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The impact of hypoxia on mercury methylation in bottom sediment of northern Gulf of Mexico

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THE IMPACT OF HYPOXIA ON MERCURY METHYLATION IN BOTTOM SEDIMENT OF NORTHERN GULF OF MEXICO

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ABSTRACT

Widespread concern has developed about high mercury content in fish in the Gulf of Mexico and adjacent estuaries and bays. Among the areas implicated as possible sources of the mercury that moves up the food chain from the methylmercury formed in sediments and anoxic waters is the seasonal hypoxic zone in the northern Gulf. This research was designed to determine if methylmercury formation is stimulated by the anaerobic sediment conditions accompanying the onset of summer hypoxia in the Gulf. Both field and laboratory studies were carried out. For the field study sediment samples were collected at three stations (i.e. C4, C6B and C8) along hypoxic transect C established by Nancy Rabalais at a monthly interval from April 05 to April 06 for total and methyl mercury analyses. *In-situ* concentrations of dissolved oxygen, temperature and salinity of bottom waters were measured using a hydro-lab. For the laboratory study the effects of anaerobic conditions and organic matter contents on methylation rate were investigated using sediment-water columns. The most probable number (MPN) of the sulfate reducing bacteria in sediments was enumerated to obtain insights into the microbial mechanism of mercury methylation. Seasonal variations in methylmercury concentrations were observed at three stations with the peak at C4 and C6B in spring 2005. This seasonal pattern might be attributed to the seasonal inputs of freshwater from the Mississippi river into the gulf. Annual average concentrations of methyl mercury were 0.31, 0.47 and 0.12 μg/kg at C4, C6B and C8, respectively. Annual averages of total mercury concentrations were 37.79, 41.06 and 11.02 μg/kg for C4, C6B and C8, respectively. Sediment texture may explain the spatial variations of methyl and total mercury among the stations. Statistical analyses indicate that the dissolved oxygen

($P<0.0001$), temperature ($P=0.03$) and sediment texture ($P=0.001$) significantly affected the methyl mercury concentration. The laboratory study indicates that organic matter was an important factor in controlling the rate of mercury methylation.

CHAPTER 1 INTRODUCTION

Elevated mercury level in fish in freshwater lakes in Louisiana and elsewhere along the gulf coast has raised the public concern about the health effect of consuming mercury contaminated fish. It has also prompted the issuance of many advisories on fish consumption by various public health agencies. Fish caught in the gulf such as king mackerel and tilefish were found with mean concentrations of 0.73 and 1.45ppm, respectively (Food and Drug Administration). These mercury levels exceeded the safety consumption limit set by WHO, USEPA and USFDA, which are 0.5, 0.3 (non-commercial fish) and 1ppm, respectively. Lewi (2002) also reported the mercury levels in fish ranging from 0.08 to 0.85ppm with an average of 0.40ppm, some of which exceeded the Florida health-based standard of 0.5ppm. Although some Gulf States had issued fish advisories, the information about the distribution of mercury in gulf fish (e.g., the location with highest methyl mercury level, species and sizes of fish that contain highest mercury level) is scarce (National Science and Technology Council Committee on the Environmental and Natural Resources, 2004). About 85-90% of the mercury that is found in the fish tissue is methyl mercury (Bloom, 1992). Methyl mercury is capable of passing through placenta and blood brain barrier; consequently, it poses a greater risk to the neurological system of the unborn fetus and young children (Environmental Protection Agency, 2002). High mercury level found in fish would also influence the fishery economy and the interest of sport fishing. Therefore, knowledge of methylation of mercury in the sediment is important because this process increases the solubility, mobility and bioaccumulation in the food chain (Ullrich et al., 2001).

Information about the source of methyl mercury in the gulf is insufficient and the detailed cycling of mercury is not known up to date (National Science and Technology Council Committee on the Environmental and Natural Resources, 2004). Atmospheric fallout

is believed to be the main contributor. The second major source comes from the freshwater inflow of the Mississippi River. Although there is some concern about the contamination of mercury in sediment from offshore oil and gas drilling activities, these activities only produce a small amount of mercury, which is less than 0.5% of the total input of mercury into the gulf (Neff, 2002). In this study, we look at another possible zone in the gulf that has condition favorable for mercury methylation, which is the hypoxic zone of the northern Gulf of Mexico.

Mercury methylation occurs mainly in anaerobic sediment through microbial activities (Berman & Bartha, 1986). Sulfate reducing bacteria are believed to be the principal mercury methylator in anoxic freshwater and estuarine aquatic environments (Compeau & Bartha, 1985; Gilmour et al., 1992). This process is influenced by the activity, distribution and physiology of sulfate reducing bacteria as well as the availability of inorganic mercury, 2000). The rate of mercury methylation has been shown to couple with the rate of sulfate reduction (King et al., 2001). A lower methyl mercury concentration in sediment was observed when sulfate was limiting (Gilmour et al., 1998). However, when sulfate was abundant, the production of methyl mercury was depressed by hydrogen sulfide through the precipitation of HgS (Compeau & Bartha, 1985). Benoit et al. (2001) suggested that the shift from the neutral sulfide complex to a more polar (negatively charged) sulfide complex had hindered the passive diffusion uptake of bacteria, which decreases production of methyl mercury.

Mercury methylation in sediment was showed to be more favorable in anaerobic condition than in aerobic condition (Compeau & Bartha, 1984; Olson & Cooper, 1976). In a controlled redox potential study, . Compeau & Bartha (1984) observed the formation of methyl mercury in estuarine sediment was favored by -200mV rather than by +110mV. Unpublished data from our laboratory study using lake sediments showed substantially active

mercury methylation at -200 mV and no methylation above -100mV (Hou, 2003). On the contrary, Trefry et al. (2002) found higher methyl mercury concentrations in sediments with moderate redox potential and high organic carbon content. They observed that the concentration of methyl mercury in sediment decreased when the redox potential was below -100mV. Conversion of sulfate to sulfide occurs in the range of 0 to -100 mV in typical seawaters (Drever, 1997). Therefore, the high sulfate concentration in seawater might inhibit mercury methylation at a low redox potential.

Organic content rich sediment with low redox potential enhances the sulfate reduction and mercury methylation rate (King et al., 2000; Lambertsson & Nilsson, 2006). Lambertsson & Nilsson (2006) recently stated that organic material was the primary control of mercury methylation and ambient methyl mercury concentration in estuarine sediment. However, the effect of organic matter on mercury methylation is complicated. It is believed that organic matter enhances the production of methyl mercury through stimulating microbial activity (Callister & Winfrey, 1986; Choi et al., 1994). But some studies observed an inverse relationship between organic matter content and methylation rate due to the complexation of organic matter with Hg2+, which reduces the bioavailability of mercury for SRB (Miskimmin et al., 1992; Barkay et al, 1997).

Knowing that abundant supply of decomposable organic matter would create a reducing environment that favors mercury methylation, we investigated the status of total and methyl mercury in the bottom sediment in the hypoxic zone of the northern Gulf of Mexico. This area experiences seasonal oxygen depletion in the water column due to the input of freshwater and nutrients from Mississippi and Atchafalaya rivers (Rabalais, Turner, & Wiseman, 2002) and its condition is susceptible to mercury methylation. We also carried out a laboratory study to determine the effect of organic matter on mercury methylation under

aerobic and anaerobic conditions. The possible role of the hypoxic zone in increasing mercury methyation was evaluated.

CHAPTER 2 LITERATURE REVIEW

2.1 Effects of Mercury on Human Health and Ecosystem

According to the survey conducted by CDC's National Health and Nutrition Examination Survey in 1999-2002, the blood levels in young children and women of childbearing-aged were usually below the reference dose of 5.8ppb. The geometric mean concentrations of total blood mercury found in all childbearing-aged women (16-49 years) and children (1-5 years) were 0.92 ppb and 0.33 ppb, respectively (CDC, 2004). However, approximately 6% of childbearing-aged women had blood mercury levels at or above 5.8ppb, which posed an increase risk of neurological disorder in more than 300,000 newborns in U.S per year (Environmental Protection Agency). Consuming fish and shellfish containing mercury is the dominant route of human exposure to methyl mercury (Environmental Protection Agency, 2001). Nearly 90-100% of mercury found in fish tissue is methyl mercury (Environmental Protection Agency, 2001). Methyl mercury present in the body is mainly water-soluble complexes or attached to the sulfur atom of thiol ligands (Clarkson, 2002). It is found with highest concentration in kidney and has a half life of 40 to more than 80 days in human body (Environmental Protection Agency, 2001.). It is excreted from the adult body through fecal and urine excretion and breast milk (Environmental Protection Agency, 2001). The major toxic effect of methyl mercury is on the central nervous system (Clarkson, 2002). The adverse effect depends on the dose and period of exposures. The consequences of acute and high dose exposure include impaired central nervous system function, kidney damage and failure, gastrointestinal damage, cardiovascular collapse, shock and death (Environmental Protection Agency, 2002). On the contrary, low doses can cause numbing sensation, which would lead to cerebellar ataxia, dysarthria, constriction of the visual fields and loss of hearing

(Clarkson, 2002). Recent finding further suggested that methyl mercury may increase the risk of myocardial infarction (Harris et al., 2003).

Previous tragedies that occurred in 1953 in Minamata's Bay in Japan and in 1971 in Iraq showed that consumption of methyl mercury containing fish and mercury-treated wheat, respectively, caused permanent neurological damage to a fetus (EPA, 2001). These incidents showed the susceptibility of methyl mercury to developing brain through prenatal exposure. It was found that the fetus suffered severe brain damage when they were born although their mothers only showed mild symptoms of poisoning. Therefore, methyl mercury is considered as the most potent form of mercury because it is capable of passing through the blood brain barrier and placenta which caused neurological damage in unborn fetuses (Environmental Protection Agency, 2001)

Besides the inhalation of mercury vapor from atmosphere, which is relatively low in concentration and is negligible, dental amalgam is the main source of human exposure to mercury vapor with the exception of certain occupational exposure (Clarkson, 2002). Amalgam filling was demonstrated to release mercury vapor into the oral cavity and later was carried into the lung through mouth breathing and absorbed and distributed to tissue (Clarkson, 2002). However, the inorganic mercury concentration produced by the mercury vapor released from the dental amalgam is well below those associated with obvious toxic effects or even with subtler neurobehavioral and renal effects (Clarkson, 2002). Inhalation of high concentration of mercury vapor causes acute, corrosive bronchitis and interstitial pneumonitis, tremors or increase excitability and death (Klassen, 2001). The symptoms in workers who were exposed chronically to mercury vapor and mercury nitrate in the fur, hat and felt industries were observed with increased excitability, tremors, gingivitis and erethism (memory loss, emotional lability, depression, insomnia, and shyness) (Goldwater, 1972).

Other than adversely impacted human health, some wildlife especially those fish-eating species are in high risk to methyl mercury exposure. Eagles, otters and endangered Florida panthers are found to contain methyl mercury in their bodies (Environmental Protection Agency). Methyl mercury could cause mortality, reduce fertility, retard growth and disrupt development and behaviors that affect survival of wildlife (Environmental Protection Agency). The impairment of reproductive system was observed in fish and birds exposed to methyl mercury (Drevnick & Sandheinrich, 2003; Heinz & Hoffman, 1998). Aquatic invertebrates such as Daphnia magna are also very sensitive to mercury especially in the larvae stage (Boening, 2000). The no-observed-effect-level for reproductive impairment of daphnia magna is 3μg/l for inorganic mercury and <0.04μg/l for methyl mercury. In a laboratory study, the aquatic plant, floating water cabbage, exposed to 20mg/l of mercuric chloride for two days showed reduced chlorophyll content, protein, RNA, dry weight, catalase and protease activity and increased production of free amino acid (De, 1985). Other aquatic plants such as Hydrilla verticillata presl, Pistia stratiotes L. and Salvinia molesta were observed with foliar injury and low chlorophyll content and phytomass when exposed to mercury with increasing concentrations (Mhatre & Chaphekar, 1985).

2.2 Sources of Mercury in the Environment

Mercury (Hg) exists naturally in the environment at low concentrations. It can be categorized as elemental mercury, inorganic mercury and organic mercury. Elemental mercury is a white-silver liquid at room temperature with melting temperature of -38.9^{o}C, and it easily vaporizes in the air. Inorganic mercury is formed when mercury binds with sulfide, chloride, or oxide. For example, cinnabar, a mercuric sulfide ore is found naturally in the earth crust. Organic mercury is formed when mercury binds with carbon. Three major forms of organic mercury compounds are phenyl mercury, methoxy mercury and alkyl mercury with alky mercury being the most dangerous one especially methyl mercury (Laws,

2003). Due to its multipurpose usages, the mining of cinnabar was peaked at 10^4 tonnes per year around 1970 in the world (Laws, 2003). Mercury had been widely used in chlor-alkali industry to produce Cl_2 and NaOH, in dentistry to make amalgam for dental filling, in electrical apparatus such as fluorescent, neon and highway light, arc rectifier, power control switch, and oscillator, in measuring and controlling devices such as thermometer, manometer, barometer, pumps and gauges, in pharmaceutical drug to treat syphilis and diuretic, in agricultural to control mold and turf grass disease, and in extraction of Au and Ag (Laws, 2003). Realizing the health and ecological effects from the mercury pollution, mercury production was reduced to about 2000 tonnes per year during early 1990s and stabilized at about 3000 tonnes per year (Laws, 2003).

About 4400 metric tons and 7500 metric tons of mercury are emitted into the atmosphere annually via natural and anthropogenic sources, respectively (United Nations Environmental Programme, 2002). Mercury is naturally released into the atmosphere through processes like evasions from the mineralized soil (O. Lindqvist et al., 1991), volcanic eruptions, geothermal activities (Varekamp, 1986), and bedrock fracture of the earth's crust (Rasmussen, 1994). Besides, volatilization of supersaturated aqueous Hg (0) and photoreduction of inorganic mercury during summer from the surface water also release some amount of mercury into the atmosphere (Amyot et al., 1997; Amyot et al., 1997; Schroeder et al., 1992). Anthropogenic sources of mercury in the atmosphere come from combustion of coal-fired power plants, smelters and solid waste incinerators. Mercury that is deposited onto the earth and oceans may not come solely from local and regional fallout but also from long range transportation fallout. Since mercury has a long residence time, about1.1-1.4 years in the atmosphere, it is capable of traveling a long distance and develops the global cycling of mercury (Slemr & Scheel, 1998). For example, it has been found that lake sediment in the

remote area received a significant amount of mercury through long range transportation (Fitzgerald et al., 1998).

Mercury enters the aquatic system through either direct discharges of fungicides, slimicides, and catalysts from chlor-alkali plant into the stream in the past (Craig, 1986) or through indirect pathways such as atmospheric fallout and terrestrial runoff. About 40% of the oxidized form of Hg (0) in the atmosphere is deposited into the ocean and 60% into the land (Mason et al., 1994). Oceans and lakes receive inorganic mercury mainly through precipitation (Mason et al., 1994), but drainage lakes receive mercury mainly from terrestrial runoff (Lee & Hultberg, 1990). Other than the external inputs of mercury, internal cycling of mercury through methylation/demethylation process also plays an important role in producing various species of mercury in the aquatic environment (Rudd, 1995).

2.3 Mercury Cycling and Chemical Species in the Aquatic Environment

When mercury enters into the natural environment, it transforms into many different species through chemical or biological processes and moves around in water, air, and sediment compartments forming a complex cycle. In the atmosphere, 95% of the total mercury is as Hg (0) and it is slowly oxidized to form Hg (II) (Morel et al., 1998) and later, about 40% of the dissolved Hg (II) would precipitate into the water column (Mason et al., 1994). In the water column, mercury forms various species according to the redox and pH conditions as well as the concentration of complexing agents (Ullrich et al., 2001). The solubility, mobility, toxicity and potential for methylation of mercury in the aquatic ecosystem are determined by the species and reaction of this species in the water column (Ullrich et al., 2001).

The partition of mercury is affected by the environment parameters such as pH, temperature, redox changes, availability of nutrients and complexing agents (Ullrich et al., 2001). Distribution of dissolved, colloidal and particulate – mercury varies widely spatially,

seasonally and with depth in the water column (Morel et al., 1998). Particulate mercury species consist of inorganic particle, particulate organic matter and biogenic particles such as bacteria, algae, and phytoplankton (Ullrich et al., 2001), which are responsible for vertical transport of mercury into the low oxygen water column in ocean (Mason & Fitzgerald, 1993) and for delivery of mercury between sediment-water interface (Hurley et al., 1991). Inorganic mercury has a propensity to bind with mineral particle and detrital organic matter, whereas methyl mercury tends to bind with biogenic particles (Hurley et al., 1994; Meili, 1997). Fe and Mn oxides influence the concentration and distribution between the dissolved and particulate species through precipitation and dissolution processes under proper redox condition and oxygen content in water and sediment (Fagerstr & Jernelov, 1972; Gagnon et al., 1997). Dissolved mercury species that are mainly distributed in water column are: Hg (0) that accounts for 10-30% in ocean and freshwater (Mason & Fitzgerald, 1993; Vandal et al., 1991), complexes of Hg (II) with inorganic and organic ligands, and organic mercury (methyl mercury and dimethyl mercury) (Ullrich et al., 2001). During summer, Hg (0) is supersaturated with the surface water and volatilized into the atmosphere (Fitzgerald et al., 1994). Production of Hg (0) in the surface water is driven by the reduction of Hg (II) via photochemical reaction in unpolluted water and via microbial merA reductase in polluted water (Morel et al., 1998). Oxidation of Hg (0) to Hg (II) occurs mainly in ocean or coastal water with high chloride concentrations and particulate matter loadings (Amyot, Gill et al., 1997). The oxidation/reduction reaction near the air-water interface controls the volatilization/precipitation of mercury between the atmosphere and surface water (Morel et al., 1998).

