5-2011

Geochemical Controls on Mercury Methylation in Backwaters of a Gulf Coastal Plain River System, Implications for Water Column Processes

Liam N. Schenk
University of Arkansas, Fayetteville

GEOCHEMICAL CONTROLS ON MERCURY METHYLATION IN BACKWATERS OF A GULF COASTAL PLAIN RIVER SYSTEM, IMPLICATIONS FOR WATER COLUMN PROCESSES

GEOCHEMICAL CONTROLS ON MERCURY METHYLATION IN BACKWATERS OF A GULF COASTAL PLAIN RIVER SYSTEM, IMPLICATIONS FOR WATER COLUMN PROCESSES

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Geology

By

Liam Schenk
Colorado School of Mines
Bachelor of Science in Mining Engineering, 2000

May 2011
University of Arkansas

ABSTRACT

The abundance and distribution of mercury and methyl mercury were investigated at three sites in the lower Ouachita River in the summer of 2010 in an effort to provide the first characterization of the extent of mercury contamination in this river system, and to investigate the potential for mercury methylation in the water column of backwaters off of the main channel. Results showed that filtered methyl mercury was positively correlated to dissolved organic carbon (r^2=0.76) for water samples taken from the bottom 1 ft of the water column at three sites, suggesting the importance of dissolved organic carbon in mercury methylation. Concentrations of filtered methyl mercury and filtered total mercury in the bottom-water were significantly different (P=0.039 and P=0.022 respectively) at two of the sample sites located approximately 14 river miles apart. Sulfide concentrations of 74.0-142.7 micrograms/liter indicate sulfate reduction was occurring in the bottom water or at the sediment-water interface, yet filtered and particulate methyl mercury concentrations were not significantly correlated to sulfide concentrations. The occurrence of sulfides in the bottom-water is important as sulfate-reducing bacteria are most commonly associated with mercury methylation. Water chemistry results for one site including total iron (39.8 milligrams/liter), high dissolved organic carbon (13.52 milligrams/liter), the highest filtered methyl mercury concentration observed for the study (1.90 nanograms/liter), and no detectable sulfate suggests the predominance of iron reduction at this site. Microbial iron reduction is also a known mercury methylation pathway. Total mercury concentrations for two of seven samples exceeded the Arkansas numeric water quality standard for total recoverable mercury in water (12 nanograms/liter), at concentrations of 13.76 and 13.99 nanograms/liter. These

data provide evidence that availability of dissolved organic carbon affects mercury methylation at all three of the sites, and that iron reduction may contribute to mercury methylation at one of the sites. No correlation between sulfide and dissolved methyl mercury was observed, suggesting sulfate reduction may not be the driving process for mercury methylation at all our study sites, and indicating the presence of multiple controls on mercury methylation in this river system.

Keywords: methyl mercury, mercury, biogeochemistry, contaminant hydrology

This thesis is approved for
Recommendation to the
Graduate Council

Thesis Director:

Dr. Phillip D. Hays

Thesis Committee:

Dr. Ralph Davis

Dr. Thad Scott

THESIS DUPLICATION RELEASE

I hereby authorize the University of Arkansas Libraries to duplicate this Thesis when needed for research and/or scholarship

Agreed _____
Liam Schenk

Refused _____
Liam Schenk

ACKNOWLEDGEMENTS

Thanks are due to the Arkansas Water Research Center for providing funding for this project through the USGS 104(b) funding program. The USGS Wisconsin Water Science Center personnel accommodated me with training on low level mercury sampling in March of 2010 in the Upper Peninsula of Michigan. In particular, John deWilde, mercury lab manager, and Charlie Thompson, hydrologic technician fielded numerous phone calls and provided all sampling equipment in a timely manner. Their patience was extremely helpful. Dave Krabbenhoft, mercury studies chief for the USGS, provided assistance at the inception of this project, reviewed abstracts at the end of the process, and always made time for my questions. The guidance of my committee members Phil Hays, Thad Scott, and Ralph Davis was also instrumental to the success of this project.

Byron Winston and Renee Vardy provided invaluable assistance in the field for the sampling events. This project would not have been possible without their time and efforts.

A very special thanks to my partner Siobhan, whose has been by my side through the last seven years through multiple moves and life changes. I am so proud of your achievements in medical school and know that you will be an excellent doctor. I am fortunate to spend my life with such a compassionate, selfless, and hard-working woman. Your support, encouragement, and patience through this thesis process made the challenging times much less difficult.

CONTENTS

1 Introduction 1
1.1 Problem Statement 3
1.2 Objective and Scope 5
1.3 Project Location 6
1.4 Site Characterization 8
1.4.1 RL-2 8
1.4.2 OR-2 10
1.4.3 OR-11 11
2 Background 12
2.1 Mercury Methylation 12
2.2 Mercury and Sulfide 13
2.3 Water Column Processes 14
2.4 Mercury and DOC 15
2.5 Mercury in Arkansas 19
2.5.1 Historical Cinnabar Mining 19
2.5.2 Aerial Deposition of Hg 20
2.5.3 Aquatic Hg contamination in Arkansas 21
3 Methodology 22
3.1 Reconnaissance and Site Selection 22
3.2 Sampling Strategy 25
3.3 Sampling and Processing Methods 25
3.3.1 Field Parameters 25
3.3.2 Mercury and DOC 26
3.3.3 Sulfide, Sulfate, Iron 28
3.3.4 Bed Sediment Sampling 28
3.3.5 Diurnal Sampling 29
3.4 Analytical Procedures 30
3.4.1 Total Mercury 30
3.4.2 Methyl Mercury in Water 31

3.4.3 Methyl Mercury in Sediment 31
3.4.4 DOC Analysis 32
3.4.5 Sulfide, Sulfate, Iron 32
3.5 Statistical Analysis 35
4 Results 36
4.1 Field Parameters 36
4.2 Sulfide, Sulfate, and Iron 39
4.2.1 Sulfide 39
4.2.2 Sulfate 42
4.2.3 Iron 44
4.3 Dissolved Organic Carbon (DOC) 46
4.4 Mercury 47
4.4.1 Mercury and pH 53
4.4.2 Mercury and Sulfides 53
4.4.3 Mercury and Iron 54
4.5 Mercury and DOC 56
4.6 Diurnal Study 57
5 Discussion 60
5.1 RL-2 60
5.2 OR-2 66
5.2.1 OR-2 Diurnal Study 68
5.3 OR-11 69
5.4 Effect of sulfide on Hg methylation 72
5.5 Effect of DOC on Hg methylation 75
5.6 MeHg Flux Rate Modelling 77
5.7 Regional Comparison of Hg contamination 82
5.8 Conceptual Model 89
5.9 Conclusion 91
References 96
6 Appendix 105

1 Introduction

Increased scientific knowledge on mercury (Hg) sources, transport, deposition and cycling, and the toxic effects of Hg species on human populations has led to growing concern over Hg contamination of aquatic systems in recent years. Hg is naturally present in the environment, but human activities such as the combustion of fossil fuels for power generation have increased the amount of Hg cycling through land, atmosphere, and ocean systems (N. E. Selin 2009). As such, atmospheric deposition of Hg is increasing in marine systems (Sunderland, et al. 2009), as well as in riverine systems (Delongchamp, et al. 2009). Riverine systems in some regions such as those draining cinnabar mining districts are at even greater risk of contamination due to exposure and mobilization of Hg during cinnabar mining (Holloway, et al. 2009).

Of primary concern is the formation of the most toxic form of Hg, methylmercury (MeHg). In aquatic systems, Hg can be deposited by either wet or dry deposition in its elemental (Hg^0) and ionic (Hg(II)) forms. Hg^0 and Hg(II) can then be transformed into toxic and highly bioavailable MeHg, the result of processes largely carried out by anaerobic bacteria (Marvin-Dipasquale, et al. 2009). Epidemiological studies have linked exposure to MeHg in pregnant women to neurological and developmental effects in their offspring (Mergler, et al. 2007), (Clarkson 1990). High degrees of human exposure to MeHg most commonly results from the consumption of high trophic-level predaceous fish such as tuna and swordfish in marine systems, and black bass and piranha in freshwater systems.

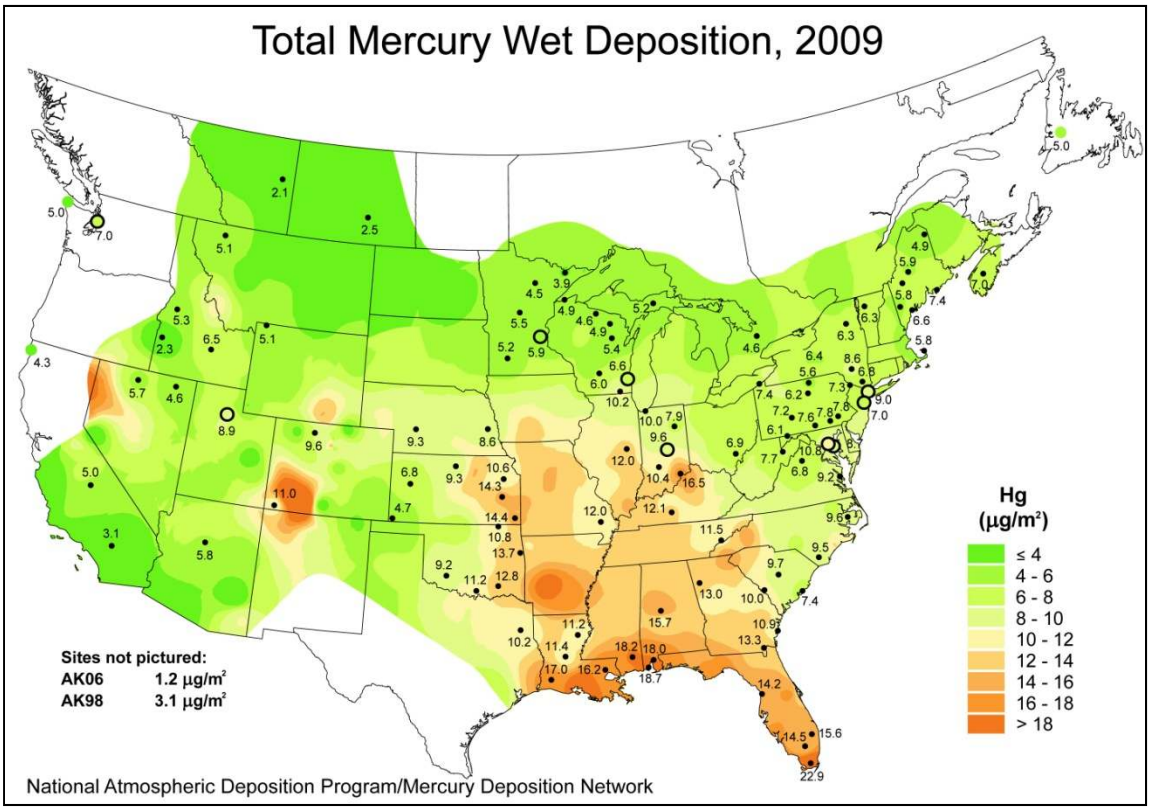


Figure 1: Total Mercury Wet Deposition 2009: http://energy.er.usgs.gov/health_environment/mercury/mercury_network.html

Arkansas has the only mid-continent/eastern U.S. cinnabar mining district and has also been shown to have a considerable Hg aerial deposition issue (Figure 1) (Facemire, et al. 1995). Fish consumption advisories are currently in effect for 20 water bodies in the state in 13 different counties because of high levels of Hg in fish tissue (Arkansas Department of Health n.d.). A number of studies have been conducted in Arkansas examining Hg occurrence in fish, sediment, rocks and soils (Lin and Scott 1997, Scott and McKimmey 1997, Stone, Nix and McFarland 1995). However, recent analytical advances, newly developed sampling methodologies, and recent improvements in the understanding of Hg processing give strong impetus to examine contemporary Hg contamination issues in Arkansas with improved outcome and better understanding of Hg cycling in the State.

1.1 Problem Statement

In riverine systems, different species of Hg can enter the water column through different pathways , can move into zones in the system that have conditions that are amenable to methylation (Figure 2), and can be transported to estuaries and wetlands where high rates

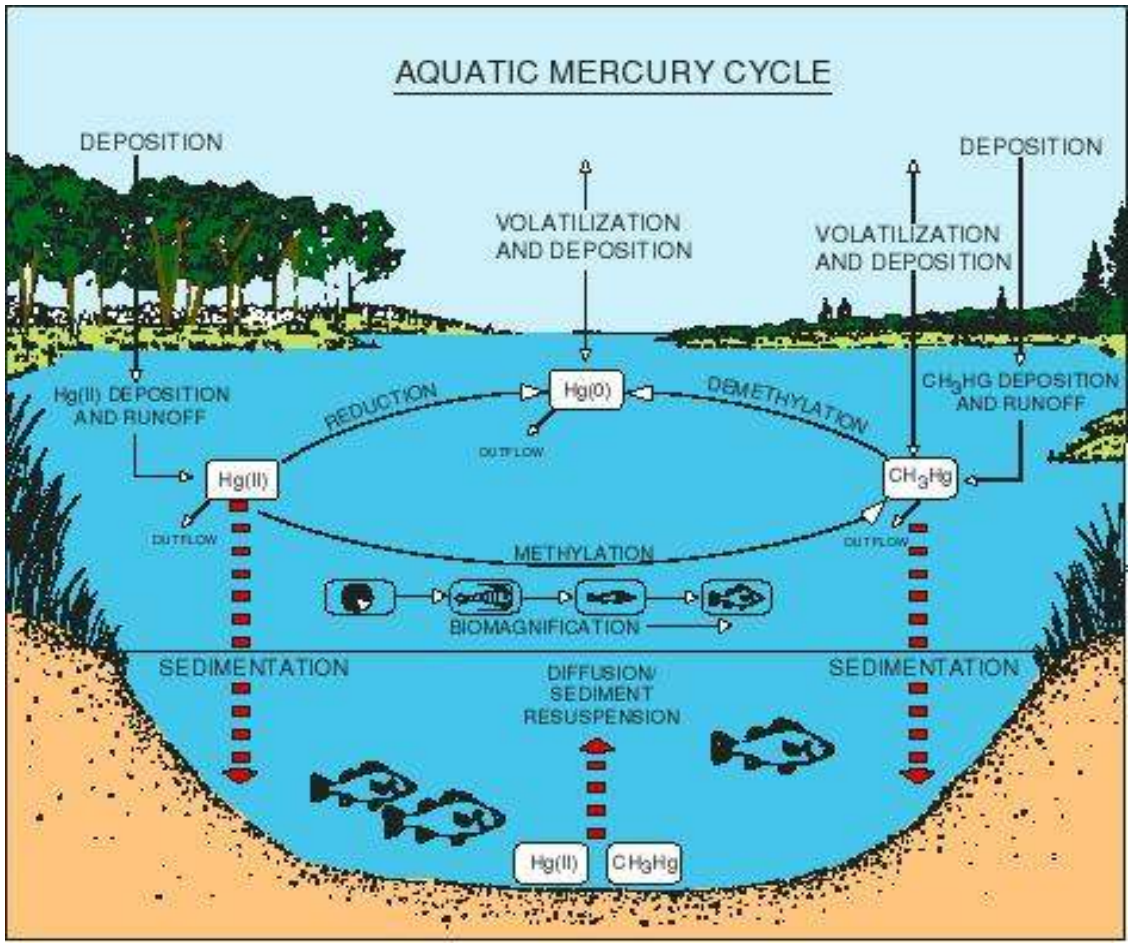


Figure 2. Aquatic Mercury Cycle, copyright Lewis Publishers, an imprint of CRC Press

of methylation have been documented (Hall, et al. 2008). For riverine systems, the conventional perspective is that most of the MeHg generated occurs in sediment where redox conditions are most favorable for the conversion of Hg (II) to MeHg by anaerobic bacteria (J. Benoit, et al. 1999). MeHg can then move into the water column during the trophic enrichment process (Marvin-Dipasquale, et al. 2009). The bioaccumulation of

MeHg in fish populations has been well documented (Zhou and Wong 2000), (Sonesten 2003), but the role of dissolved organic carbon (DOC) in the methylation process in riverine systems, and DOC effect on the fate and transport of MeHg are poorly understood. Although rivers in some environments often have very high total organic carbon and DOC content and exhibit DOC and redox stratification (Stanley and Nixon 1992), the potential importance of DOC in Hg methylation within the water column of rivers has been little studied. Recent studies of Hg distribution in marine settings by U.S. Geological Survey (USGS) scientists have documented positive correlations between organic carbon remineralization and MeHg concentrations in the water column (Sunderland, et al. 2009). These studies suggest that distribution and transport of organic carbon may play an important role in the production of toxic MeHg in marine environments. Additionally, studies on riverine systems in the U.S. have also shown relations between Hg (II) and DOC, suggesting DOC as a carrier of Hg in aquatic systems (Brigham, et al. 2009). These findings highlight the potential importance of Hg methylation in the water column—possibly even for riverine systems.

The linkage of mercury with organic carbon in MeHg production, transport, and cycling in river systems points out a weak point in our understanding of Hg contamination, and calls for a more comprehensive characterization of Hg behavior; therefore, this project tests the following hypothesis:

Hypothesis: The occurrence of MeHg in the water column of backwaters of the lower Ouachita River is controlled by a variety of geochemical factors with organic carbon availability playing a critical role in MeHg distribution.

An increased understanding of Hg fate and transport is of utmost importance to aid in our understanding of the behavior of toxic MeHg in the environment as there are hazardous ecosystem and human health implications with increasing levels of contamination. Only by accurately assessing MeHg in the environment, and determining where the transition from less toxic and less mobile forms of Hg to the much more toxic and more mobile MeHg occurs will scientists and environmental regulators be able to control human exposures. These research efforts are necessary to help enhance the accuracy of assessments of MeHg contamination, and to aid in the development of remediation techniques to reduce the human exposure.

1.2 Objective and Scope

The objective of this project is two-fold. The first objective is to provide the first detailed characterization of the occurrence and extent of Hg contamination in backwaters of the lower Ouachita River system, and second, to examine the geochemical controls on MeHg in the water column. A detailed multimedia, multi chemical-species sampling scheme was implemented to characterize the relation between organic carbon and Hg methylation. Field parameters along with sulfide, sulfate, and iron concentrations were assessed in the bottom 1-ft of the water column (hereafter referred to as bottom water) to provide insight into oxidation-reduction (redox) conditions that dominate the system and provide evidence for the presence of anaerobic bacteria known to be responsible for Hg methylation. Determination of the controls on MeHg occurrence in the bottom water is achieved by comparing total Hg (THg) and MeHg concentrations to field parameters, DOC, sulfide, iron, sulfate and MeHg sediment concentrations. The characterization of

Hg contamination at three backwater sites on the lower Ouachita River provides detailed, state-of-the-science Hg data that give insight into the extent of Hg contamination.

1.3 Project Location

The project area is located in south Arkansas on the lower Ouachita River bordering

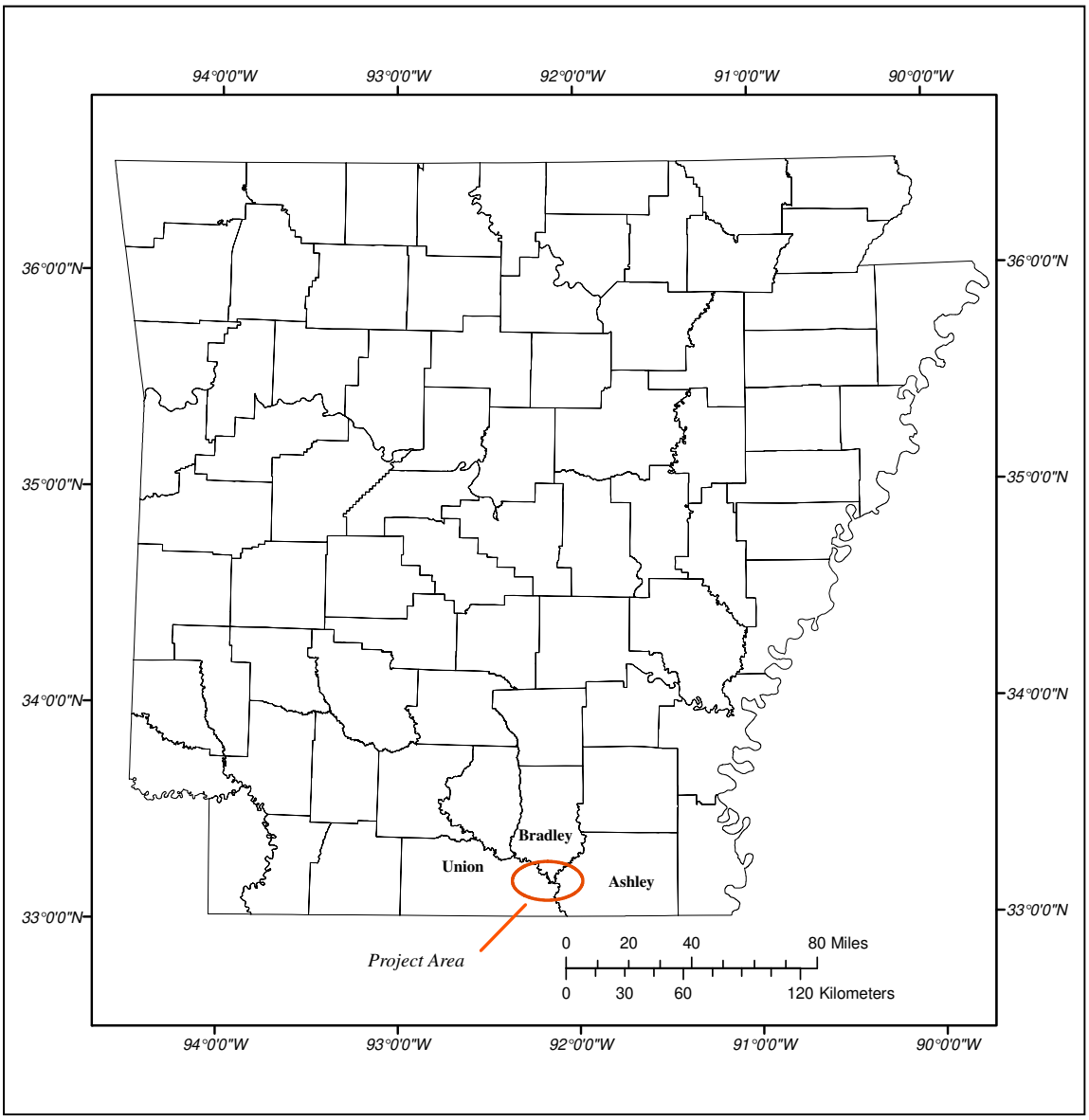


Figure 3: Project Area Map

Union, Ashley, and Bradley Counties (Figure 3). The lower Ouachita-Bayou de Loutre watershed (HUC 08040202) in this area contains three river reaches listed as impaired

water bodies for Hg on the 2008 United States Environmental Protection Agency (USEPA) 303(d) list based on Hg levels in fish tissue samples, including Lake Felsenthal and all oxbow lakes down gradient of the town of Camden (Arkansas Department of Environmental Quality 2008).

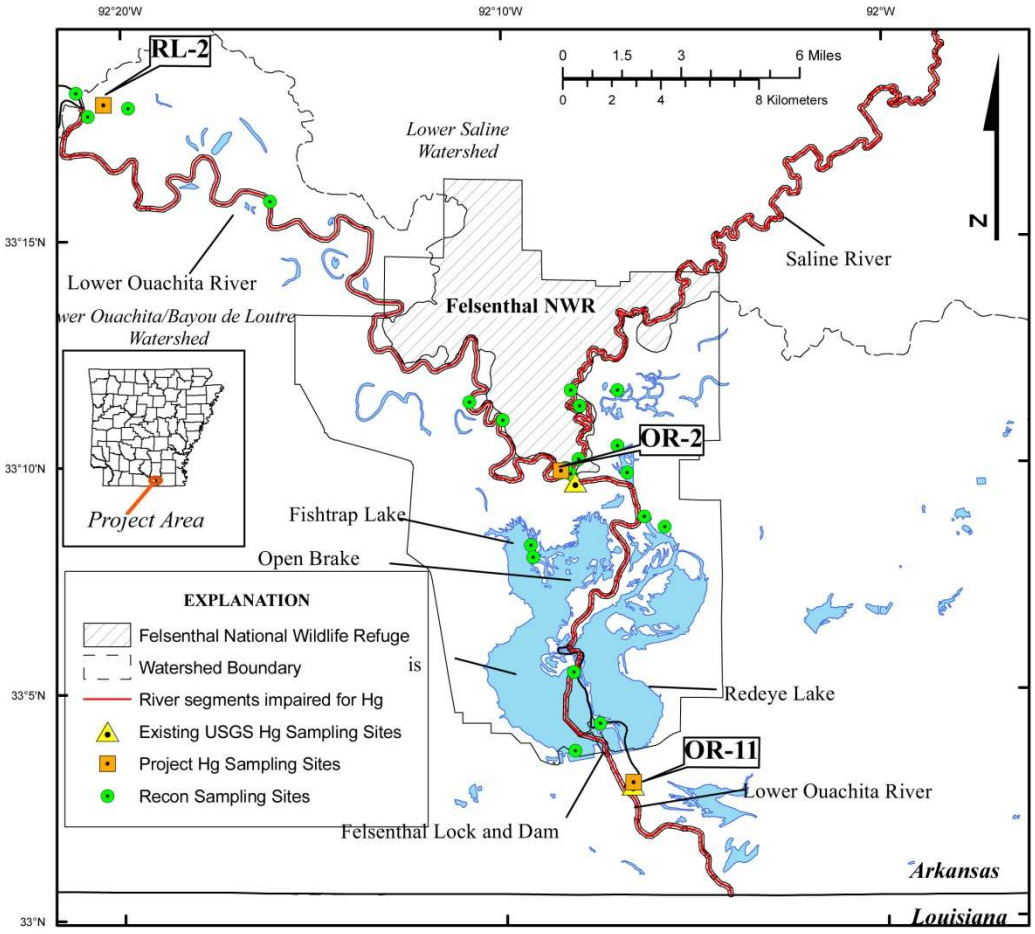


Figure 4: Project Area Map with Sampling Locations

For purposes of this report, all water bodies upstream of the Felsenthal lock and dam to highway 82.including Fishtrap Lake, Grand Marais, Redeye Lake and Open Brake will be collectively referred to as Lake Felsenthal. The river segments and reaches on the Ouachita River listed as impaired for Hg in fish tissue constitute the entire length of the

river in this watershed (Figure 4). The lower Ouachita River has been on the EPA 303(d) list for Hg since 1998 (Jim Wise, ADEQ, personal communication.).

The EPA impairment classification for the lower Ouachita river in Arkansas, coupled with existing USGS Hg data (see *Background*) from the river indicate that methylation of Hg is occurring in these specific reaches of the lower Ouachita River. For these reasons, the river segments impaired for Hg on the lower Ouachita River in south Arkansas were targeted for this project.

1.4 Site Characterization

1.4.1 RL-2

Site RL-2 is the furthest upstream site in the project area, and is located in Raymond Lake, a backwater location that is hydraulically connected to the main channel of the Ouachita River (Figure 5). Raymond Lake is east of Morrow Bay State Park off of highway 63, northeast of El Dorado, Arkansas. The sampling location is approximately ½ mile upstream of the lake's confluence with the Ouachita River. The channel is narrow, and has a prominent canopy. Raymond Lake receives stream inputs upstream of RL-2 during periods of high precipitation and runoff. The canopy provides shade in the spring and summer months, limiting the amount of solar radiation at the site as compared to the other two sampling sites. The site is located in a deep pool (depth =23 ft) just upstream of the Highway 63 bridge, and has a gravel bottom (Figure 6). The gravel at the bottom is believed to be derived from historical construction projects, evidenced by large concrete waste structures on the left and right bank at the site, most likely the remnants of the old Highway 63 bridge. As such, sediment samples were not collected at this site.

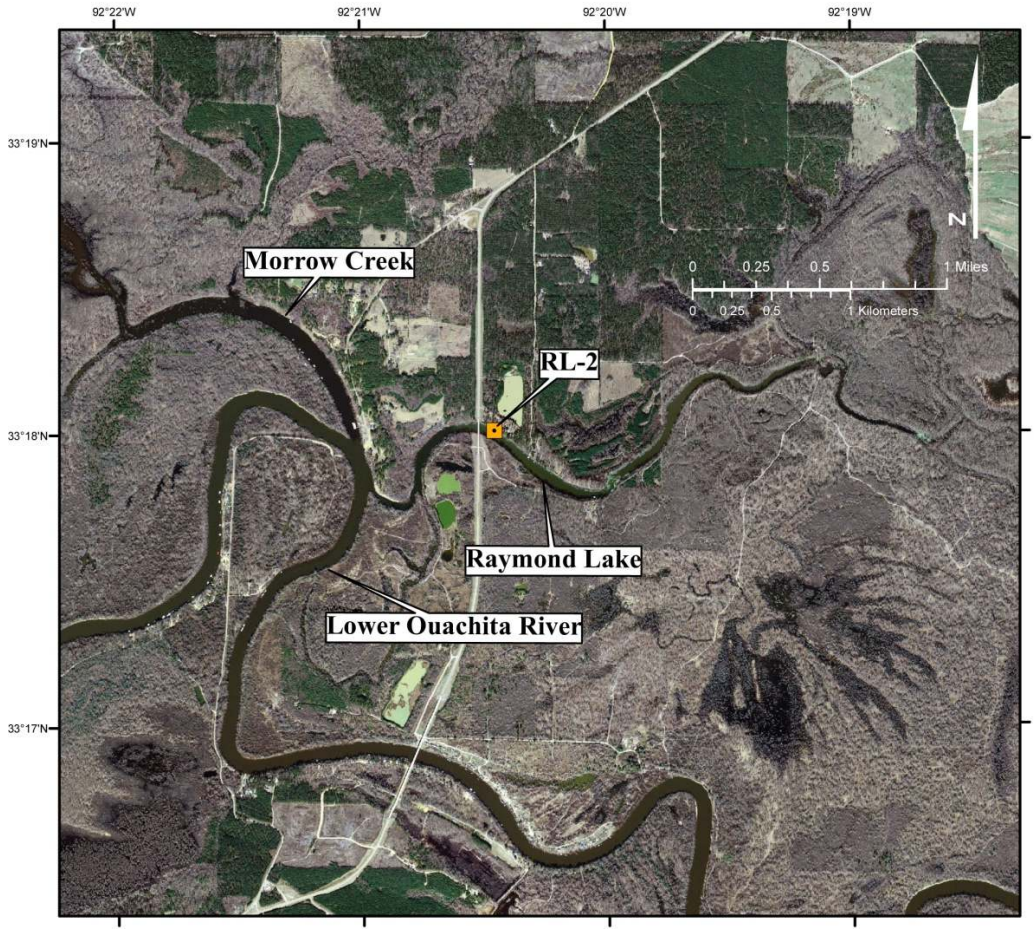


Figure 5: Orthographic map of RL-2 site location.

