PBM (pg/L)	
1	34°.08.687	89°49.033'	11.8	4.9	42.3	9.1	331.5	48.3	39.6	10.1	6.6	157	6.2	157	6.2	4.4
2	34°.08.552'	89°48.967'	12.3	6.7	37.7	10.3	281.6	43.8	24.3	8.1	8.4	164	6.2	164	6.2	4.3
3	34°.08.894'	89°49.053	12.6	10.4	42.9	9.3	313.7	50.7	60.4	8.9	7.4	125	5.8	125	5.8	4.2
4	34°.09.174'	89°49.054'	12.4	5.1	43.6	9.1	332.3	50.9	38.9	7.4	7.8	106	4.1	106	4.1	4.1
5	34°.09.469'	89°49.142	11.7	4.6	42.9	9.5	338.4	49.5	33.7	6.6	9.4	108	5.8	108	5.8	4.1
6	34°.09.291'	89°48.527'	12.1	4.6	43.6	9.1	341.8	50.4	48.1	7.9	7.6	124	3.2	124	3.2	4.2
7	34°.09.328'	89°47.855'	12.1	3.4	37.7	8.7	343.2	44.0	65.6	12.5	9.0	140	3.2	140	3.2	4.2
8	34°.09.358'	89°47.136'	11.8	2.5	33.2	8.3	347.5	38.3	55.2	16.3	12.3	170	7.5	170	7.5	4.1
9	34°.09.439'	89°46.424'	12.4	2.3	34.5	8.9	347.9	40.0	55.7	12.8	8.6	159	4.1	159	4.1	4.3
10	34°.09.175'	89°46.445'	11.6	2.3	33.2	9.1	347.5	38.3	44.3	14.3	14.4	135	3.6	135	3.6	4.0
11	34°.09.023'	89°46.961'	11.4	2.3	33.2	8.3	344.7	37.6	50.2	14.8	12.0	163	6.0	163	6.0	4.1
12	34°.08.816'	89°47.806'	12.6	2.8	35.1	8.3	341.3	41.4	45.2	12.7	5.3	227	9.2	227	9.2	4.6
13	34°.08.656'	89°50.280'	11.6	3.7	39.0	9.9	329.6	44.3	20.5	4.9	4.3	150	9.4	150	9.4	4.5
14	34°.08.393'	89°51.268'	11.1	3.7	37.7	9.7	332.1	42.4	15.2	6.0	3.9	224	8.9	224	8.9	4.8
15	34°.08.594'	89°51.663'	10.7	3.9	37.7	9.7	330.1	42.5	11.7	5.9	4.5	224	6.0	224	6.0	4.7
16	34°.09.246'	89°51.662'	10.6	3.9	38.4	9.7	330.1	43.0	14.6	5.8	4.6	201	8.2	201	8.2	4.6
17	34°.09.700'	89°50.657'	10.5	4.3	39.7	9.5	322.9	44.0	14.3	8.0	4.2	170	10.3	170	10.3	4.6
18	34°.09.580'	89°49.644'	11.1	5.0	40.3	9.2	272.5	45.3	8.6	6.2	3.8	371	3.1	371	3.1	5.0
Mean			11.7	4.2	38.5	9.2	329.4	44.2	35.9	9.4	7.5	173.1	3.4	173.1	3.4	4.4
Median			11.8	3.9	38.0	9.2	332.2	43.9	39.3	8.1	7.5	160.9	2.6	160.9	2.6	4.3
SD			0.7	1.9	3.6	0.6	21.2	4.3	18.5	3.6	3.2	61.8	2.9	61.8	2.9	0.3
Min			10.5	2.3	33.2	8.3	272.5	37.6	8.6	4.9	3.8	106.5	2.4	106.5	2.4	4.0
Max			12.6	10.4	43.6	10.3	347.9	50.9	65.6	16.3	14.4	370.6	3.2	370.6	3.2	5.0

PBM = Particle Bound Mercury (>0.45 μm)

Table 20 Pearson's correlation matrix for water parameters measured in Enid Lake on March 12, 2013.

Only p-values <0.05 are shown.

	T (°C)	Cl^- (mg/L)	TDS (mg/L)	DO (mg/L)	ORP (mV)	Cond. (µs/cm)	TSS (mg/L)	Total-Hg-Filtered (ng/L)	Total-Hg Unfiltered (ng/L)	PBM (ng/g)	PBM (ng/g)
TDS (mg/L)		0.67									
ORP (mV)		-0.58		-0.54							
Cond. (µs/cm)		0.71	0.98								
TSS (mg/L)	0.74			-0.71	0.53						
Total-Hg- Filtered (ng/L)			-0.68	-0.82	0.49	-0.58	0.75				
Total-Hg- Unfiltered (ng/L)				-0.48	0.08		0.68	0.77			
PBM (ng/g)					-0.55		-0.56		-0.52		
PBM (ng/g)					-0.55		-0.56		-0.52		
Log Kd (L/Kg)	-0.53				-0.49		-0.75	-0.55	-0.88	0.84	0.84

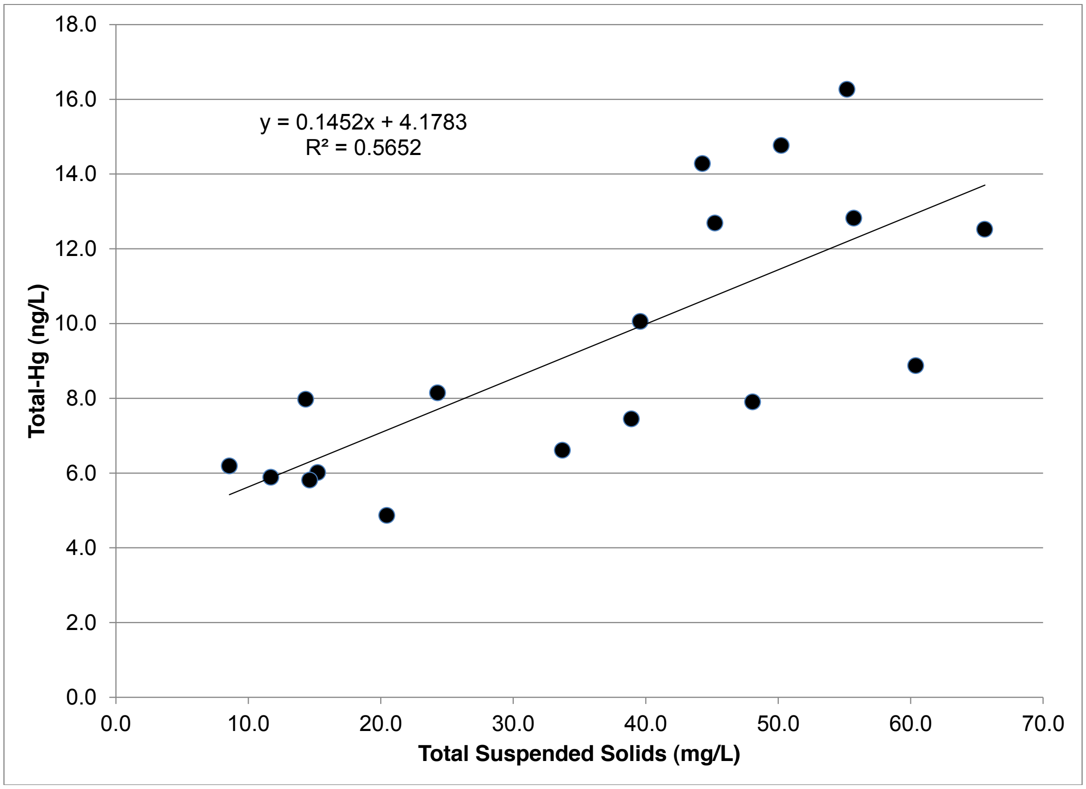

Figure 51 Relationship between Hg levels in the water and TSS for Enid Lake on March 12, 2013.

7.4.1 Relationship between Hg with particle size and organic matter

Results for Hg concentration, particle size distribution (PSD) and loss-on-ignition (LOI) in sediments from Enid Lake is given in Table 21. The PSD of sediment by location in the lake

is shown in figure 52. The sediment is dominated (>80%) by fines (<125 µm) in diameter. Not surprisingly there are more fines in the sediment closest to the dam compared to near the inflow of the Yocona River, though the middle two points are in between (Figure 52). Levels of Hg in the sediment varied by size-class, with concentrations greatest for particles <125 µm and lowest for particles between 250 µm and 500 µm (Figure 53). Data is not shown for "particles" larger than 500 µm because there was either too little material to analyze or it consisted of fragments of partially decomposed organic material like twigs and leaves. From figure 53 it can also be seen that the sediment closer to the river (Enid 3 and 4) had higher concentrations than open water areas nearer the dam (Enid 1 and 2). Organic matter, as determined by LOI, showed a similar trend (Figure 54), further demonstrating the strong relationship between Hg and organic matter.

Table 21 Particle Size Distribution (PSD), Total-Hg and Loss-On-Ignition (LOI) for surficial sediments from Enid Lake (5/14/13).