Hg (II) forms complexes with hydroxide and chloride ions in oxic water and with sulfide in anoxic water (Morel et al., 1998) . The dominant species found in freshwater without the presence of sulfide are $Hg(OH)_2$, HgOHCl, and HgCl (Stumm, 1996). However,

they tend to bind with dissolved organics and humics in the natural freshwater environment, which account for more than 95% in lakes (Hudson, 1994; Meili, 1997). The dominant species found in sea water include $HgCl^{+}$, $HgCl_2$, $HgCl_3^{-}$ and $HgCl_4^{2-}$, which are strongly associated with chloride ion concentration (Stumm, 1996). However, in high sulfide marine and pore waters, mercury forms soluble bi- and polysulfide- complexes like $HgSH^{+}$, $Hg(SH)_2$, $Hg(SH)^{S-}$, HgS_2^{2-} , $Hg(S_x)_2^{2-}$ under proper pH and redox conditions and S^0/S^{2-} concentrations (Gardner, 1974; Jay et al., 2000).

Organomercurial (monomethyl or dimethyl) mercury also forms various species in water as inorganic mercury complexes. They are CH_3HgOH (freshwater), CH_3HgCl (sea water) and CH_3HgS^{2-} (Ullrich et al., 2001). The main organomercurial species that exists in the aquatic environment is methyl mercury which is believed to be produced mainly in anoxic water and sediment through the methylation of Hg (II) (Morel et al., 1998). Methyl mercury found in the surface lake water is transported by diffusion and advection from the anoxic water column (Morel et al., 1998). Dimethyl mercury is normally undetectable in freshwater and estuarine systems but is the dominant species in the deep ocean (Mason & Fitzgerald, 1990; Ullrich et al., 2001). It is thought that methyl mercury found in the deep ocean is produced through the decomposition of dimethyl mercury (Mason et al., 1998). Demethylation of mercury observed at the low mercury concentration and oxic water is mainly through photodegradation (Morel et al., 1998). Some studies demonstrated the bacterial degradation of methyl mercury under both aerobic and anaerobic water and sediment (Oremland et al., 1991; Matilainen & Verta, 1995).

Mercury is brought to the sediment through the settling of particulate matter. Particulate mercury species settled to sediments are thought to be solid HgS, and organic matter bound and inorganic particles bound mercury (Morel et al., 1998). At low pH, mercury tends to adsorb to humus in sediment, whereas, in neutral and alkaline sediment, mercury

prefers to adsorb to mineral particles like Fe oxides and clay minerals (Bringmark, 1997). Methyl mercury found in sediment is produced through methylation of inorganic mercury by chemical and biological reaction. But, an equilibrium state of methyl mercury was observed in the combination effect of production and degradation of methyl mercury, therefore, the actual concentration of methyl mercury is a net result of methylation and demethylation processes (Pak & Bartha, 1998).

2.4 Concentration of Mercury in the Aquatic Environment

The global baseline values for total and methyl mercury were determined from the surface water of Antarctic lakes and glacial streams, which ranged from 0.4-1.9 ng/l and from <0.08 to 0.4 ng/l, respectively (Vandal et al., 1998). Total mercury concentration in contaminated water was in the order of μg/l, compared to the uncontaminated freshwaters, which was < 5 ng/l (Craig, 1986). Meili (1997) found that total mercury in the humic or mercury particulate-rich river was much higher than that in the normal freshwater system, which was up to 10 or 20 ng/l. Total mercury concentration in marine water ranged from 0.1-0.8 ng/l in the Mediterranean and North Atlantic (Cossa et al., 1997).

Total mercury in uncontaminated ocean sediments ranged from 0.02 to 0.1 μg/g (Lindqvist et al., 1984) and Craig (1986) reported unpolluted sediments in the range of 0.2-0.4 μg/g. Methyl mercury in the freshwater sediment is about 1-1.5% of total mercury and < 0.5% in the marine and estuarine sediment (Olson and Cooper, 1974). On the other hand, total mercury was observed to be 908 μg/g (d.w) in the contaminated mud of Minimata Bay (Fujiki & Tajima, 1992).

The uncontaminated freshwater fish, marine fish and marine shellfish contained mercury levels ranging from 0.2-1 μg/g, 0.01-1.5 μg/g, and 0.14-0.75 μg/g, respectively (Craig, 1986). The maximum permissible concentrations set by WHO, USFDA and USEPA were 0.5, 1 and 0.3 μg Hg/g edible tissue, respectively. About 85-90% of the mercury that

found in the fish tissue is methyl mercury (Bloom, 1992) due to its high stability, lipid solubility and tendency to bind with –SH protein group (Ullrich et al., 2001). Fish accumulate methyl mercury in their tissues through the food chain or direct intake from water. Therefore, higher trophic level organisms tend to bioconcentrate more mercury, in the order of 10^4-10^7 compare to the mercury found in the water column (World Health Organization, 1989).

2.5 Methylation and Demethylation of Mercury in the Aquatic Environment

Methylation of mercury occurs through chemical (abiotic) and biochemical (biotic) reactions. The purely chemical reactions involve transmethylation and photochemical processes (Celo et al., 2006; Hamasaki et al., 1995). The biochemical reactions involve enzymatic and nonenzymatic metabolic methylation of microorganism (Wood, 1968; Choi & Bartha, 1993). Biomethylation is known to be the dominant pathway for methyl mercury synthesis in the anoxic saltmarsh sediment, which is an order of magnitude higher compared to the abiotic methylation (Berman & Bartha, 1986). Conversion of inorganic mercury, Hg (II), to methyl mercury is actively involved in the surface sediment compared to the water column (Olson & Cooper, 1976). A maximum methylation rate was observed at the reducing boundary between the sediment-water interfaces that varies seasonally (Korthals & Winfrey, 1987). The concentration of methyl mercury decreased with increasing sediment depth (Korthals & Winfrey, 1987). Other environmental factors that influence the methylation process will be discussed in Section 2.6. Ecosystems that are vulnerable to methylation consist of wetland, peatland, tropical flood plain region, acidified lake, and reservoir (Stlouis et al., 1994; St Louis et al., 2004; Hurley et al., 1995; Guimaraes et al., 2000; Xun et al., 1987; Kelly et al., 1997).

Demethylation of methyl mercury occurs through biological and abiological pathways. Reductive and oxidative processes are two types of bacterial mediated pathway known to degrade methyl mercury. One reductive process involves mer-operon in bacteria

where merB lyase hydrolyzes organomercury to form methane and Hg (II) ion and followed by merA reductase that reduced Hg(II) ion to Hg(0) (Schroeder et al., 1992). The other reductive process occurs in sulfate reducing bacteria, *Desulfovibrio desulfuricans*, where methyl mercury reactes with sulfide to form unstable dimethylmercury sulfide and then further is decomposed to methane and ionic mercury (Baldi et al., 1995). Oxidative pathway of demethylation is not well known yet, but methonogens were showed to degrade methyl mercury in the low methyl mercury environment (Marvin-DiPasquale et al., 2000). Oremland et al. (1991) found the production of CO_2 from demethylation of methyl mercury in the aerobic and anaerobic freshwater sediment and anaerobic estuarine sediment. The abiological pathways include photodegradation and reaction between methyl mercury with sulfide to form volatile dimethyl mercury and HgS (Craig and Moreton, 1983). Photodegradation of methyl mercury is the main mechanism of methyl mercury loss from the oxic lake and sea waters (Sellers et al., 1996).

2.5.1 Abiotic Methylation

Methyl mercury can be formed in the aquatic environment through transmethylation process in the presence of suitable methyl donor. Several organometallic compounds including dissolved methylsilicon, trimethyllead, methylcobalt(III) compounds, methyltin and methyl iodide were found to react with Hg(II) or Hg (0) to form methylmercury under proper conditions (Beijer, 1979; Celo et al., 2006; Desimone, 1972). Environmental factors such as pH, temperature, and chloride concentration influence the rate and yield of methyl mercury. Methylcobalt(III) compounds were found to react rapidly with free Hg(II) in the laboratory at pH ~1.5 and 21.1 ^{o}C , which is thought to be a potential methylator in freshwater (Celo et al., 2006). In seawater, methyltin is considered to be a potential methylator because of the high concentration of monomethyltin (~1200ng/l) and constant methylation rate at pH 8 and 20 ^{o}C (Celo et al., 2006; Hamasaki et al., 1995). The formation of methylmercury by the reaction of

Hg (0) with methyltin is still ambiguous. Although Hall et al. (1995) reported no significant amount of methyl mercury formed when methyltin reacted with aqueous Hg (0), Celo (2003) found out that the yield of methyl mercury based on Hg (0) was similar to that from the methylation by SRB in the laboratory, which is about 1.1%. Other chemical reactions that produce methyl mercury were reported by Hamasaki et al., 1995. They observed the methylation of mercury chloride with methanol, ethanol, acetic acid, and propionic acid through photochemical reactions. Organic matters such as humic acid were also shown to be methylating agents in the environment (Weber, 1993).

2.5.2 Biomethylation

Mercury methylation by microorganisms (biomethylation) was first discovered by Jensen & Jernelov (1969) in the sediment from aquaria and lakes as well as in the coastal water in Sweden. Baldi et al., (1993) found at least 16 genera of aerobic and anaerobic microorganisms that were capable of methylating Hg (II) after they isolated the bacteria from a river that collected cinnabar mine waters. Methanogenic bacteria were first assumed to be the major mercury methylator due to their capability to donate methyl group nonenzymatically from methylcobalamin to mercuric ion (Wood, 1968). However, several researchers later showed that sulfate-reducing bacteria (SRB) were the principal methylator in the anoxic estuarine (Compeau & Bartha, 1985) and freshwater sediments (Gilmour et al., 1992). The formation of methyl mercury in the aquatic environment is thought to be governed by the activity, distribution and physiology of SRB (King et al., 2001; Macalady et al., 2000) as well as the availability of mercury. Concentrations of sulfate and sulfide, organic matter, redox potential, temperature, pH, and salinity would influence the rate of SRB methylation and the bioavailability of inorganic mercury (Ullrich et al., 2001).

The relationship between SRB and mercury methylation was studied using a specific inhibitor of sulfate reduction, molybdate, and the result indicated no methyl mercury

production in the presence of molybdate (Compeau & Bartha, 1985). Through a study done on *D. desulfuricans* LS, the acetyl-CoA pathway is commonly accepted as the major metabolic pathway that SRB use to methylate mercury (Choi et al., 1994). King et al. (2000) showed that SRB methylated mercury in pure cultures as the following order: *Desulfobacterium* sp. strain BG-33 >> *Desulfobacter* sp strain BG-8 ~*Desulfococcus multivorans* >> *Desulfovibrio Desulfuricans* ~ *Desulfobulbus propionicus*. They indicated that the acetate-utilizing SRB is able to methylate mercury at higher rates than other mercury-methylating SRB groups. No much information is known about how other SRB strains methylate mercury or what other enzymatic pathways are involved in methylating mercury. While some SRB that methylate mercury are known to utilize the acetyl-CoA pathway, there are mercury-methylating-SRB, such as *D. africanus*, *D.propionicus* 1pr3, and *D.propionicus* MUD, which do not use this pathway (Ekstrom et al., 2003). Thus, Ekstrom et al. concluded that the mercury methylation mechanism is independent of the acetyl-coA pathway and B_{12} – containing methionine synthase.

Recently, another type of bacteria was first reported to be capable of methylating mercury as effectively as SRB. Fleming et al., (2006) showed that an iron-reducing bacterium, Geobacter sp strain CLFeRB, was able to produce a significant amount of methylmercury in the freshwater sediment in Clear Lake, CA.

2.6 Factors Affecting Methylation in the Aquatic Environment

2.6.1 Effects of Sulfate and Sulfide Concentration on Mercury Methylation

The sulfate reduction rate is limited in the freshwater sediment at sulfate concentrations less than0.1mM (Ingvorsen et al., 1981). On the other hand, when sulfate is plentiful, the product of sulfate respiration, H_2S, would interfere with the methylation of Hg2+ through precipitation of HgS (Compeau & Bartha, 1985). This could explain the lower methyl mercury formation in ocean because of the high sulfate concentration in sea salts

(Compeau & Bartha, 1983). Gilmour et al. (1992) suggested that there was an optimum sulfate level in the sediment (200 – 500 μM sulfate) for SRB to methylate mercury, below which the mercury methylation was below maximum. In the freshwater lake with a low concentration of sulfate, an addition of 200μM sulfate was found to stimulate the production of methyl mercury (Gilmour et al., 1992). The addition of 1mM sulfate to the freshwater sediment was found to depress methylation rate because of the abundant dissolved sulfide (Winfrey & Rudd, 1990). However, mercury methylation was still observed in the presence of 30mM sulfate with a milimolar range concentration of dissolved sulfide in pure cultures and marine sediments (King et al., 1999).

Sulfide inhibits mercury methylation by controlling the bioavailability of mercury. High sulfide usually builds up in the anaerobic sediment with high organic matter and sea salt as well as in the discharges of industrial and domestic wastewaters (Compeau & Bartha, 1984). It is formed during the respiration of sulfate reducing bacteria. Increased sulfide concentration was observed to decrease the production of methylmercury. Methylation was inhibited by dissolved sulfide greater than 10 μM due to the precipitation of mercury sulfide minerals (Gilmour et al., 1998). Berman & Bartha (1986) also observed reduced methylation in the freshwater sediment with 1.98mg/g free sulfide and the methylation was recovered after the sulfide was being diluted in the sediment. Twenty percent decrease in methylation was demonstrated when 5μM of sulfide was added in pore water relative to the control sulfide concentration of 1μM (Lambertsson & Nilsson, 2006). Other than decreasing the bioavailability of Hg2+ by precipitating it as HgS, high sulfide concentration could lead to the reaction of H_2S with methyl mercury to form volatile dimethylmercury (Baldi et al., 1993; Craig and Moreton, 1983), which resulted in lower methyl mercury concentration.

Instead of Hg2+ or dissolved total mercury, Benoit et al. (1999) hypothesized that neutral dissolved mercury complexes controlled the bioavailability of mercury in the

sediment. This is because mercury was more readily diffused across the cell membrane of microorganism passively (Benoit et al., 1999). HgS(0) was suggested to be the dominant complex in the sulfidic sediment. Thus, the concentration of this complex affected the mercury methylation rate (Benoit et al., 1999). When the concentration of sulfide was increased, the neutral sulfide complex species may shift to a more polar (negatively charged) sulfide complex, which would hinder the bacterial uptake (Benoit et al., 2001). Therefore, sulfide influences methylation by affecting the speciation of dissolved inorganic mercury (Benoit et al., 2001) instead of precipitating it as HgS as discussed before. In addition, the presence of polysulfide in the sulfuric waters was showed to reduce the methylation rate in the cultures of *Desulfovibrio desulfuricans* ND132 due to the speciation shift of HgS(0) to charged complexes (Jay et al., 2002).

2.6.2 Effect of Organic Material on Mercury Methylation

Organic matter affects the level of methyl mercury through influencing the microbial activity and controlling the partition of Hg between solid and dissolved phase by serving as complexing agents for Hg2+ and methyl mercury (Lambertsson & Nilsson, 2006). Organic rich sediment with low redox potential enhanced the sulfate reduction and mercury methylation rate, and thus, increased the accumulation of methyl mercury in the sediment (King et al., 2000; Lambertsson & Nilsson, 2006). Several studies exhibited a positive effect of organic content in the fresh and marine sediment on mercury methylation (Callister & Winfrey, 1986; Choi et al., 1994; Lambertsson & Nilsson, 2006). Miskimmin (1991) found that the concentration of $CH_3{}^{203}Hg^+$ in the water overlying sediments increased with increasing dissolved organic carbon concentration (550, 2800, and 3640 μmol/L) after a 24 hr's incubation at 22^{o}C; meanwhile, an inverse relationship was observed between the partition coefficient, Kd for $^{14}CH_3Hg^+$ in sediment-water solutions, and the dissolved organic carbon concentration. Lambertsson & Nilsson (2006) showed that the ambient methyl

mercury concentration exponentially increased with the increasing organic matter content (%LOI) (R^2=0.64). This indicated the importance of the supply of organic matter to the SRB community in governing the methylation rate and methyl mercury concentration. They also indicated that spatial and seasonal variations in methylation process and methyl mercury concentration can be determined through the distribution of organic matter in sediment and the seasonal production of fresh autochthonous carbon, respectively. Jackson (1993) reported a greater microbial methylating activity due to the plentiful production of organic nutrient substrates resulting from the phytoplankton bloom and higher proportion of organic matter to mineral detritus. Jackson (1993) suggested that the high methyl mercury concentration found in the flooded forest sediments was because of the labile organic matter. However, Kainz et al. (2003) found that the amount of terrestrial organic matter compounds could not predict methyl mercury concentration and that a complex microbial dynamics involved in the methylation/demethylation process in sediment cannot be predicted solely by the fatty acid biomarkers in *D. desulfuricans* or biomarker in organic matter.