Figure 6: Picture of bottom-gravel from RL-2, collected with an Eckman Grab Sampler

1.4.2 OR-2

OR-2 is located in the backwater of a meander cutoff of the main channel of the lower Ouachita River, and is approximately 0.1 miles from the main channel, ¼ mile upstream of the confluence with the Saline River (Figure 7).

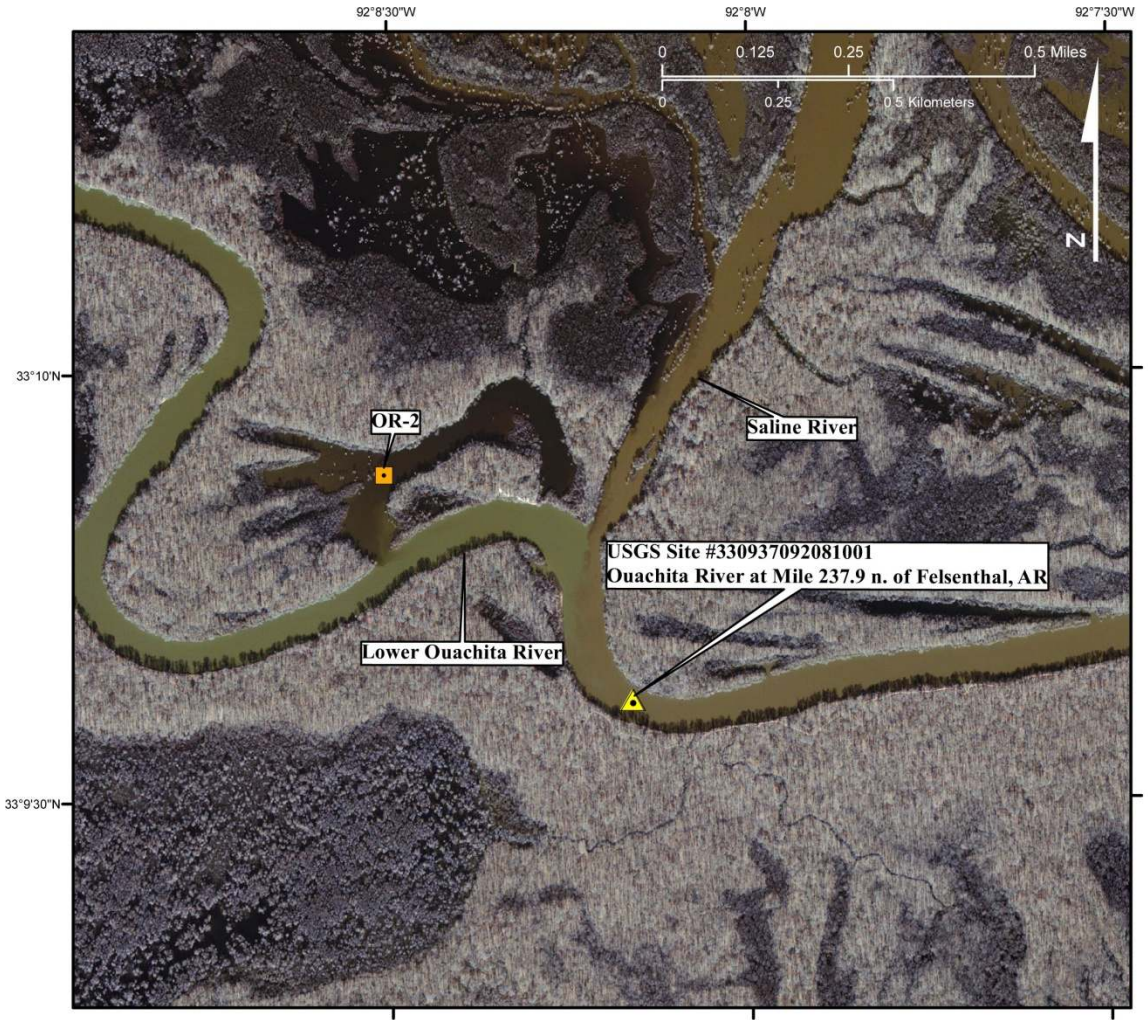


Figure 7: Orthographic Map of site OR-2

The meander cutoff is presently being filled with sediment at the confluence of the two water bodies as evidenced by increasing shallow depths on the downstream side of the backwater entrance resulting from the accumulation of unconsolidated sediments. The site is not subject to stream influences during high runoff events, i.e. no tributaries enter

along the meander, is in a much wider channel as compared to RL-2, and is subject to greater solar radiation because of the lack of canopy. The water depth at OR-2 was 14-ft, and the backwater becomes more shallow north of the sampling location into a backwater cypress swamp. A USGS site is located 0.2 miles downstream of the Saline River confluence where Hg sampling has been conducted since 2003 in the main channel of the Ouachita River.

1.4.3 OR-11

OR-11 is located downstream of the Felsenthal Lock and Dam in the former channel of the lower Ouachita River. Upstream of the site is an earthen dam that obstructs the former

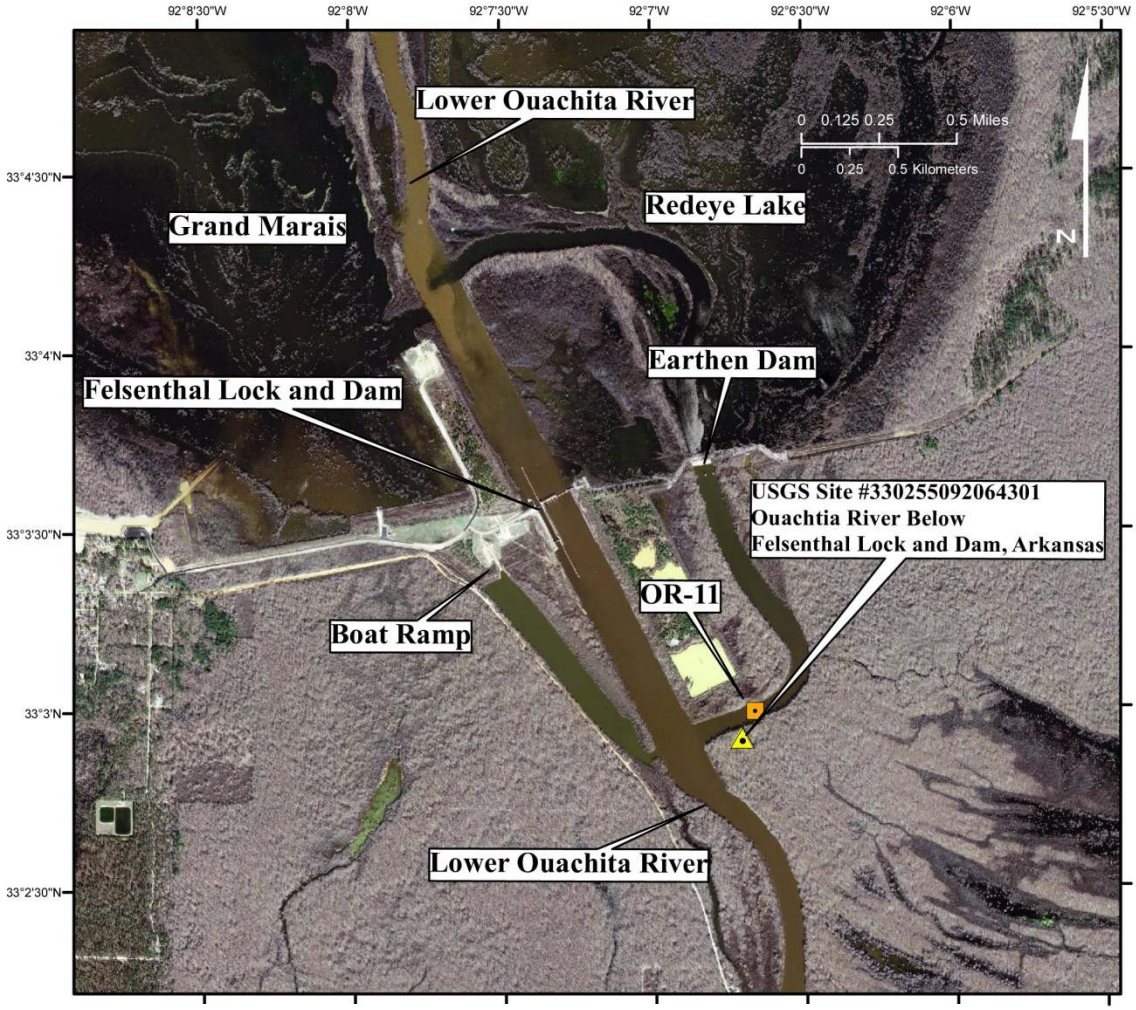


Figure 8: Orthographic map of site OR-11

main channel (Figure 8).

The water from the Ouachita River is diverted through the lock and dam, so no through flow moves past OR-11. The site is located in a deep pool much like RL-2 (depth=23-ft), and is a backwater location because of the lack of through flow. However, the site is hydraulically connected with the main channel and lies in close proximity to the active lower Ouachita channel much like OR-2. OR-11 lacks a prominent canopy, resulting in more solar radiation influence as compared to RL-2. A USGS sampling site is located near OR-11 (Figure 8). The USGS site is shown to be on land south of the OR-11 backwater (see USGS National Water Information System (NWIS) website, http://wdr.water.usgs.gov/nwisgmap/?state=ar). The actual location of this site is assumed to be on the main channel of the lower Ouachita River at the confluence with the backwater where OR-11 is located.

2 Background

The processes controlling the occurrence of MeHg in aquatic systems are complex and are influenced by physical and geochemical factors. A thorough understanding of methylation processes and controls, and potential sources of Hg, and is necessary when assessing Hg contamination in any system.

2.1 Mercury Methylation

The processes that convert inorganic Hg to MeHg are carried out largely by bacteria that thrive in anoxic environments. The mechanisms for conversion of Hg to MeHg are poorly understood, but two classes of sulfate-reducing bacteria (SRB)--complete and incomplete oxidizers--are involved in Hg methylation. Complete oxidizer SRB are the

more important methylators because they methylate Hg via an accidental side reaction of the acetyl-CoA pathway in the cytoplasm which attaches a methyl group to Hg (Ranchou-Peyruse, et al. 2009), (Benoit, Gilmour and Mason 2001(a)). However, other organisms shown to be involved in Hg methylation do not have this pathway, so the acetyl-CoA pathway cannot be the only Hg methylating mechanism for these bacteri (J. Benoit, et al. 2003). SRB are primarily responsible for this conversion in anoxic sediments (King, et al. 2000). Iron reducing bacteria (FeRB) have also been shown to methylate Hg (Kerin, et al. 2006).

2.2 Mercury and Sulfide

In addition to DOC, Hg also forms strong complexes with dissolved inorganic sulfide that are more thermodynamically favored than Hg-DOC complexes (Miller, et al. 2007). The dominant Hg-sulfide complexes in natural conditions are HgS^0, $Hg(SH)_2^0$, $HgHS_2^{-1}$, and HgS_2^{-2} (Miller, et al. 2007), with the presence of polysulfides (S_x^{-2}, where x=3-6) also potentially forming Hg complexes (Jay, Morel and Harold 2000). A study by Benoit, et al. (1999) suggested that SRB take up inorganic Hg by passive diffusion across the cell membrane, and that neutral Hg species such as HgS^0 dominate the form of Hg methylated by SRB. Hg-DOC complexes are typically too large to cross cell membranes by passive diffusion, and are therefore typically not utilized by SRB and FeRB in Hg methylation processes (Ravichandran 2004). Benoit, et al. (1999) developed a chemical equilibrium model for Hg complexation in sediments with sulfidic pore waters for two biogeochemically different ecosystems and found that neutral Hg species are accumulated by methylating bacteria in sulfidic sediments. For SRB, the presence of inorganic sulfides resulting from high sulfate concentrations can also inhibit Hg

methylation by decreasing its bioavailability to methylating bacteria (Benoit, et al. 2001a). In a separate study using pure cultures of sulfate reducting bacteria and rocks from the Ouachita mountains in Arkansas to provide a solid-phase source of Hg, Benoit, et al. (2001b) hypothesized that the amount of MeHg produced in their laboratory experiments should be proportional to the concentration of HgS^0 in solution. These findings suggested that HgS^0 is the dominant dissolved Hg-sulfide species utilized by sulfate reducing bacteria for their experiments. Additionally, increased sulfide concentrations decrease the bioavailability of Hg by causing a shift in speciation away from HgS^0 toward charged complexes that are not readily used by sulfate reducing bacteria (Benoit et al. 2001b). In a study in an estuary in southern California, Rothenberg, Ambrose and Jay (2008) predicted Hg-S spectiation as a function of pH assuming a total sulfide concentration of 272 μg/L. At near neutral pH, (6-7) neutral dissolved HgS dominated the mole-fraction of Hg-S species in the presence of sulfides and polysulfides, suggesting sulfide speciation as an important control on Hg-S speciation and related Hg methylation (Rothenberg, Ambrose and Jay 2008). At total sulfide concentrations less than 10 μM (340 μg/L), the dominant Hg-S species is $HgS^0_{(aq)}$ as described by Rothenberg, Ambrose and Jay. (2008) and Benoit et al. (1999). Sulfide concentrations in water are therefore important as they control the formation and speciation of Hg-S complexes, which are utilized by methylating bacteria depending on the speciation of Hg-S complexes.

2.3 Water Column Processes

Although Hg methylation is a process primarily driven by SRB in anoxic surface sediments, some studies have shown methylation potential in the water column.

Monperrus, et al. (2007) showed evidence for water-column methylation using isotopically labeled Hg for *in situ* incubation experiments in a shallow coastal lagoon in France. In their study, Hg methylation in the water column was attributed to the presence of micro-plankton species and light-induced processes. Methylation in the water column has also been linked to light-driven processes in an ultraoligotrophic lake in Northern Patagonia (Guevara, et al. 2008). Eckley et al (2005) determined that Hg methylation was occurring in the anoxic hypolimnion of two deep lakes in Wisconsin, citing sulfide concentrations in the water column as evidence for SRB involvement in the methylation process. Conversely, Sellers et al. (2001) cited strong stratification in summer months as evidence for diffusive MeHg flux from the sediment into the overlying column as the source of MeHg in anoxic hypolimnetic waters of an oligotrophic drainage lake in the Precambrian Shield in northwest Ontario.

2.4 Mercury and DOC

The role of DOC in Hg fate, transport, and methylation is complex. Studies have shown DOC to have a positive correlation with THg in stream systems (Brigham, et al. 2009), and positive linear correlations of organic carbon remineralization to MeHg in marine systems (Sunderland, et al. 2009). Systems with high levels of organic matter production such as wetlands or periodically flooded river plains can exhibit high rates of methylation due to the influx of fresh organic matter (J. Benoit, et al. 2003).

Understanding the role of DOC in Hg biogeochemical cycling requires an understanding of different types of DOC and how these interact with Hg in natural systems. DOC can be classified into two major groups (Figure 9); refractory compounds such as humic substances (HS), and labile, well defined compounds that include carbohydrates,

peptides, amino acids, carboxylic acids and alcohols (Sachse, et al. 2005). Refractory DOC molecules in water are largely derived from allochthonous sources, and labile DOC is typically associated with autochthonous production by heterotrophs such as bacteria, algae, and macrophytes (Bertilsson and Jones 2003). Humic substances represent a significant fraction of organic matter in most soils and waters, constituting between 6-30% of total organic matter in seawaters (Stumm and Morgan 1996), and 60-90% of DOC in fresh waters (Sachse, et al. 2005). As the name would imply, refractory DOC does not present a readily accessible food energy source and takes much longer to decompose as compared to the lighter labile fractions of DOC, thus, the refractory DOC pool is much larger than the labile pool in aquatic systems (Ostapenia, Parparov and Berman 2009). Hydrophobic and hydrophilic acids are the most reactive fractions in trace metal binding, and constitute about 80-90% of humic substances (Ravichandran 2004).

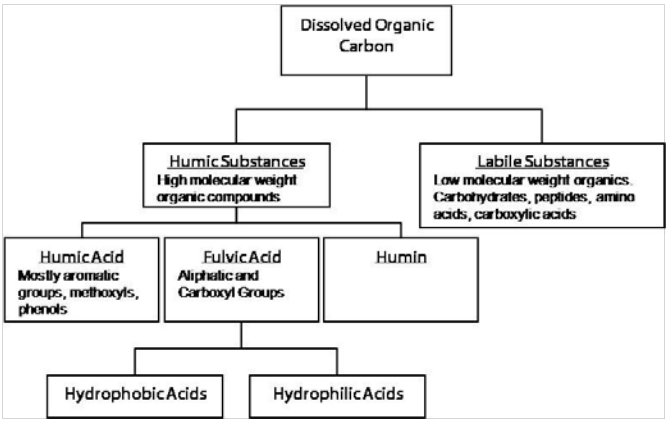


Figure 9: DOC Classification

DOC-Hg interaction affects Hg speciation, solubility, mobility and toxicity in the environment (Ravichandran 2004). Hg and other trace metals are generally bound at the acid sites in dissolved and particulate organic matter, including carboxylic acids, phenols, ammonium ions , alcohols, and thiol functional groups, with carboxylic acids and phenols contributing as much as 90% of acidity to organic matter. Despite the abundance of carboxylic acids and other oxygen-bearing functional groups in DOC, Hg has been found to interact very strongly with thiol and other sulfur-containing functional groups by

coordination at reduced sulfur sites. Thiol groups are typically present only in trace amounts in organic matter. Because hydrophobic acid fractions of DOC have significantly higher reduced sulfur content than low molecular weight DOC, Hg will preferentially bind with dissolved humic substances in aquatic environments.

The preferential binding of Hg with thiol groups can be attributed to its classification as a B-type metal cation, characterized by a "soft-sphere" of highly polarizable electrons in its outer shell, creating a preference for ligands of sulfur, nitrogen, or less electronegative halides such as iodine (Stumm and Morgan 1996). The binding of Hg at reduced sulfur sites has been proven experimentally (Benoit, et al. 2001), (Lamborg, et al. 2003), and has been proposed as a primary mechanism for Hg transport (Waples, et al. 2005). Studies examining binding constants of Hg with hydrophobic acid fractions of humic and fulvic acids under natural conditions (low Hg/dissolved organic matter (DOM) ratios) have shown that the binding of Hg to DOM is controlled by a small fraction of DOM molecules containing reactive thiol functional groups (Haitzer, Aiken and Ryan 2002). Conditional stability constants from this study were reported in the range of $10^{23.2\pm1.0}$ L/kg for 1µg of Hg/mg of DOM at pH=7.0 and ionic strength (I)=0.7 and $10^{10.7\pm1.0}$ L/kg for approximately 10 µg of Hg/mg of DOM at pH = 4.9-5.6 and I=0.1, evidence of the dependence of Hg-DOM concentration ratios on Hg binding. Data from the same experiment in a separate publication by Haitzer, Aiken and Ryan (2002), also showed low pH values resulting in decreased conditional stability constants for Hg-DOM binding because of proton competition for strong Hg binding sites. The conditional stability constant for Hg complexation with organic thiol groups can be best described by the following equation (modified from Ravichandran 2004).

$$Hg^{+2} + RS^{-} \leftrightarrow HgRS^{+} \quad K = \frac{[HgRS^{+}]}{[Hg^{+2}][RS^{-}]}$$

As mentioned previously, DOC and Hg have been shown to be positively correlated in some situations; however, null correlations have also been found in some studies possibly due to the fact that only a small fraction of DOC molecules containing reactive thiol groups that are responsible for Hg complexation (Hurley, et al. 1998).

The role of DOC and Hg cycling is complex in that DOC can both limit the bioavailability of Hg, and is an important factor in the transport of THg and MeHg. In aquatic systems with neutral pH, DOC is likely to complex with Hg(II), inhibiting its availability for methylation, but in low pH waters, excess hydrogen ions can compete with Hg(II) for negatively charged binding sites in DOC, leaving more Hg(II) available for methylation (Barkay, Gillman and Turner 1997).

The interactions of DOC and MeHg are equally complex. MeHg is characterized as a "soft" Lewis Acid that forms strong covalent bonds with "soft" Lewis Bases such as reduced sulfur, and these reduced sulfur ligands in DOC are important binding sites for MeHg (Amirbahman, et al. 2002). Studies have found that humic and fulvic acids are important in the fate and transport of MeHg (Hintelmann, Welbourn and Evans 1997), and that MeHg can also have a strong association with low molecular weight DOC (Cai, Jafe and Jones 1999). The presence of organic matter in an aquatic system can also have stimulating effects on microbial communities that are responsible for Hg methylation (Ullrich, Tanton and Abdrashitova 2001). For example, an experiment that involved the flooding of an experimental reservoir in a boreal forest catchment by Kelly, et al. (1997)

showed that Hg methylation rates increased due to the availability of organic carbon to microbial communities.

2.5 Mercury in Arkansas

2.5.1 Historical Cinnabar Mining

The only historical cinnabar (HgS) mining district in the southern mid-continent region is located in Arkansas. HgS was discovered in southwest Arkansas in 1930, and mining activities produced the ore from 1931 to 1944 (Clardy and Bush 1976). The cinnabar district lies in the Athens Plateau, which is a province of the Ouachita Mountain Region. The occurrence of Cinnabar in Arkansas is attributed to hot fluids migrating up through the folded and faulted Paleozoic rocks of the Ouachita Mountains, most likely the result of epithermal emanations from igneous rocks south of the mineralized area. The term "epithermal" implies low temperature (50-200 °C) and shallow depth (near surface to 1500m) (Schmitt 1950), (Evans 1980) fluid flow. The cinnabar occurs as fine- to medium-crystalline coatings on fractured surfaces and as coarsely crystalline cinnabar filling larger fractures and open spaces. Deposits of cinnabar are sparse in the mining district, and occur mainly in the large-displacement, high-angle reverse faults that developed during the Ouachita orogeny in middle Pennsylvanian time. As the hot fluids traveled northward up the fault planes and into fractured sandstones and shales, cinnabar precipitated in the open spaces under favorable geochemical conditions. All of the known cinnabar mineralization occurs in the Stanley shale and Jackfork sandstone formations of Pennsylvanian age, but all of the deposits that were mined were associated with sandstone units. Total production of Hg from the mining district is estimated at 12,500 76-pound flasks (Clardy and Bush 1976).

2.5.2 Aerial Deposition of Hg

The long range transport and deposition of atmospheric Hg has long been a concern because of past discoveries of Hg contaminated fish in remote lakes in the northern United States (Pai, Heisler and Joshi 1998). Three types of Hg exist in the atmosphere: gaseous elemental Hg (Hg_0), gaseous ionic divalent Hg(II)--termed reactive gaseous Hg (RGM) -- and particulate Hg (Hg_p) (Mason and Sheu 2002). These types of Hg are the primary form emitted anthropogenically (Lindberg, et al. 2007). Anthropogenic releases of Hg contribute about 1/3 of emissions to the atmosphere, and are primarily from coal fired power plants, metal smelting, and waste incineration (Selin, et al. 2007). Hg_0 is the dominant form of atmospheric Hg (~95%), is relatively insoluble, and is generally assumed to be oxidized by either ozone or OH in the atmosphere (Seigneur, et al. 2004). Oxidation of Hg_0 forms Hg(II), which can partition between the gas and particulate phases, and is the primary deposited form of Hg in terrestrial and aquatic systems (Selin, et al. 2007). Hg(II) is much more soluble than Hg_0 and are likely to be scavenged by cloud droplets (Seigneur, et al. 2004). Hg in the atmosphere can also be derived from terrestrial sources (e.g. soils), but these fluxes are difficult to quantify due to the challenges in differentiating between naturally occurring Hg in the soil, and emission of Hg previously deposited on the terrestrial landscape (Lindberg, et al. 2007).

The southeastern United States show a maximum wet deposition flux of Hg (Figure 1). Chemical cycling models by Selin et al. (2007) attribute these maxima to photochemical oxidation of Hg_0 and frequent precipitation. These high rates of wet deposition have also been attributed to consistently high concentrations of atmospheric Hg in the southeastern US (Prestbo and Gay 2009). In 2009, the national Atmospheric Deposition Program

(NADP) released an annual summary showing maximum wet deposition in the United States occurring in south-central Arkansas and states bordering the northern Gulf of Mexico (National Atmospheric Deposition Program 2010).

2.5.3 Aquatic Hg contamination in Arkansas

The aquatic Hg contamination issue in Arkansas came to light in the mid 1990's when several cases of chronic Hg intoxication were found in people and high levels of Hg in fish tissue (above the 1 mg/kg action level in Arkansas) were discovered in the Saline and Ouachita Rivers. (Joe Nix, personal communication). As a result, a Hg "task force" was created by then-governor Mike Huckabee. Subsequent investigations resulted in the 303(d) listing of water bodies in the state, including the lower Ouachita River in 1998. In 1995, the Arkansas Geological Commission released Information Circular 32 entitled "A Regional Survey of the Distribution of Mercury in the Rocks of the Ouachita Mountains of Arkansas" (Stone, Nix and McFarland 1995). The purpose of the study was to establish the background level of naturally occurring Hg in the rocks of the drainage basins of the Ouachita and Saline Rivers to corroborate another study which examined Hg in Ouachita River sediment. This study from Information Circular 32 involved the collection of 728 samples from seven counties in the Ouachita Mountain region of Arkansas. Concentrations of Hg in the sampled rocks ranged from 3 parts per billion (ppb) to 6,100 ppb, with a reported geometric mean of 88 ppb, a median of 78 ppb, and a geometric deviation of 2.8. Analyses were performed at Ouachita Baptist University in Arkadelphia, AR utilizing EPA method 245.5. The reported detection limit was "well below 5 nanograms of mercury" (Stone, Nix and McFarland 1995).

In 2002, a total maximum daily load (TMDL) document was completed for Hg in the lower Ouachita River, giving it a 4(a) status on the 303(d) list; which states that the river segments in question are not attaining their designated uses, but that a TMDL has been completed for the listed parameter (Arkansas Department of Environmental Quality 2008). The TMDL included not only the lower Ouachita River, but also impaired water bodies in the lower Saline watershed (HUC 08040201), lower Ouachita-Smackover watershed (HUC 08040204), and Bayou Bartholomew watershed (HUC 08040205) (FTN Associates, Ltd. 2002). The chronic criteria numeric water quality standard for Hg in all ecoregions in Arkansas is 12 ng/L expressed as total recoverable Hg (Arkansas Pollution Control and Ecology Commission (APCEC) 2007). Hg data have been collected by the U.S. Geological Survey (USGS) at two sites on the lower Ouachita River since 2001 (Figure 4) and show high THg values, though never exceeding the water quality standard of 12 ng/L. The TMDL also set a standard of 0.8 mg/kg Hg in fish tissue. Prior to the TMDL, fish tissue concentrations of 1 mg/kg Hg have prompted fish consumption advisories in the state (FTN Associates, Ltd. 2002).

3 Methodology

3.1 Reconnaissance and Site Selection

In order to target areas in the lower Ouachita River where production of MeHg is most likely to occur, a reconnaissance and water quality characterization was conducted on the week of July 19th, 2010. 27 potential sites were (Figure 4) selected based on ease of access and proximity to backwaters and eddies. The backwaters and eddies in the river system were targeted as likely locations for MeHg production because of the lack of

flowing water and potential for stratification with anoxic conditions in the lower part of the water column. Water-quality parameters at reconnaissance sites were collected using a Hydrolab minisonde capable of real-time field monitoring of temperature, specific conductance (SpC), pH, DO, and oxidation-reduction potential (ORP). The minisonde was lowered to specified depths, determined by gradations marked on the minisonde cable. Water-quality parameters were recorded after stabilization of DO, remaining steady for 30 seconds.

Methylation of Hg occurs in anoxic environments, where sulfate- reducing bacterial activity occurs. Thus, we were seeking zones in the river that exhibited stratification with respect to dissolved oxygen, and anoxic conditions at or above the sediment water interface. As a result, reconnaissance field data were collected in the main channel of both the Ouachita and Saline Rivers, in backwaters off of the main channels, in sloughs, and in oxbow lakes in the watershed near the main channel of the Ouachita River. Vertical profiles of the water quality parameters were taken every 1-2 feet at the first three reconnaissance sites to determine if the water column was stratified at those locations. For subsequent sites, water-quality parameters were assessed only at the bottom water to determine if ORP values were in the sulfate reduction range, and if the bottom water was anoxic. Full water-quality depth profiles were then performed only if the water column exhibited anoxic conditions and low ORP values at the bottom water.

ORP was used as a proxy for sulfate-reducing microbial activity. In the hierarchy of redox reactions, absolute values of ORP can be used as an indicator of the specific redox reaction that is occurring in the system at the time of the measurement (Schlesinger 1997). The ability to measure ORP quickly during site selection was of utmost

importance as we were targeting ORP values in the range of sulfate reduction (~-250 mV), and were attempting to assess many river miles in a short period of time. Once a site was identified as having potential for mercury methylation (i.e. low ORP), sulfide analysis was conducted in the field to determine sulfide concentration at the bottom water, which was considered a proxy for sulfate reduction. Methodology for sulfide analysis is included in section 3.3.3.

After completion of the reconnaissance, three sites were chosen as the most likely candidates for MeHg production based on the field data. These sites exhibited the lowest ORP values, anoxic conditions at the bottom-water, and measureable sulfide. From upstream to downstream order, the selected site names and abbreviations are: Raymond Lake #2 (RL-2), Ouachita River #2 (OR-2), and Ouachita River #11 (OR-11) (Figure 4). RL-2 is the furthest upstream site in the lower Ouachita River system, and is hydraulically connected to the main channel of the Ouachita River. The site is located approximately 0.7 river miles east of the Ouachita River main channel in Raymond Lake with Spring and Green Slough as tributaries. RL-2 has a gravel bottom as opposed to the soft sediment substrate at OR-2 and OR-11. OR-2 is located approximately 32 river miles downstream of RL-2 in a backwater/eddy also hydraulically connected to the main channel of the Ouachita River. The OR-2 sampling site is approximately 0.2 river miles from the main channel. The last site, OR-11 is located downstream of the Felsenthal lock and dam (L&D) in the former main channel of the Ouachita River upstream of where it flows into the tail water of the Felsenthal L&D (See Section 1.4, Site Characterization).