Sample ID:	**Enid 1**			**Enid 2**			**Enid 3**			**Enid 4**		
Coordinates:	34°10'05.4"N 89°43'49.7"W			34°9'06.35"N 89°47'13.1"W			34°9'1.20"N 89°50'18.24"W			34°08'51.8"N 89°53'22.5"W		
Size Fraction (µm)	PSD % of Total	Total-Hg (ng/g)	LOI (%)	PSD % of Total	Total-Hg (ng/g)	LOI (%)	PSD % of Total	Total-Hg (ng/g)	LOI (%)	% of Total	Total-Hg (ng/g)	LOI (%)
Bulk Sample	-	40.7 ± 3.1	2.6 ± 2.5	-	41.9 ± 1.4	3.6 ± 1.5	-	89.7 ± 1.7	14.1 ± 6.9	-	88.5 ± 1.8	9.4 ± 2.9
<125	93.5	70.9 ± 1.8	6.3 ± 0.6	81.6	68.6 ± 4.1	4.9 ± 0.5	92.9	140 ± 1	12.3	83.4	136 ± 4	24.5 ± 2.2
125-250	4.6	6.4	3.5	14.2	46.6	0.7	4.7	96.3	15.4	8.1	106	16.4
250-500	0.9	0.6	10.9	3.6	79.0	35.5	2.3	91.9	10.6	4.2	97.5	12.3
500-1000	0.9	0.0	55.2	0.3	233	32.5	0.0	-	-	4.3	89.6	21.7
>1000	0.2	80.0	25.2	0.2	361	71.2	0.0	-	-	0.0	-	-

Sample 1 and 2 were nearer the dam, sample 3 and 4 nearer the Yocona River. Uncertainty is ± 1 standard deviation

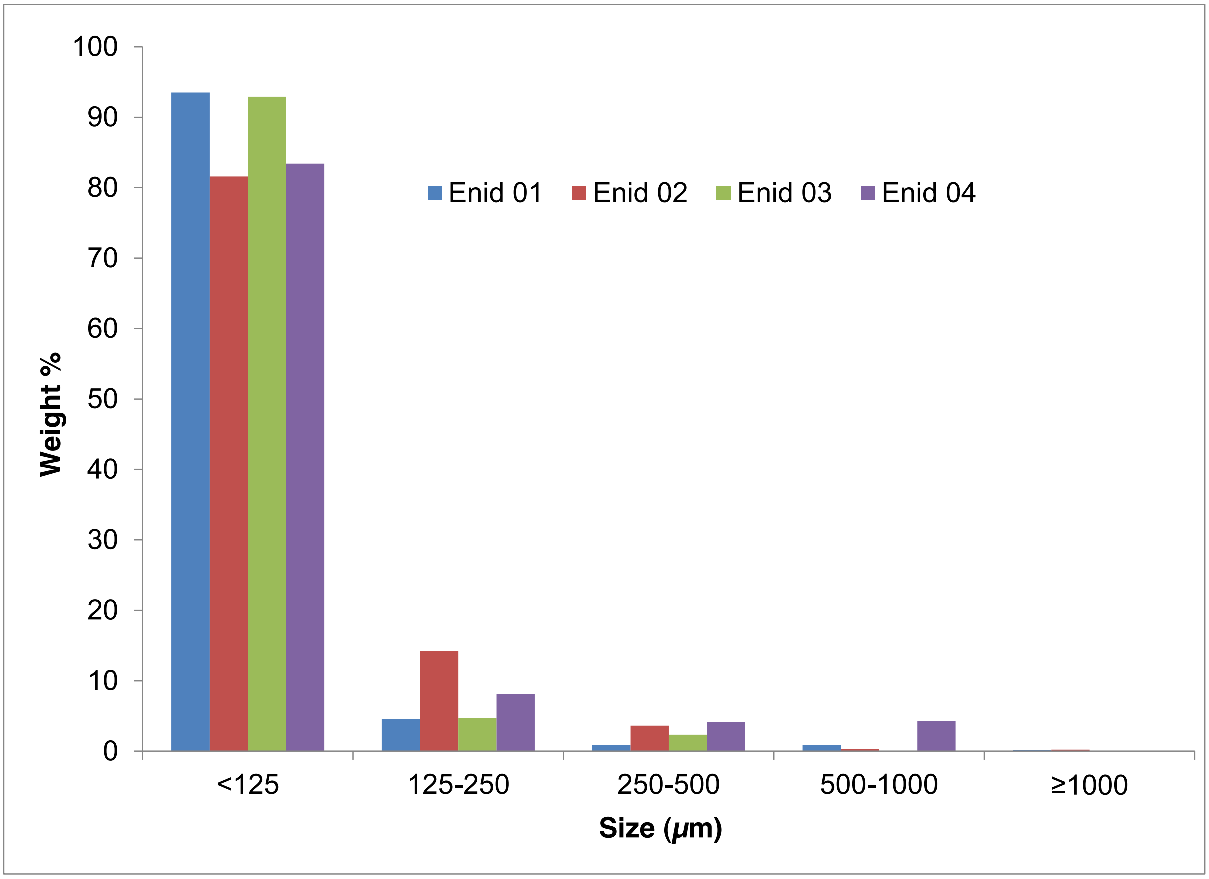

Figure 52 Particle size distribution in sediment collected from Enid Lake. Enid 1 is nearer the dam, Enid 4 nearer the Yocona River.

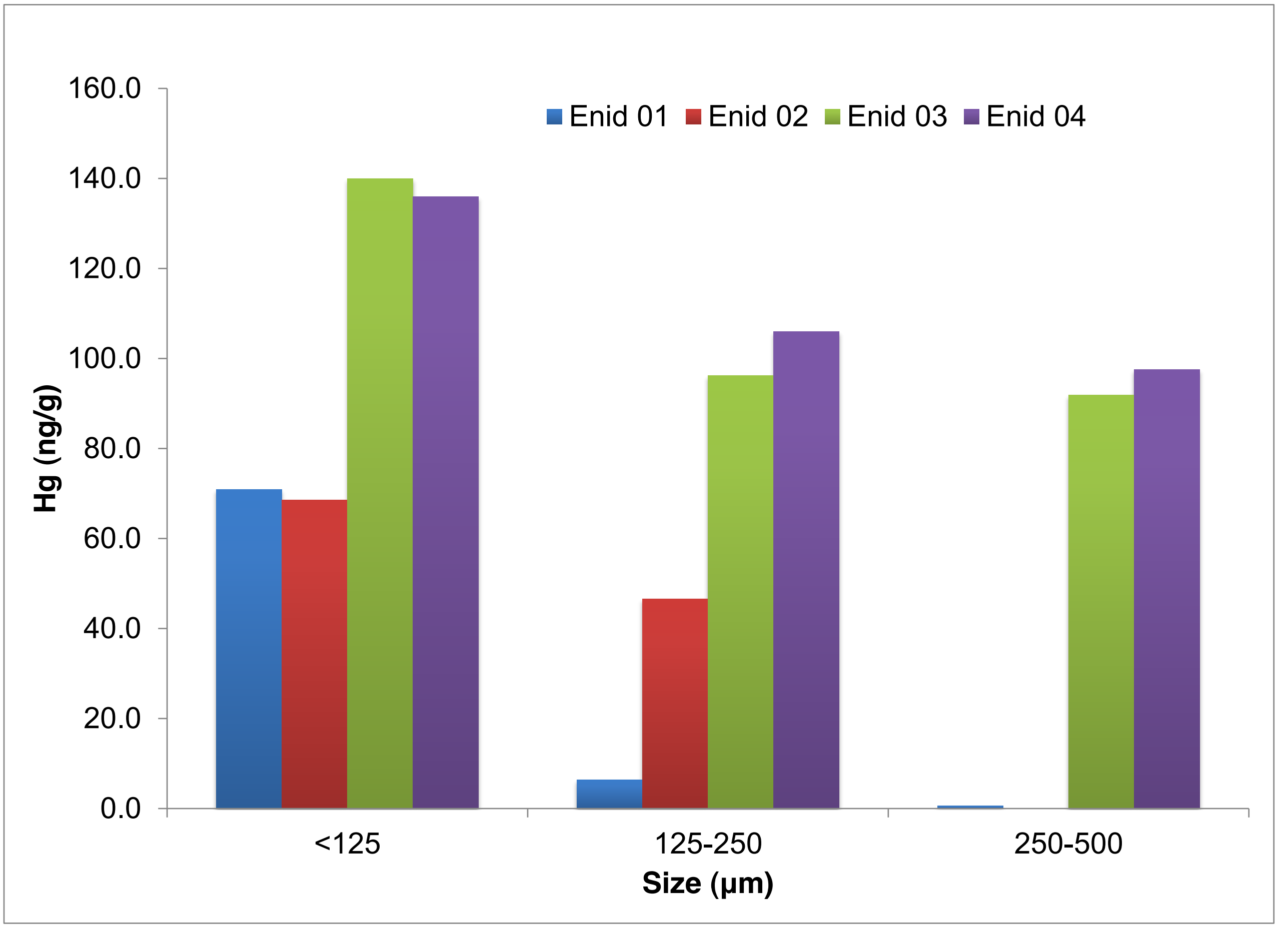

Figure 53 Mercury versus particle size for sediment from Enid Lake.