As mentioned above, organic matter in sediment commonly generates higher methyl mercury concentration. However, dissolved organic matter in water has been shown to render the methylation activity. High dissolved organic carbon (DOC) reduced the availability of inorganic mercury for methylating bacteria through the binding of free Hg2+ to DOC (Barkay et al., 1997; Miskimmin et al., 1992). Miskimmin et al. (1992) demonstrated the decreased methylation rate and increased demethylation rate when the DOC concentration was raised from 500 to 2600 μmol C/L. However, the net methylation rate decreased with increasing DOC content. At low pH, DOC was less severely complexed with free Hg2+ because of the competition of H+ with Hg2+ for negatively charged sites in DOC (Barkay et al., 1997; Miskimmin et al., 1992).

2.6.3 Effect of Redox Potential on Mercury Methylation

Bisogni & Lawrence (1975) previously demonstrated that the production rate of methyl mercury in the simulated aerobic sewage treatment reactor was 2 times more rapid than those under anaerobic condition. However, today it is established that methyl mercury mainly occurrs under anaerobic condition than aerobic condition (Compeau & Bartha, 1984; Olson & Cooper, 1976). Olson & Cooper (1976) observed higher methyl mercury concentrations and methylation rate in sediment under anaerobic condition compared to aerobic condition. In a controlled redox potential study, Compeau & Bartha (1984) showed that methylation of Hg2+ and formation of methyl mercury in estuarine sediment was favored at -200mV than at +110mV. Callister & Winfrey (1986) found the higher rate of methylation in the anaerobically incubated sediment than the aerobically incubated sediment. Gagnon et al. (1996) also detected the higher concentration of methyl mercury in pore water extracted from anoxic sediment than that from oxic sediment. They further suggested that oxic sediment served as an efficient barrier to methyl mercury diffusing freely through the sediment-water interface to the overlying waters.

On the contrary, demethylation of mercury or degradation of methyl mercury occurred more rapidly under aerobic condition (Compeau & Bartha, 1984; Olson & Cooper, 1976). Compeau & Bartha (1984) observed that the Hg2+ methylation was strongly repressed at +110 mV in low saline sediment.

2.6.4 Effect of Temperature on Mercury Methylation

Increased temperature stimulates the growth of SRB (Fukui & Takii, 1989), thus possibly affecting the production of methyl mercury. Several studies have shown greater methylation activities during mid or late summer months (Callister & Winfrey, 1986; Korthals & Winfrey, 1987). Callister & Winfrey (1986) observed an optimum temperature of 35^{o}C for mercury methylation. However, temperature is not the only factor that controls the

seasonal peak in mercury methylation. Other variables such as demethylation activity, nutrient loading, and aerobic conditions also contribute to the methylation rate (Callister & Winfrey, 1986). Although temperature is directly related to the methyl mercury in surface sediment, it only accounts for 30% variation (Callister & Winfrey, 1986; Korthals & Winfrey, 1987). It is suggested that the increased net production of methyl mercury may be caused by the low demethylation activity instead of the actual methylation and the effects of temperature and anoxic conditions. A reduction of 50-70% in methyl mercury concentration was observed in sediments at $4^{o}C$ compared to $20^{o}C$, suggesting the lower net production of methyl mercury in winter (Wright & Hamilton, 1982). Recently Lambertsson & Nilsson (2006) suggested that the temporal addition of fresh organic matter to the sediment surfaces is governed by the seasonality of primary production. The seasonal variations in methyl mercury concentration may be due to the differences in the supply of fresh organic matter.

2.6.5 Effect of pH on Mercury Methylation

The effect of pH on methylation and demethylation is important in understanding the tendency of bioaccumulation in fish in aquatic system (Miskimmin et al., 1992). Acidic lakes are known to associate with the bioaccumulation of methyl mercury in fish (Gilmour et al., 1998; Winfrey & Rudd, 1990). However, the reason why fish tend to bioaccumulate higher methyl mercury concentration in lakes with lower pH values is not well known. It was suggested that the high mercury methylation rate in acidic lake water or surface sediment might be responsible for that (Bloom, 1992; Winfrey & Rudd, 1990; Xun et al., 1987). However, Pak & Bartha (1998) showed that the bioaccumulation of mercury in fish at low pH was not directly caused by the increased methylation activity or decreased demethylation activity in the sediment. Recently, Kelly et al. (2003) demonstrated that bioaccumulation was positively correlated with the concentration of H^{+}. They observed that the uptake of

both charged and uncharged Hg (II) species by bacteria was enhanced in the presence of high concentrations of H^+ (Kelly et al., 2003).

The effect of pH on mercury methylation in sediment is contradictive. Some studies showed that decreased pH would inhibit the methylation process but others showed that decreased pH would enhance the formation of methyl mercury. (Pak & Bartha, 1998) found out neither methylation nor demethylation was affected by pH between 5.5 and 7 in anoxic lake sediments. However, Fagerstr & Jernelov, (1972) observed an optimum methyl mercury production at pH 7 and 5 and reduced with increasing pH in sediment. Xun et al. (1987) also showed increased methylation rate in surface sediment at low pH. On the contrary, Ramlal et al. (1985) observed the lower synthesis of methyl mercury in sediment when decreasing pH from 7 to 4.5. The same effect was observed by Steffan et al. (1988). They found that more than 65% of methylation was inhibited when the sediment was acidified to pH 4.5. The formation of insoluble HgS by acid-mobilized H_2S or increased availability of mercury binding sites on sediment particles decreased the availability of soluble inorganic mercury for methylation in pore water (Ramlal et al., 1985; Steffan et al., 1988). Another factor that caused lower methyl mercury production in sediment was the active demethylation at low pH (Ramlal et al., 1985; Steffan et al., 1988).

Miskimmin et al. (1992) showed that pH plays an important role in affecting the rates of net methyl mercury production in lake water. Increased methyl mercury was observed at low pH lake water due to the reduced binding of inorganic mercury to dissolved organic carbon, and therefore increased the availability of Hg (II) for methylation (Miskimmin et al., 1992; Xun et al., 1987). The level of methyl mercury was low in high pH lakes due to the volatilization of Hg (0) (Winfrey & Rudd, 1990) and the formation of volatile dimethylmercury (Craig, and Moreton, 1983).

2.6.6 Effect of Salinity on Mercury Methylation

Salinity has a negative effect on mercury methylation (Blum & Bartha, 1980; Compeau & Bartha, 1987). Thus, synthesis of methyl mercury is generally lower in estuarine and marine environments compared to freshwater systems (Barkay et al., 1997). At high salinity condition, reduced mercury methylation was observed because the production of H_2S limits the availability of Hg^{2+} by forming insoluble HgS (Compeau & Bartha, 1987). For example, sulfide concentrations of 5.9 and 7.1 mg/g dry sediment decreased the production of methyl mercury significantly (Compeau & Bartha, 1987). However, methylation still took place at a salinity of 2.4% with 7.1mg sulfide/g dry sediment (Compeau & Bartha, 1987). High salinity and aerobic conditions appear to cause demethylation of methyl mercury and are less favorable for monomethyl mercury synthesis (Compeau & Bartha, 1984). The effect of Cl^- on methylation activity is unclear. It was suggested that in full strength seawater (0.5M Cl^-), mercury solubility was affected by the formation of negatively charged $HgCl_4^{2-}$complex (Compeau & Bartha, 1987). A study using a mer-lux assay showed that uncharged mercuric chloride more readily permeated the bacterial cytoplasm than did electrochemically charged form (Barkay et al., 1997). Besides sulfate, other anionic sea salts were shown to affect the speciation of mercury by controlling the transfer of methyl groups from methylcobalamin (Blum & Bartha, 1980). Formation of $HgCO_3$ from the bicarbonate under anaerobic and aerobic conditions was thought to reduce the production of methyl mercury (Compeau & Bartha, 1983).

2.7 Hypoxia in the Northern Gulf of Mexico

Based on previous laboratory and field observations, an oxygen deficient environment that poses stress to organisms ranges from 3.0 -0.2mg/l (Rabalais et al., 2002). Hypoxia in the northern Gulf of Mexico is specifically described as oxygen levels below 2mg/l, with about 20% oxygen saturation at 25^{o}C and salinity of 35 in summertime bottom water (Rabalais et

al., 2002). Renaud (1986) stated that demersal fish or shrimps are usually not captured using the dragging trawl when the oxygen concentration is below 2ppm.

Hypoxia occurs naturally and anthropogenically worldwide. A permanent hypoxic water caused by upwelling event has been found along the continental margins of the eastern Pacific, Indian and western Atlantic Oceans (Helly, 2004). Oxygen minimum zones have been also observed in the coastal area of Baltic Sea (Mee, 2001), northern Gulf of Mexico (Rabalais et al., 2002) and northwestern shelf of Black Sea (Rosenberg, 1985). The hypoxic zone of Baltic Sea rankes the world largest hypoxia region.

The inner- to mid continental shelf (depths of 5-60m) of the northern Gulf of Mexico forms the second largest coastal hypoxia zones in the world, especially during mid July and mid August (Rabalais et al., 2001). The average area of hypoxia in mid summer from 1985-2004 on the Louisiana/Texas continental shelf was 13000km^2 and peaked at 22,000km^2 in 2002 (Rabalais et al., 2002). From the sedimentary record, hypoxia was not a dominant feature of the continental shelf prior to 1900; it appeared that some level of hypoxia may have occurred during 1940-1950 and worsened after a tripled increased of nitrate flux from the Mississippi River into the gulf of Mexico beginning in 1960 (Rabalais et al., 2002). Generally, the distribution depth of hypoxic bottom waters ranges from 5-30m (Rabalais et al., 2001).The bottom waters near the sediment often become anoxic with the release of hydrogen sulfide from the sediment (Rabalais et al., 2001). According to Rabalais et al. (2001), the hypoxic water was not only detected near the bottom water but also it could account for 10-80% of the total water column.

Freshwater input and nutrient loads are two major factors that caused the oxygen depletion in the bottom water of the northern Gulf of Mexico (Rabalais et al.,. 2002). Approximately 580 km^3 (~420 billion gallons) of freshwater from Mississippi River is discharged into the Gulf of Mexico each year (Meade, 1995). Dunn (1996) reported the gulf

receives about 80% of the freshwater from Mississippi and Atchafalaya rivers, an estimate of 91% of nitrogen and 88% of phosphorus. The spring-summer hypoxic zone is formed and maintained through the salinity and temperature stratifications between surface and bottom water column. (Rabalais et al.,2002). Stratification forms when less dense seasonal-warm surface freshwater that discharges from river is resided above and separated from the denser and colder saltwater of gulf (Rabalais et al., 2002). The stratification goes through a well-defined seasonal cycle, which occurs most intensely during summer and weakest during winter. Processes that influence the stratification are` the strength and phasing of river discharge, wind mixing, regional circulation, and air-sea heat exchange (Rabalais et al., 2002). Increased nutrient loads stimulate the growth of phytoplankton and may result in increased commercial fishery productivity. However, when the phytoplankton is not consumed into the food web, it would sink together with the fecal material generated from the food web into the bottom water. As a result, decomposition of organic material by bacteria depletes the oxygen level in the bottom water when the respiration rate of bacteria happens more rapidly than it could be replaced by vertical diffusion of oxygen through the stratified water column (Rabalais et al., 2002). Eadie et al. (1994) stated that the organic matter found at the lower water column of the northern Gulf of Mexico was mostly derived from the phytoplankton that grow because of the riverine-delivered nutrients instead of the river-borne organic matter carried from Mississippi river.

The effect of hypoxia on the Louisiana continental shelf food web is not well known (Rabalais et al., 2002). Although no significant fishery losses from the northern Gulf of Mexico was observed, increase level of nutrient loading and aggravating hypoxia condition may lead to potential impact on the ecologically and commercially important species and alter the coastal ecosystem (Rabalais et al., 2002). The shift of benthos community from larger, longer-lived burrowing infauna to short-lived, smaller surface deposit-feeding

polychaetes, and absence of several taxa: pericaridean crustaceans, bivalves, gastropods, andophiuroids was observed in the low dissolved oxygen bottom water column by (Rabalais et al., 2002). As a result, the sediment structure and sediment iogeochemical cycles as well as the bottom-feeding fish and crustaceans trophic states were being altered (Rabalais et al., 2002).

CHAPTER 3 MATERIALS AND METHODS

3.1 Sampling Area

Northern Gulf of Mexico from the inner- to mid Louisiana continental shelf to the west of the Mississippi and Atchafalaya Rivers formed the second largest coastal hypoxia zones in the world, especially during mid July and mid August (Rabalais et al., 2001). The average area of hypoxia in mid summer from 1985-2004 on the Louisiana/Texas continental shelf was 13,000km^2 and was peaked on 2002 at 22,000km^2 (Rabalais, 2005). The cause of hypoxia is the freshwater and nutrient loadings from Mississippi and Atchafalaya rivers (Rabalais et al., 2002). The spring-summer hypoxic zone was formed and maintained through the salinity and temperature stratification between surface and bottom water column as well as the decomposition of organic matter that produced oxygen depletion in the bottom water (Rabalais et al., 2002).

Sediments were collected from three stations in the transect C of northern Gulf of Mexico (Figure 1) from mid-April 2005 to early-April 2006. The location and water depth of each station were as followed: C4 at 28^{o}57.019' N, 090^{o}31.783'W; ~ 13m, C6B at 28^{o}52.230' N, 090^{o}28.045'W; ~19m and C8 at 28^{o}47.312' N, 090^o 16.668'W; ~ 23m, respectively. According to the survey of Rabalais et al. (2001), transect C was affected by low dissolved oxygen concentration as early as late February through early October and quite persistent from mid-may through mid-September but rare in late fall and winter. The hypoxia area observed in mid-summer (July 24-29, 2005) was 11,840 km^2 and the affected water depth of 20-85 ft, which began from near Mississippi River and extended to the Louisiana/Texas border (Figure 2; Rabalais, LUMCON Press 2005).

3.2 Field Measurements and Sample Collections

Concentrations of bottom dissolved oxygen, temperature and salinity were measured in-situ in the bottom water column at each station using a conductivity/temperature/depth

(CTD/ Rosette System) with an attached dissolved oxygen sensor (SBE 13-01-2) and the sediments were collected using Wildco-Eckman standard dredge.

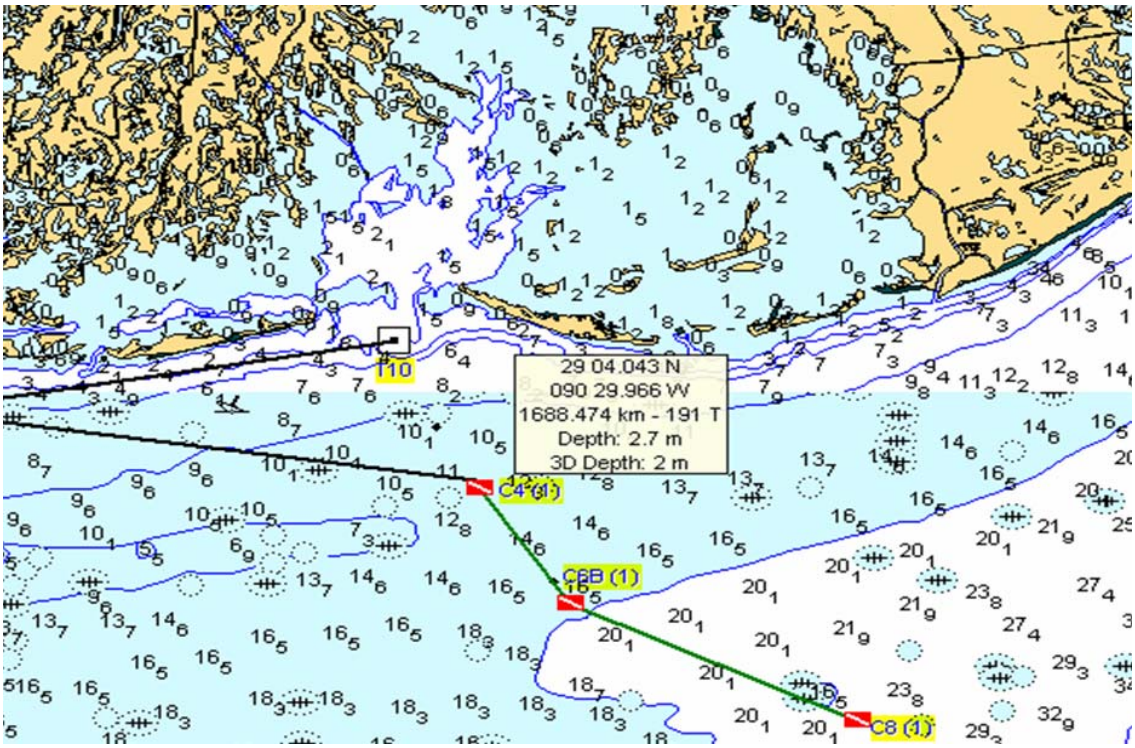

Figure 1. Sampling Location: C4, C6B, and C8 of northern Gulf of Mexico.

