MERCURY UPTAKE BY WILD RICE PLANTS IN NORTHERN MINNESOTA

A Thesis

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Dedication

This thesis is dedicated to my family.

Abstract

Mercury can be methylated by microbes into the toxic, organic form of methylmercury. Previous studies have shown that not only is MeHg a human health concern through the consumption of fish, but it is also a concern in Asian countries due to its presence in white rice. Rice plants grow in flooded conditions that, along with plant decay, enhance microbial activities, and this involves the methylation of inorganic Hg by certain species of bacteria and archaea. The MeHg produced is then exported into surrounding lake sediments, where it can then be taken up by rice plant through the same root processes used for the taking up of other heavy metals and growth substrates. Studies of white rice plants have shown that MeHg accumulates preferentially in the rice grain, which is the part of the plant that is ultimately consumed by humans. In Minnesota, it is estimated that 2-4 million lbs. of wild rice is harvested each year and is a major staple in the diet of the Native American community.

In order to determine if there is a toxicity risk involved with consuming Minnesotan wild rice, six rice lakes were studied in northern Minnesota. Two of these lakes sit on mineral-rich glacial till on the White Earth Reservation in northwestern Minnesota, while the remaining four lakes are located on organic-rich glacial till on the Fond du Lac Reservation and the 1854 Treaty Area in northeastern Minnesota. Surface water samples, surface lake sediment samples, and wild rice samples were analyzed for total Hg and MeHg. The lake sediments on the White Earth Reservation showed a significantly lower amount of THg than the Fond du Lac and 1854 Treaty Area lake

sediments, although the MeHg in the sediments across the six lakes were much more consistent. The THg and MeHg values in the wild rice grains were also consistent across the six lakes, and this suggests the geologic setting appears to have no effect on the amount of Hg taken up by rice plants in Minnesota. Using the wild rice grain data from this study and a daily intake chronic exposure formula, it was determined that bioaccumulation through the consumption of Minnesota wild rice is extremely low compared to the IRIS Toxnet oral reference doses for both THg and MeHg.

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CHAPTER 1: INTRODUCTION

1.1 A Review of Hg

Mercury (Hg) is a unique metal that exists in the environment in various physical and chemical forms, such as elemental Hg (Hg^0), inorganic Hg (Hg^{2+}) and organic Hg (Azevedo, 2012). The only metal that is in liquid state at room temperature, Hg is soluble in water and this allows it to easily move in several ecosystems (Azevedo, 2012). A naturally occurring element, Hg can be emitted into the atmosphere as well, and human activity has increased atmospheric concentrations and deposition by a factor of 7 to 10 worldwide (Krabbenhoft, 2013).

Natural sources account for only one third of atmospheric Hg concentrations and include volcanic eruptions, forest fires, biomass burning, and low temperature volatilization (Hsu-Kim, 2013). Anthropogenic sources account for the remaining concentrations of atmospheric Hg and include fossil fuel combustion, mining, waste disposal, and chemical production (Hsu-Kim, 2013). Most Hg released to the atmosphere is in its gaseous elemental form and has an atmospheric lifetime of 6 to 12 months, which allows global mixing and transport before deposition in waters and sediments, where it already occurs as a natural source (Krabbenhoft, 2013). The average crustal content of Hg is 70 ng/g and once in the right environment, Hg can be converted into organic methylmercury (MeHg), CH_3Hg^+ (Kabatas-Pendias, 2011). The major form of Hg in the aquatic environment is Hg^{2+}, and MeHg is only a small proportion of total Hg in water and sediments (Hsu-Kim, 2013).

Considered a global pollutant by the 1990 US Clean Air Act Amendment, Hg is identified as one of the trace elements listed in the legislation as “hazardous air pollutant” because of its toxicity, availability, potential bio-accumulation within the environment, and human health risk (Kabatas-Pendias, 2011). Generally a toxic metal, Hg becomes even more toxic when it is methylated and this form accumulates in wildlife (Azevedo, 2012). There has been much concern and research done on the uptake of Hg by fish and how it affects humans when the fish is consumed. Small quantities of MeHg can be hazardous to human health when it is ingested through the consumption of fish (Tangahu, 2011). However, the consumption of fish isn't the only dietary pathway of MeHg into the human body. MeHg can also be taken up by humans through the consumption of plants, and rice is a large part of certain cultures' diets. This can be wild rice (*Zizania palustris*) for Native Americans or white rice (*Oryza sativa*) for various Asian cultures, where more than 2 billion people get up to 70% of their daily dietary energy from rice (Zhang, 2010). There has been less research done on MeHg uptake by wild rice, thus research is needed to find its main pathway into the plants, which part of plants contain the most MeHg, and what possible affect this has on humans when wild rice is ingested.

1.2 Methylating Bacteria

Rice plants can grow in flooded conditions that would drown other plants, and these conditions enhance microbial activity and elevate the level of micronutrients taken up by plants (Yin, 2013). Heavy metals are natural components in soil and can be micronutrients necessary for plant growth (Tangahu, 2011). Furthermore, metal nanoparticles are the dominant metal species in the environment, and the constituents of

anoxic surface water and sediment porewater include dissolved molecules, amorphous (non-crystalline) nanoparticles, and larger (perhaps crystalline) particles (Hsu-Kim, 2013). It is believed that cinnabar (HgS) is the major form of dissolved Hg in sediment and porewater (Hsu-Kim, 2013).

Sulfur-reducing bacteria actively occur in paddy soil and are a major contributor in the methylation of Hg, along with organic matter and pH levels (Zhang, 2010). MeHg yields are elevated in rice paddies, where the flooded conditions and plant decay promote the methylation of Hg (Yin, 2013). This is done intracellularly by sulfur-reducing bacteria (Schaefer, 2014). The hgcA and hgcB genes were recently discovered and seem to be common among all known methylating microorganisms, which provides a molecular marker for these organisms (Schaefer, 2014). Therefore, not all sulfur-reducing bacteria can methylate Hg, and the methylation rates generally depend on the productivity of anaerobic microorganism and the bioavailability of Hg^{2+} that can be taken up by these bacteria (Hsu-Kim, 2013). In fact, it is likely that only a small proportion of Hg^{2+} in anaerobic settings is available for uptake by methylating bacteria (Hsu-Kim, 2013).

Since methylation of Hg is intracellular, transport from extracellular surroundings through the outer and inner membranes of microorganisms is important, and the uptake of Hg^{2+} is believed to be mediated by transport proteins or passive diffusion (Hsu-Kim, 2013). It is thought that dissolved organic matter (DOM) stabilizes small HgS particles (nanoparticles) against aggregation, and HgS is thus more bioavailable to Hg methylating

bacteria (Graham, 2012,). These "filter passing" H-S-DOM clusters are likely the dominant forms of Hg^{2+} in anoxic sediment and sediment porewater where MeHg is produced (Graham, 2012). Therefore, there are positive correlations between dissolved organic carbons (DOC) and MeHg production in many aquatic sediments, especially those associated with wetlands.