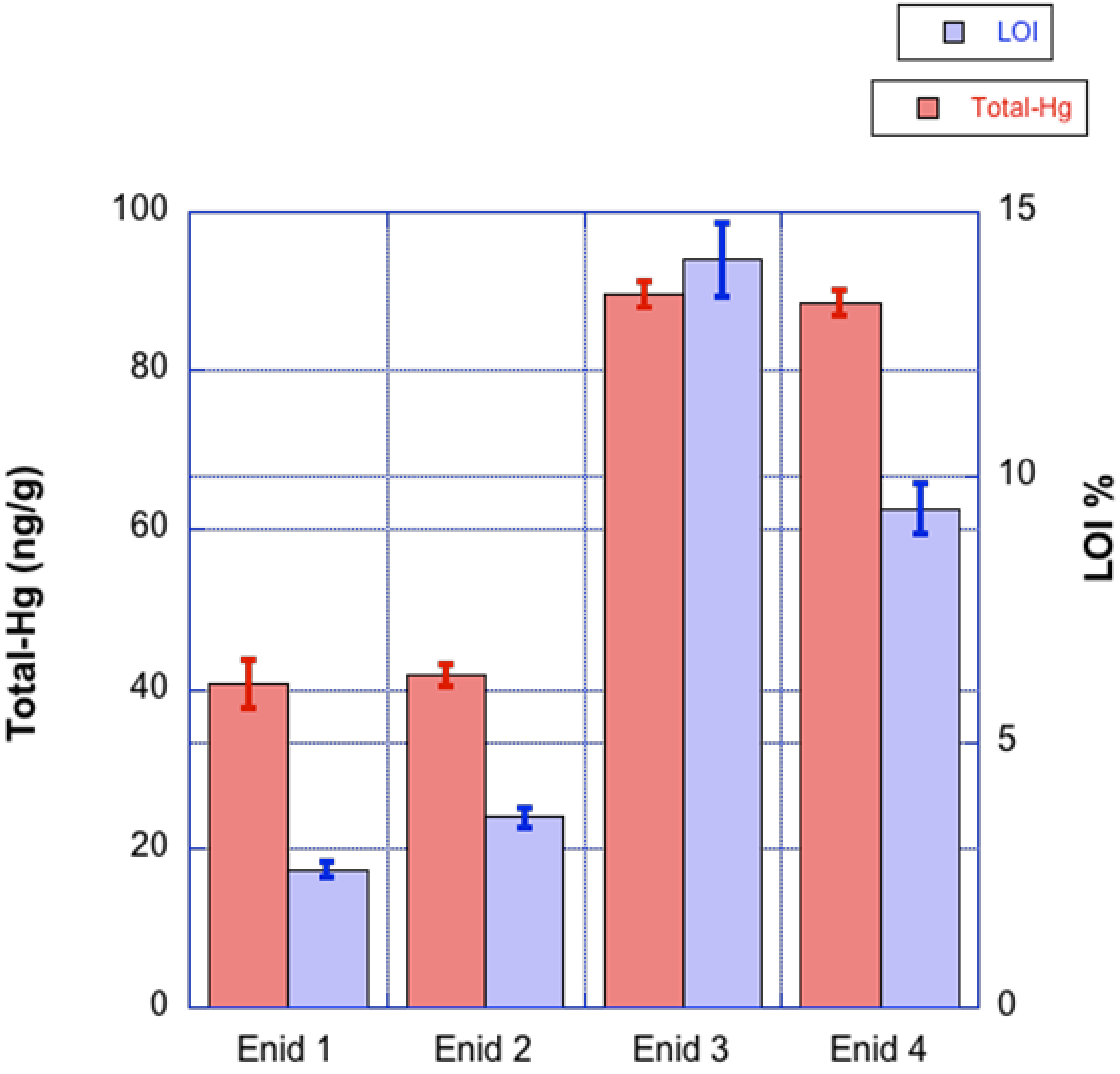

Figure 54 Mercury concentrations and Loss-On-Ignition (organic matter) data for bulk sediment transecting on a transect of Enid Lake. Enid 1 and 2 are nearer the dam, Enid 3 and 4 nearer the Yocona River.

7.4.2 Remote sensing and modeling

The relationship between reflectivity in the NIR measured by the MODIS satellite imagery and TSS and Hg concentrations are shown in Figures 55 and 56. In both cases the parameters were positively correlated with an r-square value of about 0.6. This reflects, as has been discussed elsewhere, the binding affinity of Hg with suspended solids. The data was mapped by NCCHE (Figures 57 and 58). Gradients in both TSS and Hg concentrations are clearly evident, showing the influence of the Yocona River on the distribution of suspended material and Hg. The intense red color at the dam is an artifact due to reflectance from the dam surface and should be ignored. The model does not account for the effects of wind on the surface, which can induce turbulence and alter the flow patterns. Future work will involve collecting additional measurements of TSS using a field probe, rather than the in-laboratory analysis that were conducted in this study. This will allow a large number of data points to be acquired to validate the model that was developed.

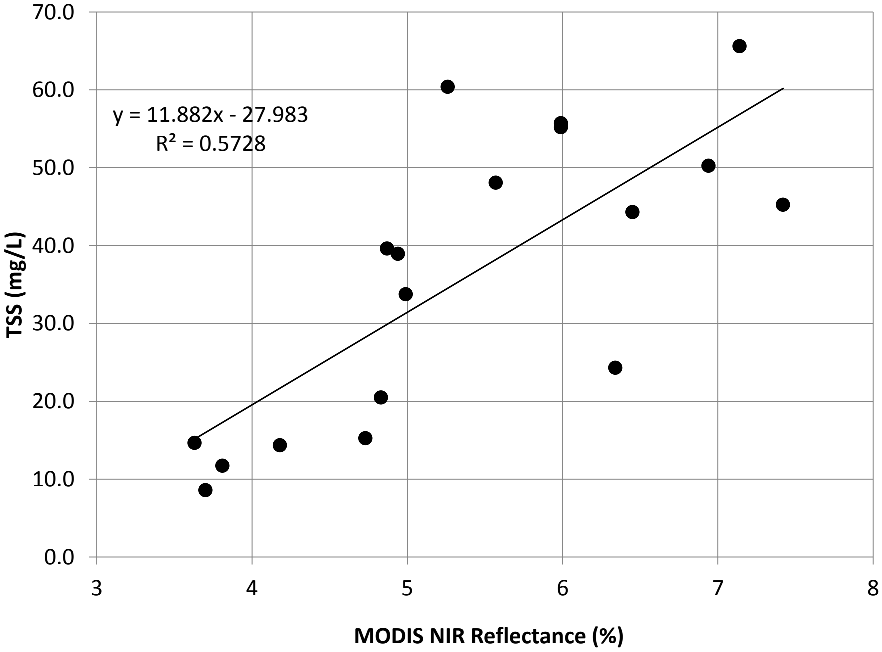

Figure 55 Correlation between MODIS NIR Band and Total Suspended Solids for water collected from Enid Lake on March 12, 2013. A similar correlation was found

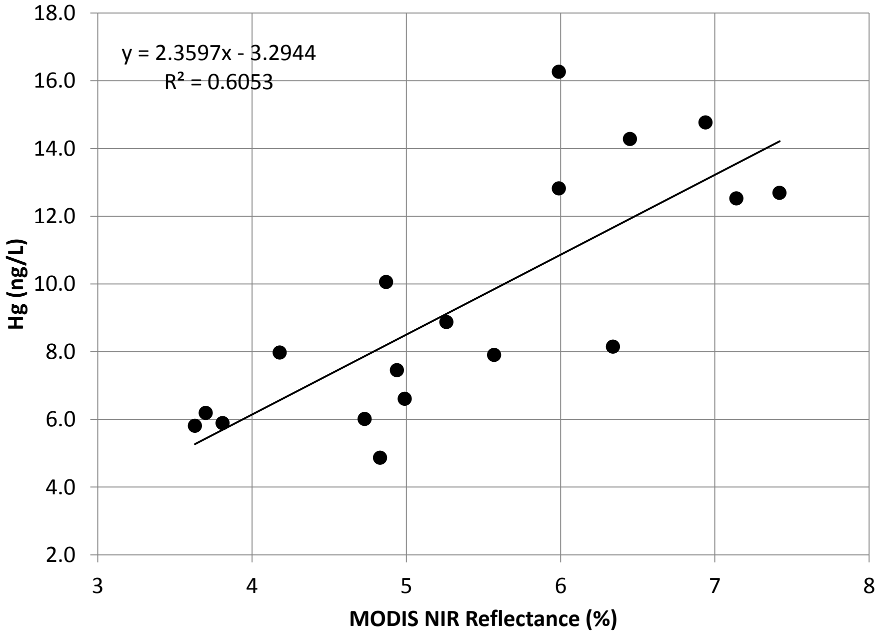

Figure 56 Correlation between MODIS NIR Band and Hg concentrations for water (unfiltered) from Enid Lake on March 12, 2013.

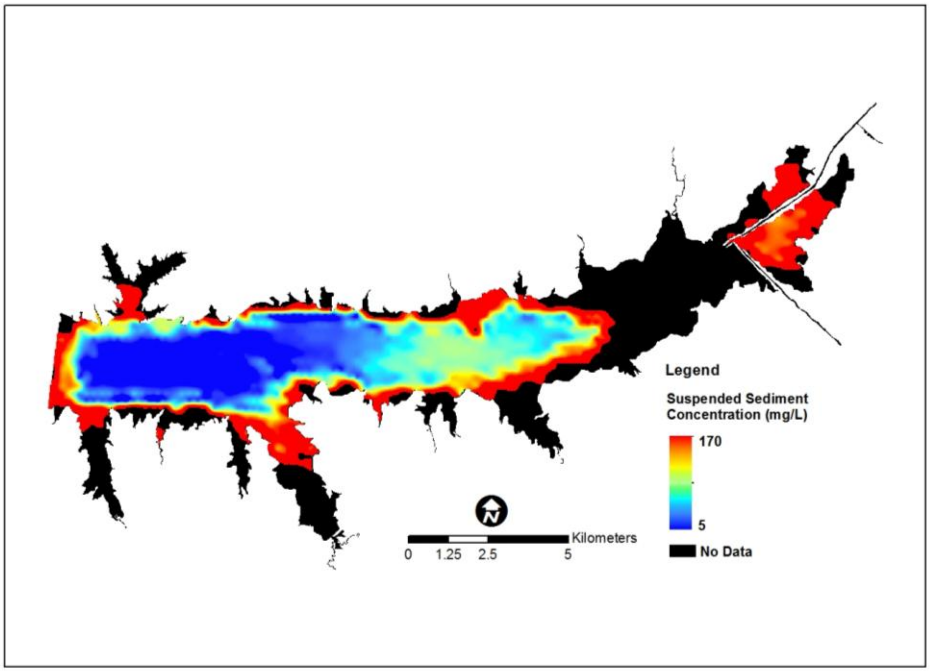

Figure 57 Distribution of suspended sediments in Enid Lake as estimated by MODIS remote sensing imagery acquired on March 12, 2013.