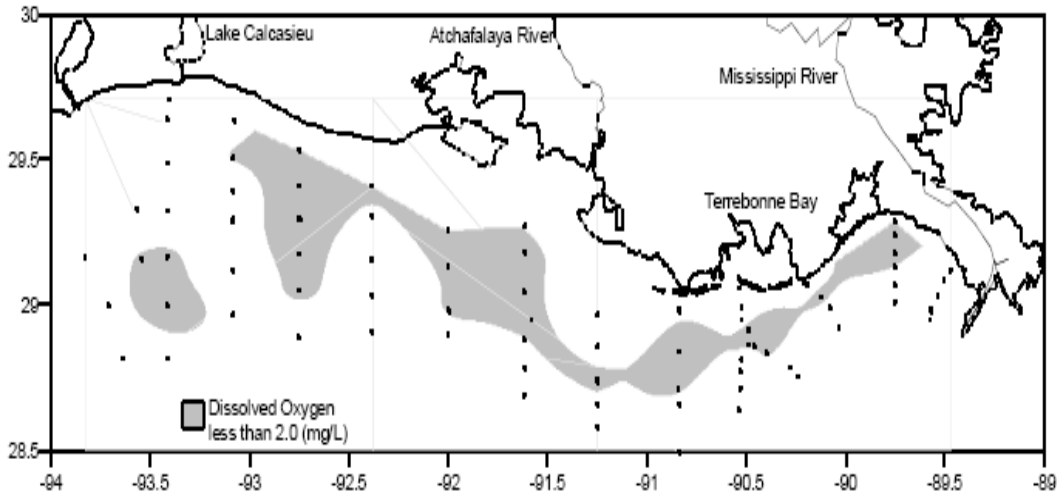

Figure 2 . Bottom hypoxia in July 24-29, 2005. Courtesy of Rabalais, 2005.

For mercury analysis, all the glass and plastic wares used for sampling and analysis were cleaned by soaking in a 2M nitric acid bath overnight and rinsed with deionized water thoroughly before use to ensure no trace heavy metal contamination. Surface sediment

samples were collected from the surface layer of 0-2cm using spoon into polyethylene cups. To investigate the depth profile of mercury concentration, core sediments were collected using a 2' x 6' PVC tubes with 11.5' rubber stoppers for April, May and July sampling trips. Core sediments were cut into 3 sections: 0-2 cm, 2-5 cm and 5-8cm into a polyethylene cup and mixed well before mercury extraction.

For microbial work, surface sediment and bottom water were collected using sterile polypropylene specimen containers and spoons. Difference spoons were used at difference stations to prevent sediment contamination. The samples were kept in a refrigerator at 4 ^{o}C in the ship immediately after each sampling. Then, samples were transported to the laboratory in a cooler and stored in a 4 ^{o}C refrigerator. Sediments and bottom waters for microbial study were stored in -20^{o}C after incubation of sulfate reducing bacteria. The sediment samples were analyzed for total and methylmercury concentrations, organic matter contents and most probable number (MPN) of sulfur reducing bacteria as well as soil texture.

3.3 Laboratory Study - The Effect of Organic Matter on Mercury Methylation

Surface sediment and bottom water used in the laboratory study were collected from station C6B on May 11th 2005 at 28^{o}52.230' N, 090^{o}28.045'W. The in-situ dissolved oxygen level was 5.32mg/l and water depth was~20m. The organic content for the upper 2cm surface sediment was 4.11% LOI. The procedure used to set up the incubation study was modified from Gilmour et al. (1992). Surface sediments collected from 3 box cores were composited and homogenized thoroughly before setting up the experiment. About 30 g of sediment was weighted into a platinum built-in tube (3cm diameter x 12cm long) and mixed with 2ml of algae solution with the following concentrations: 0.01%, 0.1% and 1%. The algae solution used was purchased from Reed Mariculture.Inc. Algae were used to simulate the phytoplankton that sink to the bottom water column and seabed in the gulf. When transferring the sediment, the tube was tapped slowly to avoid bubbles formed between the sediment's

gaps. Bottom waters was used to seal the surface sediment with the ratio of 1:2 and capped with 7' rubber stopper. The incubation was carried out for 3 weeks at 23 ^{o}C in the dark under nitrogen or air flushed. Redox potential was measured the next day after 24 hours of incubation. At the end of the experiment, redox potential and methyl mercury concentration were determined. Redox potential was measured using the Orion Model 420A bench top pH/mV/ORP/Temp Meter. Methyl mercury concentration was determined using method mentioned in section 3.5.

3.4 Total Mercury Extraction and Analysis

The method used for total mercury analysis was based on the EPA method 245.5 coupled with the LabAnalyzer 254 operating protocol. For total mercury (inorganic and organic) extraction, approximately 1.2 g of homogenized wet sediments was weighed into each BOD bottles. Then, 5 mL of HPLC grade water and aqua regia (Aqua regia = 3:1 volumes of concentrated HCl and HNO_3) were added and heated at 95 ^{o}C for 2 minutes in a water bath. Once the samples were cooled down to room temperature, 50 mL HPLC grade water and 15 mL of 5% potassium permanganate solution were added. The samples were mixed thoroughly followed by heating at 95^{o}C in a water bath for 30 minutes. The samples were cooled and 1mL of hydroxylamine hydrochloride and 55 mL of HPLC grade water were added to reduce the excess permanganate. All the mercury in the sediment will be oxidized into stable Hg (II) when potassium permanganate and concentrated acids were added.

Analysis of mercury was performed using the cold vapor method with LabAnalyzer 254. About 2-10 mL of samples was pipetted into the extractor followed by 0.5 mL of tin (II) chloride to reduce Hg (II) to Hg (0). The mercury containing sample was stripped with an adjustable 30 L/h air stream that was produced by a built-in membrane pump and was sucked into a fused silica optical cell. Mercury concentration was quantified by measuring the UV absorption of mercury atom at a wavelength of 253.7nm. The measuring range was 10ppt to

10ppb for 10 mL sample volume. The precision and accuracy of the analysis can be achieved from blanks, replicate samples and standard reference material.

3.5 Methyl Mercury Extraction and Analysis

The method used for methyl mercury analysis was based on Cai et al. (1996) and Alli et al. (1994). For methyl mercury extraction, about 4-4.5 g of homogenized wet sediments was weighted and the duplicate samples were spiked with methyl mercury chloride. 4 mL of acidic potassium bromide (1:2 volumes of $CuSO_4$ and KBr) and 5mL of HPLC grade water were added to convert CH_3HgCl to CH_3HgBr followed by an hour of shaking to facilitate the conversion process. 5.5 mL of dichloromethane (DCM) were pipetted into each vial and the samples were shaken overnight. The next day, the samples were centrifuged at 3000 rpm for 10 minutes to separate the aqueous, soil and organic solvent phases. The aqueous phases were removed and 1.3 mL of $Na_2S_2O_3$ solution was pipetted into the recovery DCM containing CH_3HgBr. Again, the samples were shaken for 45 minutes and centrifuged for 5 minutes. The top aqueous phase was recovered. CH_3HgBr was transferred back into DCM by adding acidic KBr and DCM. The samples were then shaken for 15 minutes and centrifuged for 5 minutes. The final recovery CH_3HgBr was transferred into vials containing a scoop of anhydrous Na_2SO_4 for drying.

The samples were analyzed using a GC-AFS system which is the Hewlett- Packard model HP 6890 Series plus gas chromatograph coupled with a PS Analytical Merlin Mercury Fluorescence Detector System (Model 10.023) (Devai et al., 2001).Once the sample was injected into the split/splitless injector port that was maintained at 200 ^oC, it was carried by 4mL/min of high purity argon through a 15m x 0.53mm fused silica analytical column coated with 1.5 μm thickness of DB-1 into the PSA Merlin Mercury Fluorescence Detector system (Devai et al., 2001). The oven temperature was retained at 50 ^oC for 1 minute, programmed to 140 ^oC and 250 ^oC for 3 minutes at a rate of 30 ^oC /min (Devai et al., 2001). Methyl mercury

chloride powder dissolved in methanol was used to generate calibration curve and a secondary standard solution (1000ppm methyl mercury chloride in water) was used to ensure the quality of the calibration curve.

3.6 Organic Matter Estimation Using LOI Method

Loss-On-Ignition (LOI) was used to estimate the organic matter content in the sediments following the method of Luczak et al. (1997). The moisture content of the sediments was first determined by weighing a few grams of samples into a crucible and dried in the oven at 105 ^{o}C overnight. Then, the oven-dried samples were kept in the dessicator till cooled. The weight of the crucible, wet weight and dry weight of the samples were recorded. After this, the oven-dried samples were heated in a furnace at 500 ^{o}C for 6 hours. The samples were only put in when the furnace temperature reached at constant 500 ^{o}C to avoid overheating. The samples were cooled in the dessicator before measurement.

3.7 Soil Texture Determination Using Pipette Method

The particle distributions and textures of sediments for each station were determined using pipette method (Soil Survey Staff, 2004). The analysis was performed by Coastal Wetlands Soils Characterization Lab.

3.8 Sulfate Reducing Bacteria Determination Using MPN Method

Sulfate Reducing Bacteria was cultured under anaerobic condition using a standard Hungate technique (Bryant, 1972). The Multipurpose saltwater medium was prepared as illustrated by Widdel (1999). For a 1000mL of basal saltwater medium, 1mL of nonchelated trace element mixtures, 1mL of selenite-tungstate mixtures, 30mL of 1M bicarbonate solution, 5mL of acetate solution, 5mL of propionate and 3mL of lactate solution were added together, adjusted with 2M of HCl solution to pH 7-7.3, flushed with N_2, autoclaved and poured 9mL into each tube inside the anaerobic chamber. 0.8 mL of resazurin was added into the 1L medium as an indicator of reduced/oxidized condition. 100mL of vitamin solution is

prepared separately using a 0.2um pored sterile filter and consisted of 20mL of Vitamin mixture, 20ml of thiamine solution and 20mL of vitamin B_{12} solution with 40mL of sterile water. 0.05mL of this vitamin solution and 0.075mL of autoclaved, N_2 flushed sulfide solution were added into each tube.

The Most Probably Number (MPN) technique was used to enumerate the SRB population. About 5g of surface sediments from station C6B were weighed and diluted into a 45mL sterile water serum bottles. This 10% dilution was flushed with N_2, vortexed and transferred 1 ml into the next dilution tube using the N_2 flushed and sterile syringe and needles. The serial dilution was carried up to 10^{-8} for sediment and 10^{-4} for bottom water. Lastly, 1mL of CO_2 was flushed into each tube and incubated at 23 ^{o}C for 2 months. Chemical test was used to prove the growth of SRB. Reagent used was a mixture of 5mM CuSO4 and 50mM HCl in distilled water. 0.2mL of enrichment was transferred into a 1mL reagent using a syringe. When transferring the enrichment, the tip of the syringe must be dip inside the reagent to prevent loss of volatile H_2S. Sulfide was produced when brown color was presented. The result was then calculated using the MPN table (Linquist, 2003).

3.9 Data Analysis

A multiple regression model was used to determine the effects of total mercury, organic matter, dissolved oxygen, temperature, salinity and soil texture (percentage of sand) on methyl mercury concentration found in the bottom surface sediment (0-2cm). The assumptions used in the regression model are normality, homogeneity of variance, independence and x_i measured with error. We fitted our regression model by taking a natural log of y (methyl mercury concentrations) to meet the assumptions. The analysis of variance (factorial) was used to analyze the spatial and seasonal variations observed in the total and methyl mercury concentrations. The assumptions that apply to factorial ANOVA are normality, homogeneity of variance and independence. We did not meet the assumption of

normality after taking the log-transformation of the concentrations of methyl mercury, however, the residuals were symmetrically distributed; therefore, the analysis was still robust and tended to perform well. The homogenous variance was tested using the null model likelihood ratio test. The vertical profile (top, middle and bottom) of total and methyl mercury in the sediment cores were determined using a randomized block design (factorial ANOVA). Total and methyl mercury concentrations were natural log transformed to fulfill the assumption of normality and homogeneity of variance. The difference between the populations of sulfate reducing bacteria between the sampling months at C6B was analyzed using one-way ANOVA. The population SRB was log-transformed to ensure the residuals were normally distributed. The relationship between the total mercury and clay% in the sediment was determined using a power model. The analysis of covariance was used to correlate the concentration of methyl mercury and organic matter as well as the concentration of methyl mercury and redox potential in both aerobic and anaerobic treatments in our laboratory study. We did not find any significant difference between the intercepts of both treatments when determining the relationship between the level of methyl mercury and organic matter as well as redox potential. Therefore, we pooled the data together and fitted the regression using exponential model. The assumption of independence is guaranteed through random sampling. Normality is justified using the Shapiro-wilks test, normal probability and stem left plots. The residual plot is used to examine the outlier; homogeneity of variance; and curvature. Residual plot that reflects random scatter about the regression line indicates homogeneity of variance. All the analysis was performed using alpha value of 0.05.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Seasonal Data of Dissolved Oxygen, Temperature, Salinity and Organic Matter

In-situ measurements of bottom water concentrations of dissolved oxygen, salinity and temperature were listed in Table 1. Very low levels of dissolved oxygen were observed during summer in all three stations and during spring in stations C6B and C8. This seasonal variation of dissolved oxygen was caused by the freshwater and nutrient input from the Mississippi and Atchafalaya rivers (Rabalais et al., 2002). Rabalais et al. (2001) suggested that the water column was most stable during spring because of high runoff and during summer because of weak wind mixing and strong sunlight. About 580 km^3 of freshwater from Mississippi river is discharged into the gulf every year (Dunn, 1996). An estimated 80% freshwater, 90% total nitrogen and 87% total phosphorus from the combined streams of Mississippi and Atchafalaya rivers are discharged into the gulf annually (Dunn, 1996). The discharged was carried predominantly westward along the inner shelf of Louisiana/Texas to the continental shelf, especially during spring (Rabalais et al., 1996). In the gulf, the water column is stratified through the differences in density because the freshwater from the river is lighter than the salt water. The nutrient from the river stimulates the production of phytoplankton that may eventually either be transferred into the food web or get decomposed by bacteria when it falls in the seabed as organic debris and consequently, dissolved oxygen in the bottom water is depleted by the respiration of microbial activity (Rabalais et al., 2002). Factors like strength of stratification, light limitation, nutrient availability, and rate of primary production as well as the flux of organic matter to the seabed influence the formation of the optimal distance and depth of hypoxia (Rabalais et al., 2002). The breakdown of vertical stratification occurs during events like tropical storms, cold fronts or thermal turnover (Rabalais et al., 2001). This could explain infrequent hypoxia events during late fall

and winter. Water column stratification does not persisted until October 2005 was probably caused by the vertical mixing by Hurricane Katrina occurred in the end of August 2005. Other than vertical mixing by hurricane, the stability of water column is also affected by upwelling of the deeper oxygenated waters by wind mixing and tidal advection (Rabalais et al., 2001).

Table 1. In-situ measurements of dissolved oxygen, salinity and temperature for station C4, C6B and C8.

Sampling	Dissolved Oxygen(mg/l)			Salinity (PSU)			Temperature (^{o}C)		
Date	C4	C6B	C8	C4	C6B	C8	C4	C6B	C8
4/18/2005	6.79	0.30	0.46	30.00	34.70	34.40	21.77	21.34	21.11
5/11/2005	7.53	5.13	2.77	28.12	32.83	36.09	22.77	22.55	21.14
6/3/2005	2.01	1.84	3.49	35.52	35.90	36.14	23.93	23.36	23.32
7/23/2005	3.32	0.89	4.12	33.00	33.40	34.80	28.81	27.34	27.10
8/17/2005	0.14	0.34	0.87	34.50	35.28	35.34	29.42	28.02	27.86
10/9/2005	4.69	4.55	4.30	34.18	34.69	35.07	27.57	27.72	28.36
1/11/2006	NA	NA	NA	34.29	35.65	35.87	17.65	19.65	19.90
4/5/2006	6.16	3.97	6.36	31.14	35.06	36.24	21.24	21.38	21.50

Particle size distribution for surface sediment (0-2cm) collected from each station for each sampling month was showed in Appendix. Eleven types of texture classes were determined in the surface sediment collected from all stations. Between these three stations, C4 is closer to and C8 is farther from the shore and C6B is located between these two stations (see Figure 1). Surface sediments in C8 contained mostly sand (ranging from 18.7% – 98.8%), except one sample which contained 37.5% of clay. Surface sediments in C6B and C4 contained mostly clay, which ranged from 22.8% -60.6% and 9.4% -51.3%, respectively.

The organic matter content (%LOI) in surface sediments at C4, C6B and C8 ranged from 3.219 to 8.217 with average and standard error of 5.116±0.283, 2.840 to 6.710 (average = 3.877±0.187) and 1.950 to 5.565 (average= 3.682±0.232), respectively. The average organic matter content for each station was determined using triplicate sediment samples regardless of the type of sediment texture.