Once MeHg is produced by sulfur-reducing bacteria, it is rapidly exported to surrounding sediment and porewater, which might represent a detoxification strategy for the bacteria (Graham, 2012). Microorganisms can also demethylate Hg through reductions and oxidization (Graham, 2012). In fact, the demethylation of MeHg could provide a balance to the methylation of inorganic Hg, thus reducing the likelihood of a runaway effect for MeHg production. Also, the environment plays a role as to which microbes are responsible for methylating mercury. Sulfur-reducing bacteria might be the dominant contributor, but iron-reducing bacteria and methanogens must be considered, as they also have been known to methylate mercury (Schaefer, 2014).

1.3 Plant Uptake

The state grain of Minnesota, wild rice (*manoomin* to the Ojibwe), is found in abundance in the state largely due to a suitable habitat, including a favorable climate. Wild rice has the most productivity in flowing waters with a pH of 6.0-8.0 and a depth of up to about a meter. Wild rice is an annual plant, as it develops in the early spring from seeds that dropped the previous fall, when it is harvested. From the germination of a new plant to the dropping of mature seeds, the entire process requires 110-130 days, and the

seeds are ripe for several weeks to longer than a month. Therefore, over the course of a summer, a wild rice plant will go from floating vegetation to fully emergent. (MNDNR, 2008)

Although most of the research done on Hg uptake by rice plants has been done on white rice plants, this research provides pertinent information on rice plants, exploring how they take up various forms of Hg. The uptake of MeHg by rice plants depends on sediment alteration and plant species (Azevedo, 2012). White rice plant uptake of Hg in the sediment might be via the same process in which plants uptake other growth substrates through rhizofiltration, or root process (Tangahu, 2011). Water that evaporates from plant leaves can serve as a pump to absorb soil substances into plant roots, and this process is responsible for moving contaminants into plant shoots (Tangahu, 2011). As MeHg binds with sulfur and nitrogen ligands, it is thought to enter the cell through ionic channels while competing with other heavy metals (Azevedo, 2012).

Once inside a white rice plant, MeHg is accumulated preferentially by the rice grain part of the plant (Zhang, 2010, Figure 1-1). This is compared to inorganic Hg (IHg), which is accumulated in the roots and leaves of these rice plants, the latter done so by atmospheric adsorption (Zhang, 2010, Figure 1-1). Because the rice grain is the part of the plant that is harvested and consumed, rice may become a major pathway of MeHg to humans, and the average absorption rate of MeHg by the human body is 95% (Zhang, 2010). Once in the human body, Hg binds with DNA and this may lead to chromosomal damage in humans (Azevedo, 2012).

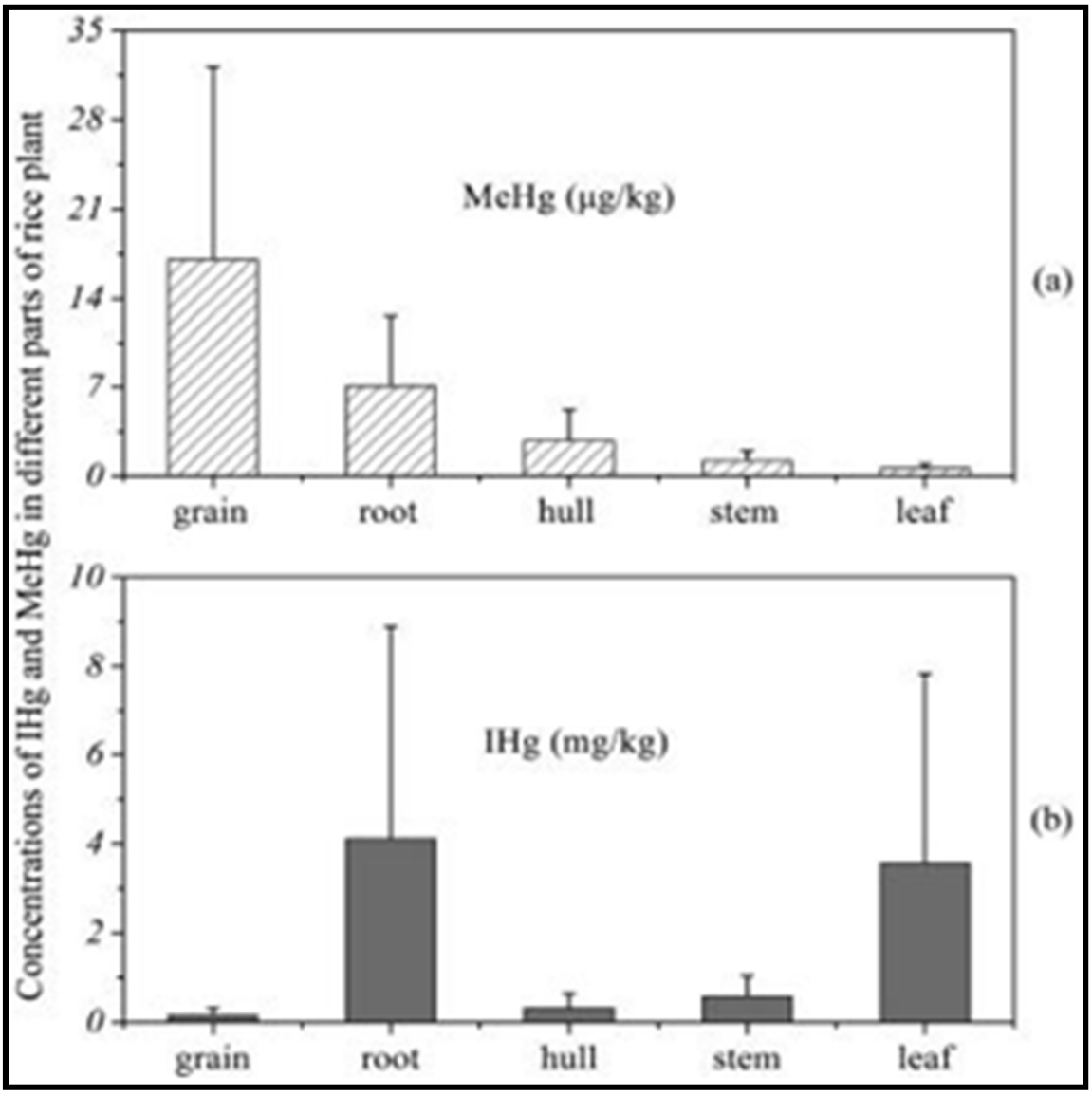

Figure 1-1: Concentrations of MeHg (a) and IHg (b) in different parts of rice plants from selected sites in Wanshan Hg mining area in China. (Zhang, 2010)

1.4 Harm to Humans

In Minnesota, it is thought that more than 3,000 tribal members participate in the harvest of wild rice in addition to approximately 1,500 state licensed harvesters in recent years. From 2004-2006, the average annual harvest per state licensed harvester was 430 pounds per individual, which suggests that 4,000-5,000 individuals harvest approximately 2 million pounds per year. In fact, from 1940-1972, it is estimated that the annual harvest of wild rice in Minnesota ranged from 20,000-4,000,000 pounds of unprocessed grain. Each pound of wild rice yields up to ten and a half cups of cooked rice. Therefore, wild rice has become a staple in the diets of many Minnesota residents, especially on Minnesota reservations, where a rise in diabetes has caused a need for a more natural diet including fish and wild rice. While fish remains a MeHg risk, it is unclear whether or not the inclusion of large amounts of wild rice adds to this risk through bioaccumulation. (MNDNR, 2008)