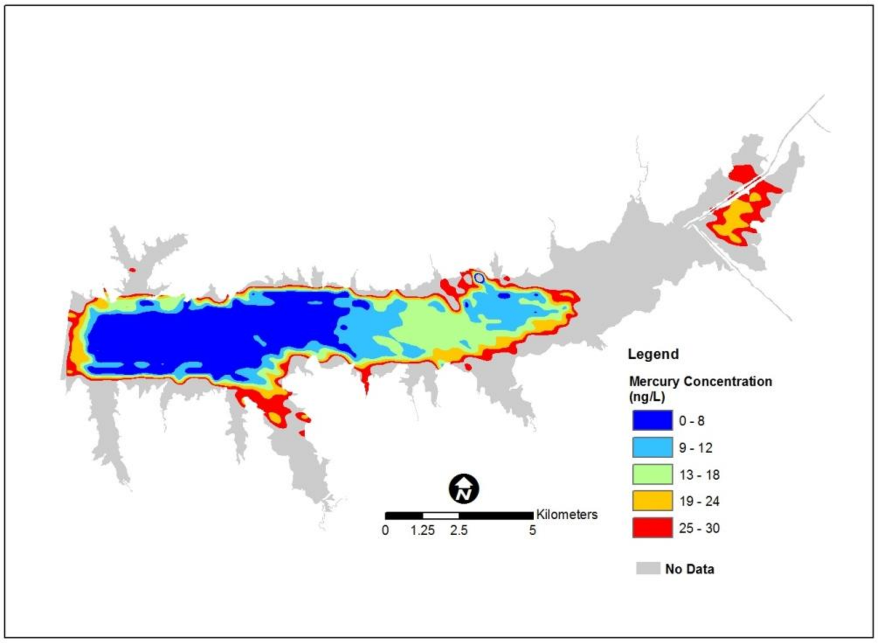

Figure 58. Distribution of Hg concentrations in Enid Lake as estimated by MODIS remote sensing imagery acquired on March 12, 2013.

7.5 CONCLUSIONS

Mercury in water, suspended sediment, and bottom sediment, as well as a wide range of water quality parameters were determined at multiple locations in Enid Lake following a heavy rainstorm event. Particle size and organic matter content and water flow were important factors controlling the distribution of Hg in the lake. Our data has shown that satellite imagery can be used to model suspended solids and Hg transport in relatively large reservoir systems, thereby providing a new tool for lake managers and stakeholders to monitor changes in the lake, assess Hg distribution fundamentals, and to evaluate the effectiveness of established TMDLs.

Acknowledgements

We are extremely grateful and acknowledge the contributions from Dr. Xioabo Chao and Dr. Azad Hossain at the NCCHE for contributing to the modeling and satellite imagery aspects of this study and Dr. Clifford Ochs for providing the sampling boat.

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CHAPTER EIGHT

8. MERCURY SPECIATION IN GULF WATERS NEAR THE DEEP WATER HORIZON OIL SPILL AND IN THE HYPOXIA ZONE NEAR THE MOUTH OF THE MISSISSIPPI RIVER, AND MERCURY IN WETLAND FISH

8.1 INTRODUCTION TO CHAPTER

The work described in this chapter includes a preliminary assessment of total-Hg and MeHg concentrations in northern Gulf of Mexico waters, focusing on the hypoxia zone that develops in the mouth of the Mississippi each summer, and in areas impacted by the Deepwater Horizon Oil Spill. The purpose was to determine whether there is enhanced methylation in the water column in areas where oxygen is depleted and carbon sources are abundant, and to conduct some of the first measurements for MeHg post Deepwater Horizon Oil Spill.

Finally, the last study that is discussed involves the capture and tagging of fish from a wetland. During this process fish were measured and a muscle plug was removed using a biopsy punch. The fish were then released and the muscle tissue was analyzed for Hg. The objective was to repeat the process at a later date to study the Hg dynamics in the same fish as it grows and ages; however we have not been successful retrieving a tagged fish.

8.2 MERCURY SPECIATION IN SEAWATER FROM AREAS IN THE GULF OF MEXICO IMPACTED BY THE DEEPWATER HORIZON OIL SPILL

8.2.1 Introduction

A recent report from the National Science and Technology Council Committee on the Environment and Natural Resources on MeHg in the Gulf of Mexico stated that it is critical to continue and expand research and monitoring efforts to better understand the chemical and biological processes that control the bioaccumulation of MeHg and its concentration in fish and shellfish (NSTC 2004). The long-term impact of the Deep Water Horizon Oil Spill in the northern Gulf of Mexico is unknown. Oil introduced into the ecosystem from the spill may have an altering effect on MeHg levels in the water.

Whereas analysis of total mercury in water is relatively routine, mercury speciation is more difficult. Levels of MeHg, often ng/L or parts-per-trillion (ppt) or less, are generally an order of magnitude lower than inorganic (Hg+2) concentrations. In addition, the MeHg must be separated from other forms of mercury prior to analysis. A number of analytical approaches have been used to measure MeHg, including liquid chromatography with cold vapor atomic fluorescence detection (LC-CVAFS) (Chiou 2001), LC coupled with inductively coupled plasma mass spectrometry (LC-ICPMS) (Bramanti 2005), and gas chromatography (GC) (Lansensa 1990).

In this study, we analyzed water collected using clean techniques from areas in the Gulf Coast impacted by the oil spill. The samples were analyzed using a new MeHg analyzer. The system employs aqueous phase ethylation, gas chromatography, and cold vapor atomic fluorescence spectrometry (CVAFS).

8.2.2 Materials and Methods

Water was sampled using clean techniques from areas in the Gulf Coast impacted by the oil spill. Samples were collected from eight stations just south of Bay Saint Louis, MS using either a teflon-coated external spring Niskin bottle or the ship's rosette sampler with metal-clean GoFlo bottles (Figure 59). The water was then transferred to acid washed Teflon bottles and shipped overnight to our lab for analysis. The samples were passed through a 0.45 μm glass fiber filter and both filtered and unfiltered samples were preserved to 0.4% H_2SO_4. Samples were analyzed using a Tekran MeHg analyzer (described in previous chapters)

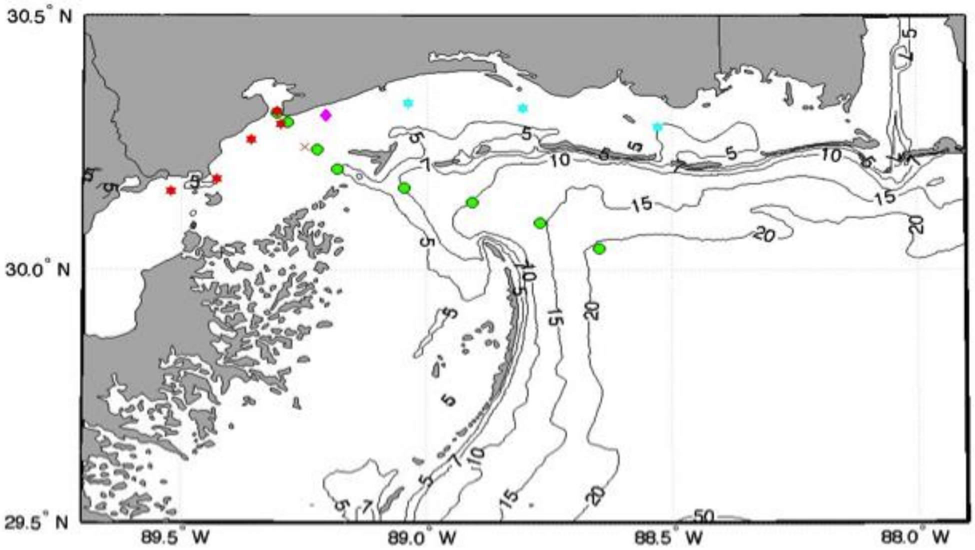

Figure 59 Map showing the Mississippi Gulf Coast (near Bay St. Louis) and sampling areas (green circles).

8.2.3 Results and Discussion

MeHg concentrations in seawater from near Bay St. Louis, Mississippi averaged 0.025 ng/L and from ranged from 0.012 to 0.051 ng/L (Fig. 60). These levels are in the range of what others have found in seawater outside the Gulf of Mexico (Bowles and Apte, 1998). There were no distinctive spatial trends (across the transect), except for high levels for the filtered sample from station 5 (which was perhaps contaminated). The average dissolved oxygen for a depth of 3 m for all transacts in the month of July in 2010 and 2011 was 5.96 mg/l.