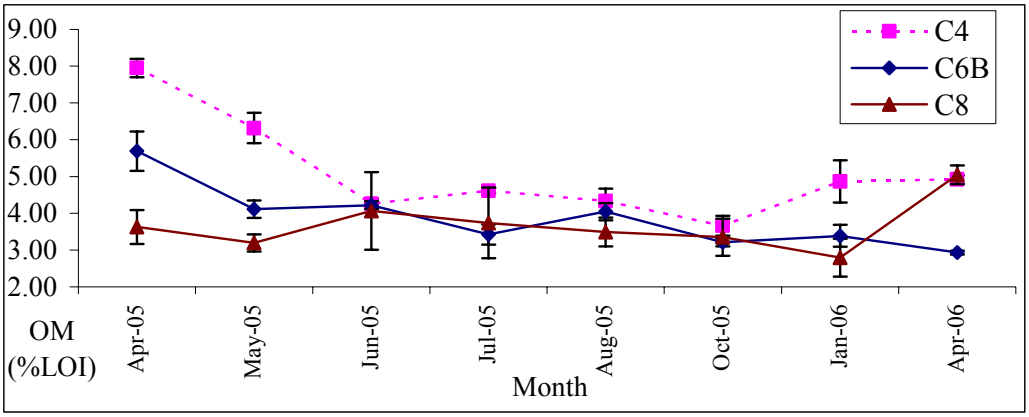

Figure 3. Seasonal variations in organic matter level (%LOI) in bottom sediment at each station.

The change of organic matter content with sampling months for each station was plotted in Figure 3. The %LOI determined in C4 was higher than C6B and C8. Higher organic matter content was observed in C4 and C6B during the spring. We did not observe any obvious seasonal effects on C8. The peak showed in C8 in April 06 was due to the differences in sampling location. This sampling location was about a few meters away from the previous sampling location. High standard deviations were observed in C8 for both June 05 and July 05 samples because of the variation in particle size distribution. The spatial and temporal variations in the organic matter found in the surface sediments for all stations probably were caused by the seasonal productivity of phytoplankton and respiration activity in the water column which was associated with the freshwater and nutrient loading from Mississippi and Atchafalaya rivers. The organic matter decomposed by bacteria in the lower water column was derived from the marine phytoplankton rather than the carbon carried from the Mississippi river (Eadie et al., 1994). Rabalais et al. (2002) reported that the nutrient flux

was moved westward from the Mississippi River Delta to the northern gulf. They observed higher respiration rates per unit chlorophyll a in the spring. The respiration rate was decreased with decreasing nutrient and chlorophyll a concentrations and with increasing distance from the west of Mississippi River Delta.

4.2 Level of Total and Methyl Mercury in Surface Sediments

The average concentrations of total and methyl mercury in surface sediments for each station are shown in Table 2. Total mercury concentrations determined in all stations were comparable to the background level of mercury in unpolluted ocean sediment, which was 0.02-0.1 μg/g (Lindqvist, 1984; Ullrich et al., 2001). This concentration was also below the total mercury level determined in the nearshore, offshore or near platform of the gulf (Table 3). Low concentrations of total mercury observed in the surface sediments in all three stations indicate that there were no significant local sources of mercury (Regnell et al., 1997). Therefore, other non-point sources such as atmospheric fallout, runoff from Mississippi river basin and direct discharge from Mississippi and Atchafalaya rivers may account for the mercury in the gulf. According to Neff (2002), approximately 55000lbs of atmospheric mercury were deposited into the gulf water surface each year , which is considered to be the main source, and followed by the loading from Mississippi river, with an estimation of 48000 lbs/year. Oil and gas drilling activities in the gulf produced about 420 lbs of mercury annually (Neff, 2002).

Methyl mercury concentration determined in the sediment was a net result of methylation and demethylation processes (Korthals and Winfrey, 1987). This is because of the high turnover of methyl mercury (residence time of 1.7 days), i.e., the formation and destruction of methyl mercury occur continually in the sediment (Hintelmann et al., 2000).

Average methyl mercury concentrations determined in all stations were in the range and below the highest concentration found in sediment close to the drilling sites in the Gulf of

Mexico (Table 3). The proportion of methyl mercury in total mercury in sediment ranged from 0.30-2.69%, which was higher compared to studies done by Olson (1974) and Kannan and Falandysz (1998). Kelly et al. (1995) reported a range of 1-90% of methyl mercury compared to total mercury in three lake sediments and they concluded that total mercury input or concentration was not practical to predict the concentration of methyl mercury.

Table 2. Average concentrations of methyl and total mercury at each station.

Station	Average meHg ± standard error , μg/kg d.w. (range)	Average THg ± standard error, μg/kg d.w. (range)
C4	0.31±0.03 (0.09-0.58)	37.79±2.49 (16.83 – 61.64)
C6B	0.47±0.06 (0.11-1.10)	41.06±2.41 (18.60 – 66.94)
C8	0.12±0.02 (0.00-0.50)	11.02±1.77 (3.03 – 30.19)

Table 3. Concentrations (dry weight based) in total and methyl mercury in sediment from other locations.

Location	n	Total mercury μg/kg	Methyl mercury μg/kg	References
Biscayne Bay	5	17 (3-66)	0.026 (<0.001-0.59)	Kannan et al., 1998
Tampa Bay	9	8.3 (1-13)	0.049 (0.009-0.127)	Kannan et al., 1998
Charlotte Harbor	3	29 (7-43)	0.074 (0.030-0.120)	Kannan et al., 1998
Florida Bay	30	12 (3-100)	0.082 (<0.001-0.318)	Kannan et al., 1998
Pine Island Sound	3	6.3 (4-9)	0.055 (0.041-0.068)	Kannan et al., 1998
Whitewater Bay	1	69	< 0.001	Kannan et al., 1998

(table continued)

Hillsborough Channels	1	219	0.490	Kannan et al., 1998
Boca Ciega Bay	2	10 (4-16)	0.052 (0.042-0.062)	Kannan et al., 1998
Sarasota Bay	2	3.5 (2-5)	0.063 (0.010-0.016)	Kannan et al., 1998
Turtle Bay	1	3	0.033	Kannan et al., 1998
Caloosahatchee River	1	60	0.035	Kannan et al., 1998
Matlacha Pass	1	3	0.183	Kannan et al., 1998
Gordon River	1	174	0.230	Kannan et al., 1998
Chokoloskee Bay	1	6	0.175	Kannan et al., 1998
Oyster Bay	1	19	0.028	Kannan et al., 1998
Card Sound	1	13	0.012	Kannan et al., 1998
Long Sound	1	33	0.062	Kannan et al., 1998
Barnes Sound	1	21	0.172	Kannan et al., 1998
Tarpon Basin	1	33	0.019	Kannan et al., 1998
Torchkey Mangroves	1	10	<0.001	Kannan et al., 1998
Cudjoe Basin	1	11	0.041	Kannan et al., 1998
Garrsion Bight	1	38	0.076	Kannan et al., 1998
Gulf of Mexico(NF)	49	136.5 (52-248)	0.51 (0.19-1.42)	Trefry, 2002
Gulf of Mexico(MF)	25	78.33 (54-106)	0.51 (0.34-0.77)	Trefry, 2002 (table continued)

Gulf of Mexico (FF)	38	59.67 (29-79)	0.55 (0.29-0.87)	Trefry, 2002
Poland (coastal marine)	NA	164 (37-880)	0.645 (0.035-2)	Kannan and Falandysz, 1998
Malaysia (off Kuala Terengganu)	NA	61 (20-127)	0.038 (0.01-0.053)	Kannan and Falandysz, 1998
Russia (Anadyr Estuary)	NA	339 (77-2100)	0.24 (0.055-0.62)	Kannan and Falandysz, 1998
Minamata Bay (0-2cm surface)	9 ,2	2922 (1410-4340)	4640 (4220-5060)	Tomiyasu et al., 2006
Fukuro Bay (0-2cm surface)	3	4660 (4460-4820)	18270 (2940-29030)	Tomiyasu et al., 2006

Relatively low methyl mercury concentrations were found in the gulf bottom sediment compared to freshwater sediment because of the abundant sulfate present in sea salt (Compeau and Bartha, 1983). In-situ measurement of salinity in our study ranged from 28.14 to 36.24 PSU (Table 1). Approximately $1.2x10^{15}$ tonnes of SO_4^{2-} is present in the sea water with salinity of 35 PSU (Open University, 1989). Benoit et al. (2001) suggested that sulfide inhibits methylation through chemical speciation of dissolved inorganic mercury. The shift from neutral sulfide complex to a more polar (negatively charged) sulfide complex had hindered the passive diffusion uptake of the bacteria (Benoit et al., 2001).

Besides the effect of sulfate, salinity was generally shown to negatively correlate with production of methyl mercury (Blum and Bartha, 1980; Compeau and Bartha, 1987). Compeau and Bartha (1984) found that high salinity and aerobic conditions appeared to have caused demethylation of methyl mercury. It was suggested that in the full strength seawater

(0.5M Cl^-), mercury solubility was affected by the formation of the negatively charged $HgCl_4^{2-}$complex (Compeau and Bartha, 1987). Barkay et al. (1997) showed that uncharged mercuric chloride permeated the bacterial cytoplasm more readily than electrochemically charged forms. Other anionic sea salts besides sulfate were shown to affect the speciation of mercury by controlling the transfer of methyl groups from methylcobalamin (Blum and Bartha, 1980). Formation of $HgCO_3$ from bicarbonate under anaerobic and aerobic conditions was thought to reduce production of methyl mercury (Compeau and Bartha, 1983).

4.3 Vertical Profile of Total and Methyl Mercury Concentrations in Sediment Cores

The sediment depth profile (i.e., top (0-2cm), middle (2-5cm) and bottom (5-8cm)) of mercury concentration was studied in sediment cores collected from station C4, C6B and C8 in the months of April 05, May 05 and July 05.

The average concentrations of methyl and total mercury are shown in Table 4. Concentration of methyl mercury was significantly different between different depths ($P<0.0001$). Methyl mercury concentrations determined in the top segment were significantly higher than the bottom segment and decreased with increasing depth. This was because of the supply of available organic matter and sulfate near the surface sediment that stimulated the activities of the sulfate reducing bacteria (Choi et al, 1994). Korthals and Winfrey (1987) reported higher methyl mercury concentrations in surface sediment, decreasing with depth in sediment core. They suggested that the decreasing methyl mercury level with depth in sediment cores was because of the decrease in microbial methylation activity. Vertical profile of total mercury did not show obvious different compared to the vertical profile of methyl mercury (Figure 4 and Figure 5). We did not observe a strong vertical profile of methyl mercury in some sediment cores (Figure 4) due to the constraint during box core sampling. Low methyl and total mercury concentrations were observed at C8 because the sediment at this station contains mostly sand.

Table 4. Average concentrations of organic matter, total and methyl mercury in sediment cores.

Station	Depth	Ave. meHg ± standard error	Ave. THg± standard error
	cm	ug/kg d.w.	ug/kg d.w.
C4	0-2	0.38±0.19	40.72±5.37
	2-5	0.29±0.01	32.04±5.62
	5-8	0.26±0.01	30.40±3.53
C6B	0-2	0.52±0.10	43.34±5.75
	2-5	0.26±0.04	39.98±9.41
	5-8	0.14±0.05	28.86±3.24
C8	0-2	0.11±0.03	13.54±5.73
	2-5	0.11±0.03	17.46±6.21
	5-8	0.09±0.01	15.29±4.81

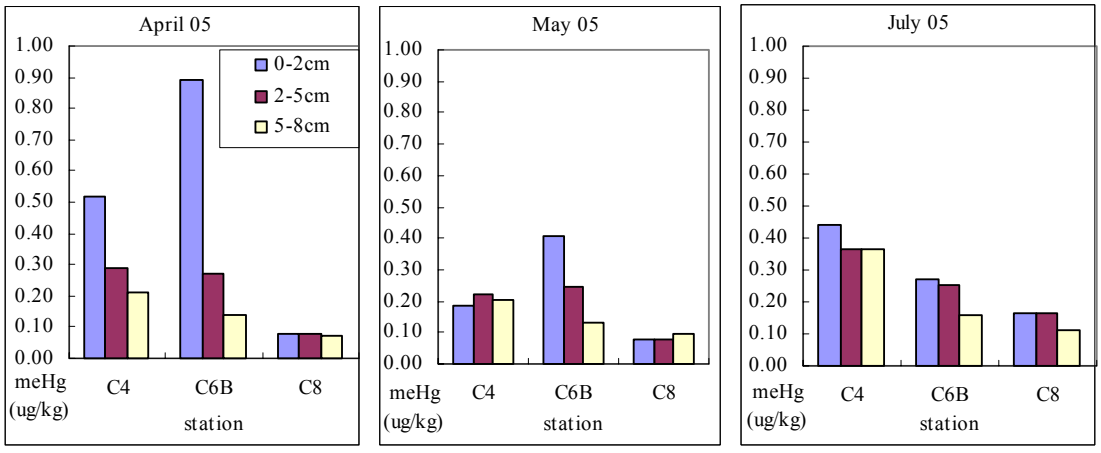

Figure 4. Vertical profile of methyl mercury concentration in sediment cores.

4.4 Spatial Variation in Total and Methyl Mercury in Surface Sediment

Comparing the average total mercury between three stations, C8 was observed to be significantly different from C4 and C6B ($P<0.0001$). As shown in the Figure 7, the total mercury level in C8 was relatively low compared to C4 and C6B. The spatial variation of total mercury between three stations was due to the differences in sediment texture. The

capacity of capturing mercury is varied in sediment texture (particle size distribution). We observed higher total mercury concentration in sediment containing high percentages of clay (Refer Appendix).

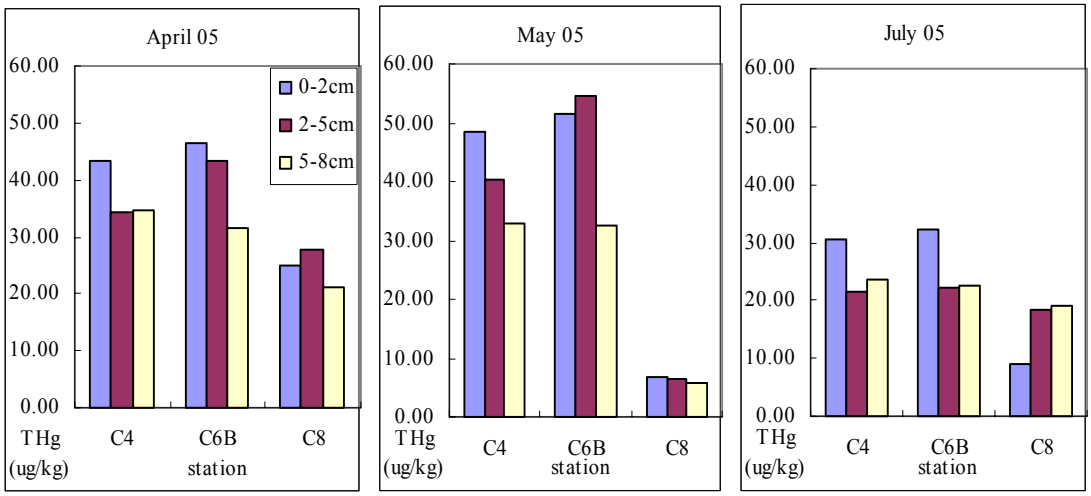

Figure 5. Vertical profile of total mercury concentration in sediment cores.

There was a significant positive correlation between the total mercury in sediment and percentage of clay as showed in figure 6. The model accounted for 62% of total variation. Hunerlach (2004) observed higher total mercury in sediment having finer particle size and indicated a positive relationship between the total mercury and proportion of clay in the sediment. Kongchum (2005) showed 54% correlation between the total mercury concentration and clay content in the sediment.

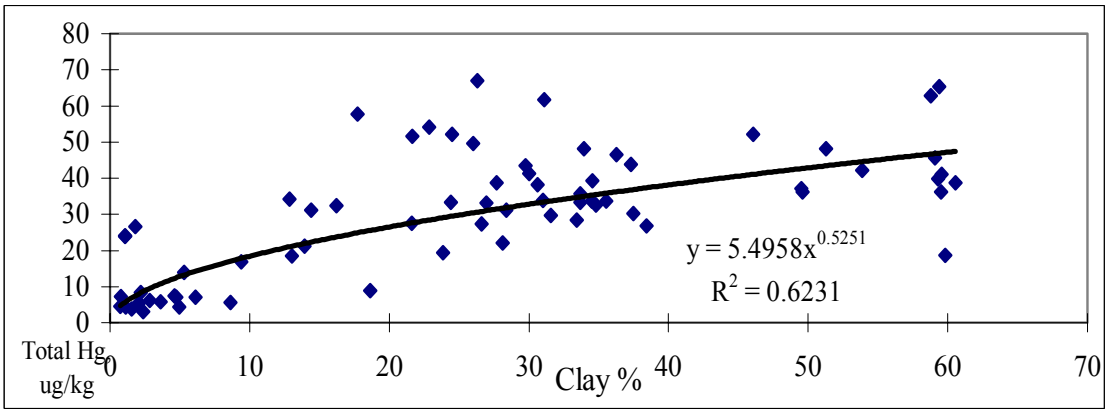

Figure 6. The effect of particle size (clay %) on total mercury concentration.