3.2 Sampling Strategy

Sampling of all three sites was conducted from late July to early August 2010. This time frame was selected to target the season that would have the highest ambient temperatures and water temperatures of the year, and lowest water flow, and thus the most likely time for stratification to occur in the water column. Both RL-2 and OR-2 were visited three times each to sample surface water for THg and MeHg, DOC, field parameters, and bed sediment for MeHg. OR-11 was sampled twice.

Field sampling was conducted out of a 16-ft flat bottom aluminum boat. The boat was equipped with a depth finder and all equipment necessary for mercury sampling and field parameter analysis. Two field personnel were always present during sampling for safety and logistical purposes.

3.3 Sampling and Processing Methods

3.3.1 Field Parameters

Field parameters were collected using a Hydrolab MS5 minisonde configured to measure temperature, pH, SpC, DO (amperometric sensor), and ORP. This minisonde was a loaned instrument from Hach Hydromet configured specifically for this project. Water depth for point analysis was determined using gradations on the MS5 cable. The MS5 was calibrated daily for pH, SpC, and DO for the duration of the project. pH calibration was conducted using a 2-point calibration at pH 4 and 7. SpC calibration was conducted using a 2-point calibration with 50 and 100 µS/cm standards. DO was calibrated with a one-point calibration using the air-saturated water method. All calibration methods followed those outlined in the USGS Field Manual for the Collection of Water-Quality

Data, chapter A6 (F. e. Wilde chapter sections variously dated). The ORP probe was calibrated by Hach Hydromet in their instrument lab in Loveland, CO before the project, and was not calibrated during the project at the suggestion of Hach personnel.

3.3.2 Mercury and DOC

Water column sampling for Hg and DOC was performed using the pump-sampling method described in (F. Wilde, et al. 2004-2009(b)). Clean Hands/Dirty Hands (CH/DH) techniques were used when handling all sampling equipment. The sampling process involved the use of a Teflon weighted depth sampler, Teflon tubing, a peristaltic pump and a Kevlar rope provided by the USGS Wisconsin District Mercury Lab (WDML). The Teflon tube and Kevlar rope was attached to the depth sampler and lowered to the desired sample depth. One section of Teflon tubing was designated for each site to avoid cross contamination. Bottom-water samples were taken approximately 5 cm above the bottom-water to avoid disturbing the bottom sediment. Whole water was pumped through the tubing using the peristaltic pump located on the sampling boat for 3-5 minutes to clear the tube, and then

Figure 10: Vacuum-desiccator filtration chamber with filtration assembly attached (Photograph by Michael E. Lewis, after F. Wilde, et al. 2004-2009(b))

into a 2-liter polyethylene terephthalate copolyester, glycol modified (PETG) bottle. Only clean, unused PETG bottles were used to collect water samples for each sampling event. The PETG bottles were triple rinsed with native water before filling, and placed in a cooler with double-bagged ice for transport. PETG bottles were discarded after each sampling event. Three bottom water samples were obtained for each site plus a water column sample except for OR-11 which had only two bottom water samples.

Water samples were processed in a clean, controlled environment (hotel rooms) using the vacuum-filtration method described in (F. Wilde, et al. 2004-2009(b)). Sample water was filtered through a 47-mm 0.7-µm pre-combusted quartz fiber filter (QFF) attached to the filtration chamber (Figure 10). The QFF's were placed on Teflon petri dishes and frozen for later analysis for particulate THg (PTHg) and particulate MeHg (PMHg). Sample water for THg analysis was filtered into a 500-mL Teflon bottle provided by the WDML, and sample water for MeHg analysis was filtered into a 250-mL Teflon bottle. All sample bottles and filtration supplies were supplied by the WDML, and undergo rigorous cleaning protocols involving 4N hydrochloric (HCL) acid baths at 65-75°C for 24-48 hours. After filtration, filtered THg (FTHg) samples were preserved with 10 mL of 6N omni pure HCL, and filtered MeHg (FMHg) samples preserved with 5 mL of 6N omni pure HCL provided by the WDML. Ultra pure reagent-grade blank water supplied by the WDML was filtered and analyzed in the controlled environment once a week prior to field sampling for a total of three blank sample runs for Hg and DOC analysis.

DOC samples were filtered through a 0.7 µm QFF using the same vacuum filtration apparatus and method as the Hg samples. Sample water was filtered into sterilized 8-oz

amber bottles and preserved with 2 ml of 4.5 *N* sulfuric acid following methods described in (F. Wilde, et al. 2004-2009 (a)).

3.3.3 Sulfide, Sulfate, Iron

Sample water for sulfide, sulfate, and iron analysis was obtained using both horizontal and vertical Van-Dorn beta samplers manufactured by Wildco, Inc. Samples from the first week of sampling were collected using a Van Dorn vertical water sampler, but resulted in inconsistent values for field triplicates, and demonstrated an inability to target a specified depth within an acceptable deviation from that depth. A horizontal Van Dorn sampler was obtained for the last two weeks of the project which allowed for sampling of more accurate, specified depths. Samples were obtained from the bottom-water in addition to one vertical profile per site with sample water collected at 5-ft and 10-ft depths in addition to the bottom-water. The purpose of these profiles was to assess any variation in the concentration of the parameters through the water column.

3.3.4 Bed Sediment Sampling

Sediment samples to be analyzed for sediment MeHg (SMHg) were obtained using a 6x6x9 Birge-Ekman grab sampler following procedures outlined in (Radke 2005). This sampler provides a penetration depth of 0-4 inches (0-10 cm), and was chosen because of its ability to obtain water samples in deep waters. Teflon vials and gloved hands were used to transfer the sediment in the grab sampler to the sample vial. Extreme care was used to ensure that the sediment collected in the Teflon vial did not come into contact with any metal surface in the grab sampler. CH/DH techniques were always used when handling Teflon vials and the grab sampler. The use of this type of sampler for this project was approved by USGS WDML via personnel communication.

3.3.5 Diurnal Sampling

On August 11th and 12th, 2010, we implemented a diurnal sampling event at OR-2 to determine any potential fluctuations of Hg and sulfides over a 24-hour period. Sampling began at 12:11 p.m. on 8/11/10 and concluded at 9:06 am on 8/12/10. The site was visited eight times, approximately every three hours. Individual trips were made from a boat ramp to the sampling site for each sampling time. After every sample was collected, we returned to the boat ramp to transfer the Hg samples into a storage cooler.

Sample collection methods for Hg for the diurnal sampling followed the procedure described in 1.3.2. However, instead of pumping into 2-L PETG bottles, water was pumped directly into the 500-mL and 250-mL Teflon bottles for unfiltered THg (UTHg) and unfiltered MeHg (UMHg) analysis, respectively. The bottles were triple rinsed with native water before filling, and placed in a cooler with double-bagged ice for transport. Samples were preserved in two groups on two occasions at the boat ramp over the 24-hour period to reduce the exposure of the preservation acid to the environment. CH/DH techniques and extreme care was taken to avoid contamination of the preservation acid. Field parameters were collected with the MS5 minisonde at 5-ft, 10-ft, and 14-ft depths (total depth at OR-2 was 14-ft) for each site visit. Sulfide samples were collected at the bottom-water and analyzed on the boat for each site visit to go along with each Hg sample.

3.4 Analytical Procedures

3.4.1 Total Mercury

Processed water samples for FTHg and UTHg were shipped to the WDML for analysis within 14 days of collection. FTHg and UTHg are analyzed using EPA Method 1631 (United States Environmental Protection Agency 2002; Olson and De Wild 2010). The method involves the use of Bromine Monochloride (BrCl) to oxidize all forms of Hg to the Hg(II) oxidation state. After 5 days at 50°C, the BrCl is neutralized with Hydroxylamine Hydrochloride, followed by the addition of Stannous Chloride to reduce the Hg from Hg(II) to Hg^0. The Hg^0 is then purged onto a gold-coated glass bead trap. The Hg vapor is then thermally desorbed to a second gold trap and from that detected by cold vapor atomic fluorescence spectrometry (CVAFS) (Olson and De Wild 2010). For this method, THg and total recoverable mercury are synonymous (United States Environmental Protection Agency 2002). The WDML utilizes both a manual THg method (described above) and an automated THg Tekran 2600 sampler (Ogorek & Thompson 2010). The method detection limit (MDL) for THg at the WDML is 0.04 ng/L

The PTHg analysis method also utilizes CVAFS by analyzing the suspended solids isolated on the QFF's from the filtering process. Analysis methods follow the standard operating procedures described by the WDML (Olund, et al. 2010). In short, the filters are transferred from the Teflon petri dishes; they are placed in to 125-mL wide mouth Teflon bottles and oxidized with a 5% BrCl solution to convert all forms of Hg to Hg(II). The samples are then placed in an oven at 50°C for a minimum of 5 days, and then

analyzed according to USEPA method 1631 (Olund, et al. 2010). The MDL for PTHg is 0.059 ng/filter.

3.4.2 Methyl Mercury in Water

All water samples for FMHg and UMHg were shipped to the WDML for analysis within 14 days of collection along with the THg samples. The WDML utilizes a method for determination of FMHg and UMHg by aqueous phase ethylation followed by cold vapor atomic fluorescence detection (CVAFD) outlined by (De Wild, Olson, & Olund, 2002). The method detection limit for FMHg and UMHg is 0.04 ng/L.

The WDML analyzes PMHg by coupling the Brooks-Rand "MERX" Automated MeHg Analytical System with an Elan Inductively Coupled Plasma-Mass Spectrometer (ICPMS) following procedures outlined by (J. Ogorek n.d.). The MDL for PMHg is 0.01 ng/filter.

3.4.3 Methyl Mercury in Sediment

Samples for SMHg are analyzed at the WDML by CVAFD with the Brooks-Rand "MERX" automated MeHg analytical system. Samples are weighed into polypropylene digestion tubes and digested in 2ml of 25% KOH/Methanol at 60°C for 4 hours. The sample extract is then diluted up to a 10ml volume with reagent water. This diluted extract is buffered to a pH of 4.5 – 5.0 and treated with Sodium Tetraethylborate (NaTEB), resulting in ethylation of oxidized Hg species. The ethylated species are stripped from liquid phase with argon gas, desorbed and separated with a gas chromatograph column, and then detected by CVAFS. Procedures for this method are

outlined in (De Wild, et al. 2004) and (Ogorek and Dewild n.d.). The MDL for SMHg is 0.08 ng/g.

3.4.4 DOC Analysis

Processed water samples for DOC were filtered through 0.7 μm QFF's as described earlier. All samples were sent to the AWRC water quality lab in Fayetteville, Arkansas. The lab uses a Skalar Formacs Carbon Analyzer for analysis and follows the American Public Health Association (APHA) method 5310 C. The AWRC lab MDL for this method is 0.3 mg/L.

3.4.5 Sulfide, Sulfate, Iron

Sulfide, sulfate, and iron analysis was conducted at the site on the deck of the sampling boat using whole water as soon as the Van Dorn sampler was brought to the surface. This was particularly important for sulfide analysis as oxidation of sulfide can occur rapidly when introduced to an oxidizing environment. Analysis was performed using a Hach DR-2800 colorimeter. For each aliquot of sample water from the Van Dorn sampler, six 10-mL sample cells were used for analysis; one blank was used to zero the instrument, two blanks were run as samples, and three sample cells of sample water analyzed for the specified constituent to provide triplicate analysis. The concentration of the specified constituent from the three sample water cells was averaged to obtain a single value with a standard deviation from the mean. Mean and Median values of triplicate analysis agreed within 10% on average for all three sites.

Sulfide concentrations were determined using Hach method 8131, a USEPA approved Methylene Blue method, with a reported detection range of 5-800 μg/L. This procedure

is adapted from *Standard Methods for the Examination of Water and Wastewater*, equivalent to Standard Method 4500-S^{2-}D. The Methylene blue method reflects concentrations of hydrogen sulfide and acid-soluble metal sulfides. A seven-point calibration curve was generated using serial dilutions of a 1,000 mg/L sulfide standard solution obtained from Aqua Solutions, Inc. to calculate sulfide concentrations (Appendix 1, Calibration curves). Standard solution concentrations for the calibration curve ranged from 100-700 µg /L. Although a seven-point calibration curve was generated, most sulfide concentrations only fell within the 0-100 µg/L range, utilizing only two points of the curve. As a result, a separate 5-point calibration curve was generated to cover the range of concentrations encountered at the three sites. The low-level calibration curve was generated from standards ranging from 20-100 µg/L. The sulfide standard was used within 2 days of delivery to minimize any effect of sulfide oxidation that might compromise the standard concentration. Absorbances from sulfide analysis in the field were recorded and applied to the standard curve to calculate concentrations.

Calibration curves for sulfide were applied to data on and after 8/10/2010. All sulfide data collected before 8/10/2010 are the result of concentrations recorded directly from the DR-2800 colorimeter, and were not calculated using a calibration curve. A comparison of calibration-curve corrected concentrations to concentrations reported by the colorimeter at site OR-11 showed an agreement between the two concentrations within 17% (n=6). This close agreement, which occurred at the low end of the detectable range, gives evidence for the validity of data reported by the colorimeter before a calibration curve was employed.

The lower limit of detection (LLD) was calculated for sulfide analysis for the DR2800 using the same 1,000 ppm sulfide standard used in the calibration curves. The standard was used within 2 days of delivery to avoid any sulfide loss from the standard due to oxidation. The LLD was calculated following procedures in the Standard Methods for Water and Wastewater, chapter 1030C, "Data Quality/Measurement Uncertainty". Unacceptably high variability was encountered from analysis of the standard at low concentrations after various serial dilutions, which exemplified the need for extreme care when handling the standards. Serial dilutions were repeated until proper absorbances were obtained for the standard concentrations. Twenty samples of 20 μg/L standard were analyzed to calculate the LLD. The standard deviation of the concentrations for the standard was multiplied by a factor of 3.29, the multiplication factor given in the Standard Methods to reduce the probability of a type II error (false non-detection). The resulting LLD was 18.43 μg/L. This value is also interpreted as the potential variability in all low-level sulfide analysis, making all reported concentrations ±18.43 μg/L. The LLD will also be referred to as the method detection limit (MDL) for purposes of this project.

Sulfate concentrations were determined using the Hach Sulfa Ver 4 method, adapted from *Standard Methods for the Examination of Water and Wastewater* by Hach, a procedure equivalent to USEPA method 375.4 for wastewater. Sulfate concentrations were also determined using a seven-point calibration curve generated using serial dilutions of 1000 mg/L sulfate standards obtained from Ricca Chemical Company (Appendix 1: Calibration Curves). Standard solution concentrations for the calibration curve ranged from 10-70 mg/L. A calibration curve for sulfates was not calculated for

the instrument until August 8th, so all data before this date reflect concentrations recorded from the colorimeter directly in the field and were not adjusted to a calibration curve. Analysis of measured sulfate concentrations versus sulfate concentrations after August 8th using the calibration curve showed an average 19% difference in concentrations. Therefore, the sulfate concentrations recorded before the implementation of the calibration curve are assumed to be accurate within 20% of the expected concentrations using the calibration curve. Hach reports the detection range for the Sulfa Ver 4 method at 0-70.0 mg/L sulfate.

Total iron concentrations were determined using Hach Ferro Ver method 8008, also adapted from *Standard Methods for the Examination of Water and Wastewater.* A seven-point calibration curve was generated using serial dilutions of 1000 mg/L iron standards obtained from Ricca Chemical Company. A calibration curve for iron was not calculated until August 8th, so all data before this date reflects concentrations recorded directly from the colorimeter without any correction. Analysis of measured iron concentrations versus corrected iron concentrations after August 8th using the calibration curve showed a 3% difference in concentrations. Therefore, the iron concentrations recorded before the implementation of the calibration curve are assumed to be accurate within 5% of the expected concentrations. Standard solution concentrations for the calibration curve ranged from 0.4 to 2.8 mg/L. Hach reports the detection range for the Ferro Ver method at 0-3.00 mg/L total iron.

3.5 Statistical Analysis

Non-parametric statistics were used to compare median values of bottom-water concentrations of Hg species and other parameters between sites. Sign-rank and rank-

sum tests were used to test the equality of median values, and one-way ANOVA's were used to test for equality of means. Statistical significance was set at α=0.05.

4 Results

4.1 Field Parameters

All three sites exhibited stratification with respect to temperature and DO, with high temperature and high DO in the shallow depths, and lower temperatures and anoxic conditions in the bottom-water (Figure 12). RL-2 exhibited the strongest stratification with respect to both temperature and DO as shown in Figure 12. OR-2 was the shallowest site with a maximum depth of 14-ft, but also exhibited strong stratification with anoxic conditions at the bottom-water.

pH values did not vary much from the water surface to the bottom water, with values ranging from 5.89 – 7.04 for all three sites at all depths (mean=6.51, median=6.56) (Figure 11). pH values did not vary significantly at the sites on different sampling days. pH at the bottom water for all three sites had a mean value of 6.56, and a median of 6.61.

Specific conductance for all three sites increased with increasing depth in the water column (Figure 13). RL-2 had the highest values for specific conductance at the bottom-water as compared to the other three sites, with a mean of 411 µS/cm compared to a mean of 109 and 121 µS/cm for OR-2 and OR-11, respectively.

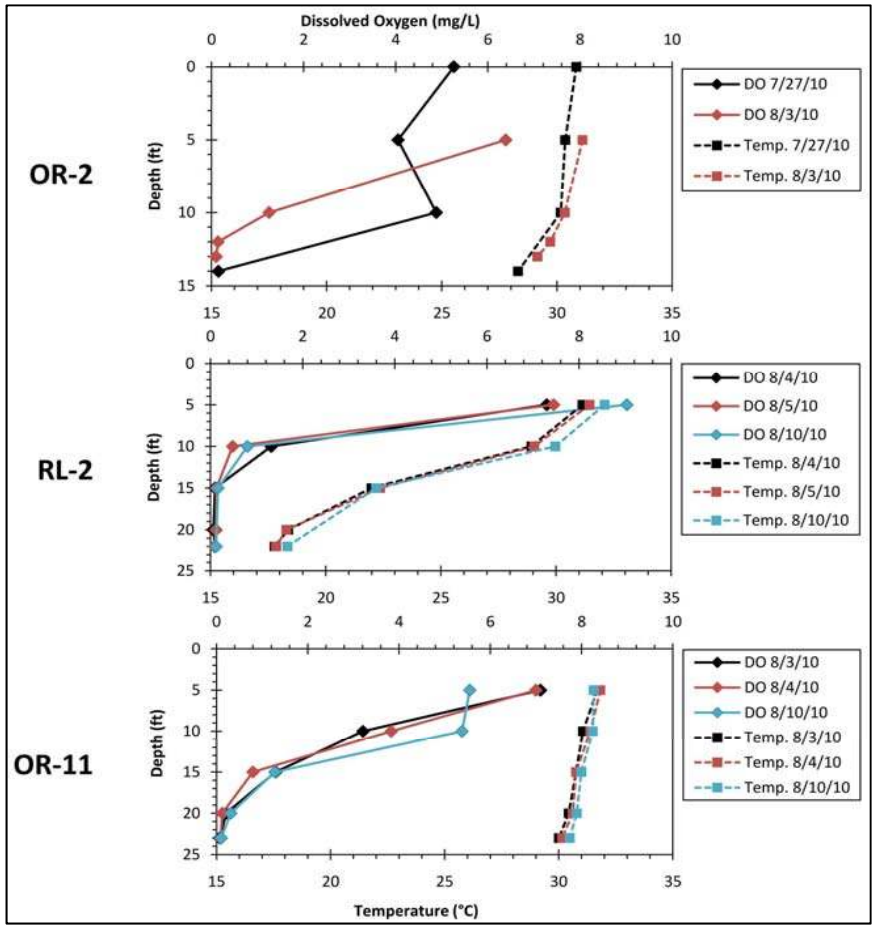


Figure 12: Dissolved Oxygen and Temperature Profiles

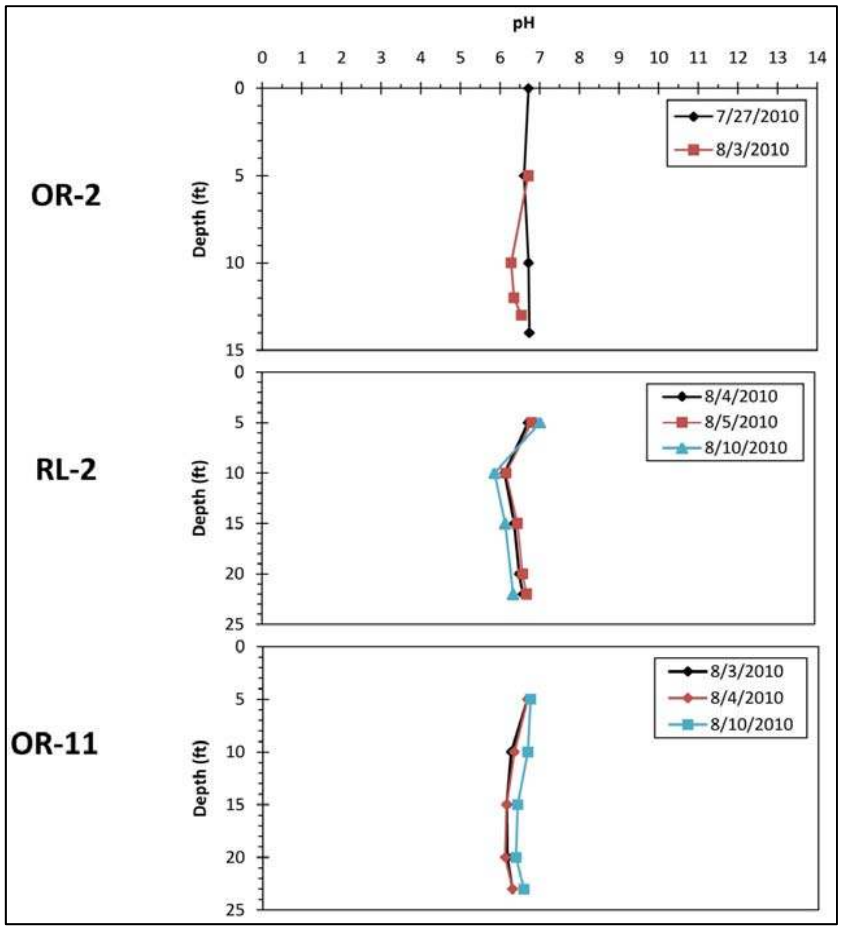


Figure 11: pH Profiles

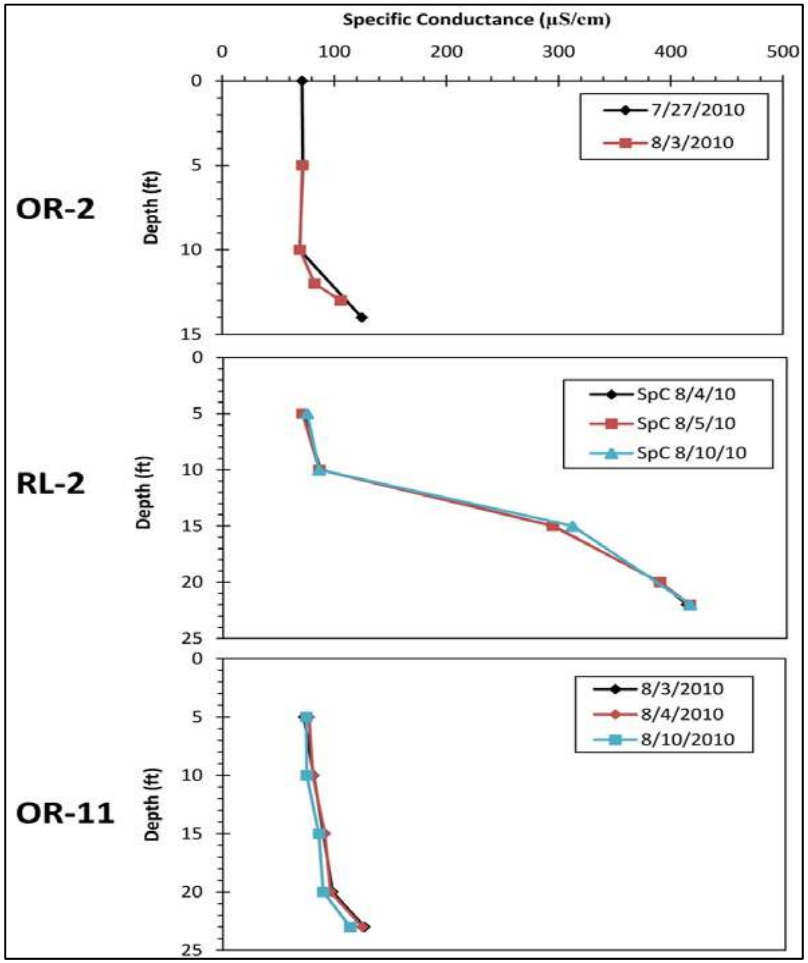


Figure 13: Specific Conductance Profiles

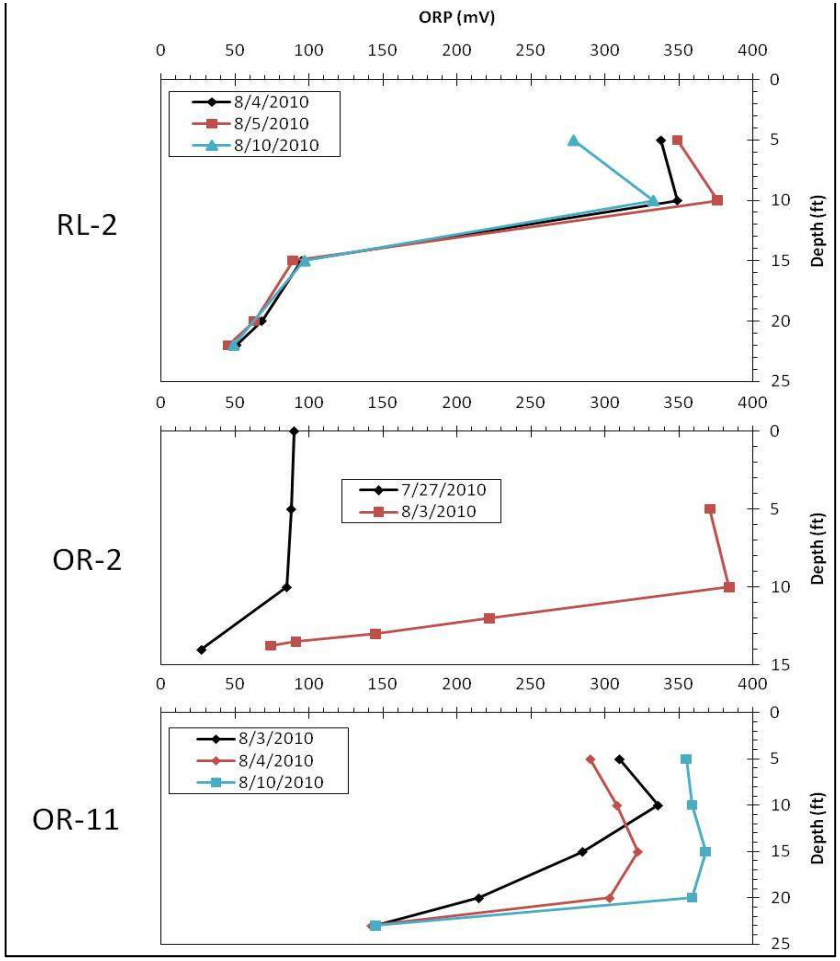


Figure 14: Oxidation Reduction Potential Profiles

Oxidation Reduction Potential (ORP) profiles were recorded at all three sites when Hg samples were collected (Figure 14). High values (+300mV) were recorded in the oxygenated water near the surface, with ORP decreasing with depth. RL-2 and OR-2 both exhibited ORP decreases of approximately 300 mV from the water surface to the bottom-water, withe a median value of 47mV in the anoxic bottom-water for RL-2 and 46mV for OR-3. The decrease in ORP at OR-11 was not as dramatic as RL-2 and OR-2, with an average drop of 184 mV, and a median ORP of 145 in the bottom-water. OR-2 exhibited the highest variability in the two ORP profiles obtained on 7/27/10 and 8/3/10, with values in the anoxic bottom water of 27mV and 74 mV respectively. A Hg sample was collected on 7/29/10 at OR-2, but ORP profiles were not collected.

4.2 Sulfide, Sulfate, and Iron

4.2.1 Sulfide

Sulfide concentrations were assessed both at the bottom water to provide evidence for the presence or absence of sulfate reduction, and at the midpoint of the water column to graphically depict the vertical change in concentrations. Sulfide vertical profiles exhibit an increase of sulfides in the water column from the water surface to the bottom-water for all three sites (Figure 15). For the profile data, site OR-11 had the highest sulfide value at the bottom-water (143 µg/L), followed by OR-2 with a concentration of 88 µg/L, and RL-2 with a concentration of 81 µg/L. Sulfide concentrations in the shallow surface waters (10 ft for RL-2 and OR-11, and 5 ft for OR-2), were at or below the MDL of 18.43 µg/L for sulfides using the DR-2800 colorimeter.