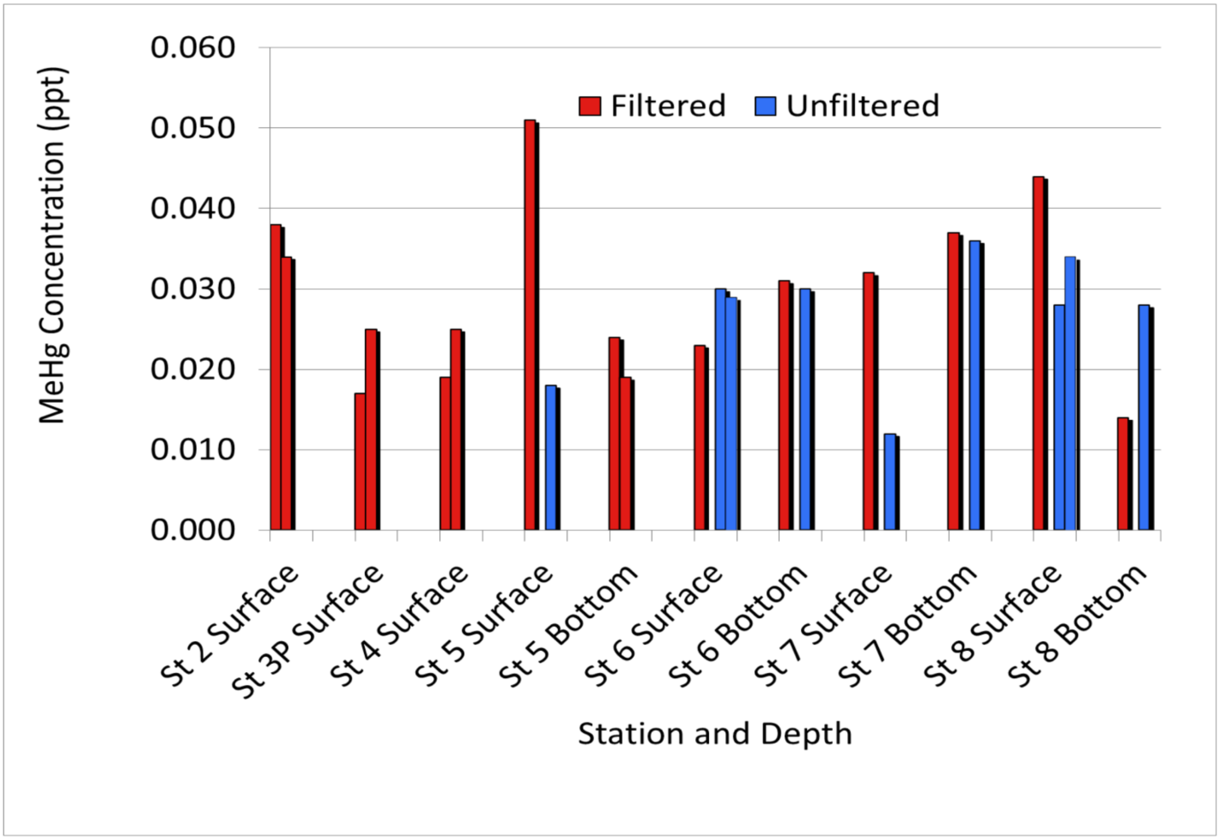

Figure 60 MeHg in the Gulf of Mexico near the Deep Water Horizon Oil Spill. "St" represents the station sampled (see figure 59).

Whereas the levels of MeHg in the Gulf samples were not particularly high, it should be noted that the impact of the Deep Water Horizon oil spill in the Gulf of Mexico on the distribution and cycling of MeHg is of continued interest. Over time the oil and dispersants may alter the element's complex biogeochemical cycling due to: proliferation of hydrocarbon-degrading- and possibly methylating microorganisms; changes in redox conditions as a result of increased microbial activity; higher levels of dissolved organic carbon which is known to affect Hg bioavailability; and the shear amount of Hg introduced into the ecosystem from the oil itself.

8.2.4. Conclusions

Concentrations of MeHg in the Gulf of Mexico do not appear to be impacted by the Deepwater Horizon Oil Spill. Both basic research and long-term monitoring efforts for MeHg at strategic locations in the Gulf should be a high priority given that the influence of the oil and dispersants on the formation and fate of MeHg is not known.

Acknowledgements

We thank the US EPA for funding this project (EPA Wetland Grant CD-95450510-0), and Alan Shiller and co-workers at the University of Southern Mississippi for providing samples from the Gulf of Mexico.

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8.3 MERCURY SPECIATION IN SEAWATER FROM THE HYPOXIA ZONE NEAR THE MOUTH OF THE MISSISSIPPI RIVER

8.3.1 Introduction

Each year tons of nutrients from the Mississippi River create hypoxia in the waters near its mouth as a result of eutrophication (Kim et al. 2013). Waters are classified as hypoxic when dissolved oxygen (DO) levels fall below 2-3 mg/L, which is insufficient to sustain much aquatic life (MR/GoM Watershed Nutri. Task Force, 2004). Normal DO levels, which support aquatic life, are between 8-10 mg/L.

Methylation of Hg occurs by sulfate reducing bacteria, which are commonly found in- and stimulated by- low oxygen environments. There is very little Hg speciation data in the Gulf of Mexico (GoM); influence of the dead zone on Hg speciation is not well-understood. In this study, samples from the GoM were collected from the Chandler Sound region of the northern GoM in the hypoxia zone near the mouth of the Mississippi. Samples were collected during the months of July, when the hypoxic zone is largest, and during the following two months when the system slowly recovers.

8.3.2 Materials and Methods

Samples of seawater were collected from Chandler Sound in the northern GoM (Fig. 61). Samples were collected using clean protocols described in chapter 6. Samples were shipped overnight to the University of Mississippi, Department of Chemistry where they were preserved to 0.4% using 12N H_2SO_4. Samples were analyzed following EPA methods 1630 using a Tekran MeHg analyzer (discussed in detail in previous chapters).

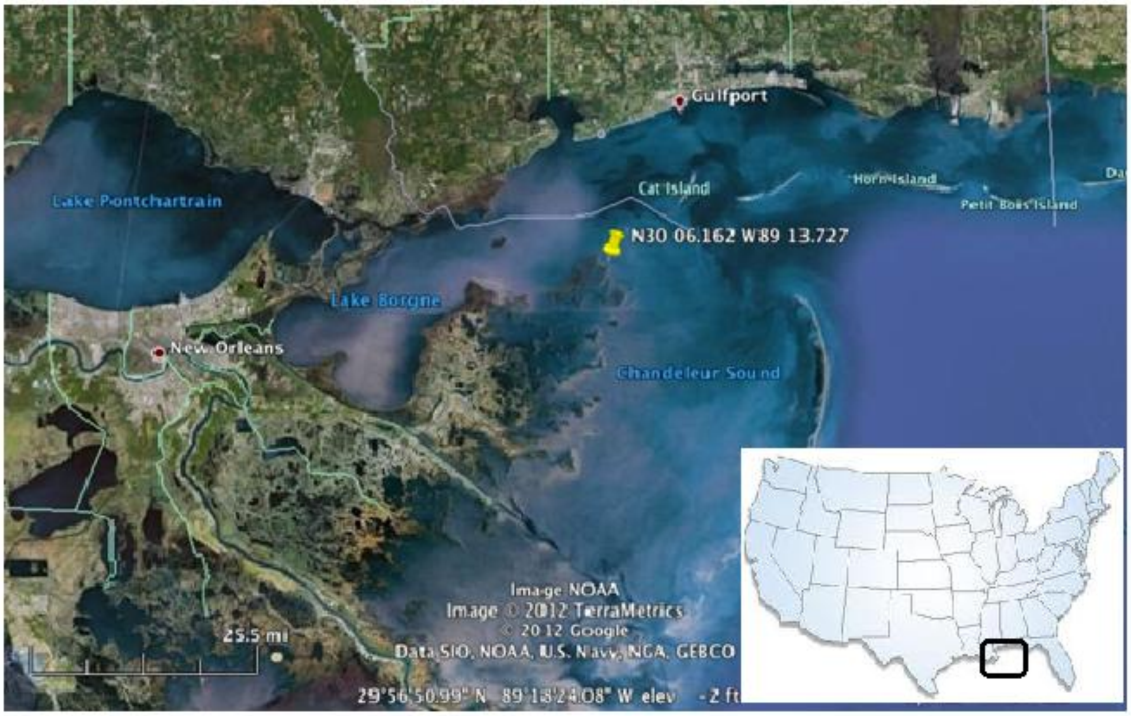

Figure 61 Map showing the Chandler Sound region of Gulf of Mexico (near Plaquemines Parish, LA). General sampling area is designated with a yellow tack.

8.3.3 Results and Discussion

Total-Hg and MeHg concentrations for unfiltered water from Chandler Sound by summer month are shown if Figure 62. Concentrations for total-Hg and MeHg were slightly higher during the height of the hypoxia season (July) compared to the subsequent two months. Total-Hg concentrations averaged ~4 ng/L and ranged from 3 to 6 ng/L; MeHg concentrations averaged ~0.07 ng/L and ranged from 0.04 to 0.08 ng/L. Levels of MeHg were similar (within error) between filtered and unfiltered samples, suggesting that most of the MeHg is in the dissolved form. This was not the case for total-Hg for July and August (Fig. 62), which may have some turbidity left over from the high flow of the Mississippi in the spring and early summer. The percentage of Hg associated with particulates decreased from about 35% in July to ~20% in August and <10% in September.

Levels of MeHg were higher than samples collected east of the mouth of the Mississippi River, further east from the hypoxia zone (near Bay St. Louis), which averaged 0.025 ng/L (see Section 8.2). The average DO for the sample location of Chandler Sound was also lower 4.9 mg/L compared to 6.0 mg/L. The relationship between MeHg and DO is shown in Figure 64. In Chandler Sound, the DO apparently remind close to DO levels in July and August and increased during the sampling month of September. Interestingly, MeHg levels dipped during August, opposite of what we hypothesized. The reason for this is unclear. More sampling during the summer and other seasons, beyond the scope of this pilot study, are needed to get a better picture of the dynamics of Hg species in the hypoxia zone. It should be noted that DO in the Chandler Sound measured in this study was low but still sufficient to sustain aquatic life (~2 ppm).