Average methyl mercury level was significantly different between C4, C6B and C8 ($P<0.0001$). The level of methyl mercury was observed to be highest in C6B and lowest in C8 as shown in Figure 8. In our calculation and statistical analysis, we decided not to include one of the highest methyl mercury concentration observed in sediment sample collected at C4 in April 05. This is because the high point may not belong to the population and was not representative of the concentration for the bottom sediment in the northern Gulf of Mexico. The high concentration observed indicated that there was a hot spot in station C4 for mercury methylation. The spatial variation of methyl mercury concentration found in three stations could be explained by the difference in the pool of inorganic mercury available for microbial methylation and the amount of decomposable organic matter. We found that concentration of methyl mercury was significantly decreased with increasing percentage of sand in the sediment ($P<0.0001$). The capacity of sandy sediment to capture mercury is relatively low. Therefore, it reduces the availability of mercury for methylating bacteria. In addition, population of microorganisms found in sandy sediments is usually lower compared to sediments containing more clay. Since mercury is the substrate for mercury methylation, a relationship between the concentration of total and methyl mercury is expected in the sediment (Hammerschmidt and Fitzgerald, 2004). So, lower methyl mercury observed in sediment C8 may be due to lower microbial activities in sandy sediment and lower total mercury concentration available for mercury methylating bacteria.

4.5 Seasonal Variations in Methyl Mercury in Surface Sediment

Concentrations of methyl mercury in surface sediment at each station was significantly different among the sampling months ($P<0.0001$). Factorial ANOVA also depicted a strong interaction between the seasonal and spatial effects ($P<0.0001$). Methyl mercury concentration observed at C8 was relative low compared to that at C4 and C6B in each sampling month as shown in Figure 8 because of the sediment texture differences.

Relatively low methyl mercury observed in summer months compared to spring months showed that temperature may not be the main factor controlling the mercury methylation process in the gulf.

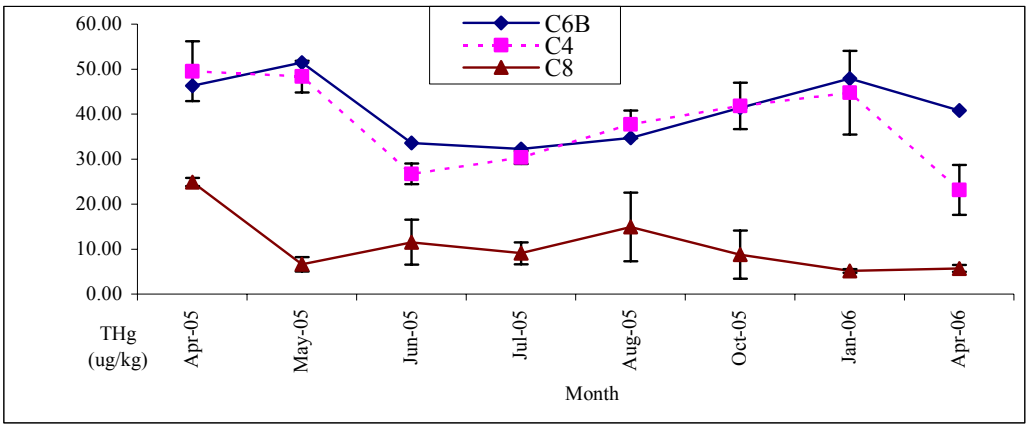

Figure 7. Spatial variation in total mercury in each station.

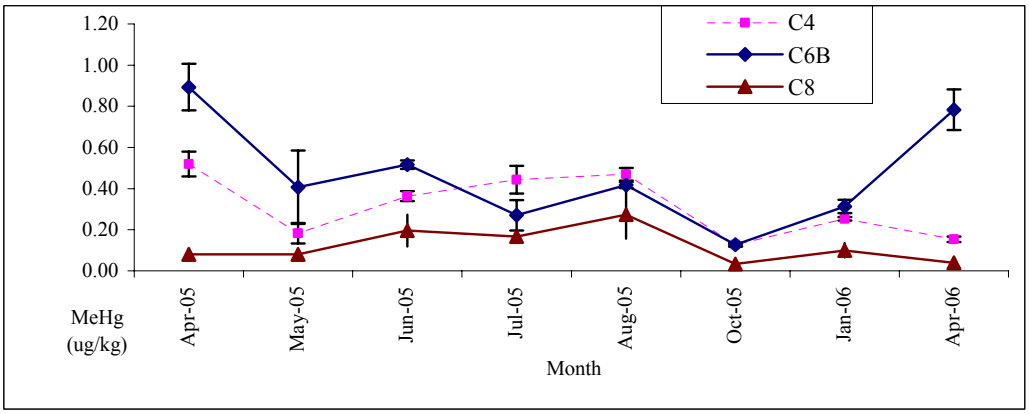

Figure 8. Seasonal variation in methyl mercury in each station.

Although high temperature was showed to favor mercury methylation, we observed negative effect of temperature on methyl mercury concentration in multiple regression model (estimated parameter = -0.042, P=0.161). This was contrary with other studies that showed greater methylation activity during mid or late summer months (Callister and Winfrey, 1986; Korthals and Winfrey, 1987; Hammerschmidt and Fitzgerald, 2004). Although Callister and Winfrey (1986) observed an optimum temperature of 35^{o}C for methylation, they suggested that temperature is not the only factor that controlled the seasonal peak in methylation. Other

variables such as demethylation activity, nutrient loading, and aerobic conditions also contribute to the methylation rate.

Methyl mercury at C6B fluctuated through the rest of the months after April 05 and increased again in spring 06 after October 05. Methyl mercury in C4 was slightly increased till August 05 after a sharp drop from April 05 to May 05. Decrease in methyl mercury at both stations C4 and C6B in May 05 after April 05 may be due to the combined effects of reduce organic matter (Figure3) and increased dissolved oxygen in bottom water (Table 1). High methyl mercury concentration appeared in April 05 at C4 and C6B as well as in April 06 at C6B was probably caused by the freshwater and nutrient loading from Mississippi river. That stimulates the phytoplankton production which results in abundant amount of organic matter settling on the seabed. We found that the concentration of methyl mercury was positively correlated to organic matter (estimated parameter = 0.158, $P=0.052$) and negatively correlated to dissolved oxygen (estimated parameter = -0.214, $P<0.0001$). A very reducing condition was created as a consequence of microbial respiration when the organic matter was abundant. This organic content-rich sediment with low redox potential was shown to enhance the sulfate reduction and mercury methylation rate, thus increasing the accumulation of methyl mercury in the sediment (King et al., 2000; Lambertsson and Nilsson, 2006). Jackson (1993) also found the greater microbial-methylating activity due to the plentiful production of organic nutrient substrates resulting from phytoplankton bloom and higher proportion of organic matter to mineral detritus. Organic content in sediment was also shown to be an important factor in controlling mercury methylation by SRB and it is thought that the temporal addition of fresh organic matter to the sediment surface was affected by the seasonality in primary production (Lambertsson and Nilsson, 2006). Our laboratory data confirmed a positive effect of organic matter on mercury methylation which would be discussed in Section 4.6.

Although anaerobic conditions favored mercury methylation, we did not observe high methyl mercury in sediment during summer months where the bottom water dissolved oxygen was low (Table 1). This may be caused by the inhibition of mercury methylation by sulfide under low redox condition. Drever (1997) showed that the conversion of sulfate to total sulfide occurs in the range of 0 to -100 mV in typical seawater. Sulfide level greater than 5-10 μM was shown to suppress the rate of methylation (Benoit et al., 1999; Benoit et al., 2001). Trefry (2002) found low methyl mercury levels with redox potential of <-100mV and > 1mM total H_2S whereas high methyl mercury levels were observed with a redox potential of ~ 0mV and high total organic carbon content.

Lowest methyl mercury level was observed in October 05 for all three stations; probably caused by the Hurricane Katrina which occurred during late August 05. The stratification of water column was stirred up by this event and resulted in increased dissolved oxygen in bottom water.

4.5 Sulfate Reducing Bacteria Population

Sulfate reducer exists abundantly in the marine sediment and plays an important role in sulfur cycling in the aquatic environment (Widdel, 1999). As shown in Table 5, the SRB number in the bottom sediment of C6B ranged between $6.9x10^3$ and $1.04x10^5$ cells/g wet sediment. This range was higher compared to the SRB numbers in bottom sediment determined in the Gulf of Gdansk, which ranged between $76x10^2$ and $1.27x10^4$ cells/g (Mudryk, 2000). Bak and Pfennig (1991) reported a maximum density of $6.3x10^6$ cells/ml. The number of SRB was found to be significantly different between the sampling months in bottom sediment of C6B ($P = 0.0009$). Increased temperature was shown to stimulate the growth of SRB (Fukui and Takii, 1989). Mudryk (2000) also observed highest number of SRB in the summer months. However, Abdollahi (1979) showed no detection response or adaptation of SRB population to seasonal environmental temperature. Our result did not show

highest SRB population in August, although this sampling month had the highest temperature (see Table 1). SRB population in Jan 06 was found to be significantly lower compared to other sampling months. This might be due to the cold weather. We expected to observe higher SRB population as the temperature rises. Organic matter availability plays an important role in controlling the production of SRB (Mudryk, 2000). About 50% of sulfate reduction correlated with the total organic matter degradation in marine sediment (Jørgensen, 1982). SRB was also determined to be the principal mercury methylator in estuarine and freshwater sediment (Compeau and Bartha, 1985; Gilmour et al., 1992). However, we did not find any significant positive correlation between the number of SRB and concentration of methyl mercury in the surface bottom sediment. This may be due to the variation in the type of SRB that are capable of methylating mercury (King et al., 2000).

4.6 Laboratory Study- Effect of Organic Matter and Redox Potential on Mercury Methylation

The average concentration of methyl mercury was higher in water overlying sediment exposed to nitrogen compared to air, which was 3.04 μg/kg d.w and 1.83 μg/kg d.w., respectively. Both overlying water sediment were incubated under very reducing condition with a range of Eh from -69.9 to -278.9 mV.

Table 5. MPN for SRB in surface sediment in C6B.

	MPN Number for SRB ($x10^5$ cells/g wet sediment)							
	April	May	June	July	August	October	January	April
	0.474	0.480	0.181	0.756	0.182	0.300	0.086	0.460
	0.161	0.462	0.083	0.780	0.298	-	0.045	0.178
	0.472	2.178	0.087	0.758	0.293	-	0.076	0.843
mean	0.369	1.040	0.117	0.765	0.258	-	0.069	0.494
std dev.	0.181	0.986	0.055	0.014	0.066	-	0.021	0.334

After 21 days of incubation, redox potential in sediment was slightly decreased in nitrogen-flushed and increased in air-flushed conditions (Figure 9). However, redox potential in 1%-added organic content sediment under air flushed was decreased. This reduced condition was probably caused by the active microbial respiration in the presence of higher organic content.

Table 6. Average methyl mercury (meHg) concentration and redox potential (Eh) under nitrogen and air incubation. [a]redox potential after 24 hours
[b]redox potential at day 21

	Nitrogen-flushed			Air-flushed		
Added OM,%	meHg,ug/kg	[a]Eh_i,mV	[b]Eh_f,mV	meHg,ug/kg	Eh_i,mV	Eh_f,mV
0%	0.89	-69.9	-165.6	0.57	-165.0	-120.6
0.01%	0.80	-125.3	-195.7	0.81	-148.2	-142.8
0.10%	2.32	-153.6	-194.8	1.62	-223.3	-190.6
1%	8.14	-132.5	-278.9	4.33	-149.2	-234.3

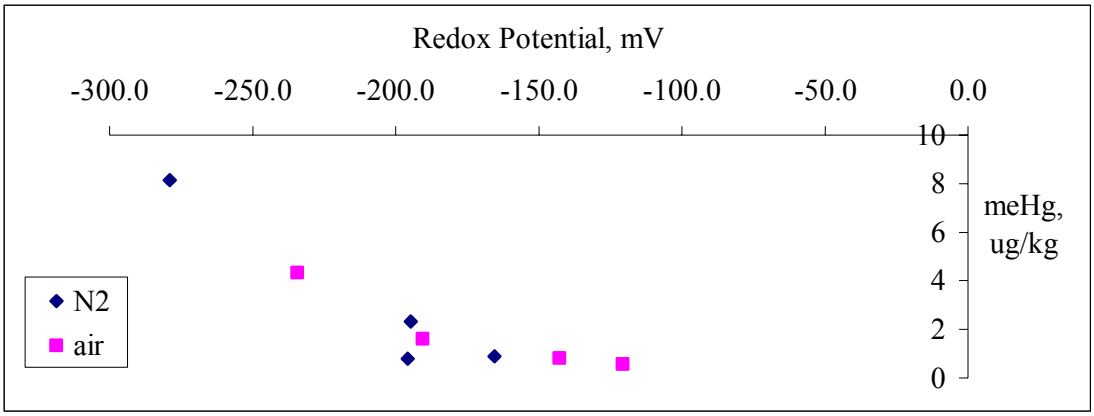

Figure 9. The effect of redox potential on methyl mercury concentration.

Redox potentials were decreased with increasing organic content added in both nitrogen- and air-flushed conditions. However, we found no significant difference between the nitrogen- and air-flushed conditions. Therefore, we combined the data to perform a

regression of methyl mercury concentration on redox potential. Our result showed that methyl mercury was significantly correlated with redox potential in a negative exponential relationship (Y = 0.1520*exp [-0.01227*X]; R^2=0.4990; P=0.0032). DeLaune et al. (2004) showed that sediment overlying with non-oxygenated water column produced more methyl mercury than oxygenated water column. In their control study, they found that increased redox potential (from -200mV to +250mV) decreased the methyl mercury content in freshwater sediment. We did not observe a positive redox potential in the air-treated water overlying the sediment because the redox potential was measured below 1cm from the surface sediment. Only a very thin sheet (~1-2mm) of the surface sediment was being oxidized. Compeau and Bartha (1984) found that controlled redox potential of -200mV was more favorable for mercury methylation than +110mV in estuarine sediment. Olson and Cooper (1976) demonstrated that increased methylation capacity in San Francisco Bay sediment was associated with anaerobic conditions. They also observed more stable production of methyl mercury in sediment under anaerobic condition than aerobic condition because methyl mercury was broken down faster under aerobic condition by most of the aerobic demethylating-microorganism. Methyl mercury was higher in the anaerobic sediment because of the activity of sulfate reducing bacteria (Compeau and Bartha, 1985).

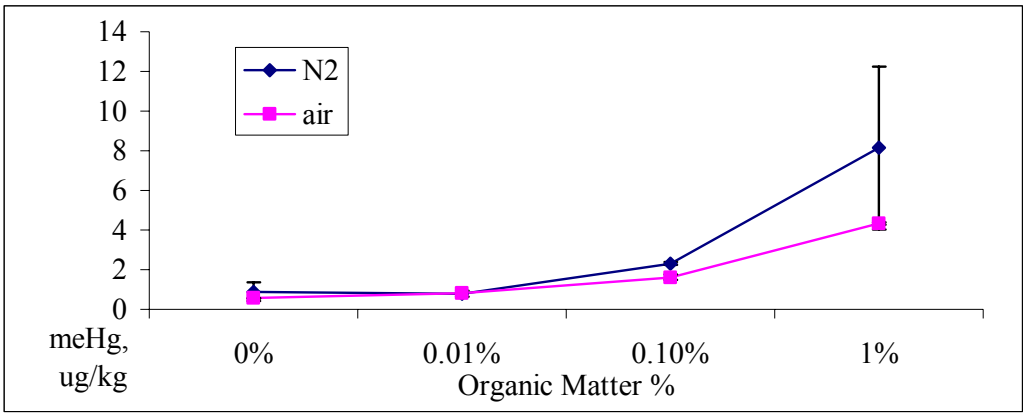

Figure 10. The effect of organic matter on methyl mercury concentration.

Mercury methylation occurred in both air- and nitrogen-treated conditions but there were no significant difference between these treatments. This is probably because the treatment effects were masked over by the organic matter effect. Again, all the data was pooled together to see the effect of organic matter on methyl mercury concentration. Increase in organic content significantly increased the methyl mercury content in sediment in our study (Figure 10). There was a significant positive exponential relationship found between the organic content and methyl mercury ($Y= 0.9349*\exp [1.8282*X]$; $R^2=0.7154$; $P<0.0001$. Concentration of methyl mercury was shown to be significantly affected by the organic content regardless of either air- or nitrogen-treated conditions. Our controlled study result was comparable with the finding of Lambertsson and Nilsson (2006). They showed that the ambient methyl mercury concentration was exponentially increased with the organic matter content (%LOI) with $R^2=0.64$.

Increased methyl mercury in sediment with increased organic content % indicated the importance of the supply of organic matter in mercury methylation by SRB under reduced environment (Lambertsson and Nilsson, 2006). Callister and Winfrey (1986) and Choi et al. (1994) also found that organic content exhibited a positive effect on mercury methylation in the freshwater and marine sediments. Organic matter helps in maintaining a low redox potential condition while providing electron donors to SRB for stimulating the methylation process (Lambertsson and Nilsson, 2006). Organic matter also controls the mercury methylation through controlling the partition of mercury between solid and dissolved phase by serving as complexing agents for Hg2+ and methyl mercury (Lambertsson and Nilsson, 2006). On the other hand, Hammerschmidt and Fitzgerald (2004) observed inverse relationship between the sedimentary organic content and potential rate of microbial mercury methylation. They found that sedimentary organic content affects mercury methylation through controlling the sediment-water partitioning of Hg(II) and so, concluded that

decreased organic content in sediment could increase microbial production of methyl mercury via increase of Hg(II) in pore water.