Research has been conducted to determine the levels of Hg in infants in the Lake Superior Basin, which includes Minnesota, Wisconsin, and Michigan. Dried fetal cord blood spots were analyzed for infants over these three states to provide information on Hg exposure to developing fetuses. Of the 1,465 samples analyzed, 1,126 of them were from Minnesota and the Hg concentrations were highest for the Minnesota samples. Eight percent of the samples analyzed were above 5.8 ug/l, which is the U.S. EPA Reference Dose for MeHg (Figure 1-2). The study suggests that further research is needed in order to investigate the exposure pathways. (McCann, 2011)

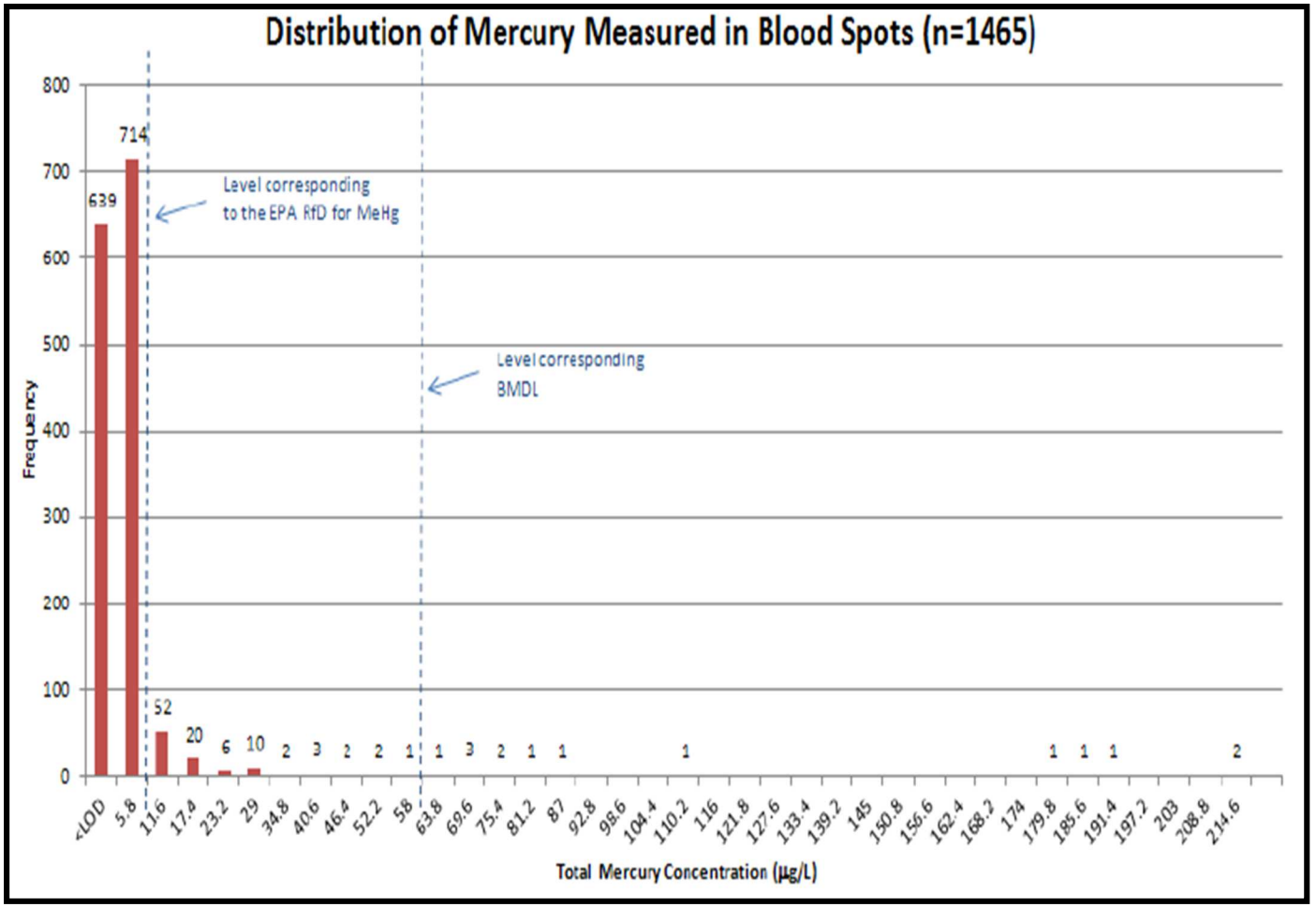

Figure 1-2: Total mercury in all blood spot specimens. (McCann, 2011)

1.5 Project Outline

This study focuses on the levels of MeHg taken up by wild rice plants on the White Earth Reservation, the Fond du Lac Reservation, and the 1854 Treaty Area. The White Earth Reservation is located in northwestern Minnesota, where woodlands meet prairie environments (Figure 1-3). The Fond du Lac Reservation is located within the 1854 Treaty Area, which covers much of northeastern Minnesota, and is dominated by woodlands on poorly developed soils (Figure 1-4). The 1854 Treaty Area consists of territory in northeastern Minnesota ceded to the United States and protected, while allowing hunting, fishing, and gathering by the Fond du Lac, Grand Portage, and Bois Forte Bands of the Lake Superior Chippewa.

The ecoregions of Minnesota can be seen in Figure 1-5 below. Many lakes in western Minnesota have a pH of around 8 and exhibit sediments containing calcium-carbonates ($CaCO_3$). These prairieland lakes exhibit a lake bottom consisting of mineral-rich sediment, and sediment is known to have low values of Hg (Engstrom, 1994). Lakes in northeastern Minnesota tend to have a pH of around 7 and the sediments are rich in organic matter and heavy metals, containing little to no calcium-carbonates and high amounts of iron. Therefore, this study compares MeHg uptake by these two different ecosystems (woodland watersheds and prairie pothole lakes) in order to see if geology plays a role in the amount of MeHg taken up by rice plants. The highest levels of blood spot Hg in the data mentioned above was collected near the Grand Portage Reservation and Ceded Territories, where rice is harvested (McCann, 2011). Due to MeHg bioaccumulation concerns, this study will also look to answer the question of whether or

not MeHg in Minnesota wild rice poses a human health risk.

This study will comprise of sampling lakes in each area, and this will include surface water samples, surface sediment samples, and wild rice grain samples. The surface water and sediment samples will be used to provide context to results obtained from the analysis of the wild rice grains. Analyzing the wild rice grains will provide an awareness of the amount of MeHg that accumulates in the part of the plant that is consumed, which will allow for any toxicity through bioaccumulation exposure to be determined. All samples will be analyzed for concentrations of total Hg and MeHg. In order to obtain insight into the microbial community, a lake core will be taken from one of the lakes and analyzed to determine the relative abundance of mercury methylating *Methanomicrobia* and *Deltaproteobacteria*, which contain clades abundant in sulfur and iron reducers.