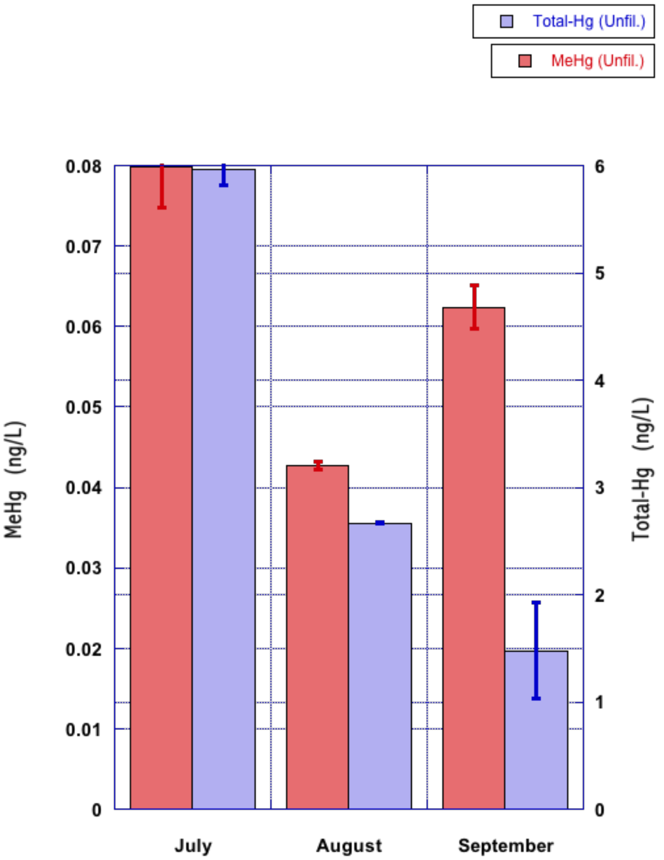

Figure 62 MeHg (red) and Total-Hg (blue) concentrations in seawater from Chandler Sound in the northern Gulf of Mexico in the summer months of 2012.

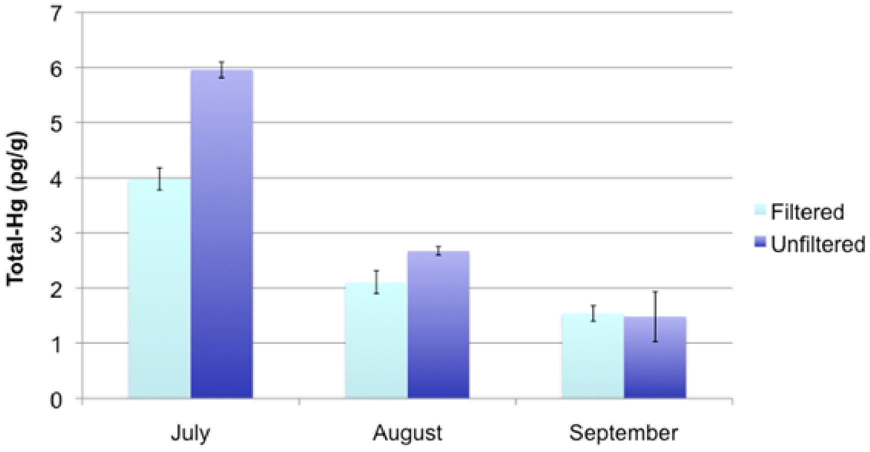

Figure 63 Concentrations of total-Hg in in seawater from Chandler Sound in the northern Gulf of Mexico in the summer months of 2012.

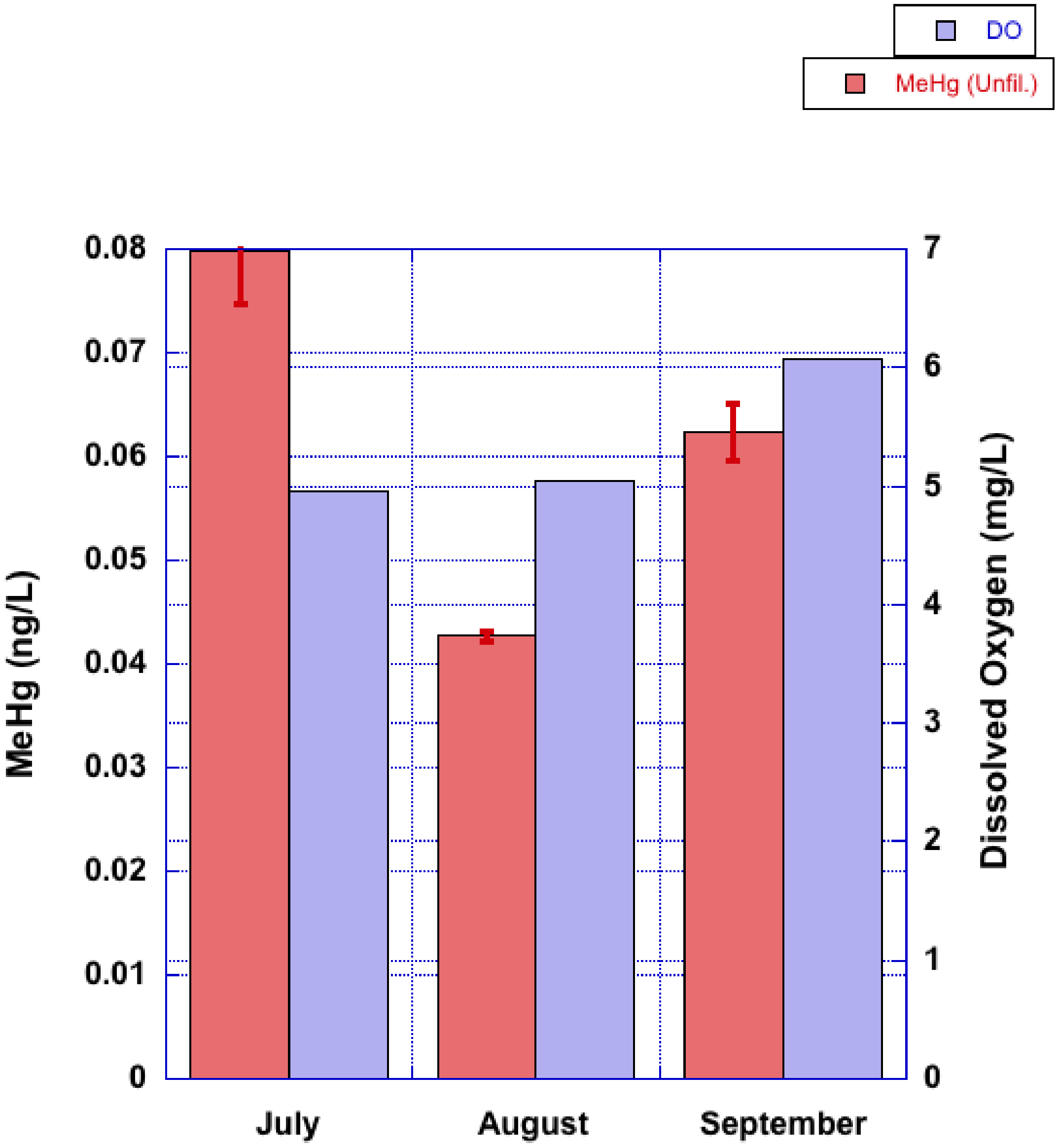

Figure 64 MeHg concentrations (red) and dissolved oxygen levels (blue) in seawater from Chandler Sound in the northern Gulf of Mexico in the summer months of 2012.

8.3.4 Conclusions

The levels of MeHg collected in the summer months from the hypoxia zone near the mouth of the Mississippi River were only slightly elevated compared to neighboring regions. The DO levels observed in this study, although low, were above the threshold level for aquatic life sustainability. Future work should target lower DO levels and sample during fall, winter, and spring seasons to obtain a more complete picture and understanding of the dynamics of Hg species in the hypoxia zone in order to determine if it serves as a net source of MeHg to Gulf waters.

Acknowledgements

We thank the US EPA (EPA Wetland Grant CD-95450510-0) and the University of Mississippi Graduate Student Council Research Grant for funding this project, and Chris Schieble and co-workers at the University of New Orleans Nekton Research Laboratory for providing samples from the Gulf of Mexico.

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8.4 TEMPORAL CHANGES IN MERCURY LEVELS IN INDIVIDUAL FISH FROM A WETLAND IN NORTH MISSISSIPPI USING TAGGING AND NON-LETHAL MUSCLE PLUG COLLECTION

8.4.1 Introduction

Fish consumption advisory was issued for the Enid Reservoir in May 1995 and Yocona River in September 1996 due to high levels of Hg in fish sampled in the waters. The FDA criteria for Hg in fish tissue are 1 ppm; many of the fish exceeded this level. There are no known point sources for the high levels of Hg in the reservoir fish. Wetland that feed into the Enid River maybe a potential source to the load of MeHg entering into the impaired river due to anoxic the conditions in which wetlands contain.

Wetlands benefit the natural environment in a number of ways, including providing habitat for wildlife and natural filtering of water impurities. Under certain conditions, however, they can also have negative impacts. For example, Kesterson reservoir, situated in Merced County, CA, resulted in severe poisoning of fish and birds because of selenium accumulation. With respect to Hg, wetlands are known to be sources of MeHg to ecosystems and may explain the high levels of Hg found in fish in remote and near-pristine regions (Mierle and Ingram 1991).

Typically fish that are analyzed for Hg are sacrificed, however sampling using biopsy punches has been shown to be a non-lethal alternative (Cizdziel 2002). The goal of this study was to use, for the first time to our knowledge, non-lethal sampling combined with fish-tagging to study the Hg dynamics in individual largemouth bass (*Micropterus salmoides*) as they grow, age and accumulate Hg from their diet. Unfortunately, to date we have not successfully recaptured tagged fish so this report focuses on the levels found in the initial sampling. 8.4.2 Materials and Methods

Largemouth bass were collected using artificial lures with a rod and reel from a wetland located near the University of Mississippi (Figs. 65 and 6). GPS coordinates for ponds 1 and 2 are 34° 21.089' N 89° 33.359' W, and 34° 21.261' N 34° 33.221' W, respectively. A muscle-plug was collected from the dorsal part of the fish using a 5 mm diameter sterile biopsy punch and put into a vial. The fish were tagged before release back into the pond. The vial was placed on ice in a cooler. The tissue was later analyzed for Hg by combustion-AAS as described in other chapters.