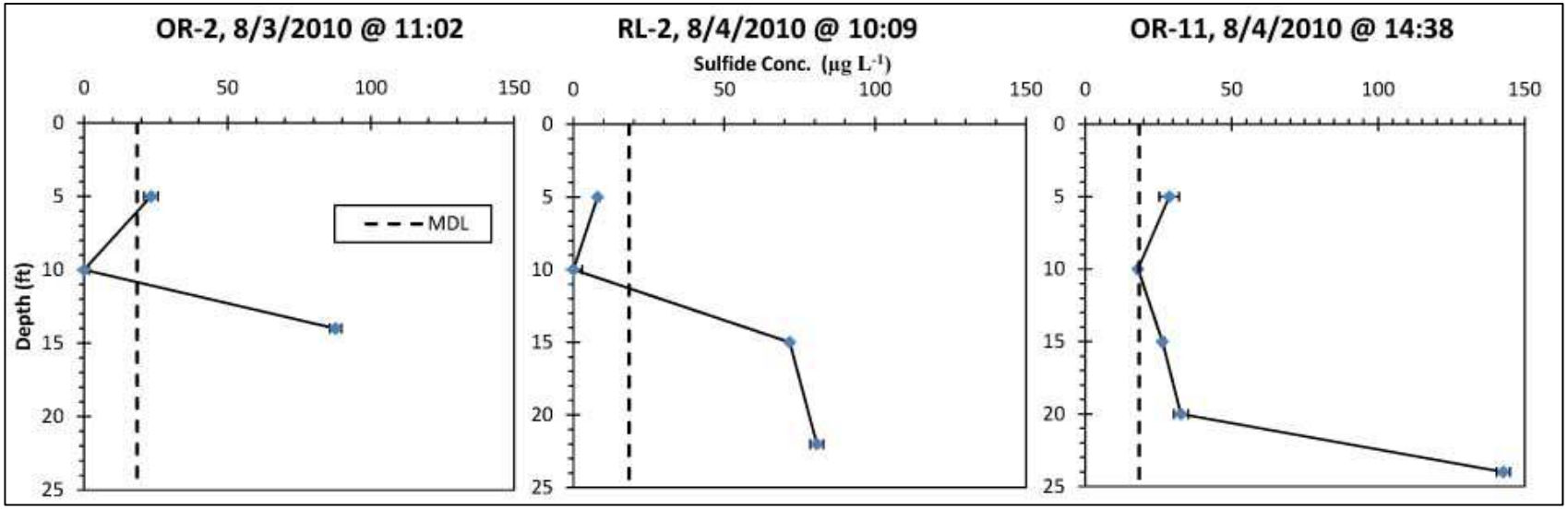


Figure 15: Sulfide Profiles for all sample sites

Sulfide concentrations at the bottom-water for all three sites assessed for each Hg sample ranged from 65-237 µg/L (Figure 16), with a mean of 110 µg/L and a median of 92 µg/L. The maximum value reported (237 µg/L) was a sample obtained from site OR-2, and was most likely biased high because of the influence of bottom sediments in the sample. A vertical van-dorn sampler was used for this sample which proved challenging in

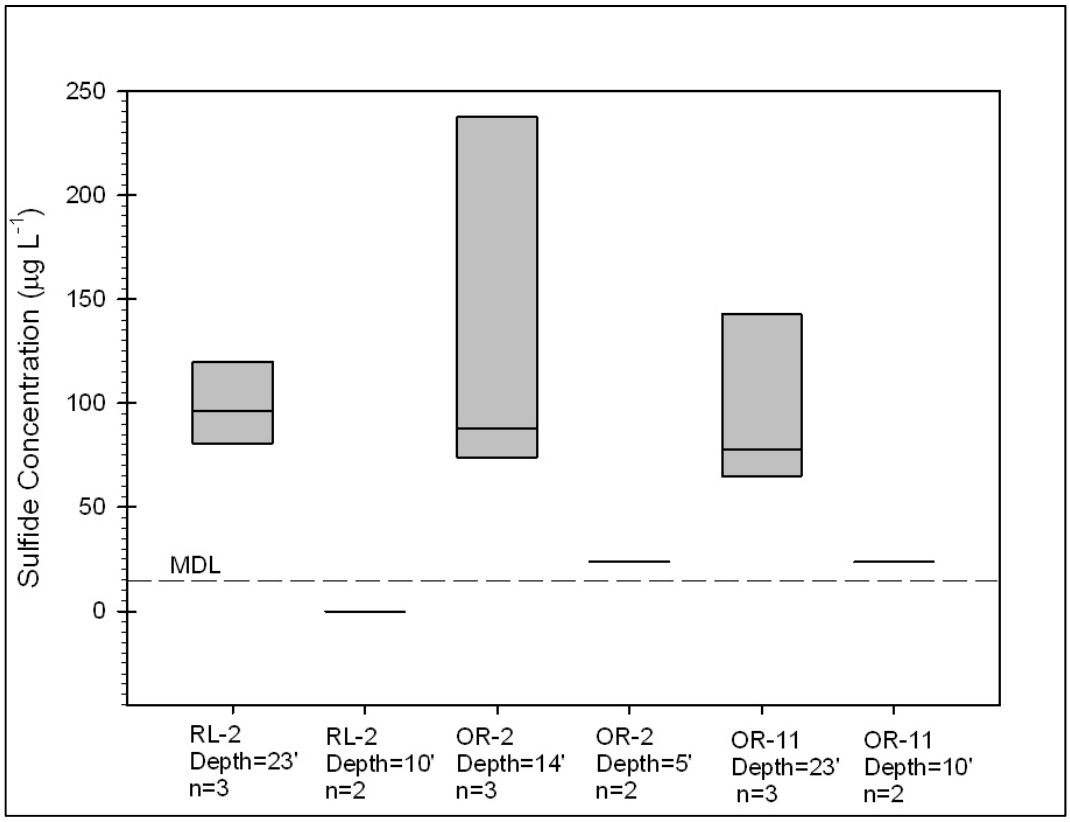


Figure 16: Sulfide concentration comparison at bottom-water and mid-point of water column. Mid-point at RL-2 and OR-11 is 10-ft, mid-point at OR-2 is 5'

obtaining representative samples of anoxic water at the bottom-water, as the sampler would agitate bottom sediments when the messenger released the caps during sample collection. Incorporation of bottom sediment into the sample would bias the sulfide concentrations toward a higher concentration because of the increased rates of sulfide production in the anoxic sediments. As a result, a horizontal Van Dorn sampler was

obtained and used for the remaining sampling events for the project on and after August 3, 2010.

Water-column samples were analyzed for sulfide to compare the potential for sulfate reduction at the mid point of the water column to sulfate reduction at the bottom-water. These comparisons are shown in Figure 16. Sulfide concentrations at the mid-point of the water column were lower than concentrations in the bottom-water. The sulfide range in the water column for all three sites was 0-29.33 µg/L, with a mean of 15.78 µg/L and a median of 20.67 µg/L, making the mean and median values for water column samples slightly above the MDL for the sulfide method.

4.2.2 Sulfate

Sulfate data were not collected as frequently as sulfide data due to time constraints in the field owing to time-intensive sulfide analysis and mercury sampling. As such, sulfate data were collected at the bottom water once for RL-2 and OR-11, and twice for OR-2. In addition, water column samples were also collected once for each site at the same depth as the sulfide water-column samples with the exception of OR-2, which had two water column samples. Some sulfate analyses showed high variability, with standard deviations of triplicate analyses nearly exceeding the mean for two samples (Figure 17). Additionally, distilled water field blanks became turbid to the point of exceeding the colorimeter's absorbance capabilities for two of the sample runs when the reagent packets were added to the blank samples. In these instances, the field blank reagent reactions were repeated until an adequate null value was obtained. At RL-2, no sulfate was detected for the one sample at the bottom water. A water column sample was obtained at a depth of 5-ft , resulting in a sulfate concentration of 16.67 mg/L, with a standard

deviation of 11.55. this large standard deviation could be attributed to instrument error , as well as the fact that this sample was one of the two samples during the project where field blanks of distilled water exceeded the absorbance capabilities of the colorimeter for this method (ABS>3.5). Two samples were collected at the bottom-water at OR-2 (14-ft depth) on two different days (7/29/10 and 8/3/10), and resulted in an order of magnitude difference in concentrations; 1.67 mg/L (σ =3.51) and 65.0 mg/L (σ=0.00), respectively. The high standard deviation on the sample from 7/29/10 could be attributed to the variability of detection at low concentrations, as the concentration range for sulfate analysis is 0-70 mg/L. The water column sample at OR-2 (depth=5') was also on the low end of the concentration range at 5.00 mg/L (σ=1.00).

One sulfate sample was collected at the bottom-water and one in the water column at OR-11 with concentrations of 4.94 mg/L (σ=1.20) and 12.67 (σ=2.52) respectively.

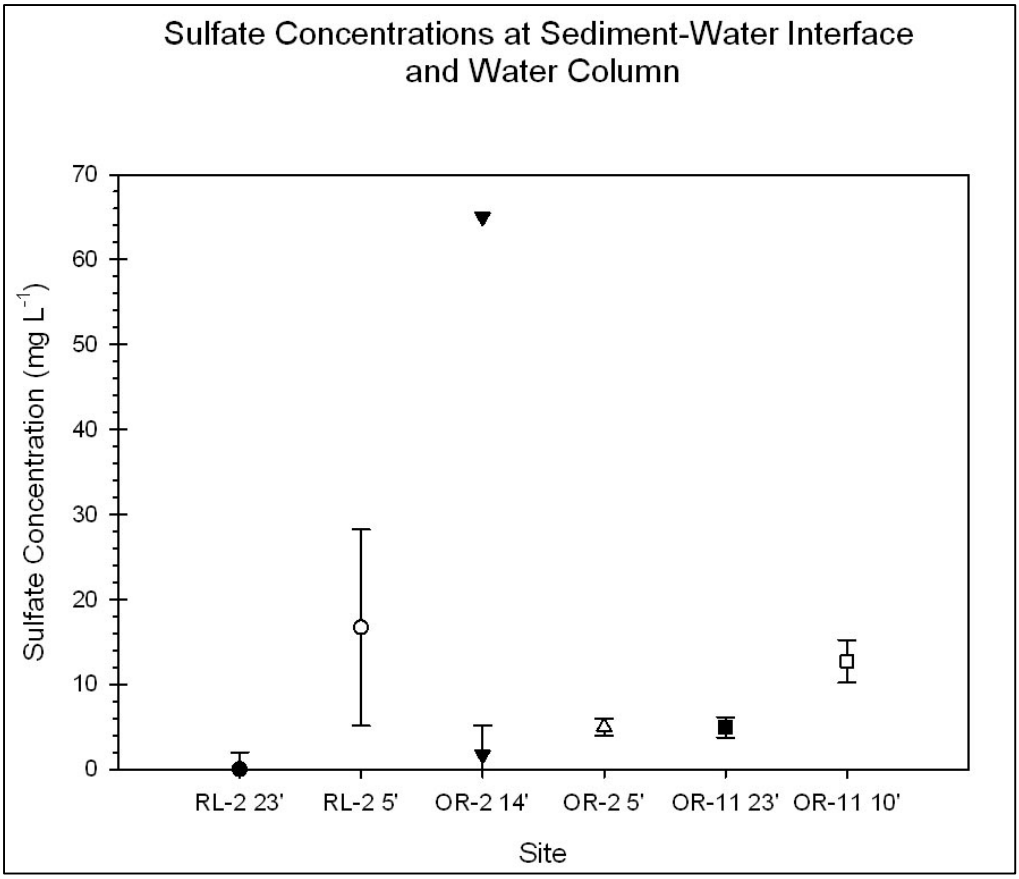


Figure 17: Sulfate Concentrations at bottom-water and mid-point of water column (when applicable). Single points represent sample days, error bars represent standard deviation of triplicate analysis in the field.

4.2.3 Iron

Iron samples were collected less frequently than sulfate, but bottom-water and water-column samples were collected and analyzed for the sites, except for RL-2, which only has one sample at the bottom-water and no samples representing the water column (Figure 18).

The one sample at RL-2, a bottom-water sample, exhibited a concentration of 39.8 mg/L (σ=7.5 for triplicate analysis), the highest iron value recorded at any of the three sites. The RL-2 sample had to be diluted by a factor of 10 because of this high concentration

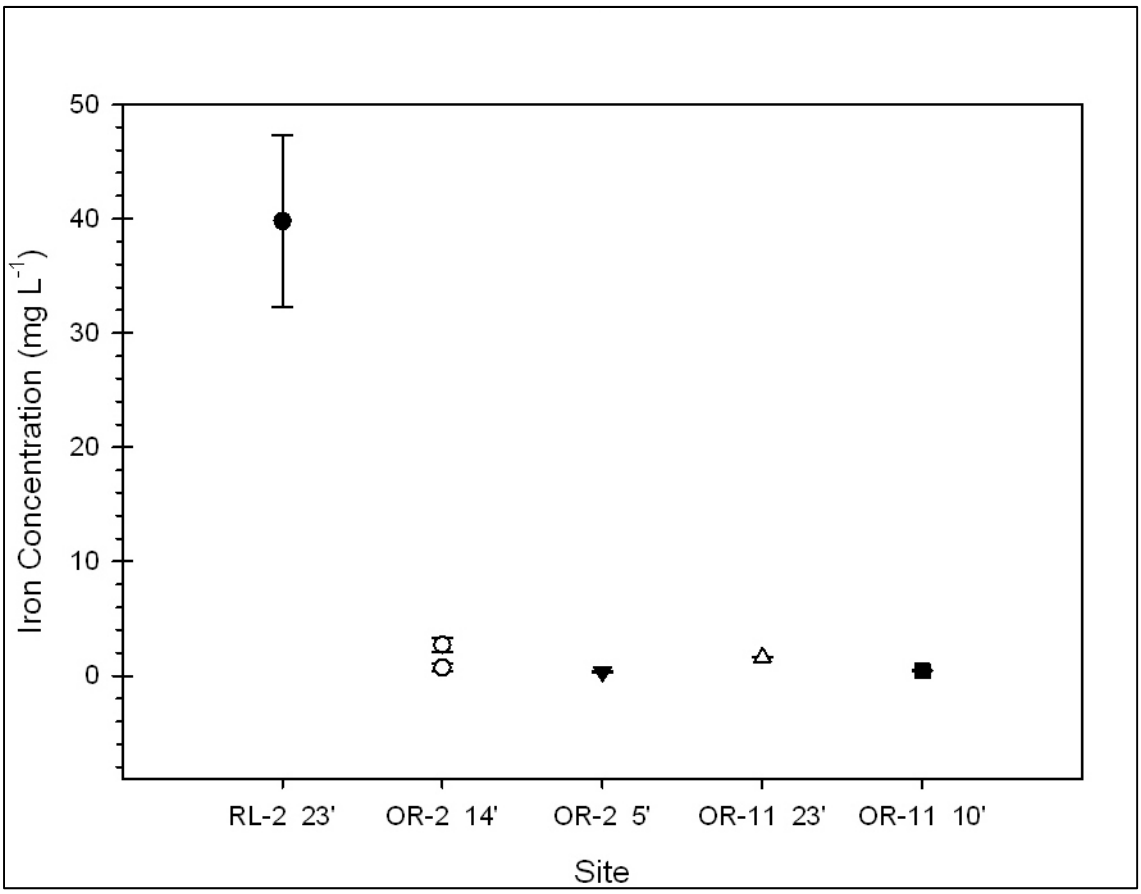


Figure 18: Iron Concentrations at bottom-water and mid-point of water column (when applicable). Single points represent sample days, error bars represent standard deviation of triplicate analysis in the field.

(detectable range is 0-3.00 mg/L for iron analysis), which may explain the high standard deviation. RL-2 also had zero sulfate concentrations at the same depth on the same date of analysis (7/29/10), suggesting the predominance of iron reduction processes at the bottom-water. Iron concentrations at OR-2 at the bottom-water were 2.69 mg/L (σ=0.58) and 0.70 (σ=0.35) on 7/29/10 and 8/3/10, respectively. Concentrations at OR-11 were 1.61 mg/L (σ=0.02) at the bottom-water, and 0.44 mg/L (σ=0.01) in the water column at a depth of 10-ft.

4.3 Dissolved Organic Carbon (DOC)

DOC samples were collected at the same time as each Hg sample for all three sites (see Methodology). DOC showed significant variation between sites (Figure 19), with RL-2

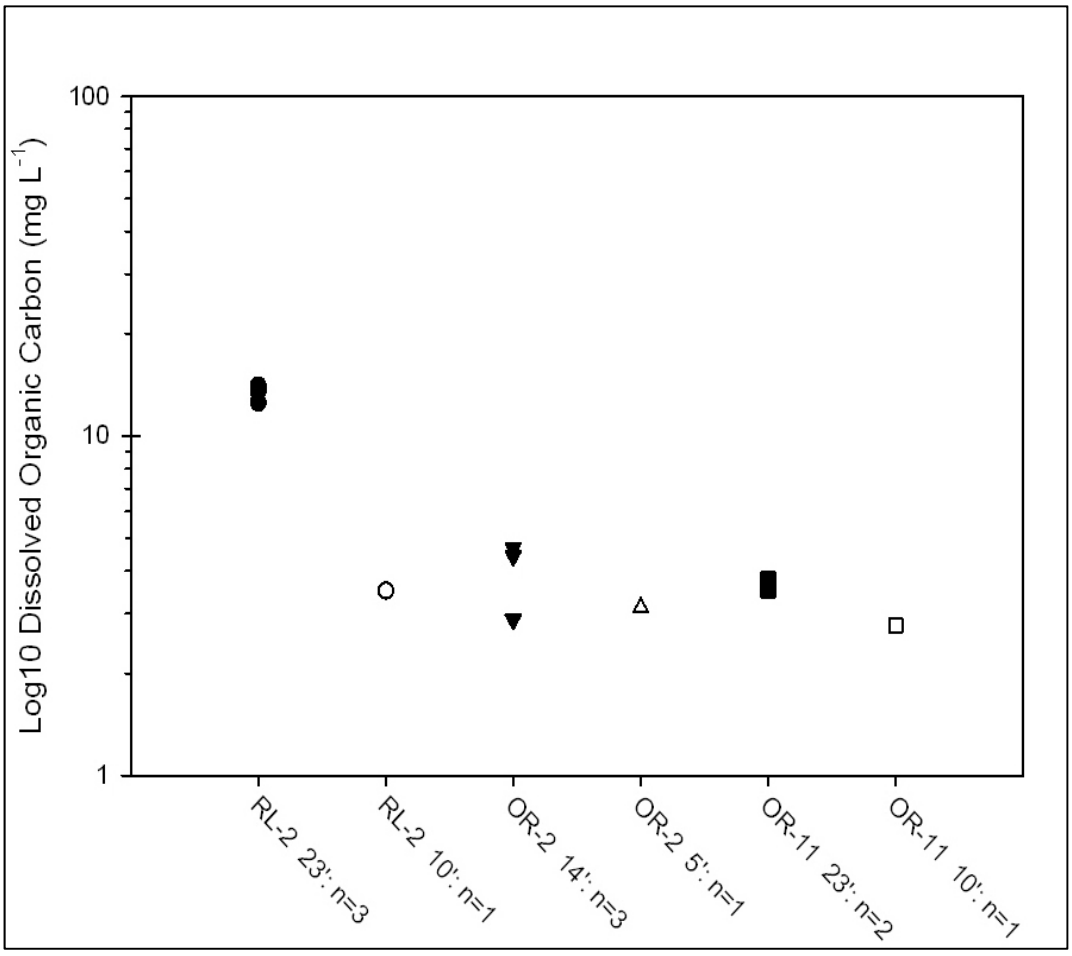


Figure 19: DOC Data at bottom water and midpoint of water column

exhibiting the highest DOC concentrations at the bottom-water (mean 13.37 mg/L for three samples collected on three different days). Median values of DOC concentrations were not statistically different at the bottom water between all three sites when employing non-parametric Mann-Whitney Rank Sum tests. However, mean concentrations were statistically different between RL-2 and OR-2 and RL-2 and OR-11 using a one-way ANOVA ($P<0.001$ for both tests), but were not statistically different

between OR-2 and OR-11 (P=0.660). The power of the latter test was 0.050, well below the desired power of 0.800, owing to the small sample sizes (n=3 for RL-2 and OR-2, n=2 for OR-11). DOC concentrations at the mid-point of the water column were similar between all sites (RL-2: 3.50 mg/L; OR-2: 3.12 mg/L; OR-11: 2.76 mg/L). Only one sample was analyzed for each water column sample at the three sites.

4.4 Mercury

Hg samples were collected and processed from the three sites over the course of 4 weeks. One processed Hg sample consists of filtered and particulate MeHg, and filtered and particulate THg concentrations, for a total of four Hg concentrations per site per sampling time. Three bottom-water samples and one sample at the midpoint of the water column were collected at RL-2 and OR-2. Two bottom-water samples and one sample at the midpoint of the water column were collected at OR-11. Concentrations of Hg compiled from all three sites show increases from the dissolved MeHg (FMeHg) fraction to total Hg (THg), with the lowest FMeHg concentrations occurring at 0.05 ng/L,just above the MDL (0.04 ng/L), and the highest Hg concentrations occurring in the THg fraction at 13.99 ng/L, calculated as the sum of filtered and particulate THg.

Site	Date	Sample Time	Sample Depth (ft)	FMeHg ng L^{-1}	PMeHg ng L^{-1}	TMeHg ng L^{-1}	FTHg ng L^{-1}	PTHg ng L^{-1}	THG ng L^{-1}	F HgD ng L^{-1}	SMeHg ng g^{-1}	DOC mg L^{-1}
RAYMOND LAKE #2	7/29/2010	12:00	23	1.90	1.67	3.57	4.89	5.38	10.27	2.99	-	13.52
RAYMOND LAKE #2	8/4/2010	11:42	23	1.20	1.24	2.44	3.27	8.28	11.55	2.07	-	12.54
RAYMOND LAKE #2	8/5/2010	10:45	23	1.20	1.11	2.31	3.56	10.20	13.76	2.36	-	14.06
RAYMOND LAKE #2	8/10/2010	14:34	10	0.15	0.18	0.33	0.87	1.641	2.51	0.72	-	3.50
OUACHITA RIVER #2	7/27/2010	12:30	14	0.98	2.46	3.44	2.98	8.60	11.58	2.00	0.8	4.68
OUACHITA RIVER #2	7/29/2010	17:00	14	0.06	0.21	0.27	1.15	3.18	4.33	1.09	0.6	2.88
OUACHITA RIVER #2	8/3/2010	13:08	14	0.56	0.94	1.50	2.32	7.28	9.60	1.76	0.64	4.43
OUACHITA RIVER #2	8/12/2010	8:45	5	0.06	0.08	0.14	0.66	1.069	1.73	0.60	1.069	3.12
OUACHITA RIVER #11	8/4/2010	16:00	23	0.34	0.70	1.04	1.18	12.81	13.99	0.84	0.78	3.78
OUACHITA RIVER #11	8/10/2010	9:11	23	0.40	0.55	0.95	0.97	5.584	6.55	0.57	0.55	3.50
OUACHITA RIVER #11	8/10/2010	9:11	10	0.05	0.05	0.10	0.66	1.067	1.73	0.61	-	2.76

Table 1: Hg data for all three study sites

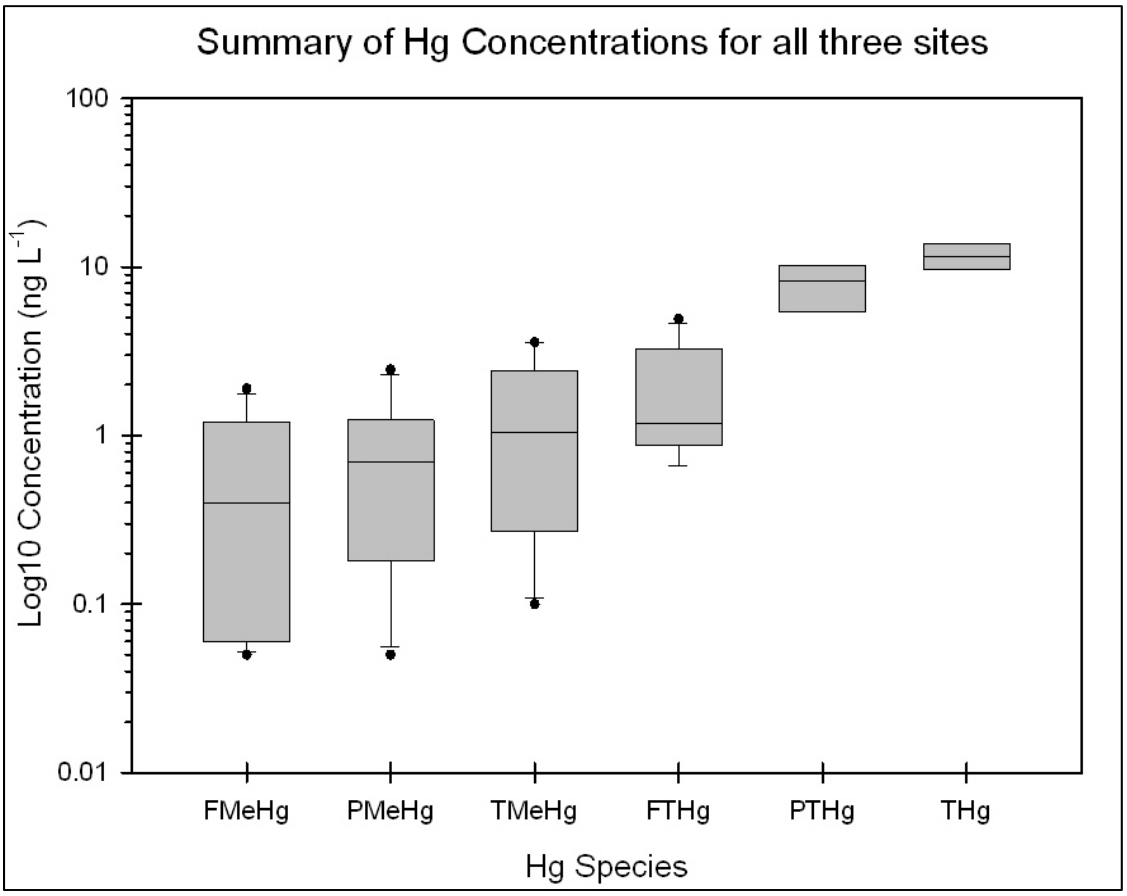


Figure 20: Hg concentrations combined from all three site including mid point water column and bottom-water samples. n=11 for FMeHg, PMeHg, TMeHg, FTHg, n=7 for PTHg, THg

Mercury concentrations in all size fractions and speciation varied between sites (Figure 21). RL-2 had the highest dissolved MeHg and THg as compared to the other sites. OR-11 had the lowest FMeHg, PMeHg, and FTHg, but had the highest PTHg and THg. Hg concentrations at OR-2 were between the other two sites for all size fractions and species. FMeHg and PMeHg at OR-2 showed variability in concentrations between sample days shown by the error bars in Figure 21 representing standard deviation of samples collected on three separate sample days.

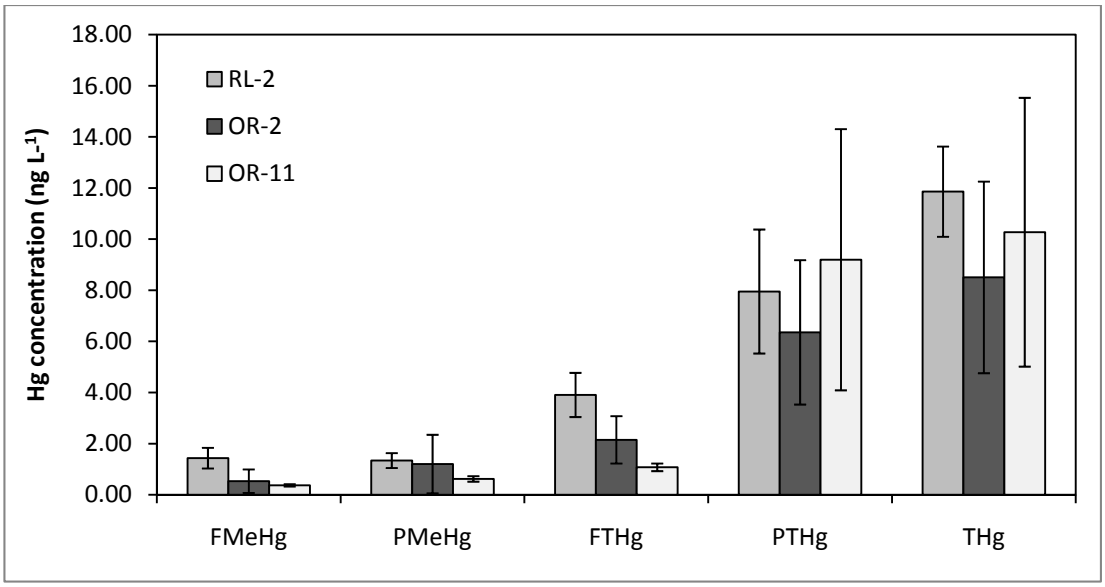


Figure 21: Bottom-water concentrations of Hg size fractions and species at all three study sites. N=3 for all sites. Error bars represent standard deviations of samples collected on separate days.

Non-parametric statistics were used to compare bottom-water samples of Hg species and size fractions between sites. There was no statistical difference of median values at the bottom-water for any of the Hg species and size fractions when using rank-sum or sign-rank statistical tests. One-way ANOVA's used to test the difference of means resulted in statistical differences of FMeHg in the bottom-water between RL-2 and OR-11 (P=0.039) and FTHg between RL-2 and OR-11 (P=0.022) only. All other species and size fractions were not statistically different at the bottom-water between sites. As with DOC analysis, statistical power was low for those tests that failed the one-way ANOVA, most likely due to small sample sizes.

MeHg and THg were positively correlated at the bottom-water for filtered samples (Figure 24), but not significantly correlated for total MeHg (TMeHg) and THg. TMeHg is the sum of the filtered and particulate fractions of the MeHg in the system. THg is

reported in the same way, as the sum of the filtered and particulate fractions of THg in the system, and can be considered as total recoverable Hg.