Figure 1-3 Map showing the locations of the White Earth Reservation in Minnesota. (Tribes: White Earth, 2012)

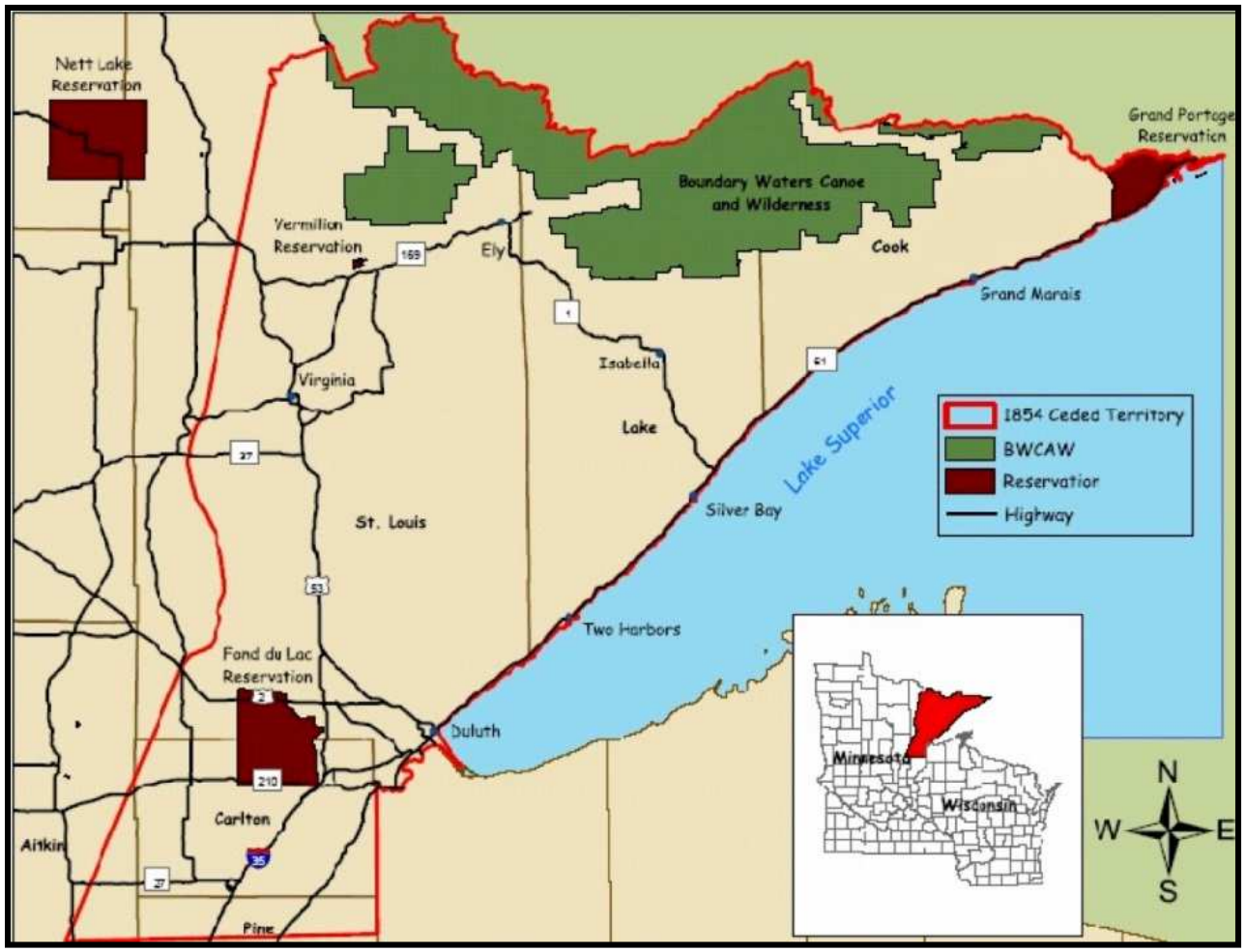

Figure 1-4 Map showing the location of the 1854 Treaty Area and the included Fond du Lac Reservation in Minnesota. (1854 Maps)

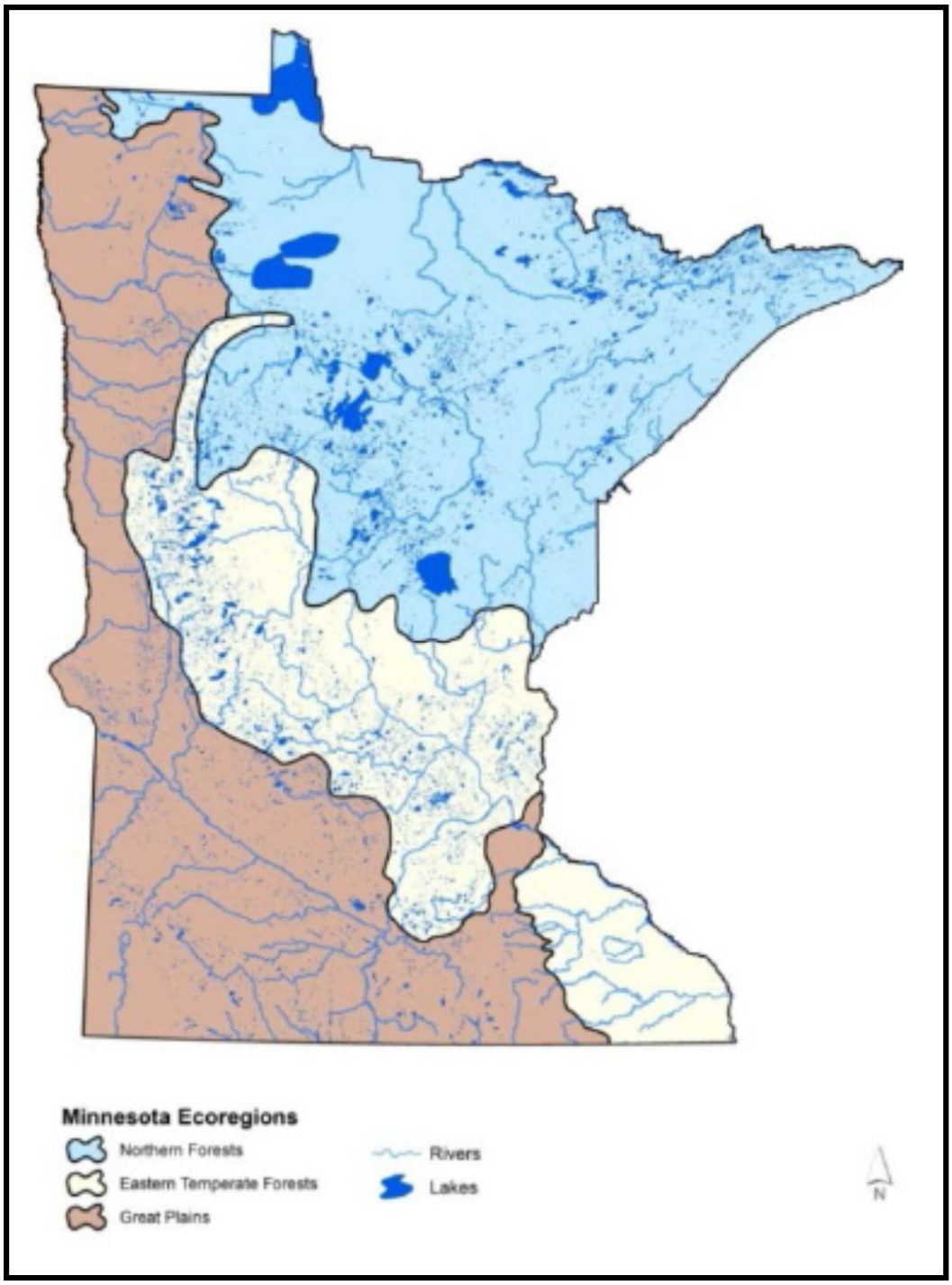

Figure 1-5 Minnesota water bodies and ecoregions from right to left, Northern Forests, Eastern Temperate Forests, and Great Plains. (MNDNR, 2008)