CHAPTER 5 CONCLUSIONS

In summary, our field data demonstrated that the discharges from the Mississippi and Atchafalaya rivers during the spring would increase the organic matter content on the seabed and result in lower dissolved oxygen levels in the bottom water column in the Gulf of Mexico. This condition would create a reduced and organic-rich environment for mercury methylating-bacteria. We observed peak concentrations of methyl mercury at C4 and C6B during the spring of 2005. Relatively lower methyl mercury concentrations were observed in the bottom sediment in comparison to freshwater sediments possibly because of the high salinity and sulfate concentration in the sea water. Lower total and methyl mercury concentrations were observed at C8 compared to that at C4 and C6B due to the differences in sediment texture. Multiple regression analysis indicated the importance of dissolved oxygen, organic matter and sediment texture in influencing the concentration of methyl mercury in the bottom sediment of the hypoxic zone. Our laboratory study showed that the concentration of methyl mercury in surface sediment increased exponentially with increasing amount of organic content but decreased exponentially with increasing redox potential. Result from our laboratory study was in agreement with the field data. Both studies implied that the coastal euthrophication and hypoxia possibly stimulates the mercury methylation process by providing a reduced and organic rich environment for SRB.

REFERENCES

Abdollahi, H., and David Nedwell (1979). "Seasonal temperature as a factor influencing bacterial sulfate reduction in salt marsh sediment " Microbial ecology **5**(1): 73-79.

Alli, A., R. Jaffe, et al. (1994). "Analysis of Organomercury Compounds in Sediments by Capillary Gc with Atomic Fluorescence Detection." Hrc-Journal of High Resolution Chromatography **17**(11): 745-748.

Amyot, M., G. A. Gill, et al. (1997). "Production and loss of dissolved gaseous mercury in coastal seawater." Environmental Science & Technology **31**(12): 3606-3611.

Amyot, M., D. Lean, et al. (1997). "Photochemical formation of volatile mercury in high Arctic lakes." Environmental Toxicology and Chemistry **16**(10): 2054-2063.

Bak, F. and N. Pfennig (1991). "Sulfate-Reducing Bacteria in Littoral Sediment of Lake Constance." Fems Microbiology Ecology **85**(1): 43-52.

Baldi, F., F. Parati, et al. (1995). "Dimethylmercury and Dimethylmercury-Sulfide of Microbial Origin in the Biogeochemical Cycle of Hg." Water Air and Soil Pollution **80**(1-4): 805-815.

Baldi, F., M. Pepi, et al. (1993). "Methylmercury Resistance in Desulfovibrio-Desulfuricans Strains in Relation to Methylmercury Degradation." Applied and Environmental Microbiology **59**(8): 2479-2485.

Barkay, T., M. Gillman, et al. (1997). "Effects of dissolved organic carbon and salinity on bioavailability of mercury." Applied and Environmental Microbiology **63**(11): 4267-4271.

Beijer, K., and Jernelöv, A. (1979). Methylation of mercury in aquatic environments, Elsevier/North-Holland Biomedical Press, Amsterdam.

Benoit, J. M., C. C. Gilmour, et al. (2001). "The influence of sulfide on solid phase mercury bioavailability for methylation by pure cultures of Desulfobulbus propionicus (1pr3)." Environmental Science & Technology **35**(1): 127-132.

Benoit, J. M., C. C. Gilmour, et al. (1999). "Sulfide controls on mercury speciation and bioavailability to methylating bacteria in sediment pore waters (vol 33, pg 951, 1999)." Environmental Science & Technology **33**(10): 1780-1780.

Berman, M. and R. Bartha (1986). "Levels of Chemical Versus Biological Methylation of Mercury in Sediments." Bulletin of Environmental Contamination and Toxicology **36**(3): 401-404.

Bisinoti, M. C. and W. F. Jardim (2003). "Production of organic mercury from Hg-0: Experiments using microcosms." Journal of the Brazilian Chemical Society **14**(2): 244-248.

Bisogni, J. J. and A. W. Lawrence (1975). "Kinetics of Mercury Methylation in Aerobic and Anaerobic Aquatic Environments." Journal Water Pollution Control Federation **47**(1): 135-152.

Bloom, N. S. (1992). "On the Chemical Form of Mercury in Edible Fish and Marine Invertebrate Tissue." Canadian Journal of Fisheries and Aquatic Sciences **49**(5): 1010-1017.

Blum, J. E. and R. Bartha (1980). "Effect of Salinity on Methylation of Mercury." Bulletin of Environmental Contamination and Toxicology **25**(3): 404-408.

Boening, D. W. (2000). "Ecological effects, transport, and fate of mercury: a general review." Chemosphere **40**(12): 1335-1351.

Bringmark, L. (1997). "Accumulation of mercury in soil and effects on soil biota." Metal Ions in Biological Systems, Vol 34 **34**: 161-184.

Bryant, M. P. (1972). "Commentary on Hungate Technique for Culture of Anaerobic Bacteria." American Journal of Clinical Nutrition **25**(12): 1324-1328.

Cai, Y., R. Jaffe, et al. (1996). "Determination of organomercury compounds in aqueous samples by capillary gas chromatography atomic fluorescence spectrometry following solid-phase extraction." Analytica Chimica Acta **334**(3): 251-259.

Callister, S. M. and M. R. Winfrey (1986). "Microbial Methylation of Mercury in Upper Wisconsin River Sediments." Water Air and Soil Pollution **29**(4): 453-465.

CDC (2004) "Blood mercury levels in young children and childbearing-aged women- United States, 1999-2002." MMWR **Volume**, 1018-1020.

Celo, V. (2003). Abiotic pathways of mercury methylation in the aquatic environment. Ottawa, University of Ottawa. **PhD**.

Celo, V., D. R. S. Lean, et al. (2006). "Abiotic methylation of mercury in the aquatic environment." Science of the Total Environment **368**(1): 126-137.

Choi, S. C. and R. Bartha (1993). "Cobalamin-Mediated Mercury Methylation by Desulfovibrio-Desulfuricans Ls." Applied and Environmental Microbiology **59**(1): 290-295.

Choi, S. C., T. Chase, et al. (1994). "Metabolic Pathways Leading to Mercury Methylation in Desulfovibrio-Desulfuricans Ls." Applied and Environmental Microbiology **60**(11): 4072-4077.

Clarkson, T. W. (2002). "The three modern faces of mercury." Environmental Health Perspectives **110**: 11-23.

Compeau, G. and R. Bartha (1983). "Effects of Sea Salt Anions on the Formation and Stability of Methylmercury." Bulletin of Environmental Contamination and Toxicology **31**(4): 486-493.

Compeau, G. and R. Bartha (1984). "Methylation and Demethylation of Mercury under Controlled Redox, Ph, and Salinity Conditions." Applied and Environmental Microbiology **48**(6): 1203-1207.

Compeau, G. and R. Bartha (1985). "Sulfate-Reducing Bacteria - Principal Methylators of Mercury in Anoxic Estuarine Sediment." Applied and Environmental Microbiology **50**(2): 498-502.

Compeau, G. and R. Bartha (1987). "Effect of Salinity on Mercury-Methylating Activity of Sulfate-Reducing Bacteria in Estuarine Sediments." Applied and Environmental Microbiology **53**(2): 261-265.

Cossa, D., J. M. Martin, et al. (1997). "The distribution and cycling of mercury species in the western Mediterranean." Deep-Sea Research Part Ii-Topical Studies in Oceanography **44**(3-4): 721-740.

Craig, P. J. (1986). Organomercury compounds in the environment, Longman, Harlow.

Craig, P. J., and Moreton P.A. (1983). "Total mercury, methyl mercury and suphide in river carson sediments." Marine Pollution Bulletin **14**: 408-411.

De, A. K., Sen, A.K., Modak, D.P., Jana, S. (1985). "Studies of toxic effects of mercury(II) on pistia stratintes." Intl. J. of Water, Air, and Soil Pollution **24**: 351-360.

DeLaune RD, J. A., Devai I, Patrick WH Jr. (2004). "Relationship of sediment redox conditions to methyl mercury in surface sediment of Louisiana Lakes." J Environ Sci Health A Tox Hazard Subst Environ Eng. **39**(8): 1925-1933.

Desimone, R. E. (1972). "Methylation of Mercury by Common Nuclear Magnetic-Resonance Reference Compounds." Journal of the Chemical Society-Chemical Communications(13): 780-&.

Devai, I., R. D. Delaune, et al. (2001). "Changes in methylmercury concentration during storage: effect of temperature." Organic Geochemistry **32**(5): 755-758.

Drever, J. I. (1997). The Geochemistry of Natural Waters. Upper Saddle River , NJ Prentice Hall**:** 436

Drevnick, P. E. and M. B. Sandheinrich (2003). "Effects of dietary methylmercury on reproductive endocrinology of fathead minnows." Environmental Science & Technology **37**(19): 4390-4396.

Dunn, D. D. (1996). Trends in nutrient inflows to the Gulf of Mexico from streams draining the conterminous United States 1972–1993. U. G. Surv.

Eadie, B. J., B. A. Mckee, et al. (1994). "Records of Nutrient-Enhanced Coastal Ocean Productivity in Sediments from the Louisiana Continental-Shelf." Estuaries **17**(4): 754-765.

Ekstrom, E. B., F. M. M. Morel, et al. (2003). "Mercury methylation independent of the acetyl-coenzyme a pathway in sulfate-reducing bacteria." Applied and Environmental Microbiology **69**(9): 5414-5422.

Environmental Protection Agency, E.

Environmental Protection Agency, E. (2001). Mercury Updata:Impact on Fish Advisories. USEPA, Office of Water 4305.

Environmental Protection Agency, E. (2002). USEPA.

Fagerstr, T. and A. Jernelov (1972). "Some Aspects of Quantitative Ecology of Mercury." Water Research **6**(10): 1193-&.

Fitzgerald, W. F., D. R. Engstrom, et al. (1998). "The case for atmospheric mercury contamination in remote areas." Environmental Science & Technology **32**(1): 1-7.

Fitzgerald, W. F., Mason, R.P., Vandal, G.M., and F. Dulac (1994). Air-water cycling of mercury in lakes. Mercury Pollution-Integration and Synthesis. C. J. Watras, and W. Huckabee. Boca Raton, Fl., Lewis Publishers**:** 203-220.

Fleming, E. J., E. E. Mack, et al. (2006). "Mercury methylation from unexpected sources: Molybdate-inhibited freshwater sediments and an iron-reducing bacterium." Applied and Environmental Microbiology **72**(1): 457-464.

Food and Drug Administration, F. Mercury level in commercial fish and shellfish.

Fujiki, M. and S. Tajima (1992). "The Pollution of Minamata Bay by Mercury." Water Science and Technology **25**(11): 133-140.

Fukui, M. and S. Takii (1989). "Reduction of Tetrazolium Salts by Sulfate-Reducing Bacteria." Fems Microbiology Ecology **62**(1): 13-20.

Gagnon, C., E. Pelletier, et al. (1997). "Behaviour of anthropogenic mercury in coastal marine sediments." Marine Chemistry **59**(1-2): 159-176.

Gagnon, C., E. Pelletier, et al. (1996). "Diagenetic behavior of methylmercury in organic-rich coastal sediments." Limnology and Oceanography **41**(3): 428-434.

Gardner, L. R. (1974). "Organic Versus Inorganic Trace-Metal Complexes in Sulfidic Marine Water - Some Speculative Calculations Based on Available Stability-Constants." Geochimica Et Cosmochimica Acta **38**(8): 1297-1302.

Gilmour, C. C., E. A. Henry, et al. (1992). "Sulfate Stimulation of Mercury Methylation in Fresh-Water Sediments." Environmental Science & Technology **26**(11): 2281-2287.

Gilmour, C. C., G. S. Riedel, et al. (1998). "Methylmercury concentrations and production rates across a trophic gradient in the northern Everglades." Biogeochemistry **40**(2-3): 327-345.

Goldwater, L. J. (1972). Mercury: A History of Quicksilver. Baltimore, York Press.

Guimaraes, J. R. D., M. Meili, et al. (2000). "Mercury net methylation in five tropical flood plain regions of Brazil: high in the root zone of floating macrophyte mats but low in surface sediments and flooded soils." Science of the Total Environment **261**(1-3): 99-107.

Hall, B., N. S. Bloom, et al. (1995). "An Experimental-Study of 2 Potential Methylation Agents of Mercury in the Atmosphere - Ch3i and Dms." Water Air and Soil Pollution **80**(1-4): 337-341.

Hamasaki, T., H. Nagase, et al. (1995). "Formation, Distribution, and Ecotoxicity of Methylmetals of Tin, Mercury, and Arsenic in the Environment." Critical Reviews in Environmental Science and Technology **25**(1): 45-91.

Hammerschmidt, C. R. and W. F. Fitzgerald (2004). "Geochemical controls on the production and distribution of methylmercury in near-shore marine sediments." Environmental Science & Technology **38**(5): 1487-1495.

Harris, H. H., I. J. Pickering, et al. (2003). "The chemical form of mercury in fish." Science **301**(5637): 1203-1203.

Heinz, G. H. and D. J. Hoffman (1998). "Methylmercury chloride and selenomethionine interactions on health and reproduction in mallards." Environmental Toxicology and Chemistry **17**(2): 139-145.

Helly, J., and Lisa Levin (2004). "Global distribution of naturally occurring marine hypoxia on continental margins." Deep Sea Research **51**: 1159-1168.

Hintelmann, H., K. Keppel-Jones, et al. (2000). "Constants of mercury methylation and demethylation rates in sediments and comparison of tracer and ambient mercury availability." Environmental Toxicology and Chemistry **19**(9): 2204-2211.

Hou, A. (2003).

Hudson, R. J. M., Gherini, S. A., Watras, C. J., and D. B. Porcella (1994). Modeling the biogeochemical cycle of mercury in lakes: The Mercury Cycling Model (MCM) and its application to the MTL study lakes. Mercury Pollution — Integration and Synthesis. C. J. a. J. W. H. Watras. Boca Raton, FL, Lewis Publishers**:** 473-523.

Hunerlach, M. P. C. N. A., Mark Marvin-DiPasquale, Howard E. Taylor, and John F. De Wild (2004). Geochemistry of Mercury and other Trace Elements in Fluvial Tailings Upstream of Daguerre Point Dam, Yuba River, California, August 2001. Sacramento, U.S. GEOLOGICAL SURVEY.

Hurley, J. P., J. M. Benoit, et al. (1995). "Influences of Watershed Characteristics on Mercury Levels in Wisconsin Rivers." Environmental Science & Technology **29**(7): 1867-1875.

Hurley, J. P., C. J. Watras, et al. (1991). "Mercury Cycling in a Northern Wisconsin Seepage Lake - the Role of Particulate Matter in Vertical Transport." Water Air and Soil Pollution **56**: 543-551.

Hurley, J. P., Watras, C. J., and N. S. Bloom (1994). Distribution and flux of particulate mercury in four stratified seepage lakes. Mercury Pollution — Integration and Synthesis. C. J. W. a. J. W. Huckabee. Boca Raton, FL,, Lewis Publishers**:** 69-82.

Ingvorsen, K., J. G. Zeikus, et al. (1981). "Dynamics of Bacterial Sulfate Reduction in a Eutrophic Lake." Applied and Environmental Microbiology **42**(6): 1029-1036.

Jackson, T. (1993). "Effects of environmental factors and primary production on the distribution and methylation of mercury in a chain of highly eutrophic riverine lakes " Water Quality Research Journal of Canada **28**(1): 177-216.

Jay, J. A., F. M. M. Morel, et al. (2000). "Mercury speciation in the presence of polysulfides." Environmental Science & Technology **34**(11): 2196-2200.

Jay, J. A., K. J. Murray, et al. (2002). "Mercury methylation by Desulfovibrio desulfuricans ND132 in the presence of polysulfides." Applied and Environmental Microbiology **68**(11): 5741-5745.

Jensen, S. and A. Jernelov (1969). "Biological Methylation of Mercury in Aquatic Organisms." Nature **223**(5207): 753-&.

Jørgensen, B. B. (1982). "Mineralization of organic matter in the seabed—the role of sulphate reduction." Nature **296**: 643–645.

Kainz M. Lucotte M., Parrish C.C. (2003)."Relationship between organic matter composition and methyl mercury content of offfshore and carbon-rich littoral sediments in an oligotrophic lake. " Canadian Journal of Fisheris and Aquatic Sciences **60**(7):888-896.

Kannan, K. and J. Falandysz (1998). "Speciation and concentrations of mercury in certain coastal marine sediments." Water Air and Soil Pollution **103**(1-4): 129-136.

Kelly, C. A., J. W. M. Rudd, et al. (1997). "Increases in fluxes of greenhouse gases and methyl mercury following flooding of an experimental reservoir." Environmental Science & Technology **31**(5): 1334-1344.

Kelly, C. A., J. W. M. Rudd, et al. (2003). "Effect of pH on mercury uptake by an aquatic bacterium: Implications for Hg cycling." Environmental Science & Technology **37**(13): 2941-2946.

Kelly, C. A., J. W. M. Rudd, et al. (1995). "Is Total Mercury Concentration a Good Predictor of Methyl Mercury Concentration in Aquatic Systems." Water Air and Soil Pollution **80**(1-4): 715-724.

King, J. K., J. E. Kostka, et al. (2000). "Sulfate-reducing bacteria methylate mercury at variable rates in pure culture and in marine sediments." Applied and Environmental Microbiology **66**(6): 2430-2437.

King, J. K., J. E. Kostka, et al. (2001). "A quantitative relationship that remonstrates mercury methylation rates in marine sediments are based on the community composition and activity of sulfate-reducing bacteria." Environmental Science & Technology **35**(12): 2491-2496.