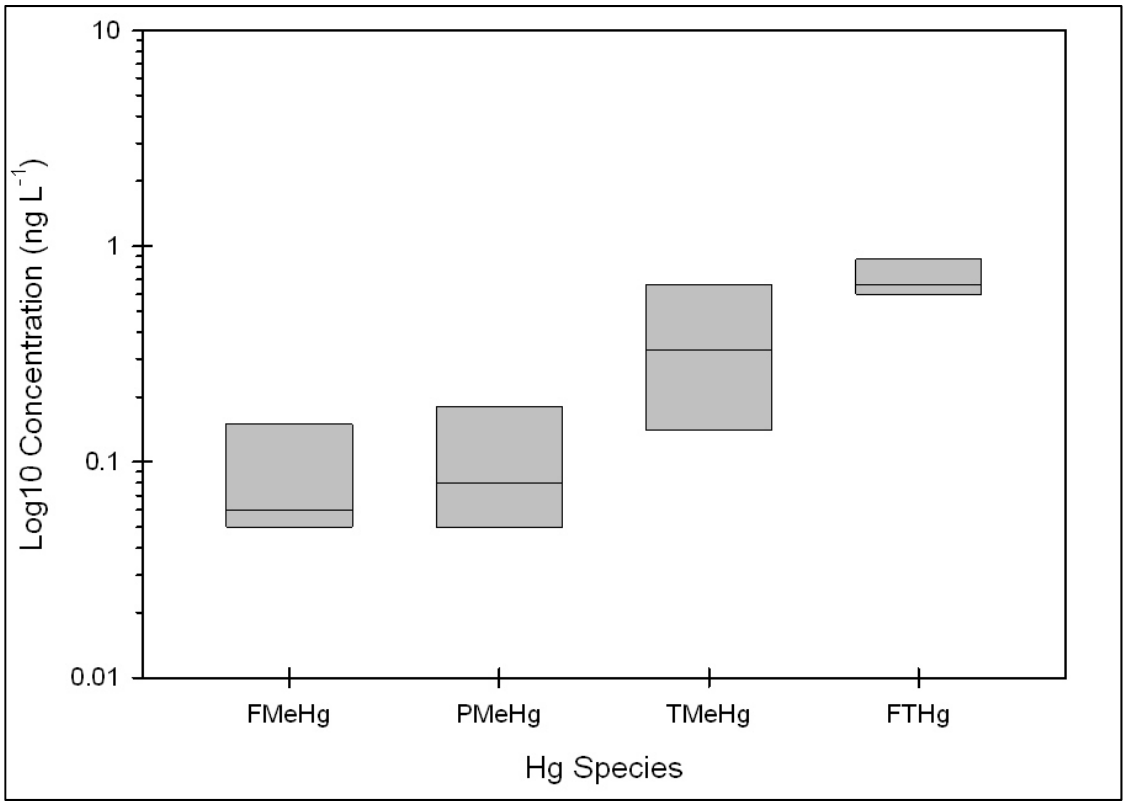


Figure 22: Distribution of Hg concentrations at the mid-point of the water column compiled from all three study sites

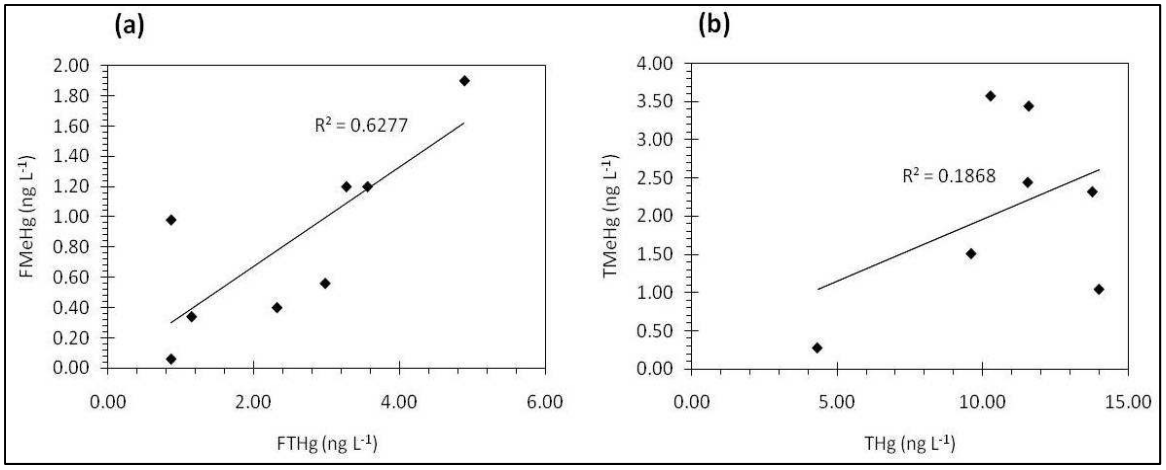


Figure 24: Linear Regressions of (a)filtered MeHg and THg and (b)TMeHg and THg. TMeHg and THg are the sum of filtered and particulate size fractions of Hg

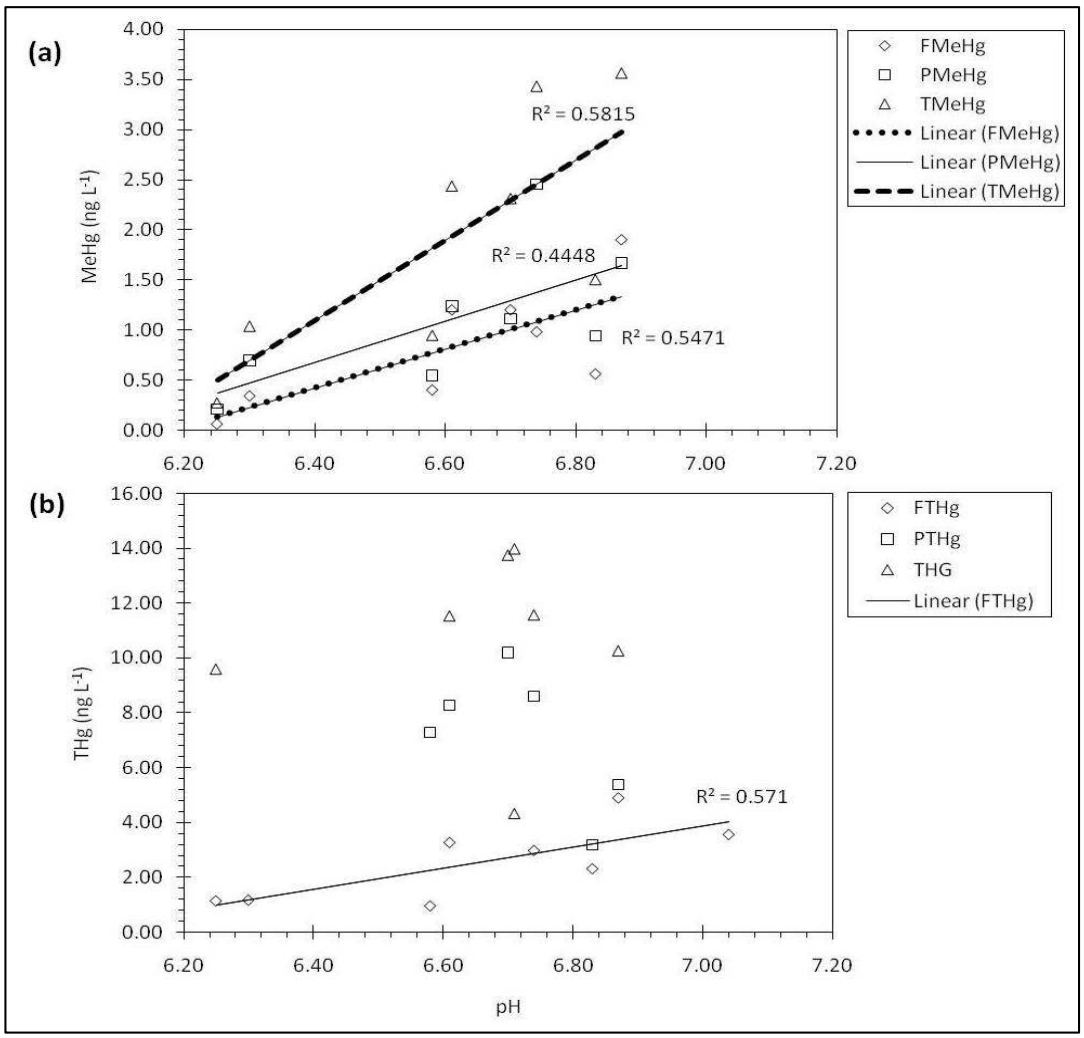


Figure 23: Linear regression of Hg and pH for (a) MeHg and (b) THg

4.4.1 Mercury and pH

All size fractions of MeHg were positively correlated with pH as pH increased from 6.3 to 7.0 at the bottom-water when combining data from all three sites. The pH-FMeHg correlation was more significant (r^2=0.55) than PMeHg (r^2=0.44) (Figure 23). FTHg was positively correlated with pH (r^2=0.57), and PTHg and THg were not correlated with pH.

4.4.2 Mercury and Sulfides

MeHg concentrations did not correlate to sulfide concentrations at the bottom water for n=7 samples, combining data from all three sites (Figure 25). Total Hg and sulfide showed similar relations at the bottom-water for dissolved and particulate size fractions with no correlation from the linear regressions.

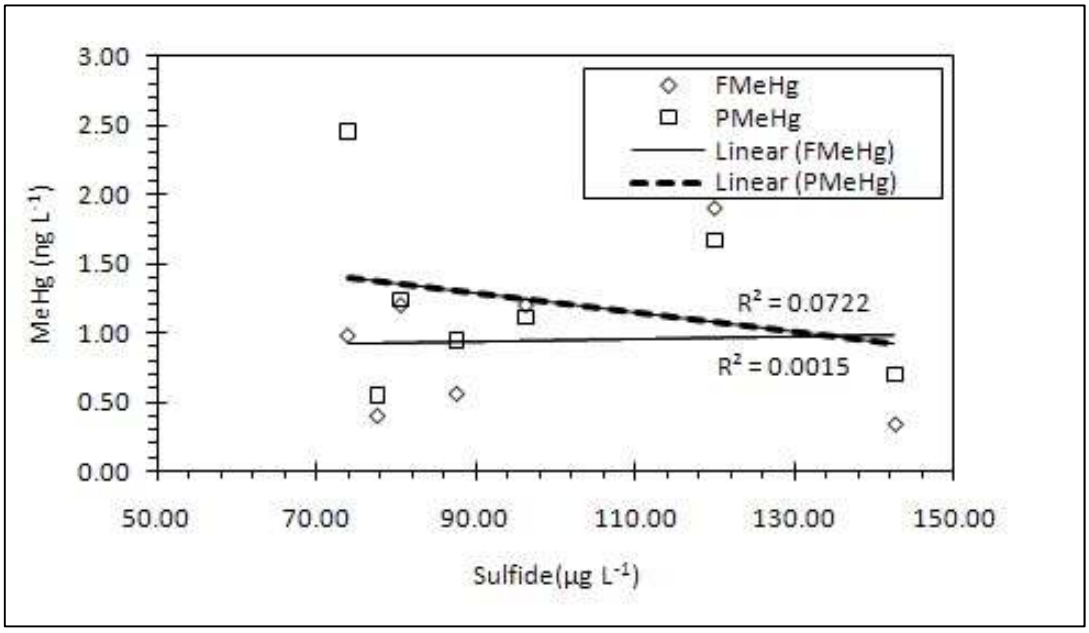


Figure 25: MeHg and Sulfide linear regressions for the bottom water at all sites

4.4.3 Mercury and Iron

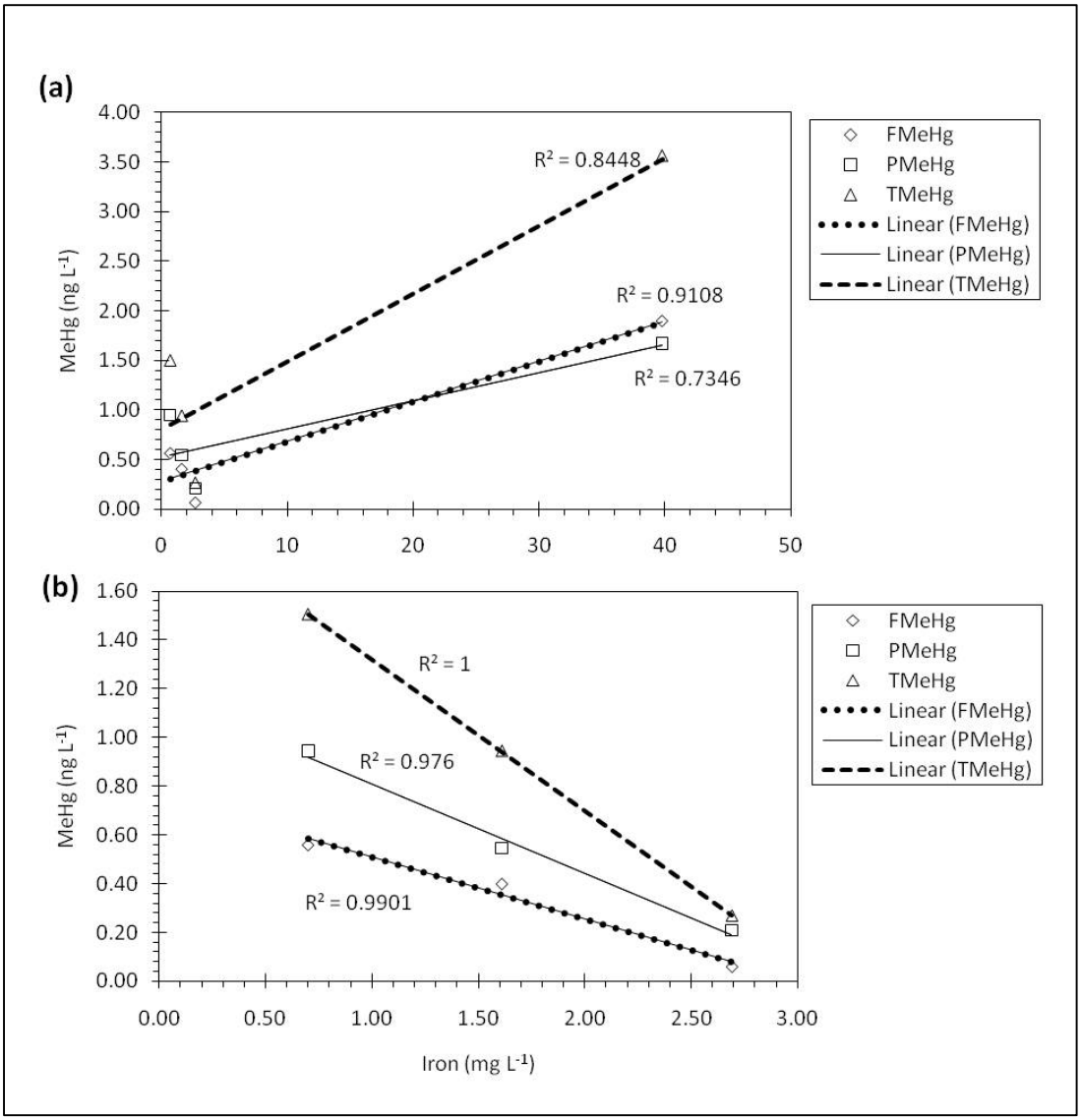


Figure 26: Linear Regressions of MeHg and Iron (a) including the high concentration at RL-2 and (b) excluding the high concentration at RL-2

When assessing iron concentrations and MeHg at the bottom-water at all three sites, a significant positive linear correlation was found for FMeHg and PMeHg (Figure 26a) due to the high iron concentration encountered at site RL-2 (39.8 mg/L). For these relations, only four data points are available for the linear regression analysis with no iron concentrations falling between 2.69-39.8 mg/L resulting in the high r^2 value for FMeHg. Assessing the relation between MeHg and iron at the lower concentrations (excluding the

39.8 mg/L concentration) resulted in a significant negative linear correlation, though these data only reflect n=3 samples, and the high r^2 values should not necessarily be considered as representative of the system. The significance of the correlation of these data are questionable due to the variance in correlations when removing the high iron concentration value at RL-2, and the lack of verification of the RL-2 value (iron was only assessed once at the bottom-water at RL-2). All size fractions of THg were not correlated with iron.

4.5 Mercury and DOC

Filtered MeHg and Total MeHg were positively correlated with DOC at the bottom water when combining data from all three sites (r^2=0.76, and 0.42 respectively), and PMeHg was less correlated with DOC (r^2=0.13). FTHg was positively correlated with DOC at the bottom water when combining data from all three sites (r^2=0.74), but PTHg was not

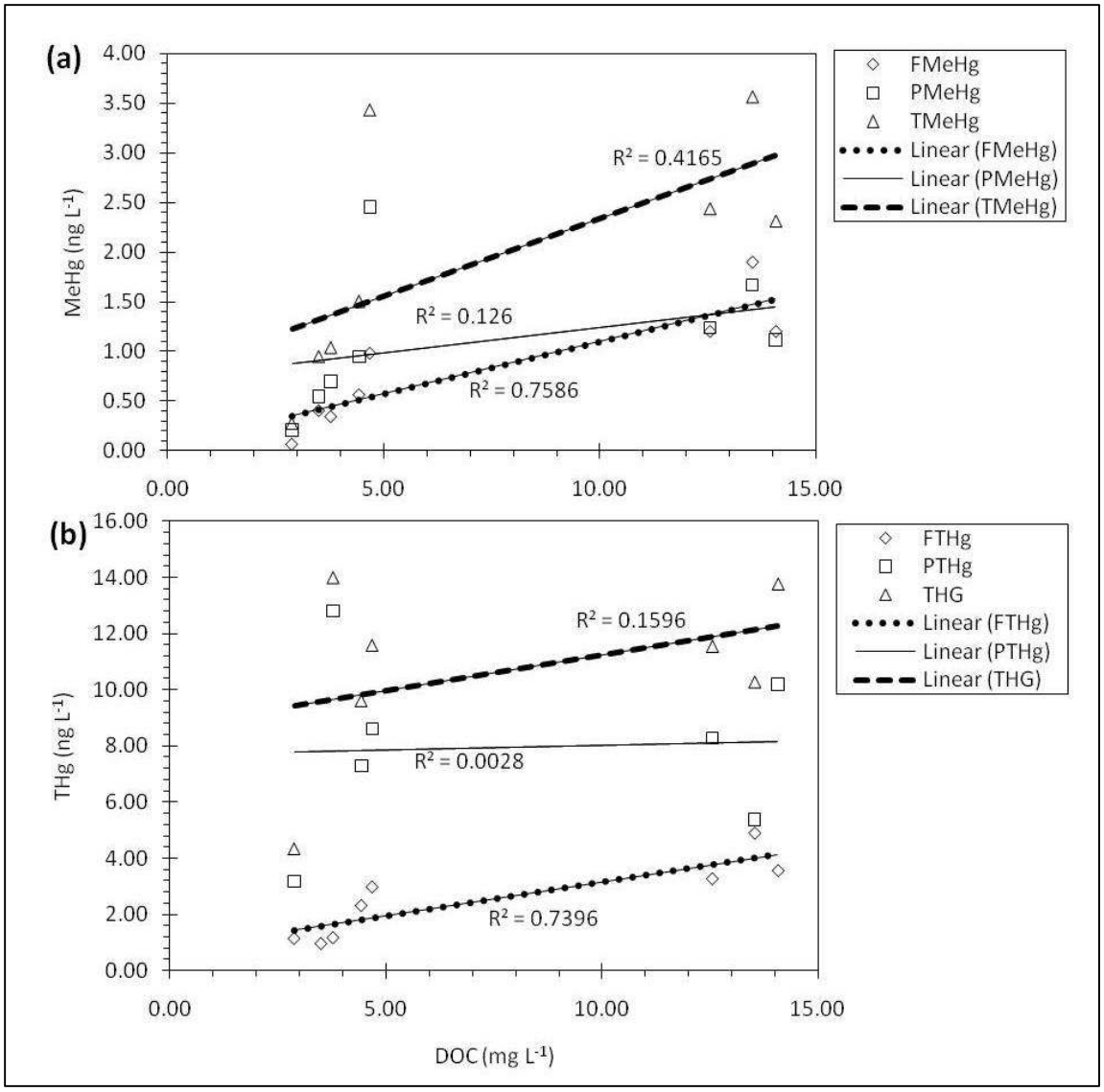


Figure 27: (a) MeHg and DOC linear regressions and (b) THg and DOC linear regressions.

correlated. THg was also positively correlated with DOC, but not strongly (r^2=0.16) (Figure 27).

4.6 Diurnal Study

Field parameters, sulfide, and unfiltered MeHg, and THg concentrations were determined approximately every three hours during the 24-hour sampling event at site OR-2 (Figure 28). Sulfide data exhibited noteable concentration peaks at 12:11 pm and 12:15 am. The minimum sulfide concentration occurred at 8:40 pm. Unfiltered MeHg (UMHg) did not

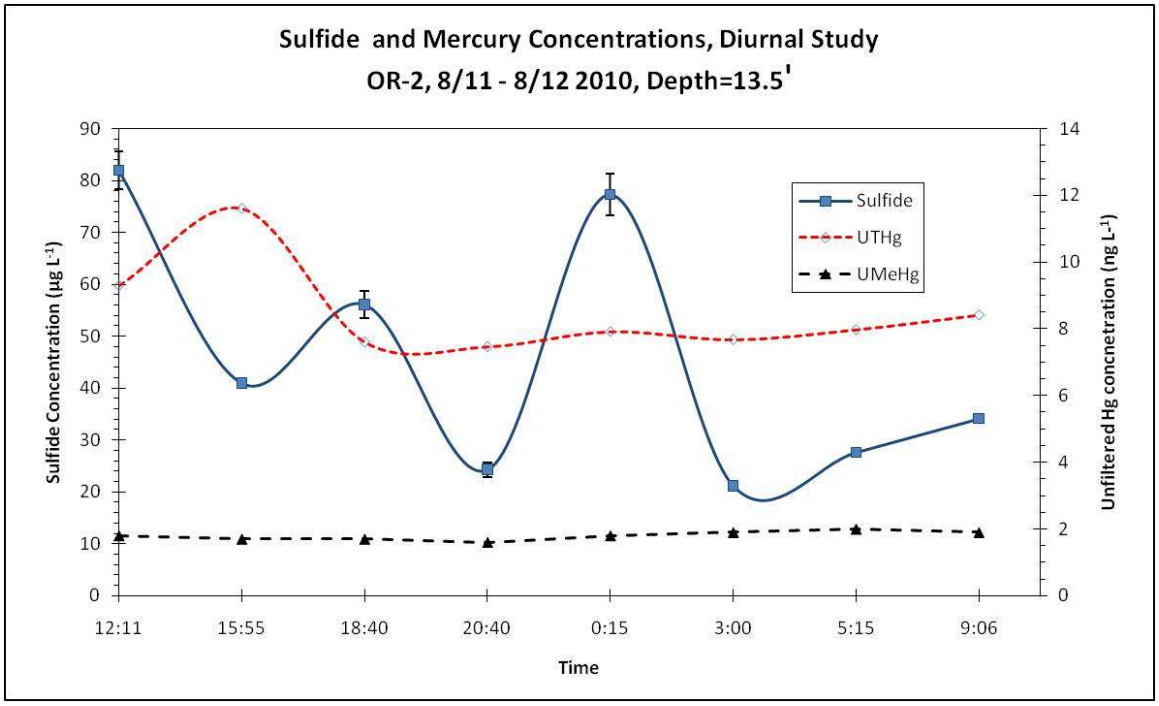


Figure 28: Sulfide and Mercury data from Diurnal study. Error bars represent standard deviation of triplicate analysis in the field.

fluctuate significantly throughout the 24-hour period, but unfiltered THg (UTHg) exhibited a peak of 11.6 ng/L at 3:55 pm, and then decreased to 7.6 ng/L for the duration of the sampling, staying relatively constant fluctuating between 7.6 amd 8.41 ng/L. Field parameter data were collected at 5-ft, 10-ft, and 14-ft depths to show the changes in water quality over the sampling period (Figure 29). Temperature, DO, pH, and specific conductance all remained relatively constant at the bottom of the water column where the diurnal mercury samples were obtained. At 5-ft depth, DO and pH followed typical

patterns of productive aquatic systems with peaks in DO and pH at about 6 pm due to photosynthetic oxygen production consuming hydrogen ions in the form of bicarbonate and carbonic acid, increasing the pH of the system**.**

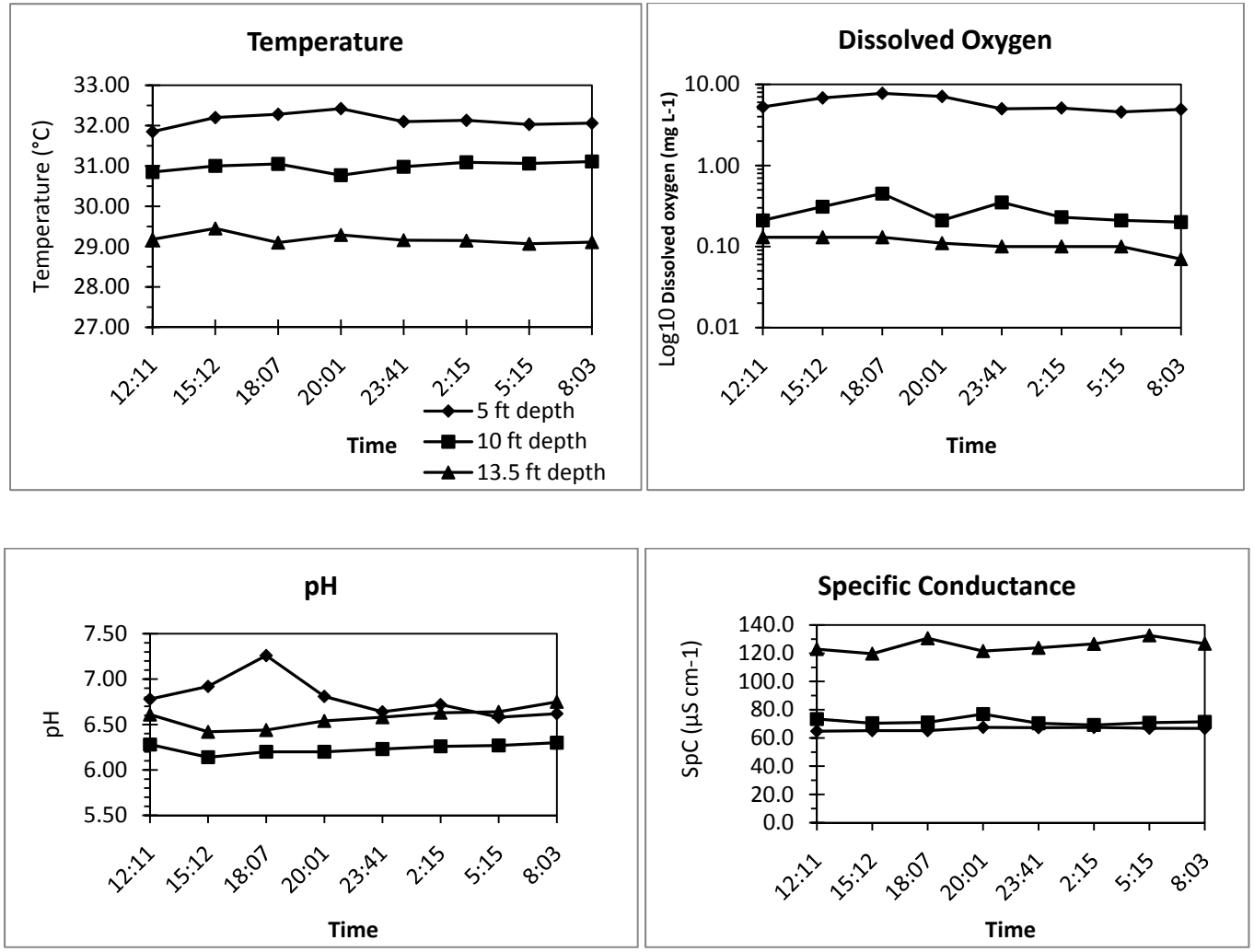


Figure 29: Field Parameter data from Diurnal Sampling, 8/11/2010 – 8/12/2010

5 Discussion

Field parameters, sulfide, sulfate, iron, and mercury data for each of the sites give insight into the primary controls on Hg methylation. Interpretation of these data highlights the differing geochemical characteristics between the sites, and how these differences affect Hg speciation and distribution. Examination of these data provides site-specific characterizations and insight into Hg methylation processes.

5.1 RL-2

Of the three study sites, RL-2 exhibited the highest dissolved MeHg and THg, and particulate MeHg concentrations in the bottom water (Figure 21). Additionally, the highest DOC concentrations were encountered at this site (mean 13.37 mg/L, n=3), and the highest total iron concentration (39.8 mg/L). These data provide evidence for geochemical controls on Hg methylation at this site, as high DOC is often related to high rates of Hg methylation, and high iron concentration gives evidence for redox processes that may have been controlling methylation.

The high DOC concentrations at RL-2 can be explained by location characteristics and channel morphology as described in the "Site Characterization" and "Field Parameters" sections of this report. This site is located in a narrow channel approximately ¾ of a river mile upstream of the confluence of the main channel of the lower Ouachita River, and receives stream inputs during high-flow events. This provides a source of terrestrial organic carbon. The leaf canopy also allows for ready input of leaf litter, further adding to the organic carbon content and potentially increasing the tannin content of the water. Visual inspection of water samples taken at 5 foot depth increments at RL-2 show chemical stratification with respect to DOC (Figure 30). Whole water samples from the bottom-water exhibited a tea-color, indicating the presence of tannins in the bottom water. DOC concentrations observed at RL-2 (mean 13.37 mg/L, n=3) are much higher than the single DOC sample obtained from the mid-point of the water column at a depth of 10' (3.50 mg/L) (Figure 31). The high DOC encountered at RL-2 in the bottom water

Figure 30: Whole water samples obtained from depths of 5', 10', 15', and 22' at RL-2

can most likely be attributed to the settling and degradation of organic particles (Kim, et al. 2000).

Channel conditions at RL-2 can also explain the site's stratification characteristics with respect to temperature and DO. The influence of the canopy creates low ambient temperatures, which result in the development of a thermocline at approximately 15-ft. Stratification is also due to the lack of significant wind effects, which will typically keep a water column mixed. RL-2 is located in a deep pool, which is stagnant because of the lack of flow related to the large distance from the site to the main channel of the lower Ouachita River and the lack of flowing tributaries at baseflow conditions. A drop in DO to anoxic conditions occurs at 15-ft (Figure 12) which can be explained by higher biochemical oxygen demand evidenced by the high DOC concentrations in the bottom-

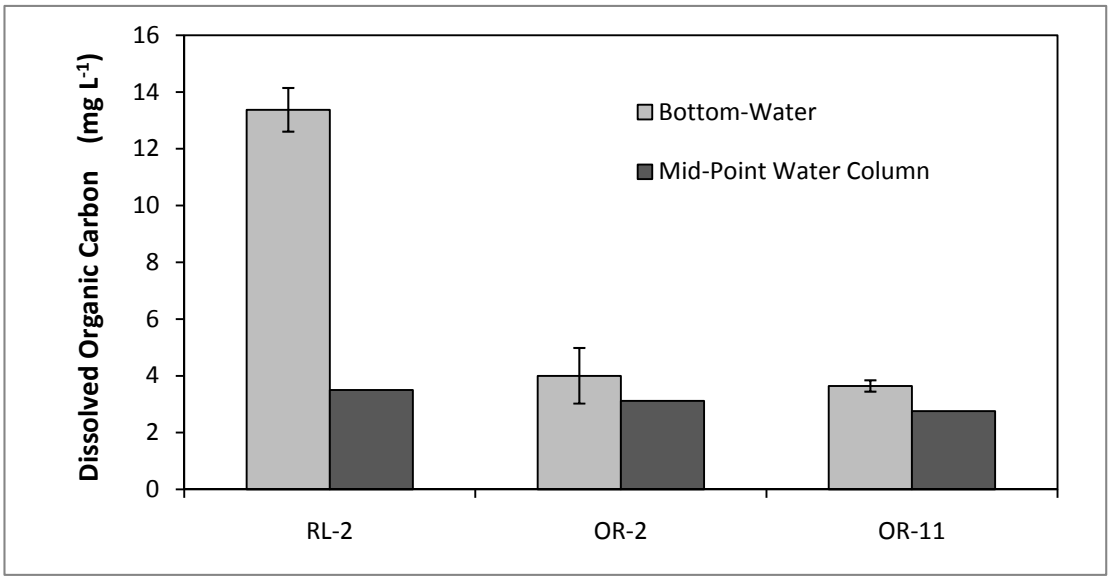


Figure 31: DOC concentrations at bottom water and mid-point of water column. n=3 for Bottom water at RL-2 and OR-2, n=2 for bottom-waer at OR-11, n=1 for all three sites at mid-point water column.

water. This high DOC can supply an energy source to respiring organisms, depleting the oxygen content.