CHAPTER 2: METHODS

2.1 Sampling Locations

Sampling was conducted in the summer and fall of 2014 in northern Minnesota (Figure 2-1). The three major areas sampled included: the Fond du Lac Reservation (July 17, 2014), the White Earth Reservation (September 19, 2014) and the 1854 Treaty Area (October 4, 2014). Six lakes were sampled (two per area): Perch Lake and Dead Fish Lake (Fond du Lac Reservation), Lower Rice Lake and Long Lake (White Earth Reservation), and Clark Lake and Wolf Lake (1854 Treaty Area). The six lakes were sampled in the order listed above and four sites were sampled in each lake, as seen below (Figure 2-2 through Figure 2-7).

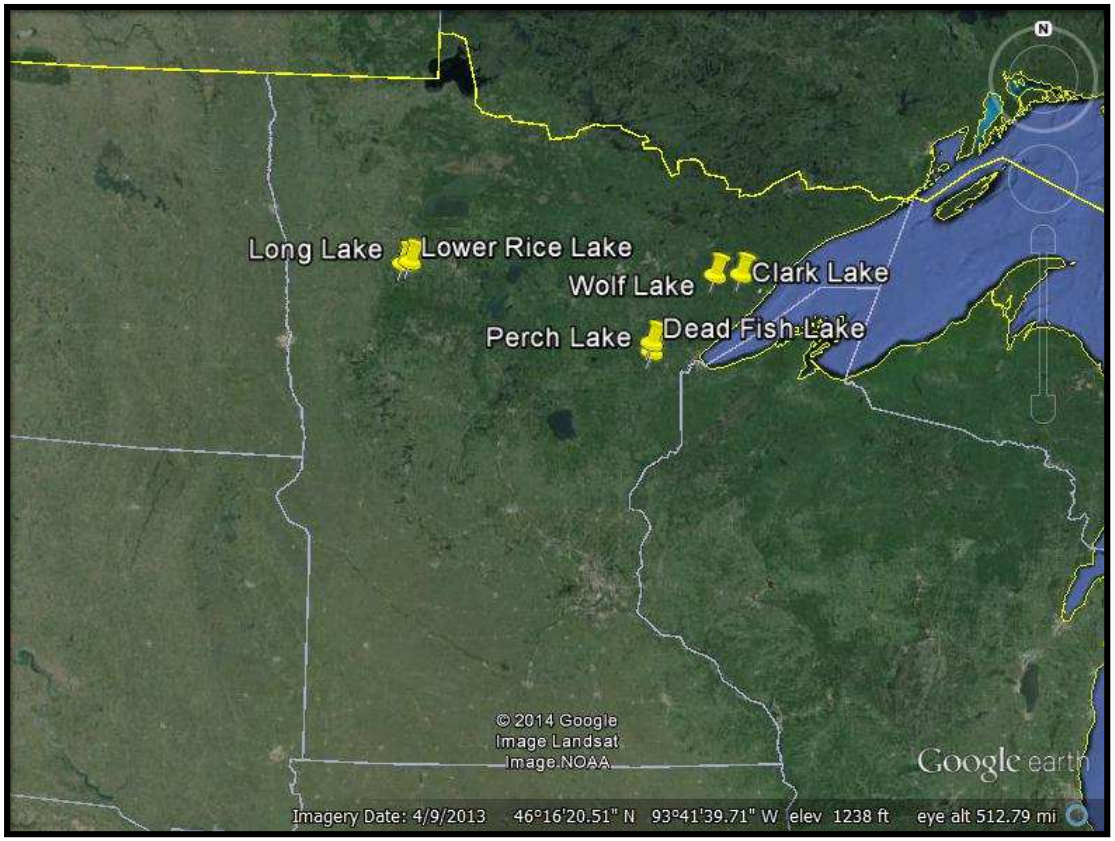

Figure 2-1 Google Earth image of the location of all six lakes sampled (Lower Rice Lake, Long Lake, Perch Lake, Dead Fish Lake, Clark Lake, and Wolf Lake).

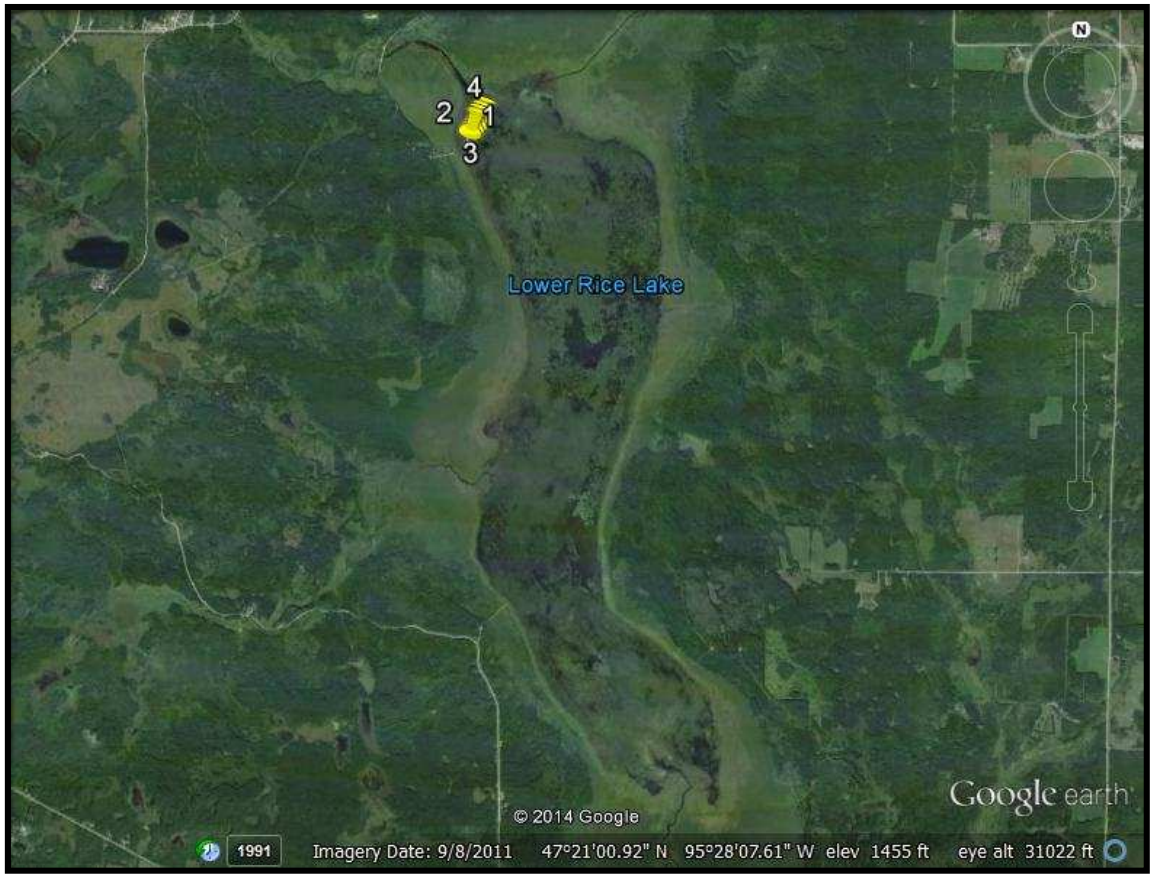

Figure 2-2 Google Earth image of the four sampling locations within Lower Rice Lake.