Figure 65 Map showing the location of the wetland (upper left), picture of wetland II (upper right), fish “sampling” (lower left), and fish tagging lower (right).

8.4.3 Results and Discussion

The largemouth bass had an average weight was 734 g (35.4 cm) and ranged between 175 to 1446 g. Mercury concentrations in the muscle-tissue ranged from 973 to 1744 ng/g, fresh weight (Table 22). The Food and Drug administration criteria for the maximum level of Hg composed in fish tissue is set as 1.0 µg/g for individual consumption. The concentrations of Hg in all but one of the bass from the wetland exceed this amount. Comparing largemouth bass from the wetland to those from Enid, Sardis, and Grenada reservoirs (Chapter 3) shows that the fish from the wetland have significantly higher concentrations ($p<0.05$) (Figs. 66 and 67). This likely reflects the higher methylation rate found in wetlands, which are known to be hotspots for MeHg (see Chapter 6).

Table 22 Mercury concentrations in fish muscle plugs from wetlands in North Mississippi.

Date	Tag No.	Length (cm)	Weight (g)	Mercury (ng/g) dry wt	Mercury (ng/g) wet wt
11/25/09	2453	NA	1362	8718	1744
11/25/09	2454	24.1	175	7450	1489
11/25/09	2455	48.3	1446	7738	1547
11/28/09	2456	35.6	565	4863	973
12/17/2009	2457*	31.4	325	5328	1066
12/22/09	2458	36.8	667	6356	1271
12/22/09	2459	36.3	599	6714	1343
*All fish collected from pond II, except tag # 2457 (pond I)					

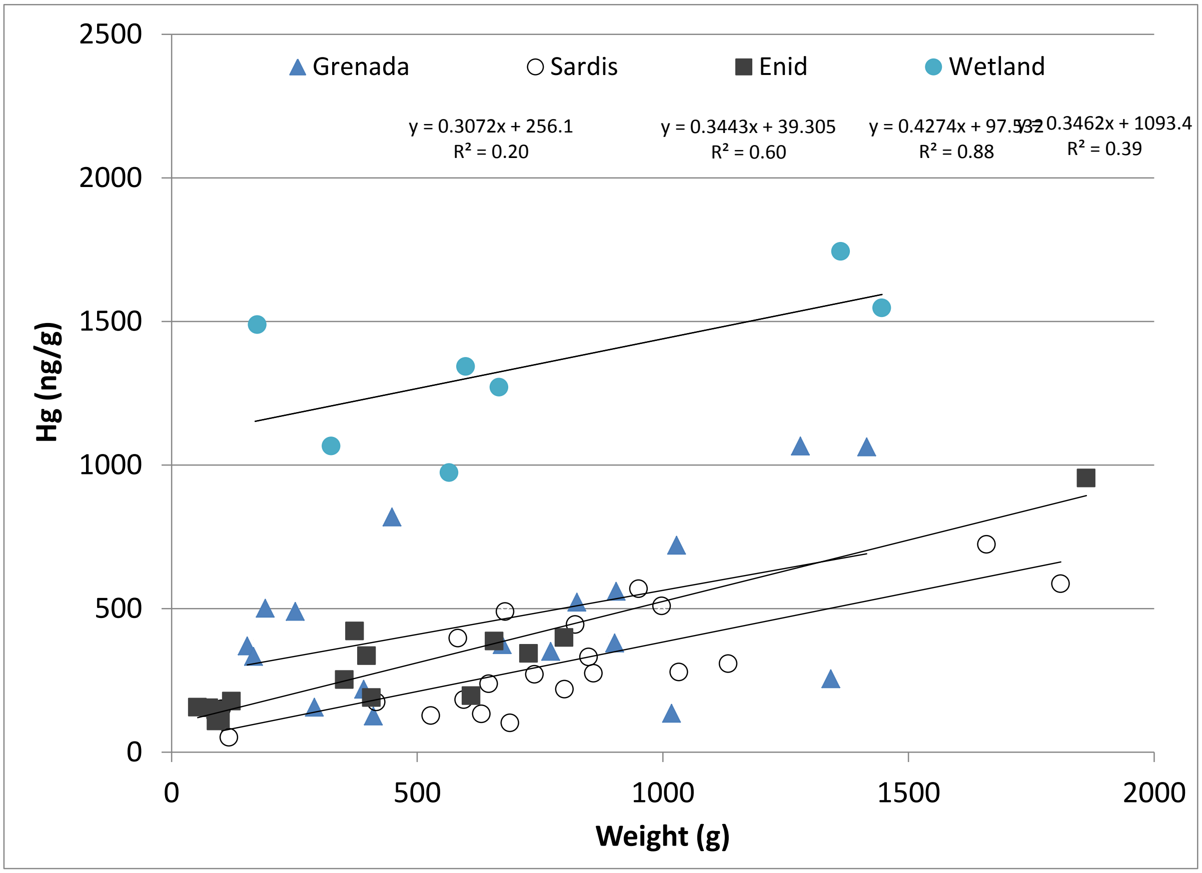

Figure 66 Total-Hg concentrations in fish-muscle vs. weight for Largemouth Bass collected from Grenada, Sardis, and Enid Lakes and a wetland in north Mississippi.

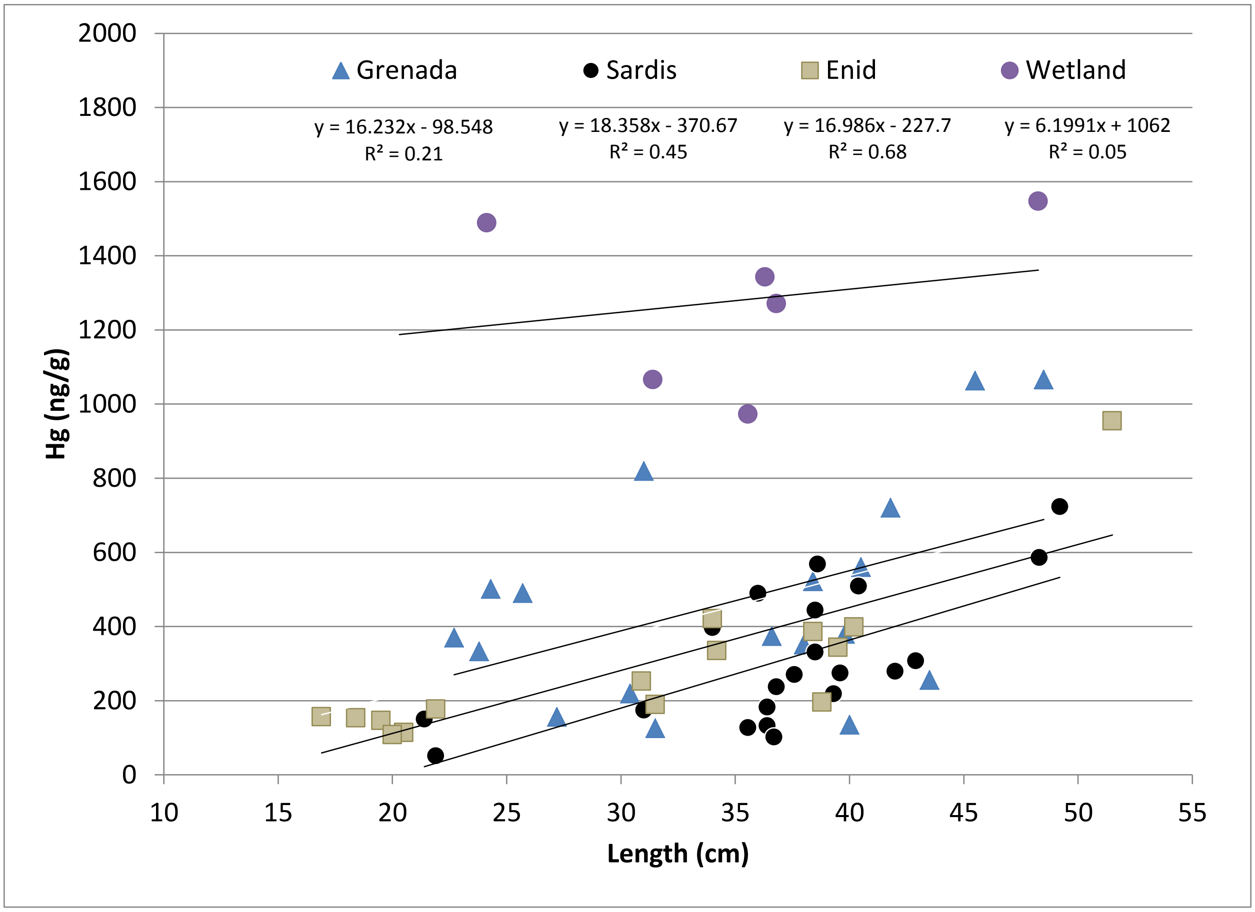

Figure 67 Total-Hg concentrations in fish-muscle vs. length for Largemouth Bass collected from Grenada, Sardis, and Enid Lakes and a wetland in north Mississippi.

8.4.4 Conclusions

Concentrations of Hg in muscle-tissue of largemouth bass from a wetland area in the Yocona River watershed are statistically higher ($p<0.05$) than bass from nearby larger reservoirs. The levels in most of the bass exceed the US EPA and FDA action levels for consumption of fish due to the toxic effects that may result. The primary factor associated with the high levels of Hg in wetland bass is likely higher methylation rates in the wetlands by sulfate reducing bacteria. Future studies should re-capture the tagged fish to study the changes that have occurred in the fish and to assess Hg loads in wetland areas. Ongoing measurements of Hg in the air will allow study of changes in Hg deposition, and the impact on the fish.