At RL-2, total iron and sulfate were assessed in the field on one day and compared to sulfide concentrations. The sulfate concentration was zero, and the sulfide concentration was 120 μg/L, indication that available sulfate in the bottom-water was utilized for sulfate reduction. A high total iron concentration (39.8 mg/L) was detected at the same time as the sulfate and sulfide analysis at RL-2, which would suggest the dominance of iron reduction. For iron data, whole water samples were used for analysis, so the dissolved and particulate fractions were non-discriminate. Based on the clarity of the samples and the high iron concentration, a significant portion of the total iron is assumed to be in the dissolved phase. However, with only one data point for iron and sulfate to characterize this relation, further geochemical analysis is necessary to confirm the absence of sulfate and the presence of high iron at this site.

ORP and SpC data were used to corroborate evidence for redox processes at RL-2, specifically for iron and sulfate reduction. ORP declined from the water at the surface to the bottom-water, with an average decline of 274 mV for three profiles taken on separate days, the lowest ORP value encountered in the bottom-water being 45 mV. The bottom-water absolute values are well above the starting values for the range of iron and sulfate reduction (0 and-250 mV, respectively), yet measurable sulfides were detected in the bottom-water (mean 99 μg/L,n=3) as well as the high iron concentration (39.8 mg/L), bringing into question the validity of absolute measurements of ORP or the source of sulfide. Field measurements of redox potential using water quality probes requires the assumption of equilibrium in redox reactions, and a constant pH of 7 at 25°C (Stumm and Morgan 1996). As most aquatic species are not in equilibrium with regards to redox, these absolute values can be questionable when attempting to determine the presence of

specific redox reactions. High SpC values (>400 µS/cm) were recorded in the bottom water as compared to water at the surface (<80 µS/cm), another indication that redox processes are occurring in the bottom-water at this site mobilizing ions, and resulting in high SpC values.

The highest dissolved MeHg concentrations of all three study sites occurred at RL-2 (mean 1.43 ng/L, n=3) (Figure 32), which also had the highest DOC concentrations (mean 13.37 mg/L, n=3), highlighting the importance of DOC in Hg methylation processes at this site. The positive linear correlation with DOC and dissolved MeHg (Figure 34) indicates that DOC may not be inhibiting Hg methylation at this site, and is providing an energy source to methylating bacteria, potentially iron reducers, owing to the high iron concentration encountered at this site.

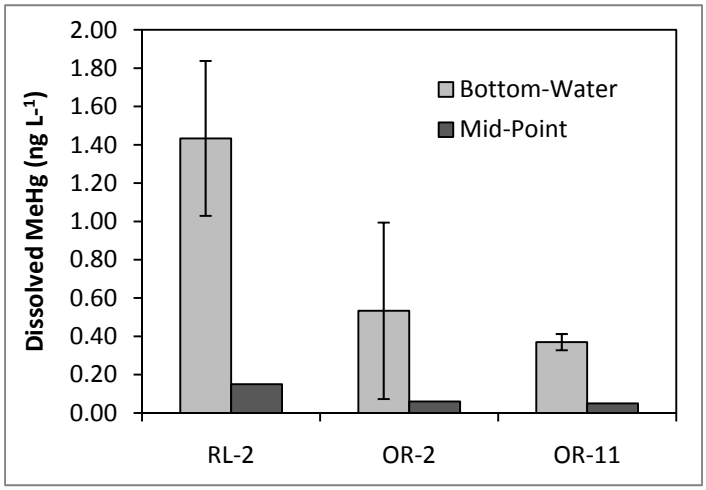


Figure 32: Dissolved MeHg concentrations at bottom-water and mid-point of water column, n=3 for bottom-water at RL-2 and OR-2, n=2 for bottom-water at OR-11, n=1 for mid-point at all three sites

Certain strains of iron reducing bacteria (FeRB) have been shown to methylate Hg. Kerin et al (2006) demonstrated in lab experiments with pure cultures of FeRB that Hg methylation may be common for some *Geobacter* strains, but that the ability to methylate is not ubiquitous among all iron reducers. Kerin et al (2006) used high concentrations of Fe(III) as an electron acceptor for these laboratory studies (55mM, ~3,000 mg/L) to

measure methylation rates in these specific microbial strains. Lab experiments have shown that pure cultures of specific FeRB have methylation rates more active than known SRB under high concentrations of Hg (1ppm as $HgCl_2$) in freshwater sediments (Fleming, et al. 2006). The work by Fleming et al (2006) also encountered soluble iron in freshwater sediments despite the presence of sulfide from sulfate reduction, emphasizing the potentially high rates of iron reduction in the sediments. In a similar study using sediments from the Mobile-Alabama River Basin (MARB), Warner, Roden and Bonzongo (2003) provided evidence for the potential for Hg methylation in sediments where iron reduction was the dominant terminal electron acceptor process (TEAP) at a dam site. Pore-water concentrations of total iron (expressed as the sum of Fe(II) and Fe(III) concentrations reported in the paper) at the MARB dam site were 4.59 mg/L for the top 2 cm of sediment, and 5.49 mg/L for the sediment between 2-4 cm. The total iron concentration at RL-2 was well above the pore water concentrations reported by Warner, Roden and Bonzongo (2003), indicating that iron reduction has the potential to be the dominant TEAP in this system. Measureable sulfide at this site indicates that sulfate reduction may be occurring concurrently with iron reduction. It is therefore possible that multiple microbial communities that methylate Hg are responsible for the high concentrations of Hg at RL-2, potentially even FeRB. The research cited above assessed dominant TEAP's in freshwater sediments and pore water. Little research if any has been conducted on FeRB Hg methylation in anoxic surface waters, highlighting a gap in the literature and in our understanding of these processes.

High concentrations of dissolved THg at RL-2 (Figure 21) suggest that a large amount of dissolved inorganic Hg (Hg_D) is available at RL-2 for methylation as compared to the

other two sites. Additionally, one sample for total recoverable Hg (13.76 ng/L) exceeded the Arkansas numeric water quality standard for Hg in water (12 ng/L).

5.2 OR-2

Average concentrations of dissolved MeHg, particulate MeHg, and dissolved THg at the bottom water for Site OR-2 were intermediate to concentrations at RL-2 and OR-11, whereas particulate THg and THg were lower. The range of dissolved MeHg (0.06-0.98 ng/L) and particulate MeHg (0.21-2.46 ng/L) show the potential for variation of controls on MeHg production, and temporal fluctuations in MeHg production. None of the three THg samples at OR-2 exceeded the numeric water quality standard of 12 ng/L.

OR-2 is located in a backwater close to the main channel, in a meander cutoff of the lower Ouachita River. No prominent canopy is in place at this sight, it does not receive stream inputs, and local topographic gradient is low. Low concentrations of DOC were encountered at this site as compared to RL-2 (Figure 31), which can be explained by these features. The site has less influence of leaf litter, and stream input provides no source of DOC. DOC must therefore be mainly autochthonous, the product of photosynthesis, and some degradation of organic matter in the bottom sediments. DOC concentrations in the bottom-water and the mid-point of the water column were not noticeably different (mean 4.00 mg/L for n=3 bottom-water samples, 3.12 mg/L for n=1 mid-point sample), suggesting that DOC concentrations are relatively consistent throughout the water column at this site, and that less DOC is present at OR-2 as compared to RL-2. The anoxic conditions in the bottom water can be explained similar to RL-2, with respiration occurring in the hypolimnetic waters, resulting in anoxic conditions below 12-ft with an average DO concentration of 0.11 mg/L. Thermal

stratification was not significant as the maximum depth at OR-2 is 14-ft, and water temperatures there did not drop below 28°C from the water near the surface to the bottom water.

At OR-2, two samples were analyzed for sulfate, sulfide and total iron. A sulfate concentration of 1.67 mg/L (σ=3.51) and a sulfide concentration of 237.4 µg/L (the anomalously high sulfide concentration that incorporated bottom sediments) were measured on one day, and a sulfate concentration of 65 mg/L (σ=0.0) and a sulfide concentration of 87.67 µg/L were measured on a separate day. Sulfide concentrations at OR-2 were not markedly different from the other sites, but the high standard deviation associated with the 1.67 mg/L sulfate concentration, along with the order of magnitude difference between the two sulfate concentrations bring these data into question as to the validity of representation of sulfate occurrence at this site. However, if these data are representative, they show the potential for temporal shifts in sulfate reduction in the anoxic bottom-water, which may be another important control on sulfate reduction and Hg methylation.

Total iron concentrations at OR-2 on the two sampling days were 2.69 and 0.70 mg/L respectively. OR-2 samples were turbid, which may have result in the particulate iron phase dominating over dissolved iron at these low concentrations. As previously mentioned, MeHg concentrations for n=3 samples ranged from 0.06-0.98 ng/L at OR-2. The lowest dissolved MeHg (0.06 ng/L) occurred on the day with the lowest sulfate (1.67 mg/L) and highest total iron (2.69 mg/L) at this site, and the middle concentration (0.56 ng/L) occurred on the day when the anomalously high sulfate concentration (65 mg/L) and low total iron (0.70 mg/L) was reported. Iron and sulfate data were not collected on

the day with the highest dissolved MeHg. If sulfate reduction was occurring in the bottom water we would expect to see measureable sulfide with low sulfate concentrations, yet sulfide was measureable at this site at 87.67 μg/L and sulfate was high (65 mg/L), making the high sulfate concentration anomalous. Also, if iron reduction processes were producing MeHg, we would expect to see high total iron concentrations, but total iron was low (0.70 mg/L) when sulfate was high.

As with the other sites, absolute values of ORP at the bottom-water (mean 50.5 mV, n=2) were well above the ranges of iron and sulfate reduction, yet detectable sulfide was measured at the bottom-water when Hg samples were collected. SpC also increased in the water column from the water near the surface to the bottom-water, indicating higher total dissolved solids. Given the variability in sulfate data, and the uncertainty of the dissolved iron at this site, a more detailed characterization of redox products is necessary to assess the dominant redox processes at this site, and give further insight into geochemical controls on Hg methylation. Of note at OR-2 is the variability in Hg concentrations on the three sample days.

5.2.1 OR-2 Diurnal Study

The 24-hour sampling scheme was implemented from 8/11/2010 to 8/12/2010 at OR-2 in an effort to assess diurnal fluctuations of Hg occurrence in the bottom-water. Sulfide data were collected along with whole water samples for THg and MeHg analysis. Figure 28 shows no significant changes in MeHg concentrations over the 24-hour period (mean 1.80 ng/L, σ=0.12, n=8), indicating that methylation/demethylation processes at the bottom water were either in equilibrium, or that there was no net MeHg production in this time period. THg also remained relatively constant (mean 8.49 ng/L, σ=1.30, n=8),

except for an excursion to 11.6 ng/L at 3:55 pm. Fluctuation in sulfide concentrations throughout the 24-hours at the bottom water is assumed to only be influenced by changes in the rates of sulfate reduction in the sediment or the water column. If the data represent sulfide flux from the sediment, and that is the principal zone of methylation, we might also expect to see MeHg concentrations in the bottom-water fluctuate with the sulfide concentrations. However, these data do not reflect these processes. As such, the diurnal study does not give insight into MeHg flux or water-column production, nor insight into principal zones of sulfate reduction.

5.3 OR-11

Hg speciation and distribution among size fractions at OR-11 are different from the other two sample sites. Dissolved and particulate MeHg, and dissolved THg concentrations were lowest at OR-11, yet particulate THg concentration was highest, resulting in the highest THg out of all three sites (THg is total recoverable Hg, expressed as the sum of filtered and particulate THg). This suggests that geochemical conditions at OR-11 are not suitable for high rates of methylation as compared to the other two sites because the majority of THg is in the particulate phase and not available for methylation (Brigham, et al. 2009). At OR-11, 92% of the total Hg was in the particulate form (Table 2).

	FMeHg	PMeHg	FTHg	PTHg	n
RL-2	52%	48%	33%	67%	3
OR-2	31%	69%	25%	75%	3
OR-11	33%	67%	8%	92%	1

Table 2: Filtered-Particulate Hg Distributions, FMeHg and PMeHg are percent of total MeHg, FTHg and PTHg are percent of total Hg (THg)

Hg is a well-known particle-reactive metal (C. L. Babiarz, et al. 2001), and particulate forms of Hg can easily adsorb onto suspended particles, including particulate organic

carbon (POC) and suspended solids (TSS) (Brigham, et al. 2009). POC and TSS were not assessed at any of the three sites, so controlling factors on size (organic or sediment particles) at OR-11 can only be inferred. A study on Lake Champlain showed increasing PTHg with increasing organic content of TSS (Scherbatskoy, Shanley and Keller 1998), suggesting the importance of organic material in the transport of inorganic Hg. Partition coefficients (K_D) for dissolved and particulate phases of Hg were assessed in seven Wisconsin rivers and showed that K_D decreased (more Hg in the particulate phase) with increasing TSS (Babiarz et al. 1998). These studies show the affinity of Hg to bind to both POC and TSS in freshwater aquatic systems, suggesting that one of the two binding agents may dominate at OR-11. The assertion that only the dissolved fraction of Hg is available for methylation (Brigham, et al. 2009) is consistent with observations at OR-11, which had the lowest average dissolved THg concentrations and the lowest average dissolved MeHg concentrations. Additionally, use of a 0.7µm filter for Hg samples as was used for this project does not allow for determination of the colloidal phase. High particulate loads typically contain a significant colloidal fraction which has also been found to bind with Hg, and not assessing this fraction can lead to overestimation of the dissolved fraction, also known as the particle concentration effect (PCE) (Babiarz et al. 2001). OR-11 showed different stratification characteristics as compared to RL-2 even though the two sites have the same maximum water depths (24-ft). Water temperatures did not drop below 30°C from the water at the surface to the bottom-water, which suggests more vertical mixing throughout the water column. The differences in thermal stratification characteristics at these two sites can be explained by differences in geomorphic characteristics. In the hottest times of the year (late July and August, when

the project sampling occurred) surface temperatures at OR-11 remain warmer throughout the water column because of the higher ambient temperatures experienced at that site, largely due to the lack of significant canopy influence and more solar radiation.

Anoxic conditions were evident in the bottom-water at OR-11 below 20-ft, suggesting that respiration is occurring in the bottom-water (at a greater rate than O_2 can be supplied), much like the other two sites. ORP at the bottom water was highest out of all three sites (average 144 mV, σ=1.73, n=3), yet measureable sulfides were detected in the bottom water as with the other two study sites, again bringing into question the validity of absolute values of ORP as measured. SpC also increased in the water column from the water near the surface to the bottom water, indicating the introduction of ions into solution from redox processes.

Average DOC in the bottom-water at OR-11 averaged 3.64 mg/L (σ=0.2, n=3), higher than the one DOC sample collected at the mid-point of the water-column (2.76 mg/L), suggesting that DOC concentrations are consistent throughout the water column at this site. Channel geomorphology at OR-11 is similar to OR-2, experiencing less influence of leaf litter. Fresh terrestrial organic matter has no way of entering the system via direct stream inputs, as is the case for OR-2. DOC at OR-11 must therefore be mainly the product of photosynthesis and some degradation of organic matter in the bottom sediments.

At OR-11, only one total iron and one sulfate analysis was performed for the bottom water, resulting in a total iron concentration of 1.61 mg/L and a sulfate concentration of 4.00 mg/L. Neither of these concentrations gives evidence for the predominance of

sulfate or iron reduction. As mentioned previously, if iron reduction was dominant, we would expect to see high total-iron concentrations, and if sulfate reduction was dominant, we would see no sulfate and appreciable sulfides. The sulfide concentration obtained along with the total iron and sulfate was 77.7 µg/L, which is below the average for sulfides detected at all of the sites (110.1 µg/L). Filtered and particulate MeHg samples collected at the same time as the total iron and sulfate samples were low (0.40 and 0.55 ng/L respectively), suggesting minimal Hg methylation at this site.

5.4 Effect of sulfide on Hg methylation

Measurable sulfide detected at the bottom water at all three sites suggest that sulfate reduction and associated MeHg production may be occurring either in the anoxic water at the base of the water column, at the sediment-water interface, or in sediment pore water with sulfide and MeHg moving out of sediment into the overlying water column. The pH of the anoxic bottom water with measureable sulfide for all three sites ranged from 6.25 to 6.87, characteristic of sulfate reducing environments (Morel and Hering 1993). Additionally, sulfide profile data show an increase in sulfide concentrations from the top of the water column to the bottom-water at all three sites. Studies have linked the occurrence of sulfides in the water column with sulfate reduction in the water column. Methylation assays performed on water samples from an anoxic hypolimnion in a Wisconsin lake by Eckley, et al. (2005) showed a peak in methylation activity in the water column that coincided with the appearance of sulfide at 0.9 µM (30.6 µg/L) and increased as sulfide reached 14.6 µM (496.4 µg/L), and these sulfide concentrations were cited as evidence of sulfate reduction in the anoxic hypolimnion. Bottom-water concentrations of sulfide at the three study sites on the lower Ouachita were in the range

of (74.0-142.7 µg/L) (2.18-4.20 µM), well within the range reported by Eckley, et al. (2005), highlighting the likely occurrence of sulfate reduction at these backwater sites. However, ORP values do not show the potential for sulfate reduction in the anoxic bottom waters. Given the conflicting evidence of absolute ORP , further data are needed at corroborate the occurrence of sulfate reduction in the bottom water at these sites including a larger sulfide data set, dissolved iron analysis, and a larger sulfate data set .

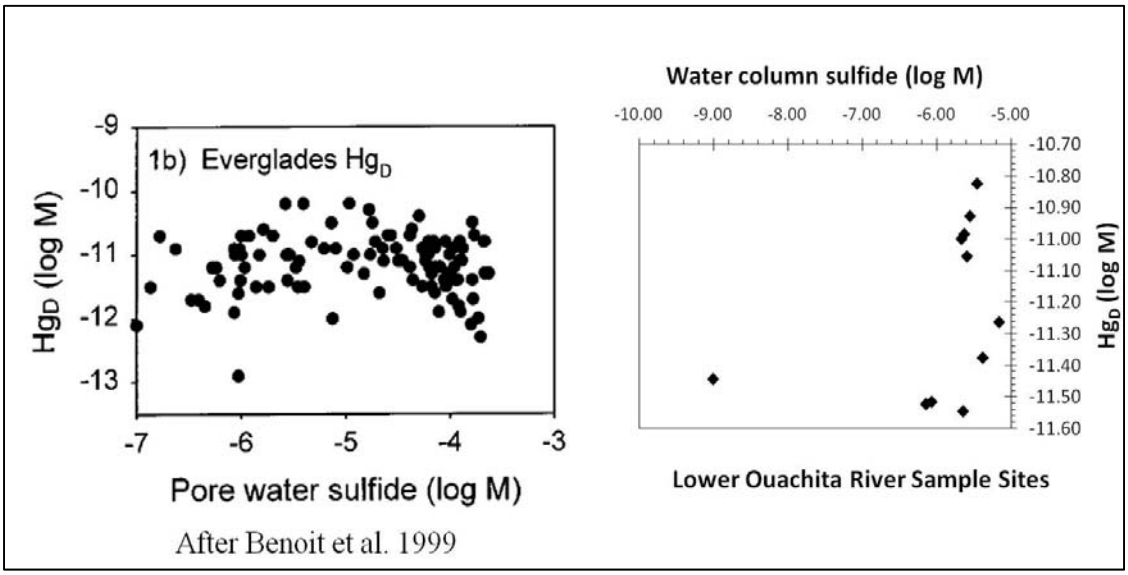


Figure 33: Comparison of sulfide and HgD from lower Ouachita River samples to pore water sulfide and HgD from the Florida Everglades.

Regardless of the source of sulfide, measureable concentrations were detected in the bottom water. Comparing sulfide concentrations to dissolved MeHg compiled from all three study sites did not result in a significant correlation (r^2=0.059); this lack of correlation may be explained by free sulfide combining with dissolved inorganic Hg (Hg_D) to form neutral bio-available Hg-sulfide complexes (Hg_D is calculated as the difference between filtered THg and filtered MeHg). Sulfide concentrations at the bottom-water from all three sites (65-237.4 µg/L, 1.91-6.98 µM) are comparable to

sulfide encountered in pore waters in the Florida Everglades by Benoit et al. (1999), a study where no correlation was observed between sulfide and dissolved inorganic Hg (Figure 33). Benoit et al. (1999) used cinnabar solubility models and concluded that high sulfide (> -5 log M, ~350 μg/L), Hg speciation shifts away from dominant neutral Hg-sulfide complexes preferentially used by sulfate reducing bacteria towards free inorganic Hg in sediment pore waters, potentially reducing Hg methylation. Sulfide concentrations from the anoxic bottom water of sites for this study ranged from 74.0 to 142.7 μg/L. Based on the work of Benoit et al. (1999, 2001b) it is therefore possible that neutral bio-available Hg-sulfide complexes dominate at these sites instead of charged Hg-S species or free inorganic Hg. This may explain why we see detectable MeHg at all three sites and no correlation with low sulfide concentrations, as the sulfides are preferentially combining with Hg in the system creating neutral Hg-S species. Because of the fact that sulfide concentrations at the sites for this study do not reach high levels that would inhibit Hg methylation, we would also not expect to see a negative correlation between MeHg and sulfide as shown by Benoit, et al. (1999). Additionally, if sulfate reduction is occurring in the anoxic bottom-water as evidenced by presence of sulfide, e.g. (Eckley, et al. 2005), the bottom-water may be a candidate for Hg methylation since the sulfide concentrations are low enough to form neutral Hg-sulfide species utilized by SRB's during sulfate reduction.

Another explanation for the lack of correlation between sulfide and MeHg is the combination of iron with sulfide generated from sulfate reduction. FeS is one of a variety of compounds formed from iron and sulfur, and is highly soluble at 25°C and moderate ionic strength (Liu, et al. 2008).The oxidation of aqueous FeS (mackinawite) by H_2S

produces pyrite (FeS_2) and is very rapid between 25 - 125°C (second order rate constant k = $1.03x10^{-4}$ l/mol/s at 25°C) as represented in the following reaction (Rickard 1997):

$$FeS_{(s)} + H_2S = FeS_2 + H_{2(g)}$$

In natural systems, this process is favored in anoxic environments rather than pyrite-forming processes involving HS^-, which requires an additional oxidizing agent to maintain electron balance (Rickard 1997). For the Ouachita River sites, these processes may explain low sulfide and the lack of correlation between dissolved MeHg and sulfide in the bottom water. As sulfide is generated by SRB's and MeHg concentrations increase, pyrite precipitates can form from the oxidation of FeS by sulfide. To examine the occurrence of FeS_2, sediment samples from intact cores at OR-2 were dried and analyzed by scanning electron microscopy (SEM) at the optics laboratory in the Physics department at the University of Arkansas. After heavy mineral separation using Tetrabromoethane, grains containing Silica, Aluminum, Oxygen, and Titanium were observed in the SEM, yet no pyrite minerals were observed. However, nanometer-size particles of sulfur were detected using EDX scanning on the surfaces of these grains, but the SEM could not obtain the resolution necessary to identify these grains individually.

5.5 Effect of DOC on Hg methylation

The observed positive linear relation between dissolved MeHg and DOC at the bottom water at all three sites indicates an important influence of DOC on Hg methylation (Figure 34). The role of DOC in Hg methylation is complex. DOC can both stimulate methylation (Benoit, et al. 2003) and inhibit methylation by binding inorganic Hg at reduced sulfur sites in DOC which would otherwise combine with free sulfide and

become bioavailable (Barkay, Gillman and Turner 1997). The positive linear relation of DOC and dissolved MeHg can be explained two ways. First, DOC at the three sites may be acting as an energy source that stimulates microbial activity responsible for methylation. Second, the median pH value of the bottom waters at the sites was 6.66, which may provide enough free protons to compete for negatively charged binding sites in DOC, leaving Hg(II) available for methylation as described by Barkay, et al. (1997).

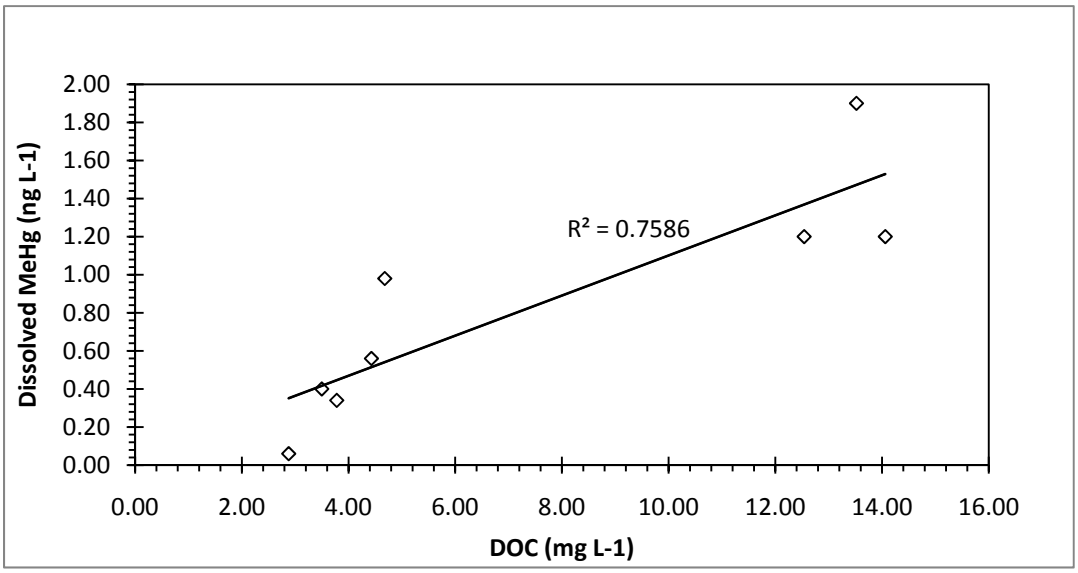


Figure 34: Dissolved MeHg and DOC, bottom-water samples compiled from all three sites

The DOC-Hg relation leads to some conclusions about the type of DOC that may be dominating the system. If a large fraction of the DOC molecules contain reactive thiol groups and the hydrogen ion activity is so low as not to supply protons to compete for binding sites, we would expect to see a null correlation between MeHg and DOC, as was encountered in the northern Florida Everglades by Hurley, et al. (1998). However, this was not the case for the lower Ouachita study sites. In general, the reactivity of Hg with DOC depends on chemical and structural differences of DOC and the presence of other competing ions, such as hydrogen, in water (C. L. Babiarz, et al. 2001). Therefore,

understanding the quality of DOC is essential in drawing conclusions pertaining to DOC interactions with Hg in any aquatic environment. For this study only bulk DOC was assessed, which can lead to inferences as to how DOC affects Hg speciation and transport in this system, but does not give insight into the quality of DOC and how that effects Hg methylation at the three study sites. Further characterization of DOC would give more insight into dominant species of DOC at the three study sites, and provide data for determining the dominant source of DOC to these sites (i.e. allocthonous or autocthonous sources).

5.6 MeHg Flux Rate Modelling

As stated previously, Hg methylation generally is thought to occur in the anoxic sediments of aquatic systems where redox conditions are amenable for reductive bacteria. MeHg can then be fluxed out of sediments into the overlying water column (Goulet, et al. 2007), where uptake can occur by diatoms and algae, beginning the bioaccumulation process (Moye, et al. 2002). The flux across the sediment-water interface is therefore important in being able to predict primary sources of Hg in an aquatic system (i.e. sediment-derived or water-column derived). Diurnal studies have shown that the flux of MeHg across the sediment water interface is not always constant, with MeHg concentrations declining during dark periods in the Florida Everglades (Krabbenhoft, et al. 1998), and in a mine-impacted stream in Montana (Nimick, et al. 2007). Advective flux of MeHg into an aquatic system can also play an important role in MeHg sourcing. A comparison of both direct (benthic chamber) measurement of benthic flux to diffusive flux calculations by Choe, Heim and Coale (2004) showed that the use of concentration gradients can account for only a minor portion of measured fluxes of MeHg, suggesting

the importance of advective processes on exchanges of MeHg at the sediment-water interface.

Flux rates can be determined by using measured concentration gradients of a constituent through the pore-water and into the sediment-water interface, employing equations of Fick's first law (Stumm and Morgan 1996), or by direct measurement of flux using benthic chamber deployments (Choe, Heim and Coale 2004). Flux rates calculated using Fick's first law can vary by orders of magnitude for different aquatic systems (Table 3).

MeHg Flux rate	Units	Time of Year	Location	Citation
-4.90E-21	$mol/cm^2/s$	July	St. Lawrence River Wetland, Canada	Goulet et al. (2007)
-2.85E-20	$mol/cm^2/s$	November	St. Lawrence River Wetland, Canada	Goulet et al. (2007)
-9.00E-22	$mol/cm^2/s$	May	St. Lawrence River Wetland, Canada	Goulet et al. (2007)
8.82E-22	$mol/cm^2/s$	April	Northern Minnesota, diffusive calculated	Hines (2004)
5.78E-20	$mol/cm^2/s$	May	SF Bay Delta, measured	Choe et al. (2004)
7.75E-21	$mol/cm^2/s$	May	SF Bay Delta, diffusive calculated	Choe et al. (2004)
Note: negative flux rates for Goulet et al. indicate flux rate out of the sediment per author				

Table 3: MeHg flux rates reported in the literature for select locations

For the lower Ouachita River sites, no pore-water profiles or benthic chambers were deployed to determine flux rate potentials from the sediment to the overlying water column for this project. However, by using measured concentrations of MeHg in the water column and assuming flux rates determined by previous workers, insight can be gained into source attribution of MeHg in the water column. Given the sparse amount of data available for these calculations at the lower Ouachita sites, some gross assumptions must be made.

Flux rates are reported in units of mass/area/time. Table 3 reflects the wide range of flux rates in different aquatic ecosystems. Calculating a flux rate out of a sediment area into the water column assumes a closed system, which is not the case in aquatic systems, especially for rivers. This assumption of a closed system is slightly justifiable given the nature of the backwater locations sampled on the lower Ouachita River, as there was no notable flow during sampling in July and August 2010. However, the unlikely situation of a closed system remains.