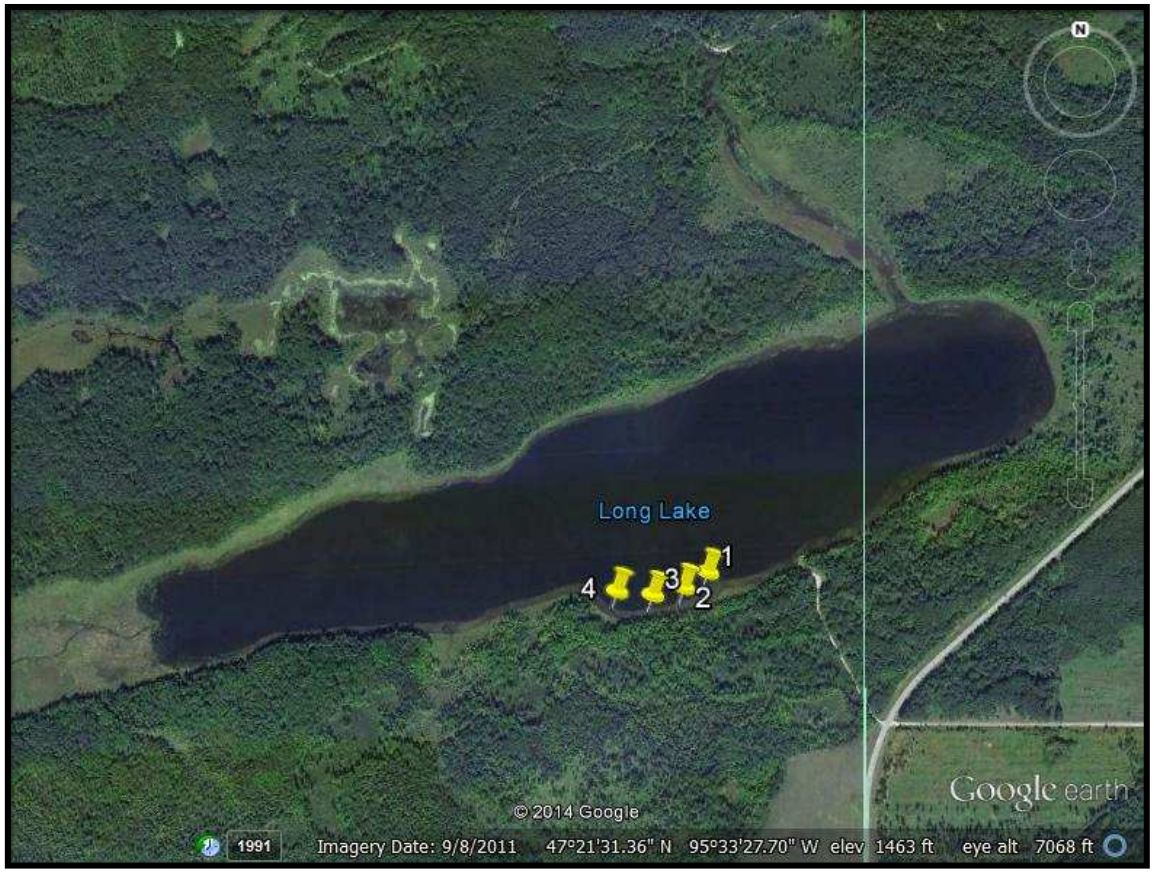

Figure 2-3 Google Earth image of the four sampling locations within Long Lake.

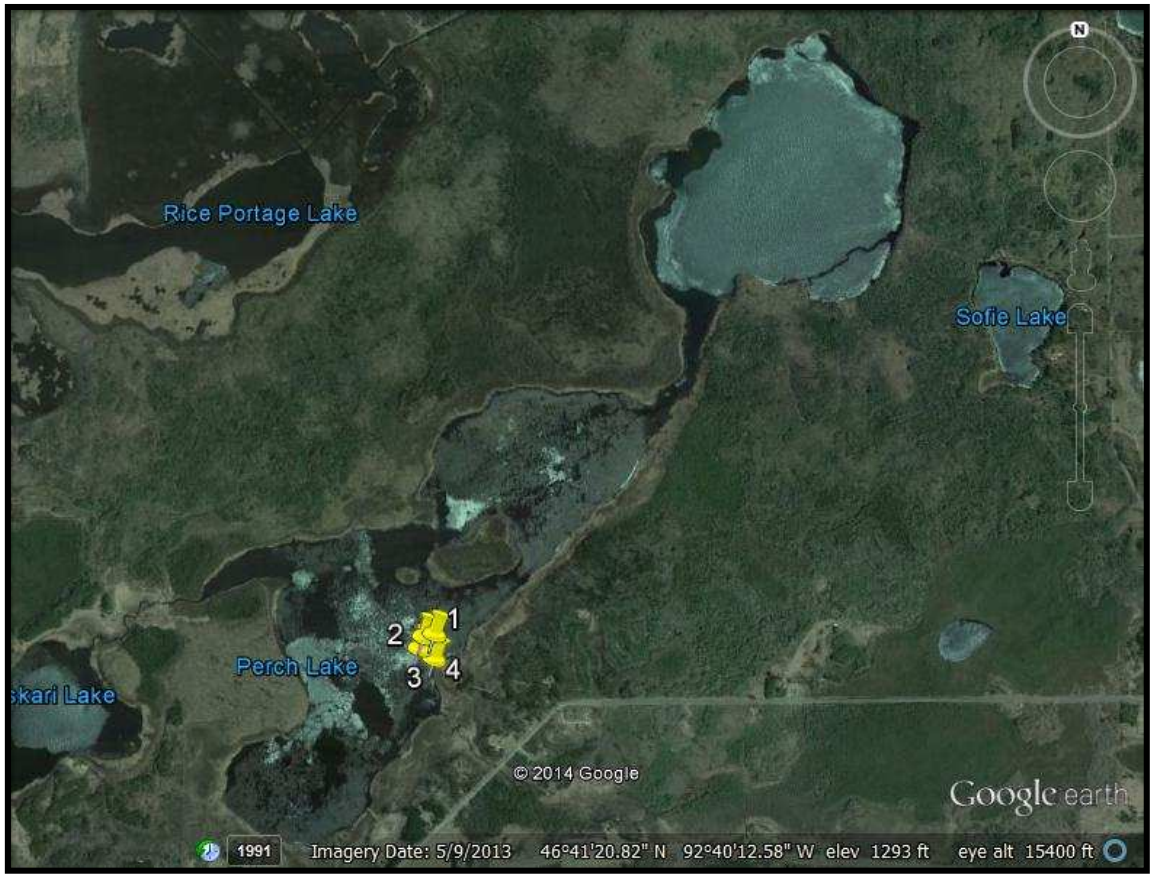

Figure 2-4 Google Earth image of the four sampling locations within Perch Lake.