King, J. K., F. M. Saunders, et al. (1999). "Coupling mercury methylation rates to sulfate reduction rates in marine sediments." Environmental Toxicology and Chemistry **18**(7): 1362-1369.

Klassen, C. D. (2001). Casarett and Doull's Toxicology: The Basic Science of Poisons, McGraw-Hill Companies, Inc.

Kongchum, M., Wayne H. Hudnall, Ronald D.DeLaune (2005). Relationship between sediment clay minerals and total mercury content. Baton Rouge.

Korthals, E. T. and M. R. Winfrey (1987). "Seasonal and Spatial Variations in Mercury Methylation and Demethylation in an Oligotrophic Lake." Applied and Environmental Microbiology **53**(10): 2397-2404.

Lambertsson, L. and M. Nilsson (2006). "Organic material: The primary control on mercury methylation and ambient methyl mercury concentrations in estuarine sediments." Environmental Science & Technology **40**(6): 1822-1829.

Laws, E. A. (2003). Aquatic PollutionJohn Wiley & Sons,Inc.

Lee, Y. H. and H. Hultberg (1990). "Methylmercury in Some Swedish Surface Waters." Environmental Toxicology and Chemistry **9**(7): 833-841.

Lewi, M. A., Scott, G.I., Bearden, D.W., Quarles, R.L., Moore, J., Strozier, E.D., Sivertsen, S.K., Dias, A.R., Sanders, M (2002). "Fish tissue quality in near-coastal areas of the Gulf of Mexico receiving point source discharges" Sci. Total Environ **284**(1-3): 249-261.

Lindqvist, O., Jernelöv, A., Johansson, K., and Rohde, H. (1984). Mercury in the Swedish Environment. Global and local sources, National Swedish Environmental Protection Board.

Lindqvist, O., K. Johansson, et al. (1991). "Mercury in the Swedish Environment - Recent Research on Causes, Consequences and Corrective Methods." Water Air and Soil Pollution **55**(1-2): R11-&.

Linquist, J. (2003). The Most Probable Number Method.

Luczak, C., M. A. Janquin, et al. (1997). "Simple standard procedure for the routine determination of organic matter in marine sediment." Hydrobiologia **345**: 87-94.

Macalady, J. L., E. E. Mack, et al. (2000). "Sediment microbial community structure and mercury methylation in mercury-polluted Clear Lake, California." Applied and Environmental Microbiology **66**(4): 1479-1488.

Marvin-DiPasquale, M., J. Agee, et al. (2000). "Methyl-mercury degradation pathways: A comparison among three mercury-impacted ecosystems." Environmental Science & Technology **34**(23): 4908-4916.

Mason, R. P. and W. F. Fitzgerald (1990). "Alkylmercury Species in the Equatorial Pacific." Nature **347**(6292): 457-459.

Mason, R. P. and W. F. Fitzgerald (1993). "The Distribution and Biogeochemical Cycling of Mercury in the Equatorial Pacific-Ocean." Deep-Sea Research Part I-Oceanographic Research Papers **40**(9): 1897-1924.

Mason, R. P., W. F. Fitzgerald, et al. (1994). "The Biogeochemical Cycling of Elemental Mercury - Anthropogenic Influences." Geochimica Et Cosmochimica Acta **58**(15): 3191-3198.

Mason, R. P., K. R. Rolfhus, et al. (1998). "Mercury in the North Atlantic." Marine Chemistry **61**(1-2): 37-53.

Matilainen, T. and M. Verta (1995). "Mercury Methylation and Demethylation Waters." Canadian Journal of Fisheries and Aquatic Sciences **52**(8): 1597-1608.

Meade, R. H. (1995). Contaminants in the Mississippi River, 1987-92. U. S. G. Survey, U.S. Geological Survey Circular 1133**:** 140.

Mee, L. D. (2001). Eutrophication in the Black Sea and a basin-wide approach to its control. Science and Integrated Coastal Management. B. R. T. von Bodungen**:** 71-91.

Meili, M. (1997). "Mercury in lakes and rivers." Metal Ions in Biological Systems, Vol 34 **34**: 21-51.

Mhatre, G. N. and S. B. Chaphekar (1985). "The Effect of Mercury on Some Aquatic Plants." Environmental Pollution Series a-Ecological and Biological **39**(3): 207-216.

Miskimmin, B. M. (1991). "Effect of Natural Levels of Dissolved Organic-Carbon (Doc) on Methyl Mercury Formation and Sediment Water Partitioning." Bulletin of Environmental Contamination and Toxicology **47**(5): 743-750.

Miskimmin, B. M., J. W. M. Rudd, et al. (1992). "Influence of Dissolved Organic-Carbon, Ph, and Microbial Respiration Rates on Mercury Methylation and Demethylation in Lake Water." Canadian Journal of Fisheries and Aquatic Sciences **49**(1): 17-22.

Morel, F. M. M., A. M. L. Kraepiel, et al. (1998). "The chemical cycle and bioaccumulation of mercury." Annual Review of Ecology and Systematics **29**: 543-566.

Mudryk, Z. J., Beata Podgorska, and jerzy Bolalek (2000). "The occurrence and activity of sulphate-reducing bacteria in the bottom sediments of the Gulf of Gdansk." Oceanologia **42**(1): 105-117.

National Science and Technology Council Committee on the Environmental and Natural Resources, N. (2004). Methylmercury in the Gulf of Mexico: State of Knowledge and

Research Needs. Interagency Working Group on Methylmercury, National Science and Technology Council Committee**:** 1-21.

Neff, J. (2002) "Influence of offshore oil and gas platforms on environmental risks of mercury in the Gulf of Mexico."

Olson, B. H., and Cooper, R. C. (1974). "In situ methylation of mercury by estuarine sediment." Nature(252): 682.

Olson, B. H. and R. C. Cooper (1976). "Comparison of Aerobic and Anaerobic Methylation of Mercuric-Chloride by San-Francisco Bay Sediments." Water Research **10**(2): 113-116.

Open University (1989). The Seawater Solution. Seawater: Its Composition, Properties and Behaviour, Pergamon Press.

Oremland, R. S., C. W. Culbertson, et al. (1991). "Methylmercury Decomposition in Sediments and Bacterial Cultures - Involvement of Methanogens and Sulfate Reducers in Oxidative Demethylation." Applied and Environmental Microbiology **57**(1): 130-137.

Pak, K. R. and R. Bartha (1998). "Mercury methylation and demethylation in anoxic lake sediments and by strictly anaerobic bacteria." Applied and Environmental Microbiology **64**(3): 1013-1017.

Rabalais, N. N. (2005). PRESS RELEASE, JULY 29, 2005
LOUISIANA UNIVERSITIES MARINE CONSORTIUM (LUMCON).

Rabalais, N. N., R. E. Turner, et al. (2002). "Nutrient-enhanced productivity in the northern Gulf of Mexico: past, present and future." Hydrobiologia **475**(1): 39-63.

Rabalais, N. N., R. E. Turner, et al. (2001). "Hypoxia in the Gulf of Mexico." Journal of Environmental Quality **30**(2): 320-329.

Rabalais, N. N., R. E. Turner, et al. (2002). "Gulf of Mexico hypoxia, aka "The dead zone"." Annual Review of Ecology and Systematics **33**: 235-263.

Rabalais, N. N., W. J. Wiseman, et al. (1996). "Nutrient changes in the Mississippi River and system responses on the adjacent continental shelf." Estuaries **19**(2B): 386-407.

Ramlal, P. S., J. W. M. Rudd, et al. (1985). "The Effect of Ph on Methyl Mercury Production and Decomposition in Lake-Sediments." Canadian Journal of Fisheries and Aquatic Sciences **42**(4): 685-692.

Rasmussen, P. E. (1994). "Current Methods of Estimating Atmospheric Mercury Fluxes in Remote Areas." Environmental Science & Technology **28**(13): 2233-2241.

Regnell, O., G. Ewald, et al. (1997). "Factors controlling temporal variation in methyl mercury levels in sediment and water in a seasonally stratified lake." Limnology and Oceanography **42**(8): 1784-1795.

Regnell, O. and A. Tunlid (1991). "Laboratory Study of Chemical Speciation of Mercury in Lake Sediment and Water under Aerobic and Anaerobic Conditions." Applied and Environmental Microbiology **57**(3): 789-795.

Renaud, M. L. (1986). "Hypoxia in Louisiana Coastal Waters during 1983 - Implications for Fisheries." Fishery Bulletin **84**(1): 19-26.

Rosenberg, R. (1985). "Eutrophication - the Future Marine Coastal Nuisance." Marine Pollution Bulletin **16**(6): 227-231.

Rudd, J. W. M. (1995). "Sources of Methyl Mercury to Fresh-Water Ecosystems - a Review." Water Air and Soil Pollution **80**(1-4): 697-713.

Schroeder, W., O. Lindqvist, et al. (1992). "Volatilization of Mercury from Lake Surfaces." Science of the Total Environment **125**: 47-66.

Sellers, P., C. A. Kelly, et al. (1996). "Photodegradation of methylmercury in lakes." Nature **380**(6576): 694-697.

Slemr, F. and H. E. Scheel (1998). "Trends in atmospheric mercury concentrations at the summit of the Wank mountain, southern Germany." Atmospheric Environment **32**(5): 845-853.

Soil Survey Staff (2004). Soil Survey Laboratory Manual. Soil Survey Investigations. R. Burt.

St Louis, V. L., J. W. M. Rudd, et al. (2004). "The rise and fall of mercury methylation in an experimental reservoir." Environmental Science & Technology **38**(5): 1348-1358.

Steffan, R. J., E. T. Korthals, et al. (1988). "Effects of Acidification on Mercury Methylation, Demethylation, and Volatilization in Sediments from an Acid-Susceptible Lake." Applied and Environmental Microbiology **54**(8): 2003-2009.

Stlouis, V. L., J. W. M. Rudd, et al. (1994). "Importance of Wetlands as Sources of Methyl Mercury to Boreal Forest Ecosystems." Canadian Journal of Fisheries and Aquatic Sciences **51**(5): 1065-1076.

Stumm, W. a. M., J. J. (1996). Aquatic Chemistry-Chemical Equilibria and Rates in Natural Waters New York, Wiley Interscience.

Trefry, J. H., Robert, P. Trocine, Michelle, L. McElvaine, and Robert, D. Rember (2002). Concentrations of Total Mercury and Methylmercury in Sediment Adjacent to Offshore Drilling Sites in the Gulf of Mexico. Final Report to the Synthetic Based Muds (SBM) Research Group**:** 1-47.

Ullrich, S. M., T. W. Tanton, et al. (2001). "Mercury in the aquatic environment: A review of factors affecting methylation." Critical Reviews in Environmental Science and Technology **31**(3): 241-293.

United Nations Environmental Programme, U. (2002). Chemicals. UNEP.

Vandal, G. M., R. P. Mason, et al. (1991). "Cycling of Volatile Mercury in Temperate Lakes." Water Air and Soil Pollution **56**: 791-803.

Vandal, G. M., R. P. Mason, et al. (1998). "Mercury speciation and distribution in a polar desert lake (Lake Hoare, Antarctica) and two glacial meltwater streams." Science of the Total Environment **213**(1-3): 229-237.

Varekamp, J. C., Buseck, P.R. (1986). "Global mercury flux from volcanic and geothermal sources." Appl. Geochem. **1**: 65-73.

Weber, J. H. (1993). "Review of Possible Paths for Abiotic Methylation of Mercury(Ii) in the Aquatic Environment." Chemosphere **26**(11): 2063-2077.

Widdel, F., Friedhelm, B. (1999). Gram-negative mesophilic sulfate-reducing bacteria. The Prokaryotes, Springer Verlag, New York.

Winfrey, M. R. and J. W. M. Rudd (1990). "Environmental-Factors Affecting the Formation of Methylmercury in Low Ph Lakes." Environmental Toxicology and Chemistry **9**(7): 853-869.

Wood, J. M., Kennedy, P. S., and Rosen, C. G. (1968). "Synthesis of methylmercury compounds by extracts of a methanogenic bacterium." Nature **220**: 173- 174.

World Health Organization, W. (1989). Mercury - Environmental Aspects. Environmental Health Criteria 86. WHO.

Wright, D. R. and R. D. Hamilton (1982). "Release of Methyl Mercury from Sediments - Effects of Mercury Concentration, Low-Temperature, and Nutrient Addition." Canadian Journal of Fisheries and Aquatic Sciences **39**(11): 1459-1466.

Xun, L., N. E. R. Campbell, et al. (1987). "Measurements of Specific Rates of Net Methyl Mercury Production in the Water Column and Surface Sediments of Acidified and Circumneutral Lakes." Canadian Journal of Fisheries and Aquatic Sciences **44**(4): 750-757.

APPENDIX: PARTICLE SIZE DISTRIBUTION

Station=C4				
		Particle Size Distribution		
Month	Texture Class	sand(%)	silt(%)	clay(%)
Apr-05	Silty Clay Loam	19.4	49.5	31.1
Apr-05	Clay Loam	28.4	43.9	27.7
Apr-05	Silty Clay	2.2	46.5	51.3
May-05	Clay Loam	21.9	48.1	30.0
May-05	Loam	39.3	39.1	21.6
May-05	Loam	28.6	46.9	24.5
Jun-05	Silty Clay Loam	13.3	55.1	31.6
Jun-05	Silty Clay Loam	15.7	50.9	33.4
Jun-05	Clay Loam	23.1	48.8	28.1
Jul-05	Loam	46.5	39.1	14.4
Jul-05	Silt Loam	24.9	53.5	21.6
Jul-05	Silt Loam	23.8	60.0	16.2
Aug-05	Silty Clay Loam	8.4	57.9	33.7
Aug-05	Silty Clay Loam	4.7	58.0	37.3
Aug-05	Silty Clay Loam	5.6	63.4	31.0
Oct-05	Silty Clay	1.1	49.3	49.6
Oct-05	Silty Clay	1.5	49.0	49.5
Oct-05	Silty Clay	6.3	47.6	46.1
Jan-06	Silty Clay Loam	6.9	54.7	38.4
Jan-06	Silt Loam	15.1	67.2	17.7
Jan-06	Silt Loam	7.7	66.3	26.0
Apr-06	Silt Loam	34.3	56.3	9.4
Apr-06	Silt Loam	16.3	70.7	13.0
Apr-06	Silt Loam	23.6	63.5	12.9

Station=C6B				
		Particle Size Distribution		
Month	Texture Class	sand(%)	silt(%)	clay(%)
Apr-05	Sandy Clay Loam	51.2	26.0	22.8
Apr-05	Clay Loam	24.8	44.6	30.6
May-05	Loam	27.5	46.2	26.3
May-05	Clay Loam	32.0	34.1	33.9
May-05	Clay Loam	28.0	37.5	34.5
Jun-05	Silty Clay Loam	17.6	46.8	35.6
Jun-05	Silty Clay Loam	17.8	47.7	34.5
Jun-05	Silty Clay Loam	17.0	49.3	33.7
Jul-05	Loam / Clay Loam	40.0	33.0	27.0
Jul-05	Clay Loam	39.1	32.5	28.4
Jul-05	Clay Loam	21.2	44.0	34.8
Aug-05	Sandy Clay Loam	49.3	26.3	24.4
Aug-05	Clay Loam	38.9	31.3	29.8
Aug-05	Sandy Clay Loam	46.3	27.1	26.6

(table continued)

Oct-05	Clay	2.8	38.1	59.1
Oct-05	Clay	0.9	38.5	60.6
Oct-05	Clay	1.5	39.2	59.3
Jan-06	Silty Clay	1.9	44.2	53.9
Jan-06	Clay	0.7	39.9	59.4
Jan-06	Clay	0.8	39.7	59.5
Apr-06	Clay	0.9	39.3	59.8
Apr-06	Clay	1.3	39.9	58.8
Apr-06	Clay	1.5	38.9	59.6

Station=C8				
		Particle Size Distribution		
Month	Texture Class	Sand(%)	silt(%)	clay(%)
Apr-05	Sand	98.8	0.1	0.8
Apr-05	Sand	98.4	0.5	0.8
May-05	Sand	96.9	0.9	1.1
May-05	Sand	94.8	3.2	1.1
May-05	Sand	98.0	1.2	1.1
Jun-05	Sandy Loam	63.5	22.6	1.6
Jun-05	Sandy Loam	66.1	15.3	1.8
Jun-05	Sand	97.5	1.8	2.0
Jul-05	Sand	98.6	0.6	2.1
Jul-05	Loamy Sand	85.4	9.3	2.2
Jul-05	Sand	93.2	3.9	2.4
Aug-05	Sand	89.3	4.6	2.9
Aug-05	Sand	92.7	2.7	3.6
Aug-05	Silty Clay Loam	18.7	43.8	4.6
Oct-05	Sandy Clay Loam	53.7	22.4	4.8
Oct-05	Sand	97.4	1.0	5.0
Oct-05	Sand	94.0	3.6	5.3
Jan-06	Sand	96.4	1.5	6.1
Jan-06	Sand	97.8	1.1	8.6
Jan-06	Sand	93.1	3.2	13.9
Apr-06	Loamy Sand	79.0	12.4	18.6
Apr-06	Loamy Sand	86.0	9.2	23.8
Apr-06	Sand	89.1	5.9	37.5

VITA

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