To calculate the flux-rate potential, site RL-2 was chosen because of the high concentrations of dissolved MeHg (mean 1.43 ng/L). MeHg at the mid-point of the water column (10-ft depth) was 0.15 ng/L. A unit area of 1 m^2 and a water-column height of 10-ft (3.048-meters) was chosen to represent the bottom 10-ft of the water column where water samples were collected. An average dissolved MeHg concentration of 0.79 ng/L was calculated to represent the occurrence of MeHg in the bottom 10-ft of the water column. Using MeHg flux rates calculated in Table 2 and an assumed time-step of 24-hours, MeHg fluxes out of the sediment for a 1 m^2 area were modeled for these different rates. Using these flux rates, calculated mass flux of MeHg at RL-2 varied by nearly two

orders of magnitude, with a range of 0.16-10.14 ng/day (Table 4). If we assume that the MeHg concentration in the hypothetical volume is zero at time zero, we can calculate the amount of time for the hypothetical volume to accrue X amount of MeHg given the mass flux rates determined for various studies. Table 4 shows low diffusive flux rates would take a much longer time to account for the MeHg in the water column, yet high flux rates that account for advection could account for all of the MeHg in the water column in a much shorter time-frame (14,465 days vs. 221 days). For the hypothetical closed system discussed here and using the flux rates in Table 3, the mass flux of MeHg ranges from 0.16-10.74 ng/day. Choe, et al. (2004) showed that at most, 65% of the measured flux could be attributed to diffusion across the sediment-water interface (minimum = 0.3%). If we focus on the maximum values of sediment-attributed MeHg (65%), then 35% of the remaining MeHg must be derived from advection processes. Residence time of water in backwaters at low-flow is likely very long given the lack of inflows to the system. Therefore, at any given point in time, 35% - 99.7% of the MeHg in the system may not be attributed to sediment flux, pointing towards uncertainty in the source attribution of MeHg.

Examining the lower Ouachita River from an open system perspective on the reach scale gives further argument for the dominance of advective transport in the system. Assuming a 7-mile reach on the lower Ouachita River and a mean monthly discharge of 3,140 cubic feet per second (cfs) as reported by the USGS for August of the 2009 water year (USGS 2010), 8.44×10^9 ng of MeHg would be present in that river reach using a concentration of 0.38 ng/L reported by the USGS at site 330937092081001 (downstream of OR-2) on 9/28/2009. Assuming that the entire substrate of the river is fluxing MeHg in this 7-mile

reach at the fastest rate reported in Table 3 (5.78E-20 mol/cm^2/s), the sediment would flux 6,012 ng MeHg/day. At that rate, it would theoretically take 3,846 years for all of the MeHg in the water column to come from the sediment in that reach. Advection must therefore account for a large amount of MeHg in the system.

This discussion makes many general assumptions about processes in this system. First, it assumes a constant flux rate from the sediment, which is highly unlikely due to the diurnal data reported by the afore mentioned researchers. Second, it assumes that the concentration of MeHg would be consistent along the hypothetical 7-mile river reach, which is also not likely given the seasonal fluctuations of MeHg in the main channel of the river (see section 5.7). The processes and occurrence of MeHg in the main channel of the lower Ouachita River were not the focus of this project, but historical data highlight a gap in our understanding of the occurrence of MeHg on large scales. Important background information is necessary regarding the onset of Hg methylation in this river system to make any conclusions regarding MeHg sources (i.e. sediment vs. water-column derived), as well as residence time of MeHg in the river in the form of methylation/demethylation rates. The importance of wetlands in MeHg production must be considered, as these ecosystems have been identified as important zones of Hg methylation (Hall, et al. 2008). Assessing the area of wetlands in the lower Ouachita River watershed that would be amenable to Hg methylation would be an important exercise in determining MeHg sources. A substantial amount of wetland area could be producing MeHg in addition to the sediments in the main channel, providing an additional source of MeHg to the main channel of the river. It is also possible that the wetland ecosystem compartments could be producing a substantial amount of MeHg that

is then transported into the main channel via advection, potentially accounting for the large mass of MeHg we see in the main channel and explaining the stark difference between measured MeHg in the main channel and potential MeHg flux from the sediment. An accurate assesessment of benthic flux using benthic chambers or concentration gradients in the main channel and in wetlands and backwaters is justifiable to give insight into the specific sediment flux characteristics of the system.

5.7 Regional Comparison of Hg contamination

THg and MeHg concentrations at all three study sites on the lower Ouachita River are typically within the range of Hg values reported in the literature for the southeastern United States (Table 5), and median and mean values exceeded reported concentrations in many areas. Dissolved and particulate MeHg concentrations were higher than all data reported in table 5. Filtered THg concentrations on the lower Ouachita were similar to concentrations encountered in freshwater rivers and wetlands in southern Louisiana by Hall, et al. (2008) in a study identifying principal zones of MeHg production. That paper reported the greatest dissolved MeHg concentrations in the surface waters of freshwater wetlands (mean 0.31 ng/L, σ=0.06). The highest dissolved MeHg concentration on the lower Ouachita River system was 1.90 ng/L at RL-2, highlighting the high rates of MeHg production. The work by Hall et al. (2008) explained the high occurrence of MeHg production in freshwater wetlands in three ways. First, oscillating water levels inundate sediments creating a reduced sulfur pool which becomes oxidized when the water levels recede giving a constant fluctuating source of sulfate to SRB's. Second, re-wetting the oxidized substrates increases the amount of labile DOC, enhancing microbial activity. Lastly, oxidation of reduced sulfur decreases pH, which can also stimulate methylation.

Although the three study sites on the lower Ouachita are not technically classified as wetlands, they can experience similar fluctuations in water levels as they are hydraulically connected to the main channel of the river, which fluctuates with seasons. These fluctuations do not expose large areas of sediment as compared to most freshwater wetlands. The concentrations of dissolved MeHg at the lower Ouachita sites are much higher than many/most of the regional concentrations, suggesting high rates of methylation, potentially due to similar conditions as explained by Hall, et al. (2008).

Flux rates are diffusive rates estimated from pore water gradients unless noted otherwise

Molecular Wt of MeHg	215	g/mol
Depth of overlying water column	300	cm
; FMeHg concentration @ RL-2 in bottom 10-ft:	0.79	ng/L
Conversion: cubic cm in one Liter	1000	cm^3

MeHg Flux rate	Units	Time of Year	Location	Citation	Assumed Time St (seconds)	Assumed area (cm^2)	Mass flux/day (ng)	*mass of MeHg in wtr column (ng)	# of days to account for mass of MeHg (days)
-4.90E-21	$mol/cm^2/s$	July	St. Lawrence River Wetland, Canada	Goulet et al. (2007)	86400	10,000	0.91	2370.00	2604
-2.85E-20	$mol/cm^2/s$	November	St. Lawrence River Wetland, Canada	Goulet et al. (2007)	86400	10,000	5.29	2370.00	448
-9.00E-22	$mol/cm^2/s$	May	St. Lawrence River Wetland, Canada	Goulet et al. (2007)	86400	10,000	0.17	2370.00	14176
8.82E-22	$mol/cm^2/s$	April	Northern Minnesota, diffusive calculated	Hines (2004)	86400	10,000	0.16	2370.00	14465
5.78E-20	$mol/cm^2/s$	May	SF Bay Delta, measured	Choe et al. (2004)	86400	10,000	10.74	2370.00	221
7.75E-21	$mol/cm^2/s$	May	SF Bay Delta, diffusive calculated	Choe et al. (2004)	86400	10,000	1.44	2370.00	1646

Note: negative flux rates for Goulet et al. indicate flux rate out of the sediment per author

* Assumes MeHg concentration of theoretical water volume is zero at time zero

Table 4: MeHg Flux rate model for RL-2

	THg (ng/L)					FTHg (ng/L)					FMeHg(ng/L)					PMeHg(ng/L)				
	n	Min	Max	Median	Mean	n	Min	Max	Median	Mean	n	Min	Max	Median	Mean	n	Min	Max	Median	Mean
Lower Ouachita River study sites	**7**	**4.33**	**13.99**	**11.55**	**10.73**	**11**	**0.66**	**4.80**	**1.18**	**2.05**	**11**	**0.05**	**1.90**	**0.40**	**0.63**	**11**	**0.05**	**2.40**	**0.70**	**0.83**
Bonzongo and Lyons 2004, Mobile-Alabama River System-Inland Waters	31	0.23	5.83	2.33	2.14	31	0.04	2.60	0.52	0.40	16	< DL	0.38	0.07	0.04	-	-	-	-	-
Bonzongo and Lyons 2004, Mobile-Alabama River System-Mobile Bay	5	0.24	2.14	1.32	1.33	5	0.20	0.50	0.41	0.43	5	0.02	0.05	0.04	0.04	-	-	-	-	-
Brigham et al. 2009, St. Mary's River, FL	-	-	-	-	-	38	2.14	14.20	4.92	-	37	*<0.04	1.03	0.32	-	38	*<0.013	0.13	<0.028	-
Brigham et al. 2009, Santa Fe River, FL	-	-	-	-	-	30	0.25	11.10	1.07	-	30	*<0.04	0.93	0.09	-	30	*<0.019	0.09	<0.028	-
Brigham et al. 2009, Little Wekiva River, FL	-	-	-	-	-	40	0.20	2.60	0.43	-	40	*<0.04	0.44	0.06	-	40	*<0.015	0.11	<0.026	-
G.Liu et al. 2008, Florida Everglades Dry Season, water (May 2005)	-	0.91	7.00	2.30	2.60	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B.D. Hall et al. 2008, Southern Louisiana Freshwater Rivers	-	-	-	-	-	-	-	-	-	2.00	-	-	-	-	0.16	-	-	-	-	0.05
B.D. Hall et al. 2008, Southern Louisiana Freshwater Wetlands	-	-	-	-	-	-	-	-	-	1.64	-	-	-	-	0.31	-	-	-	-	0.14
	* Units reported are FMeHg and PMeHg as Hg, supporting online information table S4 from ES&T publication																			

Table 5: Comparison of Hg data from bottom-water samples at lower Ouachita study sites to published values in the southeastern United States

The USGS has sampled the main channel of the lower Ouachita River for Hg since 2001 at sites within close proximity of OR-2 and OR-11 (Figure 4). The USGS site nearest to OR-2 is located downstream of the confluence of the Saline River on the main channel of the lower Ouachita, so Hg data from that site reflects inputs from both rivers. The USGS site closest to OR-11 is downstream of the Felsenthal Lock and Dam, at the confluence of the historic main channel of the lower Ouachita prior to dam construction, experiencing inputs from both stems of the river.

The site nearest OR-11(USGS site 33025509206430, Ouachita River below Felsenthal Lock and Dam, AR) shows low concentrations of THg and MeHg (<1.0 and <0.1 ng/L, respectively, samples collected in the Fall) from 2001 until 2006, followed by fluctuating concentrations after November 2006 (Figure 35). The first three samples in the data set at this site were collected once every three years, missing potential seasonal fluctuations shown by data after 2006. Hg data from the site nearest OR-2 (USGS site 330937092081001, Ouachita River at Mile 237.9 n. of Felsenthal, AR) show more noticeable fluctuations in dissolved MeHg and THg because samples were collected monthly and bi-monthly depending on the year. Filtered MeHg and THg peak between the months of March and July at both sites on the main channel near OR-11 and OR-2. These peaks show that methylation rates increase beginning in March and April during seasonal precipitation events, and continue into the dry season. Rates of Hg methylation may be directly proportional to rates of microbial growth due to increases in DOC, and the extent of methylation has been shown to be affected by other factors such as pH and temperature following flooding events (Hall, St. Louis and Bodaly 2004).

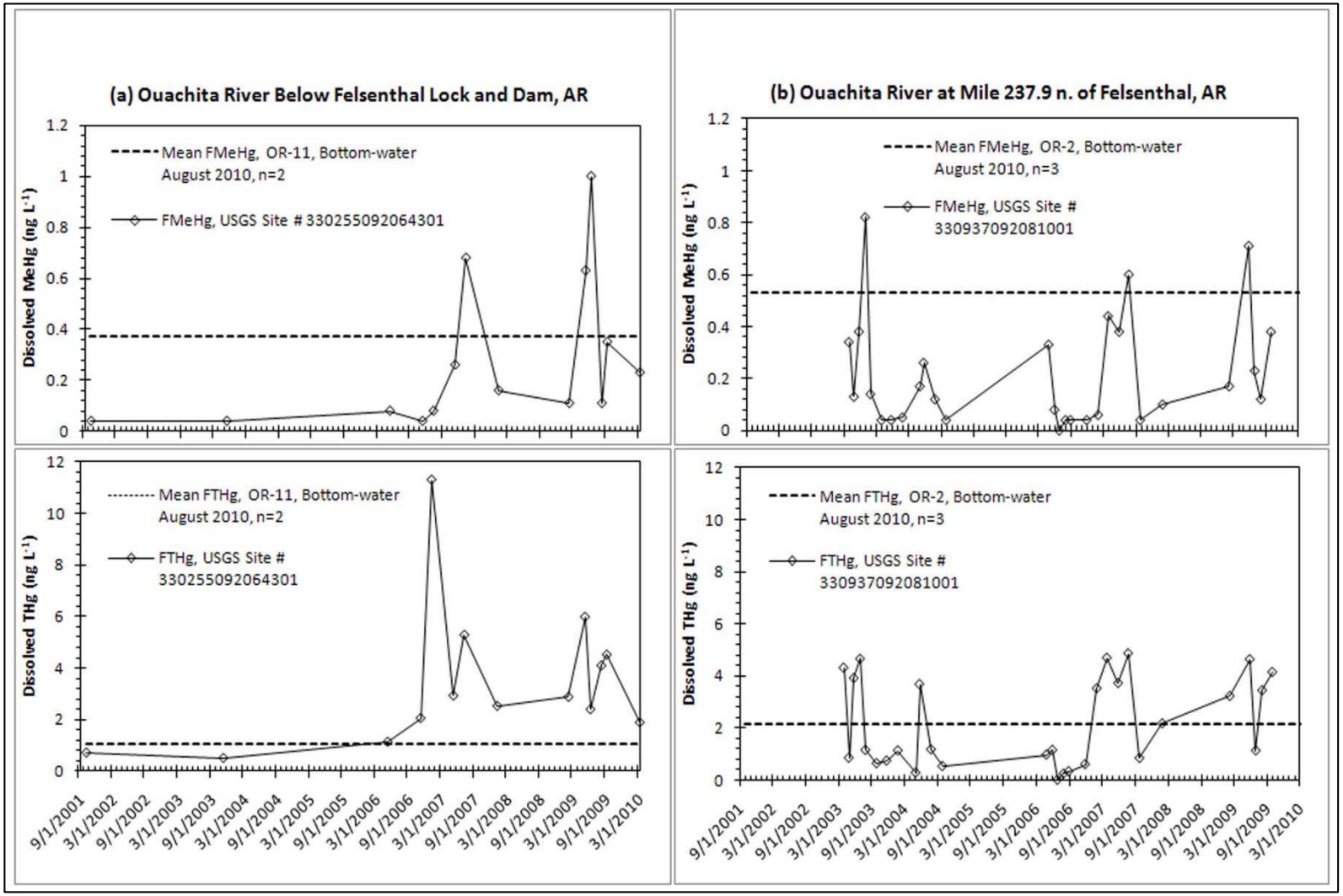


Figure 35: Historical Data for USGS sampling sites proximal to (a) OR-11 and (b) OR-2. Dashed lines indicate average MeHg and THg concentrations for bottom-water samples obtained in July and August 2010

The spring-time rains may therefore supply terrestrial DOC to the river system, providing a substrate to methylating bacteria in backwater locations and anoxic river sediments as indicated by the seasonal fluctuations of MeHg and THg. In a study assessing various ecosystem compartments as potential zones of methylation, Hall, St. Louis and Bodaly (2004) suggested that rates of substrate supply such as DOC are important ecological factors affecting MeHg production. The seasonal data from the lower Ouachita River indicate that these processes may also affect the temporal distribution of MeHg in the main channel of the river, possibly from the increased supply of substrates.

Filtered MeHg and THg data collected at the backwater sites OR-11 and OR-2 fall within the ranges depicted in Figure 35, showing that MeHg occurrence at OR-11 in August are in the middle of the range of MeHg in the main channel, and at OR-2 are at the high end of the range of MeHg concentrations in the main channel. The decline in dissolved MeHg from May/June to September can be explained by the uptake of MeHg by algae and diatoms using passive or facilitated transport mechanisms (Moye, et al. 2002). This uptake may occur after the peak in MeHg production in the spring months, which can then facilitate bioaccumulation of MeHg at all trophic levels in the food chain, beginning with diatoms and algae. These seasonal data are crucial in understanding how climate and seasonal weather patterns can affect Hg methylation rates in the lower Ouachita River system. Data collected from this project give insight into the geochemical controls of Hg methylation in ecosystem compartments amenable to methylation in this river system, enhancing the existing data collected by the USGS, and providing more insight into the occurrence and distribution of MeHg in southern Arkansas.

5.8 Conceptual Model

Data collected at the three study sites on the lower Ouachita River suggest that redox potential, geochemistry, and the occurrence of DOC control the occurrence of MeHg in back water locations in this river system, and that these controlling factors exhibit spatial variation. High concentrations of DOC are positively correlated to MeHg concentrations when compiling bottom-water data at all three sites, suggesting DOC as a major control on methylation processes in this system as a whole. The difference in MeHg concentrations between the three sites appears to be controlled by availability of DOC and the amount of filtered THg available for methylation. At RL-2, where the highest concentrations of filtered MeHg were detected (median 1.2 ng/L) and the highest DOC (median 13.52 mg/L), filtered THg accounted for 33% of total Hg, as compared to 25% and 8% for OR-2 and OR-11, respectively. OR-11 had the lowest MeHg concentration (median 0.34 ng/L), lowest DOC concentrations in the bottom water (median 3.50 mg/L), and lowest percent filtered THg (8%), highlighting these controls. Channel morphology also plays an important role in the distribution of DOC, and in site specific stratification characteristics. Sites such as RL-2, which is a narrow channel with a prominent canopy and receives stream inputs during high precipitation seasons, can have high concentrations of DOC and exhibit strong

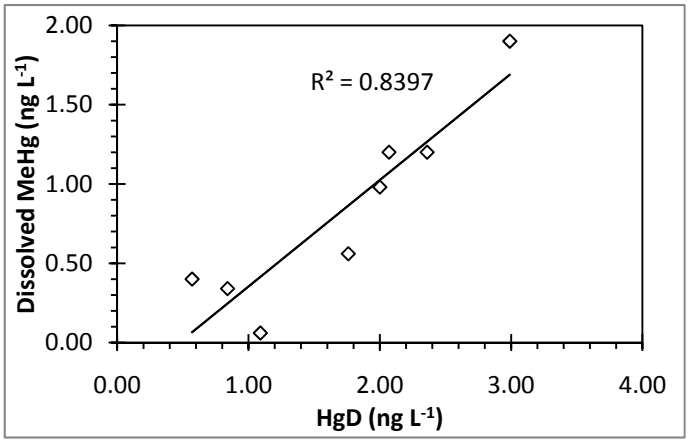


Figure 36: Dissolved inorganic Hg (HgD) and Dissolved MeHg compiled from bottom-water samples at all three sites

stratification characteristics in the summer months. Sites such as OR-2 and OR-11 may be less important in contributing MeHg as DOC and MeHg were comparatively lower at the time of sampling, although these sites should not be completely overlooked for assessments of Hg contamination.

Evidence of redox processes vary between sites, with iron reduction potentially occurring to a significant extent at RL-2 as evidenced by high total iron concentrations, but not to a significant extent at OR-2 and OR-11. High total iron at RL-2 (39.8 mg/L, n=1), coupled with measureable sulfides (median 96.3 µg/L) suggest that both iron reduction and sulfate reduction may contribtute to Hg methylation at this site. Low total iron at OR-2 and OR-11 (2.69 and 1.61 mg/L respectively), plus measureable sulfide at these sites (median 87.7 and 77.7 µg/L respectively) indicate that iron reduction may not play a significant role in Hg methylation at these sites, but that sulfate reduction may be the primary contributor to Hg methylation.

When combining Hg data from the bottom water at all three sites, Hg_D and filtered MeHg are positively correlated, indicating that the concentration of inorganic Hg substrate also mediates methylation (Figure 36). Relating site morphology to MeHg production potential can be an important time-saving step for future work in the lower Ouachita River

Assessment of seasonal fluctuations of Hg from existing USGS data at sites proximal to the lower Ouachita River sampling sites show peaks in the occurrence of filtered THg and MeHg during late spring, indicating seasonal controls on MeHg production. An increase in the supply of DOC during high precipitation or flood events may increase

MeHg production by enhancing microbial activity. These relations and seasonal processes have been noted by other researchers evaluating DOC influences on MeHg production (e.g. Hall, St. Louis and Bodaly 2004, Hall, et al. 2008).

.

5.9 Conclusion

Data generated from this study show the spatial variability in geochemistry at the study sites, which has a direct effect on MeHg production. As mentioned throughout this document, sites with high DOC had higher concentrations of dissolved MeHg as evidenced by positive correlation between these two constituents. Measureable sulfides in the bottom water at all three sites give evidence for sulfate reduction, yet high absolute values of redox potential indicate that redox potential is not low enough in the bottom-water at the study sites to allow sulfate and iron reduction. Sparse geochemical data for total iron and sulfate provide some evidence for the occurrence of redox processes at the sites, but a more detailed constituent characterization is necessary to give further insight into these processes.

The observed positive linear relation between dissolved MeHg and DOC at the bottom water at all three sites indicates an important influence of DOC on Hg methylation (Figure 34). This relation can be explained by DOC acting as an energy source that stimulates microbial activity, and by low pH in the bottom water providing protons to compete for negatively charged binding sites in DOC that would otherwise be utilized by Hg, thus leaving Hg bioavailable for methylation (Barkay, Gillman and Turner 1997). Channel morphology also plays an important role in the distribution of DOC, and in site

specific stratification characteristics. Sites such as RL-2, which is a narrow channel with a prominent canopy and receives stream inputs during high precipitation seasons, can have high concentrations of DOC and exhibit strong stratification characteristics in the summer months. For this study only bulk DOC was assessed, which can lead to inferences as to how DOC affects Hg speciation and transport in this system, but does not give insight into how the quality of DOC effects Hg methylation at the three study sites. Further characterization of DOC would give more insight into dominant species of DOC at the sites, and provide data for determining the dominant source of DOC to these sites (i.e. allocthonous or autocthonous sources).

Measurable sulfide detected at the bottom water at all three sites suggest that sulfate reduction and associated MeHg production may be occurring either in the anoxic water at the base of the water column, at the sediment-water interface, or in sediment pore water with sulfide and MeHg moving out of sediment into the overlying water column. Bottom-water concentrations of sulfide at the three study sites on the lower Ouachita were in the range of 74.0-142.7 μg/L, well within the range of sulfide concentrations cited as evidence for sulfate reduction in the anoxic hypolimnion of a Wisconsin lake (Eckley, et al. 2005). However, ORP values do not show the potential for sulfate reduction in the anoxic bottom waters at the lower Ouachita River sites. Given the conflicting evidence of absolute ORP , further data are needed at corroborate the occurrence of sulfate reduction in the bottom water at these sites including a larger sulfide data set, dissolved iron analysis, and a larger sulfate data set. Comparing sulfide concentrations to dissolved MeHg compiled from all three study sites did not result in a significant correlation (r^2=0.059); this lack of correlation may be explained by free

sulfide combining with dissolved inorganic Hg (Hg_D) to form neutral bio-available Hg-sulfide complexes (Hg_D is calculated as the difference between filtered THg and filtered MeHg) as described by Benoit, et al. (1999). Another explanation for the lack of correlation between sulfide and MeHg is the combination of iron with sulfide generated from sulfate reduction, and the precipication of these minerals (e.g. pyrite). SEM analysis of river sediments at OR-2 did not result in any noticeable authigenic pyrite, highlighting the uncertainty of the relation between sulfide and MeHg at the study sites.

Of the three study sites, RL-2 exhibited the highest filtered MeHg, THg, and particulate MeHg concentrations in the bottom water (Figure 21) as well as the highest DOC concentrations (mean 13.37 mg/L, n=3). A high concentration of total iron at RL-2 (39.8 mg/L) indicates that iron reduction has the potential to be the dominant TEAP at this site. This is an important distinction because certain strains of FeRB have been shown to methylate Hg (Kerin, et al. 2006, Fleming, et al. 2006). Measureable sulfide at this site indicates that sulfate reduction may be occurring concurrently with iron reduction. It is therefore possible that multiple microbial communities that methylate Hg are responsible for the high concentrations of MeHg at RL-2, potentially even FeRB. Filtered MeHg concentrations were lowest at OR-11, which also had the lowest percent filtered THg out of all three sites (8%), indicating that there is a small fraction of Hg available for methylation at that site. OR-2 exhibited variability of Hg concentrations between the three sampling days (min 0.06 ng/L. max 0.98 ng/L) suggesting variable controls on Hg methylation at that site. Common to all three back-water sites is measureable sulfide and stratification characteristics, showing that Hg methylation can occur in anoxic waters where sulfate reduction TEAP's dominate.

Assessment of seasonal fluctuations of Hg from existing USGS data at sites proximal to the lower Ouachita River sampling sites show peaks in the occurrence of filtered THg and MeHg during late spring, indicating seasonal controls on MeHg production. An increase in the supply of DOC during high precipitation or flood events may increase MeHg production by enhancing microbial activity. In a study assessing various ecosystem compartments as potential zones of methylation, Hall, St. Louis and Bodaly (2004) suggested that rates of substrate supply such as DOC are important ecological factors affecting MeHg production. These relations and seasonal processes have been noted by other researchers evaluating DOC influences on MeHg production (e.g. Hall, St. Louis and Bodaly 2004, Hall, et al. 2008).

Data collected from this pilot-scale study provide a baseline characterization of the extent of Hg contamination on this 303(d) listed river. High resolution data for pore-water MeHg and sulfides would make it possible to derive conclusions on other processes, including sediment-water exchange, of both of these constituents. The data produced from this study show the potential for Hg methylation in the water column given the afore mentioned occurrence of sulfides in the bottom water at all three study sites, which gives impetus for pursuing Hg research in this region of Arkansas. Further characterization of DOC would also provide insight into what types of DOC are participating in Hg cycling.

Future work to aid in addressing water column methylation potential includes incubation experiments and the use of in-situ dialysis samplers to provide pore-water data. Incubation experiments can be conducted using sample water from these sites, and spiking them with isotopically labeled HgCl to calculate methylation/demethylation rates

in the bottom-water as the water sample decreases in redox potential. Deploying an in-situ dialysis sampler (peeper) would allow pore-water samples for MeHg and sulfides to be collected in the top 5-10 cm of bed-sediment where Hg methylation occurs, and in the 5-10 cm above the bed-sediment to give data for sediment-water exchange processes, and flux rates of MeHg and sulfides.

This study provides crucial data describing the extent of Hg contamination in Arkansas, with two of eight bottom-water samples exceeding the numeric water quality standard of 12 ng/L total recoverable Hg in water. Although these samples were not collected as part of the Arkansas Department of Environmental Quality's ambient monitoring program, they were analyzed in a federal government-authorized Hg lab by the USGS, and provide the most accurate aquatic Hg data available to the State. As atmospheric Hg deposition increases across the country, the Hg issue in Arkansas only stands to become more prominent, giving impetus for additional research to be conducted on this important environmental issue.

References

Amirbahman, Aria, Andrew L Reid, Terry A Haines, J. Steven Kahl, and Cedric Arnold. "Association of Methylmercury with Dissolved Humic Acids." *Environmental Science and Technology* 36, no. 4 (2002): 690-695.

Arkansas Department of Environmental Quality. *2008 List of Impaired Waterbodies (303(d) list).* Water Quality, Little Rock: ADEQ, 2008.

Arkansas Department of Health. *Fish Consumption Notice-Mercury in Fish.* http://www.healthy.arkansas.gov/programsServices/epidemiology/Environmental/Documents/fishnotice_mercury.pdf (accessed November 14, 2010).

Arkansas Pollution Control and Ecology Commission (APCEC). *Regulation No. 2: Regulation Establishing Water Quality Standards for Surface Waters of the State of Arkansas.* Little Rock, AR: APCEC, 2007.

Babiarz, Christopher L, James P Hurley, Janina M Benoit, Martin M Shafer, Anders W Andren, and David A Webb. "Seasonal influences on partitioning and transport of total and methylmercury in rivers from contrasting watersheds." *Biogeochemistry* 41, no. 3 (1998): 237-257.

Babiarz, Christopher L., James P. Hurley, Stephen R. Hoffmann, Anders W. Andren, Martin M. Shafer, and David E. Armstron. "Partitioning of Total Mercury and Methylmercury to the Colloidal Phase in Freshwaters." *Environmental Science and Technology* 35, no. 24 (2001): 4773-4782.

Barkay, Tamar, Mark Gillman, and Ralph Turner. "Effects of Dissolved Organic Carbon and Salinity on Bioavailability of Mercury." *Applied and Environmental Microbiology* 63, no. 11 (1997): 4267-4271.

Benoit, J., C. Gilmour, R. Mason, and A. Heyes. "Sulfide Control on Mercury Speciation and Bioavailability to Methylating Bacteria in Sediment Pore Waters." *Environmental Science and Technology*, 1999: 951-957.

Benoit, J.M., C.C. Gilmour, and R.P. Mason. "Aspects of Bioavailability of Mercury for Methylation in Pure Cultures of Desulfobulbus propionicus (1pr3)." *Applied and Environmental Microbiology* 67, no. 1 (2001a): 51-58.

Benoit, J.M., C.C. Gilmour, and R.P. Mason. "The Influence of Sulfide on Solid-Phase Mercury Bioavailability for Methylation by Pure Cultures of Dusulfobulbus propionicus (1pr3)." *Environmental Science and Technology* 35, no. 1 (2001b): 127-132.

Benoit, J.M., R.P. Mason, C.C. Gilmour, and G.R. Aiken. "Constants for mercury binding by dissolved organic matter isolates from the Florida Everglades." *Geochimica et Cosmochimica Acta* 65, no. 24 (2001): 4445-4454.

Benoit, JM, CC Gilmour, A Heyes, R.P Mason, and CL Miller. "Geochemical and Biological Controls over Methylmercury Production and Degradation in Aquatic Ecosystems." Chap. 19 in *Biogeochemistry of Environmentally Important Trace Elements*, by Yong Cai and Olin C. Braids, edited by Yong Cai and Olin C. Braids, 262-291. Washington D.C.: American Chemical Society, 2003.

Bertilsson, Stefan, and Jeremy B Jones. "Supply of Dissolved Organic Matter to Aquatic Ecosystems: Autochthonous Sources." In *Aquatic Ecosystems: Interactivity of Dissolved Organic Matter*, by Stuart E Findlay and Robert L Sinsabaugh, 3-24. Academic Press, 2003.