Figure 2-5 Google Earth image of the four sampling locations within Dead Fish Lake.

Figure 2-6 Google Earth image of the four sampling locations within Clark Lake.

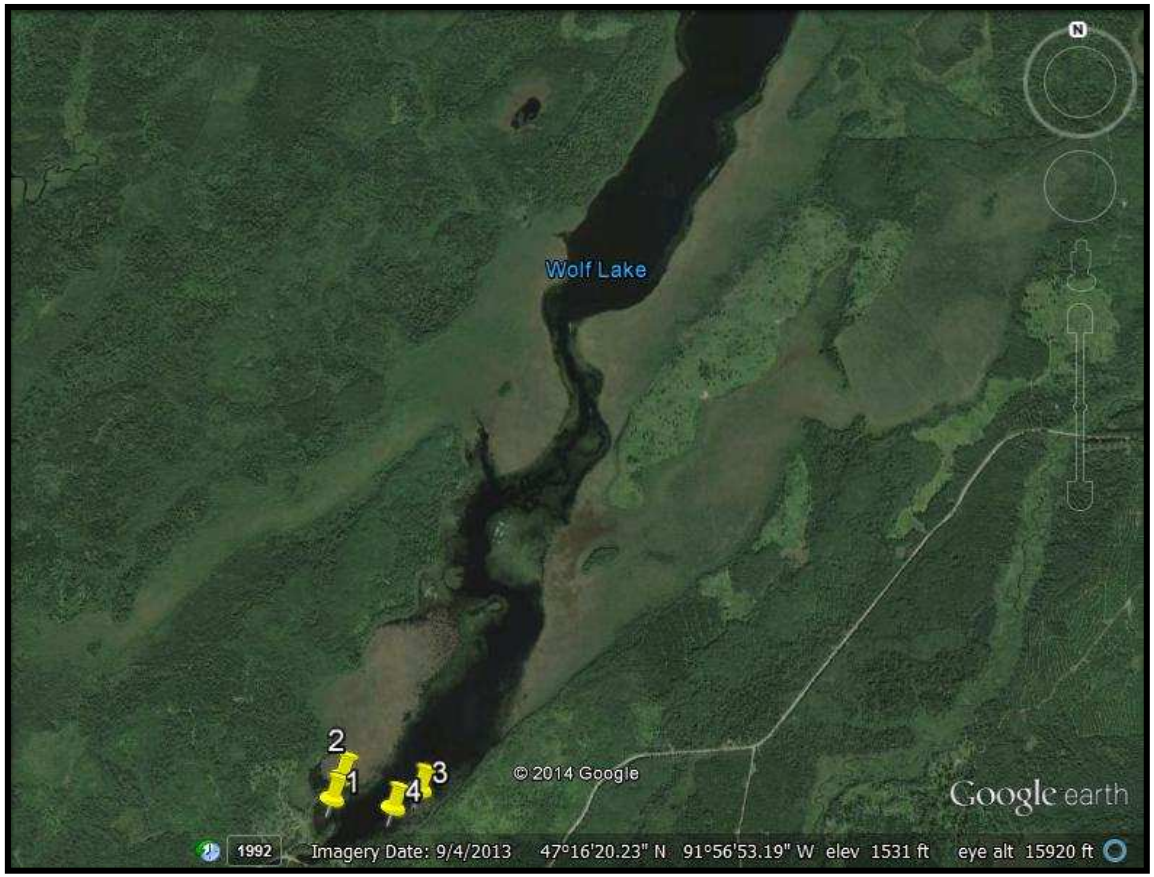

Figure 2-7 Google Earth image of the four sampling locations within Wolf Lake.

2.2 Background Geology

Minnesota exhibits a unique geology that has greatly been shaped by the most recent glaciation, or the ice age known as the Pleistocene Epoch, which took place 2 million to 10,000 years ago (Lusardi B. A., 1994). During this time, the Laurentide Ice Sheet covered much of northern North America and several ice lobes from this sheet extended into Minnesota. These ice lobes covered Minnesota as early as 1.2 million years ago and they advanced across the state several different times and from different directions, carving out the landscape and depositing glacial till over the underlying bedrock (Lusardi B. A., 1994).

The bedrock of Minnesota ranges from Mesozoic sedimentary rocks in the southwest, to Paleozoic carbonates and sandstones in the southeast and northwest, to Precambrian rocks that make up parts of the southwest (Sioux Quartzite) and the entire northern portion of the state. The youngest Precambrian rocks include Mesoproterozoic sandstones that extend southwest from Lake Superior, and also volcanic rocks, Duluth Complex gabbros, and associated rocks along the north shore of Lake Superior. Paleoproterozoic rocks include the Sioux Quartzite in the southwest, intrusive granites and various sedimentary rocks in central Minnesota, and iron formation in the northeast. The oldest rocks exposed at the surface are Archean in age and occupy the northwestern portion of the state, and these consist of greenstone belts and metamorphic rocks that range in grade up to gneiss and migmatite. (Morey, 2000)

Much of the bedrock in the state is overlain by glacial till that varies by its associated ice lobe, and these lobes include: the Des Moines Lobe, the Wadena Lobe, the Rainy Lobe, and the Superior Lobe (Lusardi B. A., 1994, Figure 2-8). The Des Moines Lobe entered the state from the northwest, bringing with it limestone and shale from Canada (Lusardi B. A., 2011, Figure 2-9). Therefore, till from the Des Moines Lobe consists of carbonates and shale-bearing sediments, and it is generally gray in color, though buff to brown where oxidized (Hobbs, 1982). The Wadena Lobe till is found in the west-central portion of the state and is also rich in carbonate rocks, and this is also gray in color, though buff to brown where oxidized (Hobbs, 1982). The Rainy Lobe deposited brown to gray, non-calcareous till in northeastern Minnesota and it consists of igneous and metamorphic rocks from the Canadian Shield (Hobbs, 1982). In the northeastern to central portion of the state, the Superior Lobe deposited non-calcareous reddish to brown till derived from volcanic bearing sedimentary rock from the Lake Superior basin, along with igneous and metamorphic rocks form the Canadian Shield (Hobbs, 1982). There were various sub-lobes involved especially with the Des Moines Lobe, and the final till deposition can be seen in a simplified map below (Figure 2-10).