Acknowledgements

We thank the US EPA for funding this project (EPA Wetland Grant CD-95450510-0).

8.4.5 References

Cizdziel, James V., Thomas A. Hinners, and Edward M. Heithmar. "Determination of total mercury in fish tissues using combustion atomic absorption spectrometry with gold amalgamation." Water, Air, and Soil Pollution 135.1-4 (2002): 355-370.

MDEQ. Phase One Mercury TMDL for the Yocona River and Enid Reservoir. (2002) Mierle, G., and R. Ingram. "The role of humic substances in the mobilization of mercury from watersheds." Water Air & Soil Pollution 56.1 (1991): 349-357.

VITA

Xavier University of Louisiana, B.S. in Biochemistry 2003 - 2007

Advisor: Professor Kathleen K. Morgan

Specific Focus: Organic/Physical Organic Chemistry

Undergraduate Thesis Title: Studies toward rearrangement of aziridines to allylic amines

RESEARCH EXPERIENCE

Mercury speciation and cycling in Mississippi waters: 1) Developing a mercury budget for Enid Lake; 2) Studying the impact of the Deep Water Horizon Oil Spill and seasonal hypoxia in the Gulf of Mexico 2008 – 2013

Advisor: Professor James V. Cizdziel, University of Mississippi

Department of Chemistry and Biochemistry, University, Mississippi 38677

Project Description:

Water and sediment from reservoirs and wetlands in North Mississippi and from the Gulf of Mexico is being examined for total-mercury, MeHg, and select trace metals. To measure ultra-trace levels, I used a wide-range of state-of-the-art instruments including, cold vapor atomic absorption spectrometry (CVAAS), cold vapor atomic fluorescence spectrometry (CVAFS), and inductively coupled plasma mass spectrometry (ICP-MS). Samples were collected from strategic locations using clean sampling techniques designed for trace levels of mercury. Standard water quality parameters, such as conductivity, dissolved oxygen, pH, and oxidative reductive potential (ORP) were determined during field sampling events. A Pearson Correlation matrix was established for the concentrations of mercury species and all ancillary data. Trace metal data was analyzed using multivariate statistics to assess similarities between sampling sites (pattern recognition) and to determine the influence of individual elements in classification of the sampling sites. The overall goal is to better understand the biogeochemical cycle of mercury in Mississippi to aid those managing the resources and developing related policy.

β-Elimination of an Aziridine to an Allylic Amine: A Mechanistic Study 2004 – 2007

Advisor: Professor Kathleen Morgan, Xavier University of Louisiana

Department of Chemistry, New Orleans, LA 70125

Project Description:

The mechanism of aziridine compounds and their rearrangement to form allylic amines was investigated. I synthesized target compounds and conducted analysis of each compound using NMR and GC/MS. Our aim was to develop easier access to an array of important allylic amines used for medicinal purposes.

The Study of Phosphido Bridging Ligands 2003 – 2004

Advisor: Professor Michael Adams, Xavier University of Louisiana

Department of Chemistry, New Orleans, LA 70125

Xavier McNair/MORE Scholar Summer Research

Project Description:

This study investigated reaction complexes of the type $Fe_2(CO)_6(\mu\text{-}PR'_2)$ where R and R' are different organic constituents on the phosphido bridging ligands. In particular, we studied reactions in which one of the bridging phosphido ligands became a terminal ligand (i.e., a “bridge-opening” reaction).

TEACHING EXPERIENCE

Graduate Teaching Assistant 2008 – 2010

University of Mississippi, Department of Chemistry and Biochemistry

As a teaching assistant my primary responsibilities were to prepare weekly lectures, administer quizzes, exams, and grade lab reports for general chemistry I and II lab classes.

PUBLICATIONS

1. Garry Brown, Jr., James Cizdziel, Ken Sleeper, Joel Blum, Marcus Johnson. "Assessment of mercury speciation and trace elements in sediment from cold seeps in the northern Gulf of Mexico" In preparation (2013).
2. Garry Brown, Jr., James Cizdziel. "Instantaneous sampling and mercury speciation determination in the Yocona River, during a high storm event: Oxford, Mississippi, U.S.A.". In preparation (2013)
3. Kathleen M. Morgan, Garry Brown, Jr., Monique A. Pichon, Geannette Y. Green. "β-Elimination of an Aziridine to an Allylic Amine: A Mechanistic Study." *Journal of Physical Organic Chemistry* (2011) 1144-1150.
4. Garry Brown, Jr., James Cizdziel. "Concentration of methylmercury in natural water from Mississippi using a new automated analysis system." *Mississippi Water Resource Conference* (2010) 2-9.
5. James Cizdziel, Garry Brown, Candice Tolbert. "Direct analysis of environmental and biological samples for total mercury with comparison of sequential atomic absorption and florescence measurement from a single combustion event." *Spectrochimica Acta Part B 65* (2010) 176–180.

PRESENTATIONS

1. The 11th International Conference on Mercury as a Global Pollutant (ICMGP), Garry Brown, Jr., James Cizdziel. *Mercury speciation in urban, agriculture, and wetland/forest runoff during storm events in a watershed impaired with mercury.* Edinburgh, Scotland 2013.

2. 2012 National Organization for the Professional Advancement of Black Chemist and Chemical Engineers (NOBCChE) Conference, Garry Brown, Jr., James Cizdziel (Oral Presentation). *Preliminary assessment of mercury speciation and trace metals in cold seeps from the northern Gulf of Mexico.* Washington, D.C. September 2012.

3. The 10th International Conference on Mercury as a Global Pollutant (ICMGP), James Cizdziel, Garry Brown, Jr., Pragya Chakravarty, Yi Jiang. *Mercury in North*

Mississippi, USA; Atmosphere Speciation, Historic Deposition, and Concentrations of MeHg in Natural Waters. Halifax, Nova Scotia July 2011.

4. 2011 National Organization for the Professional Advancement of Black Chemists and Chemical Engineers (NOBCChE) Conference, Garry Brown, Jr., James Cizdziel. *Concentration of methylmercury in natural waters from Mississippi.* Houston, TX April 2011.

5. 2010 Mississippi Water Resources Research Institute Conference, Garry Brown, Jr., James Cizdziel. *Concentration of methylmercury in natural waters from Mississippi using a new automated analysis system*. Bay, St. Louis, MS November 2010. (*1st Place Winner*)

6. 2010 National Organization for the Professional Advancement of Black Chemists and Chemical Engineers (NOBCChE) Conference, Garry Brown, Jr., James Cizdziel. *Concentration of methylmercury in natural waters from Mississippi.* Atlanta, GA April 2010.

7. 2007 NOBCChE XULA Regional Chapter Undergraduate Research Oral Presentation, Garry Brown, Jr. *Studies toward rearrangement of aziridines to allylic amines*. New Orleans, LA Spring 2007 (*2nd Place Winner*)

8. 2007 National American Chemical Society (ACS) Conference, Garry Brown, Jr., Kathleen Morgan. *Studies toward rearrangement of aziridines to allylic amines*. Chicago, IL March 2007.

9. Penn State McNair Summer Research Conference, Garry Brown, Jr., Kathleen Morgan. *Studies toward rearrangement of aziridines to allylic amines.* State College, PA July 2006.

10. Xavier University Festival of Scholars, Garry Brown, Jr. Kathleen Morgan. *Studies toward rearrangement of aziridines to allylic amines*. New Orleans, LA April 2006.

HONORS AND AWARDS

NOBCChE National Student Representative (2012-2014)

University of Mississippi Dissertation Fellowship (2013)

Graduate Assistances in Areas of National Need Fellowship (GAANN) (2013)

University of Mississippi Graduate Student Council Research Fellowship (2012)

2010 Mississippi Water Resources Research Institute "Best Graduate Poster" winner (2010)

Runner-up for NOBCChE XULA Regional Chapter Undergraduate Research Oral Presentation. (NOBCChENews OnLine. Spring 2007 vol.37, No. 2 pg. 12) (2007)

Ronald E. McNair Scholar (2005-2007)

Research Initiative for Scientific Enhancement Scholar (2003-2007)

INSTRUMENTATION/SKILLS

Cold Vapor Atomic Absorption Spectrometry (CVAAS)
Cold Vapor Atomic Fluorescence Spectrometry (CVAFS)
2700 Methyl Mercury Analyzer
Shimadzu Total Organic Carbon 5000
Inductively Coupled Plasma-Mass Spectrometry (ICP-MS)
Microwave Digestion
Nuclear Magnetic Resonance (NMR)
Gas Chromatography-Mass Spectrometry (GC-MS)
Electrospray Ionization Mass Spectrometry (ESI-MS)

Skills:

Proficient in statistical analysis and software programs: Statplus 2012, JMP IN, Principal Component Analysis, Cluster Analysis, Microsoft Word, Excel, PowerPoint and ChemDraw. CVAAS, CVAFS spectroscopy instrument repair.

SERVICE AND EXTRACURRICULAR ACTIVITIES

NOBCChE National Conference Planning Committee	(2012-2013)
AmeriCorps	(2011-Present)
Tutor at Oxford Middle School	(2011)
MLK Day of Service	(2011)
911 Day of Service	(2011)
Volunteer at Oxford Mississippi Alternative School	(2009)
Accumulated over 200 hours of community service (New Orleans, LA)	(2003-2007)
Volunteer at HIV/AIDS non-profit organizations (New Orleans, LA)	(1999-2002)

Professional Affiliations

National Organization of Black Chemist and Chemical Engineers	(2008-Present)
National Student Representative	(2012-2014)
Vice-President of U of M student chapter	(2011-2012)
American Chemical Society	(2007)