Brigham, Mark E, Dennis A Wentz, George R Aiken, and David P Krabbenhoft. "Mercury Cycling in Stream Ecosystems. 1. Water Column Chemistry and Transport." *Environmental Science and Technology*, 2009: 2720-2725.

Cai, Yong, Rudolf Jafe, and Ronald D Jones. "Interactions between dissolved organic carbon and mercury species in surface waters of the Florida Everglades." *Applied Geochemistry* 14 (1999): 395-407.

Choe, Key-Young, Wesley A Heim, and Kenneth H Coale. "Sediment-water exchange of total mercury and monomethylmercury in the San Francisco Bey-Delta." *Limnology and Oceanography* 49, no. 5 (2004): 1512-1527.

Clardy, Benjamin F, and William V Bush. *Mercury District of Southwest Arkansas.* Information Circular 23, Little Rock, Arkansas: Arkansas Geological Commission, 1976.

Clarkson, Thomas William. "Human Health Risks from Methylmercury in Fish." *Environmental Toxicology and Chemistry* 9 (1990): 957-961.

De Wild, John F, Mark L Olson, and Shane D Olund. *Determination of Methyl Mercury by Aqueous Phase Ethylation, Followed by Gas Chroatographic Separation with Cold Vapor Atomic Fluorescence Detection, Open-File Report 01-445.* Middleton, WI: U.S. Geological Survey, 2002.

De Wild, John F., Shane D. Olund, Mark L. Olson, and Michael T. Tate. *Methods for the Preparation and Analysis of Solids and Suspended Solids for Methylmercury: Techniques and Methods 5 A-7.* Reston, VA: U.S. Geological Survey, 2004.

Delongchamp, Tania M, David R.S. Lean, Jeffry J Ridal, and Jules M Blais. "Sediment mercury dynamics and historical trends of mercury deposition in the St. Lawrence River

area of concern near Cornwall, Ontario, Canada." *Science of the Total Environment*, 2009: 4095-4104.

Eckley, C.S., C.J. Watras, H. Hintelmann, K. Morrison, A.D. Kent, and O. Regnell. "Mercury Methylation in the hypolimnetic waters of lakes with and without connection to wetlands in northern Wisconsin." *Canadian Journal of Fisheries and Aquatic Sciences* 62 (2005): 400-411.

Evans, Anthony M. *Ore Geology and Industrial Minerals: An Introduction.* Malden, MA: Blackwell Science Ltd, 1980.

Facemire, C, et al. "Impacts of Mercury Contamination in the Southeastern United States." *Water, Air, & Soil Pollution*, 1995: 923-926.

Fleming, Emily J, E. Erin Mack, Peter G Green, and Douglas C Nelson. "Mercury Methylation from Unexpected Sources: Molybdate-Inhibited Freshwater Sediments and an Iron-Reducing Bacterium." *Applied and Environmental Microbiology* 72, no. 1 (2006): 457-464.

FTN Associates, Ltd. *TMDL's for Segments Listed for Mercury in Fish Tissue for the Ouachita River Basin, and Bayou Bartholomew, Arkansas and Louisiana to Columbia.* TMDL, Little Rock, AR: FTN Associates, Ltd., 2002.

Goulet, Richard R, et al. "Mercury Transformations and fluxes in sediments of a riverine wetland." *Geochimica et Cosmochimica Acta* 71 (2007): 3393-3406.

Guevara, Sergio Ribeiro, Claudia P. Queimalinos, Maria del Carmen Dieguez, and Maria Arribere. "Methylmercury production in the water column of an ultraoligotrophic lake of Northern Patagonia, Argentina." *Chemosphere* 72, no. 4 (2008): 578-585.

Haitzer, Markus, George R Aiken, and Joseph N Ryan. "Binding of Hg(II) to Aquatic Humic Substances: Influence of pH and Source of Humic Substances." *Environmental Science and Technology* 37, no. 11 (2003): 2436-2441.

Haitzer, Markus, George R Aiken, and Joseph N Ryan. "Binding of Mercury(II) to Dissolved Organic Mateer: The Role of the Mercury-to-DOM Concentration Ratio." *Environmental Science and Technology* 36, no. 16 (2002): 3654-3570.

Hall, B.D., G.R. Aiken, D.P. Krabbenhoft, M. Marvin-DiPasquale, and C.M. Swarzenski. "Wetlands as principal zones of methylmercury production in southern Louisiana and the Gulf of Mexico region." *Environmental Pollution* 154 (2008): 124-134.

Hall, Britt D., Vinvent L. St. Louis, and R.A. (Drew) Bodaly. "The stimulation of methylmercury production by decomposition of flooded birch leaves and jack pine needles." *Biogeochemistry* 68, no. 1 (2004): 107-129.

Hintelmann, Holger, Pamela M Welbourn, and Douglas R Evans. "Measurement of Complexation of Methylmercury(II) Compounds by Freshwater Humic Substances Using Equilibrium Dialysis." *Environmental Science and Technology* 31, no. 2 (1997): 489-495.

Holloway, JoAnn M, Martin B Goldhaber, Kate M Scow, and Rebecca E Drenovsky. "Spatial and seasonal variations in mercury methylation and microbial community structure in a historic mercury mining area, Yolo County, California." *Chemical Geology*, 2009: 85-95.

Hurley, James P, David P Krabbenhoft, Lisa B Cleckner, Mark L Olson, George R Aiken, and Peter S Ralick JR. "System controls on the aqueous distribution of mercury in the northern Florida Everglades." *Biogeochemistry* 40 (1998): 293-310.

Jay, Jenny Ayla, Francois, M.M. Morel, and F. Hemond Harold. "Mercury Speciation in the Presence of Polysulfides." *Environmental Science and Technology* 34, no. 11 (2000): 2196-2200.

Kelly, C.A., et al. "Increases in Fluxes of Greenhouse Gases and Methyl Mercury following Flooding of an Experimental Reservoir." *Environmental Science and Technology* 31, no. 5 (1997): 1334-1344.

Kerin, E.J., C.C. Gilmour, E. Roden, M.T. Suziki, J.D. Coates, and R.P. Mason. "Mercury Methylation by Dissimilatory Iron-Reducing Bacteria." *Applied and Environmental Microbiology* 72, no. 12 (2006): 7919-7921.

Kim, Bomchul, Kwangsoon Choi, Chulgoo Kim, U-Hee Lee, and Yoon-Hee Kim. "Effects of the summer monsoon on the distribution and loading of organic carbon in a deep reservoir, Lake Soyang, Korea." *Water Research* 34, no. 4 (2000): 3495-3504.

King, Jeffrey K, Joel E Kostka, Marc E Frischer, and Michael F Saunders. "Sulfate-Reducing Bacteria Methylate Mercury at Variable Rates in Pure Culture and in Marine Sediments." *Applied and Environmental Microbiology* 66, no. 6 (2000): 2430-2437.

Krabbenhoft, David P., James P. Hurley, Mark L. Olson, and Lisa B. Cleckner. "Diel variability of mercury phase and species distributions in the Florida Everglades." *Biogeochemistry* 40, no. 2-3 (1998): 311-325.

Lamborg, Carl H, Chun-Mao Tseng, William F Fitzgerald, Prentiss H Balcom, and Chad R Hammerschmidt. "Determination of the Mercury Complexation Characteristics of Dissolved Organic Matter in Natural Waters with "Reducible Hg" Titrations." *Environmental Science and Technology* 37, no. 15 (2003): 3316-3322.

Lin, HS, and HD Scott. *Spatial Analysis of the Cause of Mercury Contamination of Fish in Arkansas.* Water Resources Publication No. MSC-196, Fayetteville, AR: Arkansas Water Resources Center, 1997.

Lindberg, Steve, et al. "A Synthesis of Progress and Uncertainties in Attributing the Sources of Mercury in Deposition." *Ambio* 36, no. 1 (2007): 19-33.

Liu, Jianrong, Kalliat T. Valsaraj, Istvan Devai, and R.D. DeLaune. "Immobilization of aqueous Hg(II) by mackinawite (FeS)." *Journal of Hazardous Materials* 157 (2008): 432-440.

Marvin-Dipasquale, M., et al. "Mercury Cycling in Stream Ecosystems. 2. Benthic Methylmercury Production and Bed Sediment - Pore Water Partitioning." *Environmental Science and Technology*, 2009: 2726-2734.

Mason, R.P., and G.-R. Sheu. "Role of the ocean in the global mercury cycle." *Global Biogeochemical Cycles* 16, no. 4 (2002).

Mergler, Donna, Henry A Anderson, Laurie Hing Man Chan, and Kathryn R Mahaffew. "Methymercury Exposure and Health Effects in Humans: A Worldwide Concern." *Ambio* 36, no. 1 (2007): 3-11.

Miller, Carrie L, Robert P Mason, Cynthia C Gilmour, and Andrew Heyes. "Influence of Dissolved Organic Matter on the Complexation of Mercury under Sulfidic Conditions." *Environmental Toxicology and Chemistry* 26, no. 4 (2007): 624-633.

Monperrus, M, et al. "The biogeochemistry of mercury at the sediment-water interface in the Thau Lagoon. 2. Evaluation of mercruy methylation potential in both surface sediment and the water column." *Estuaring Coastal and Shelf Science* 72 (2007): 485-496.

Morel, Francois, and Janet G Hering. *Principles and Applications of Aquatic Chemistry.* Toronto: John Wiley and Sons, Inc., 1993.

Moye, Anson H, Carl J Miles, Edward J Philips, Bethany Sargent, and Kristen K Merritt. "Kinetics and Uptake Mechanisms for Monomethylmercury between Freshwater Algae and Water." *Environmental Science and Technology* 36, no. 16 (2002): 3550-3555.

National Atmospheric Deposition Program. *2009 Annual Summary.* Champaign, IL: NADP, 2010.

Nimick, David A., Blaine R. McCleskey, Christopher H. Gammons, Thomas E. Cleasby, and Stephen R. Parker. "Diel mercury-concentration variations in streams affected by mining and geothermal discharge." *Science of the Total Environment* 373, no. 1 (2007): 344-355.

Ogorek, J. http://wi.water.usgs.gov/mercury-lab/forms/Particulate%20matter%20Distillation%20and%20analysis%20by%20isotope%20dilution,%20brooks%20rand_ICPMS.pdf (accessed November 11, 2010).

Ogorek, J, and Charlie D Thompson. *Analysis of Total Mercury in Waters, Soils, and Sediments with the Tekran 2600 by Cold Vapor Atomic Fluorescence Spectrometry: WDML SOP.* Middleton, WI: U.S. Geological Survey: http://wi.water.usgs.gov/mercury-lab/forms/Tekran%202600%20HgT%20Water%20and%20Solids%20Analysis%20SOP.pdf, 2010.

Ogorek, Jacob, and John Dewild. "Analysis of Methylmercury in Plant, Sediment, and Soil Samples by Cold Vapor Atomic Fluorescence Detection with the Brooks-Rand "MERX" Automated Methylmercury Analytical System." SOP.

Olson, Mark L., and John F. De Wild. *Determination of Total Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry: WDML SOP001.* U.S. Geological Survey: http://wi.water.usgs.gov/mercury-lab/forms/hg_tsop001_rev_4.pdf, 2010.

Olund, Shane D, Mark L Olson, Michael T Tate, Charlie D Thompson, and John F De Wild. *Determination of Total Mercury in Suspended Solids by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry: SOP.* U.S. Geological Survey, http://wi.water.usgs.gov/mercury-lab/forms/Particulate%20HgT%20SOP_rev3.pdf, 2010.

Ostapenia, A.P., A Parparov, and T Berman. "Lability of organic carbon in lakes of different trophic status." *Freshwater Biology*, 2009: 1312-1323.

Pai, Prasad, Steven Heisler, and Aruna Joshi. "An emissions inventory for regional atmospheric modeling of mercury." *Water, Air, and Soil Pollution* 101 (1998): 289-308.

Prestbo, Eric M, and David A Gay. "Wet deposition of mercury in the U.S. and Canada, 1996-2005: Results and analysis of the NADP mercury deposition network (MDN)." *Atmospheric Environment* 43 (2009): 4223-4233.

Radke, D.B. *Bottom-material samples: U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap A8, June, accessed 11/10/2010 at http://pubs.water.usgs.gov/twriA8/.* U.S. Geological Survey, 2005.

Ranchou-Peyruse, M, et al. "Overview of Mercury Methylation Capacities among Anaerobic Bacteria Including Representatives of the Sulphate-Reducers: Implications for Environmental Studies." *Geomicrobiology Journal* 26 (2009): 1-8.

Ravichandran, Mahalingam. "Interactions between mercury and dissolved organic matter-a review." *Chemosphere* 55 (2004): 319-331.

Rickard, David. "Kinetics of pyrite formation by the H2S oxidation of iron (II) monosulfide in aqueous solutions between 25 and 125° C: The rate equation." *Geochimica et Cosmochimica Acta* 61, no. 1 (1997): 115-134.

Rothenberg, Sarah E, Richard F Ambrose, and Jennifer A Jay. "Mercury cycling in surface water, pore water, and sediments of Mugu Lagoon, CA, USA." *Environmental Pollution* 154 (2008): 32-45.

Sachse, A, R Henrion, J Gelbrecht, and CEW Steinberg. "Classification of dissolved organic carbon (DOC) in river systems: Influence of catchment characterisitics and autochthonous processes." *Organic Geochemistry*, 2005: 923-935.

Scherbatskoy, Timothy, James B Shanley, and Gerald J Keller. "Factors Controlling Mercury Transport in an Upland Forested Catchment." *Water Air and Soil Pollution* 105, no. 1-2 (1998): 427-438.

Schlesinger, William H. *Biogeochemistry: An Analysis of Global Change.* 2. San Diego, CA: Academic Press, 1997.

Schmitt, Harrison. "Origin of the "Epithermal" Mineral Deposits." *Economic Geology* 45 (1950): 191-200.

Scott, HD, and JM McKimmey. *Investigation of the Statistical and Spation Distributions of Mercury Contaminated Fish, Surface Waters and Soils in Arkansas.* Water Resources Publication Publication No MSC-216, Fayetteville, AR: Arkansas Water Resources Center, 1997.

Seigneur, Christian, Krish Vijayaraghacan, Kristen Lohman, Prakash Karamchanadani, and Courtney Scott. "Global Source Attribution for Mercury Deposition in the United States." *Environmental Science and Technology* 38, no. 2 (2004): 555-569.

Selin, Noelle E. "Global Biogeochemical Cycling of Mercury: A Review." *Annual Review of Environment and Resources* 34 (2009): 43-63.

Selin, Noelle E., et al. "Chemical cycling and deposition of atmospheric mercury: Global constraints from observations." *Journal of Geophysical Research* 112, no. D2 (2007).

Sellers, Patricia, Carol A. Kelly, and John W.M. Rudd. "Fluxes of Methylmercury to the Water Column of a Drainage Lake: The Relative Importnace of Internal and External Sources." *Limnology and Oceanography* 46, no. 3 (2001): 623-631.

Sonesten, L. "Fish mercury levels in lakes-adjusting for Hg and fish-size covariation." *Environmental Pollution* 125 (2003): 255-265.

Stanley, Donald W, and Scott W Nixon. "Stratification and bottom-water hypoxia in the Pamlico River estuary." *Estuaries and Coasts*, 1992: 270-281.

Stone, Charles G, Joe F Nix, and John David McFarland. *A Regional Survey of the Distribution of Mercury in the Rocks of the Ouachita Mountains of Arkansas.* Information Circular 32, Little Rock Arkansas: Arkansas Geological Commission, 1995.

Stumm, W, and James J Morgan. *Aquatic Chemistry: An introduction emphasizing chemical equilibria in natural waters.* 3rd Edition. New York: John Wiley & Sons Inc., 1996.

Stumm, Werner, and James J Morgan. *Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters.* Toronto: John Wiley & Sons, Inc., 1996.

Sunderland, E.M., D.P. Krabbenhoft, J.W. Moreau, S.A. Strode, and W.M. Landing. "Mercury sources, distribution, and bioavailability in the North Pacific Ocean: Insights from data and models." *Global Biogeochemical Cycles*, 2009.

Ullrich, Susanne M, Trevor W Tanton, and Svetlana A Abdrashitova. "Mercury in the Aquatic Environment: A Review of Factors Affecting Methylation." *Critical Reviews in Environmental Science and Technology* 31, no. 3 (2001): 241-293.

United States Environmental Protection Agency. *Method 1631, Revision E: Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectrometry: EPA-821-R-02-019.* Washington, D.C.: U.S. EPA, 2002.

USGS. *Water Data Report 2010.* http://wdr.water.usgs.gov/wy2010/pdfs/07362000.2010.pdf (accessed 03 13, 2011).

Waples, Jacob S, Kathryn L Nagy, George R Aiken, and Joseph N Ryan. "Dissolution of cinnabar (HgS) in the presence of natural organic matter." *Geochimica et Cosmochimica Acta* 69, no. 6 (2005): 1575-1588.

Warner, Kimberly A., Eric E. Roden, and Jean-Claude Bonzongo. "Microbial Mercury Transformation in Anoxic Freshwater Sediments under Iron-Reducing and other Electron-Accepting Conditions." *Environmental Science and Technology*, 2003: 2159-2165.

Wilde, F.D., D.B. Radke, jacob Gibs, and R.T. Iwatsubo. *Processing of water samples (version 2.2): U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A5, April, accessed 11/10/10 at http://pubs.water.usgs.gov/twri9A5/.* U.S. Geological Survey, 2004-2009 (a).

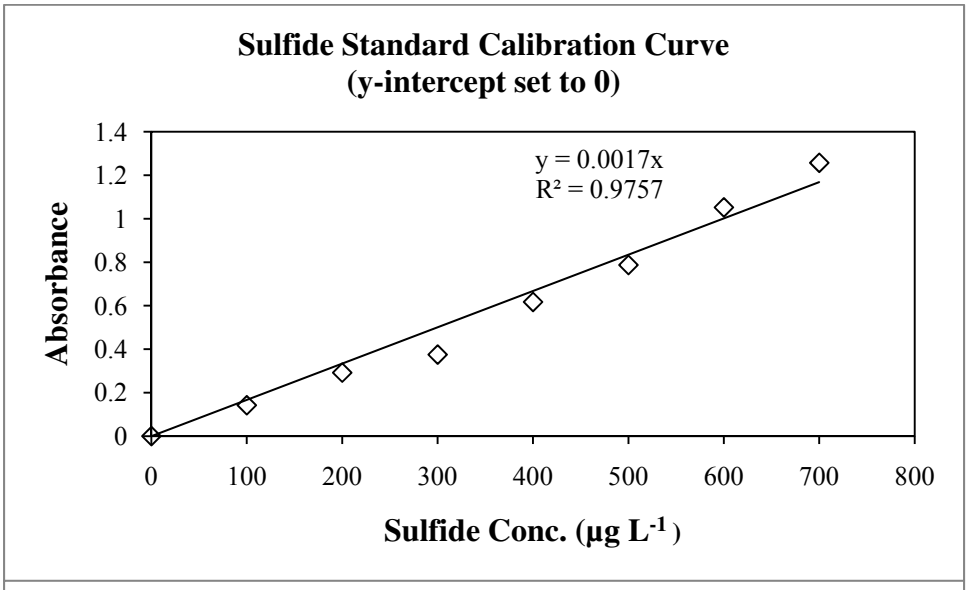
Wilde, F.D., D.B. Radke, Jacob Gibs, and R.T. Iwatsubo. *Processing of water samples (version 2.2): U.S. Geological Survey Techniques of Water-Resources Investigations,*

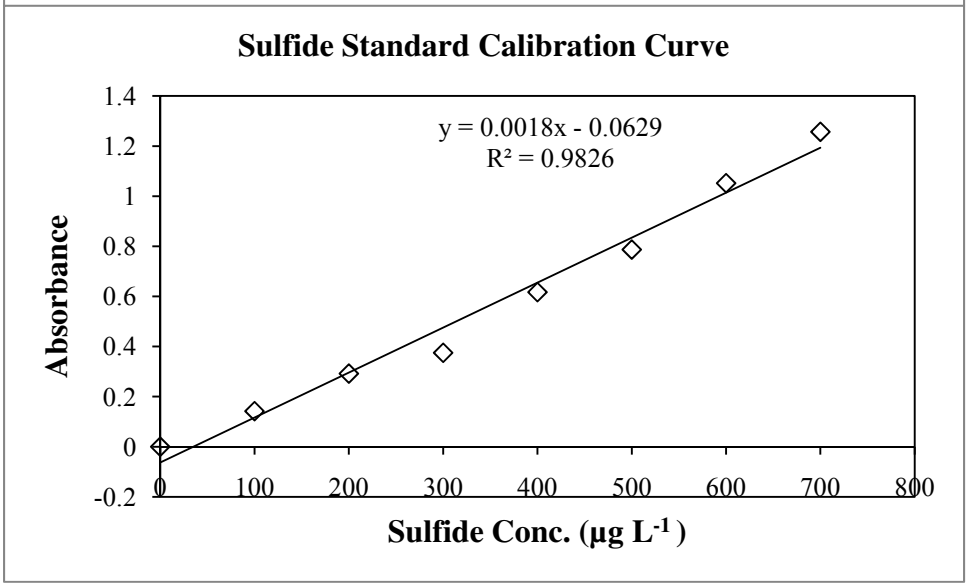
book 9, chap. A5, April, accessed 11/8/2010 at http://pubs.water.usgs.gov/twri9A5/ Chapter 5.6.4.B "Low-level Mercury". U.S. Geological Survey, 2004-2009(b).

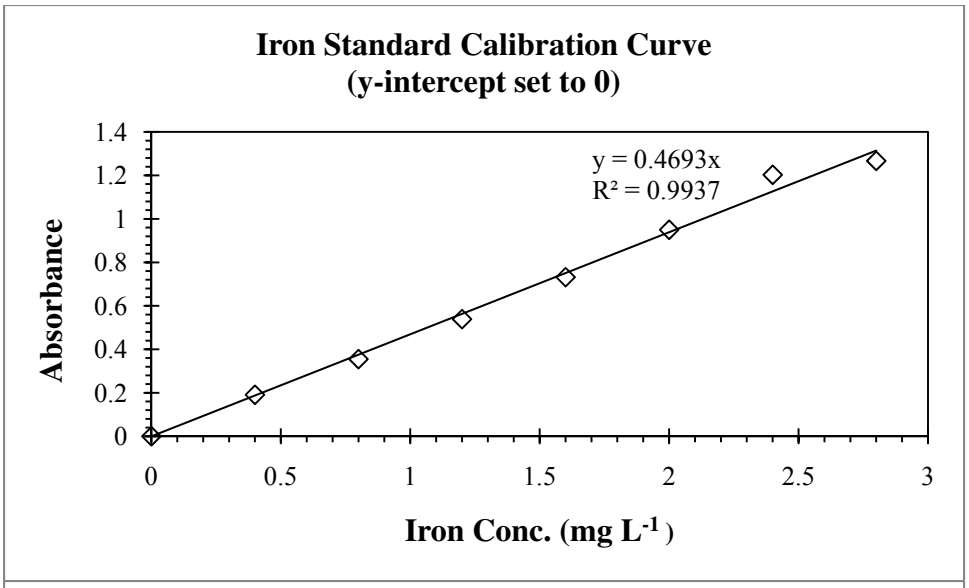
Wilde, F.D., ed. *Field Measurements: U.S. Geological Survey Techniques of Water-Resources Invesigations, book 9, chapter A6.* Reston, VA: U.S. Geological Survey, chapter sections variously dated.

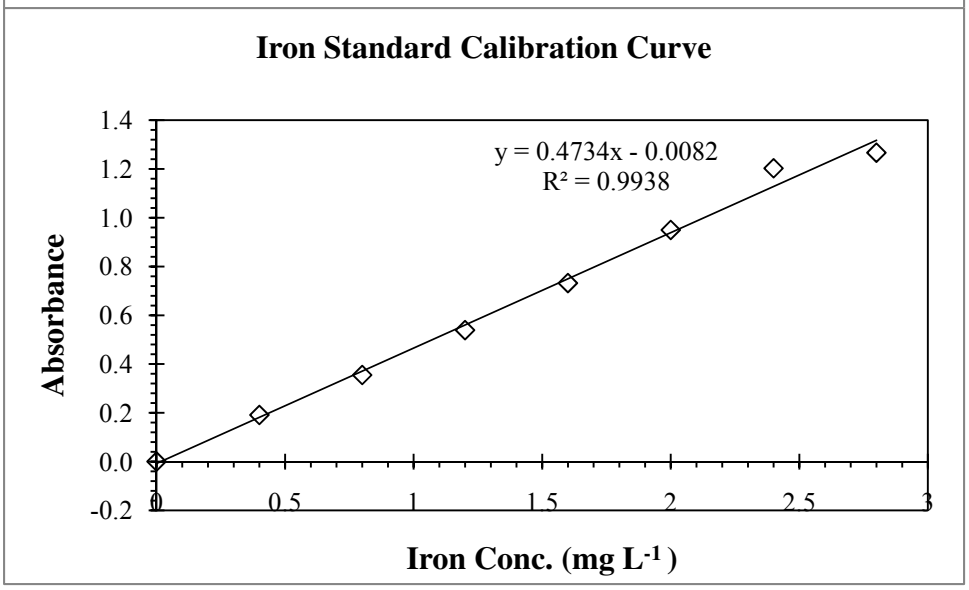
Zhou, HY, and MH Wong. "Mercury Accumulation in Freshwater Fish with Emphasis on the Dietary Influence." *Water Resources*, 2000: 4234-4242.

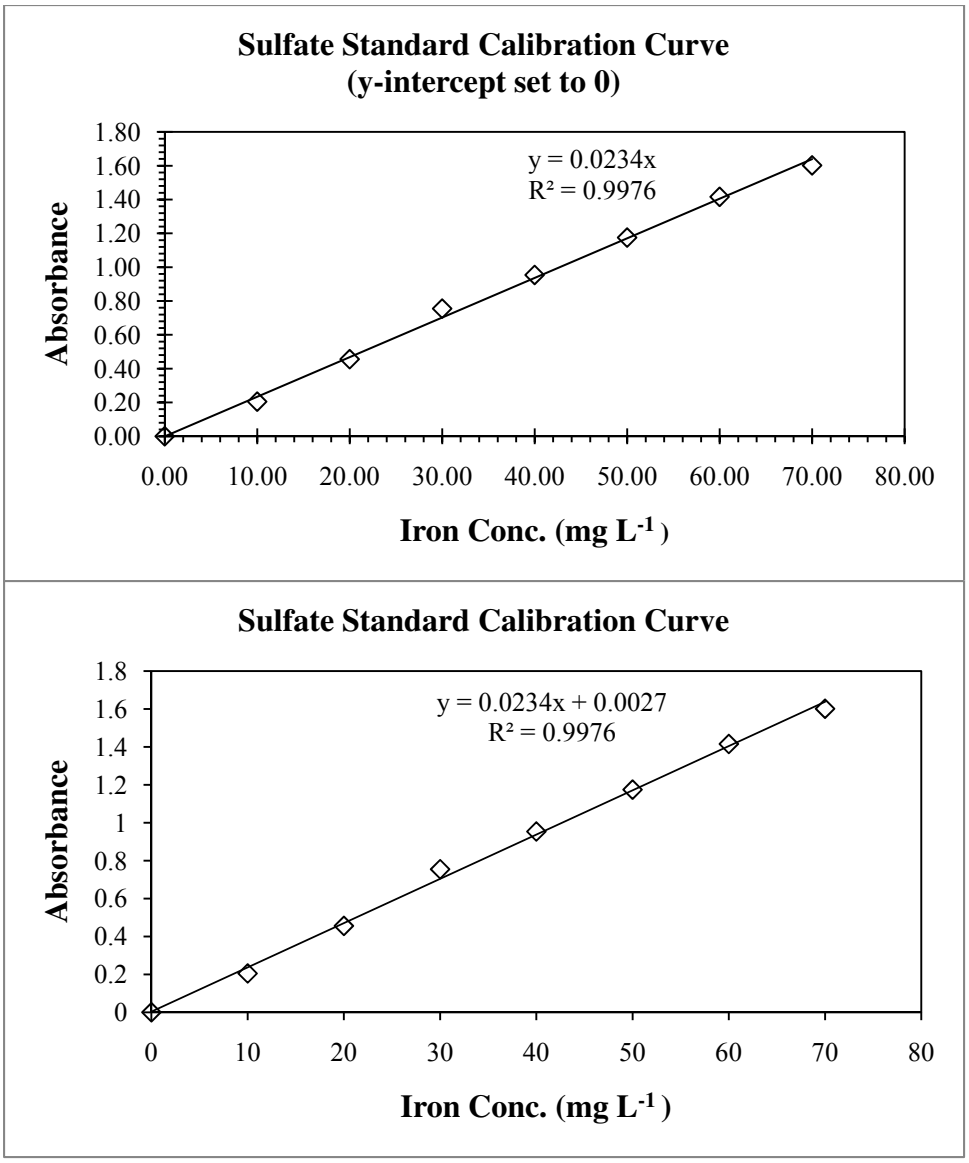
6 Appendix


Sulfide Standard Calibration Curve
(y-intercept set to 0)
y = 0.0017x
R² = 0.9757
Absorbance
Sulfide Conc. (µg L-1)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0
100
200
300
400
500
600
700
800


Sulfide Standard Calibration Curve
y = 0.0018x - 0.0629
R² = 0.9826
Absorbance
Sulfide Conc. (µg L-1)
-0.2
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0
100
200
300
400
500
600
700
800


Iron Standard Calibration Curve
(y-intercept set to 0)
y = 0.4693x
R² = 0.9937
Absorbance
Iron Conc. (mg L-1)
0
0.2
0.4
0.6
0.8
1
1.2
1.4
0.5
1.5
2
2.5
3


Iron Standard Calibration Curve
y = 0.4734x - 0.0082
R² = 0.9938
Absorbance
Iron Conc. (mg L-1)
-0.2
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0.5
1.5
2
2.5
3


Sulfate Standard Calibration Curve
(y-intercept set to 0)
y = 0.0234x
R² = 0.9976
Absorbance
1.80
1.60
1.40
1.20
1.00
0.80
0.60
0.40
0.20
0.00
0.00
10.00
20.00
30.00
40.00
50.00
60.00
70.00
80.00
Iron Conc. (mg L-1)
Sulfate Standard Calibration Curve
y = 0.0234x + 0.0027
R² = 0.9976
Absorbance
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
0
10
20
30
40
50
60
70
80
Iron Conc. (mg L-1)