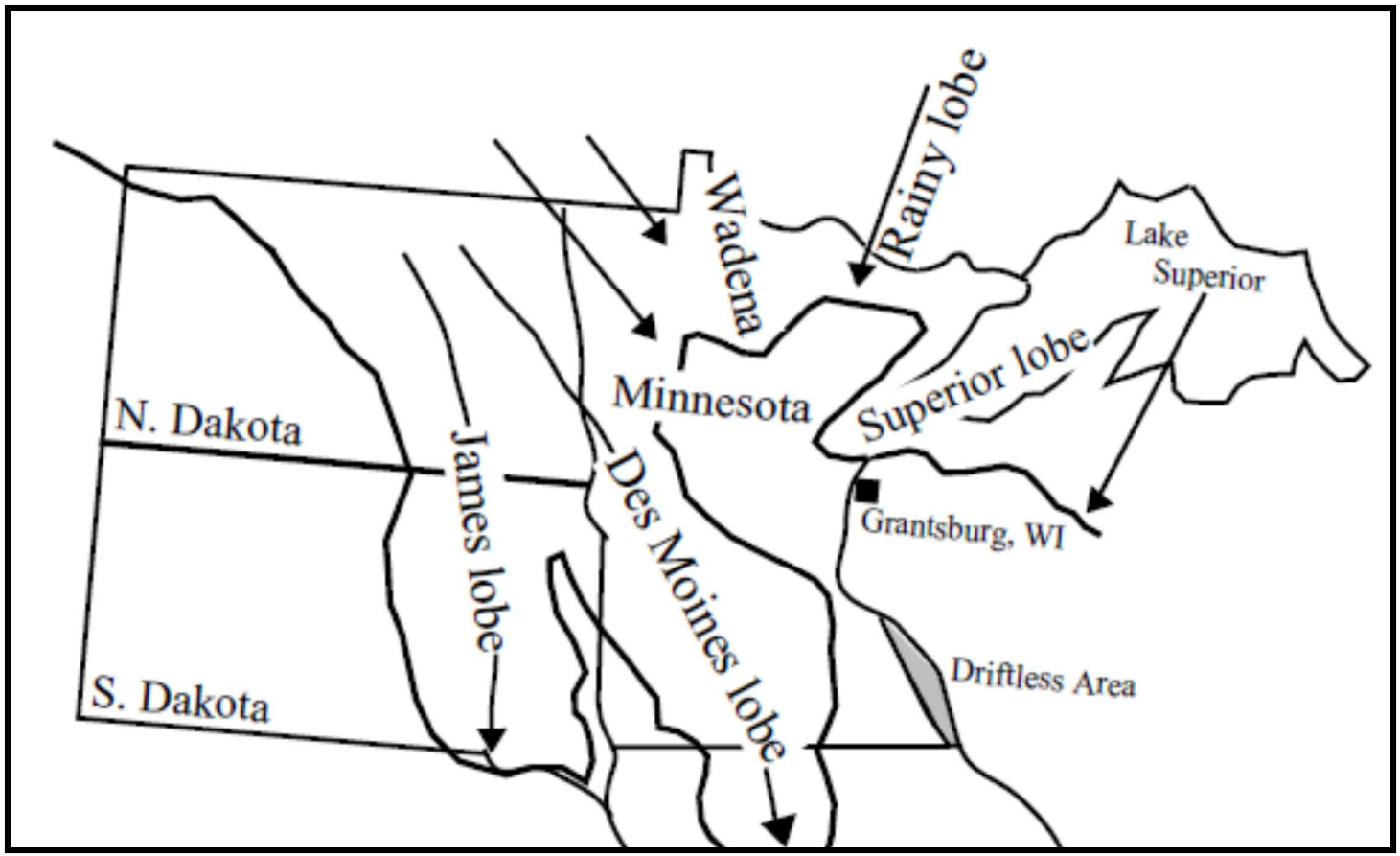

Figure 2-8 A simplified drawing of ice lobes in Minnesota about 14,000 years ago. (Lusardi B. A., 1994)

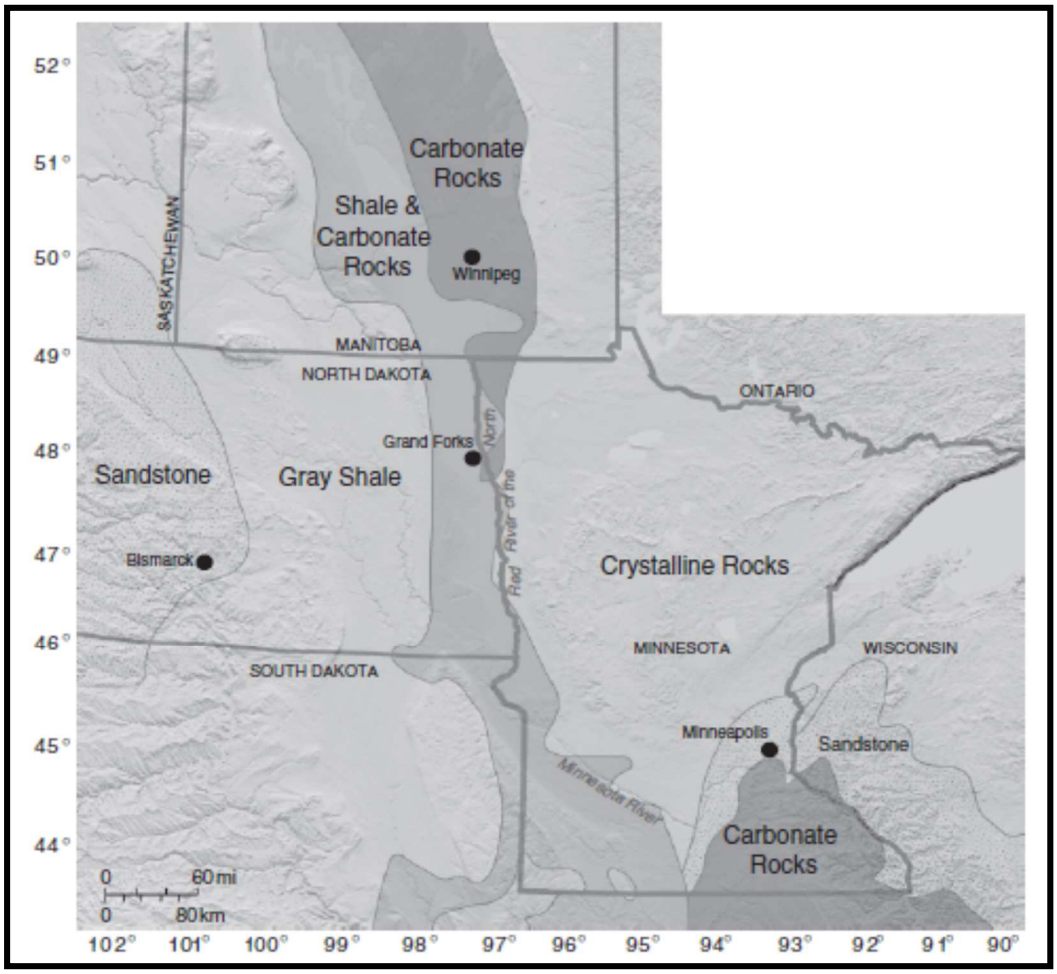

Figure 2-9 A simplified bedrock subcrop of Minnesota and surrounding regions superimposed on land-surface topography. (Lusardi B. A., 2011)

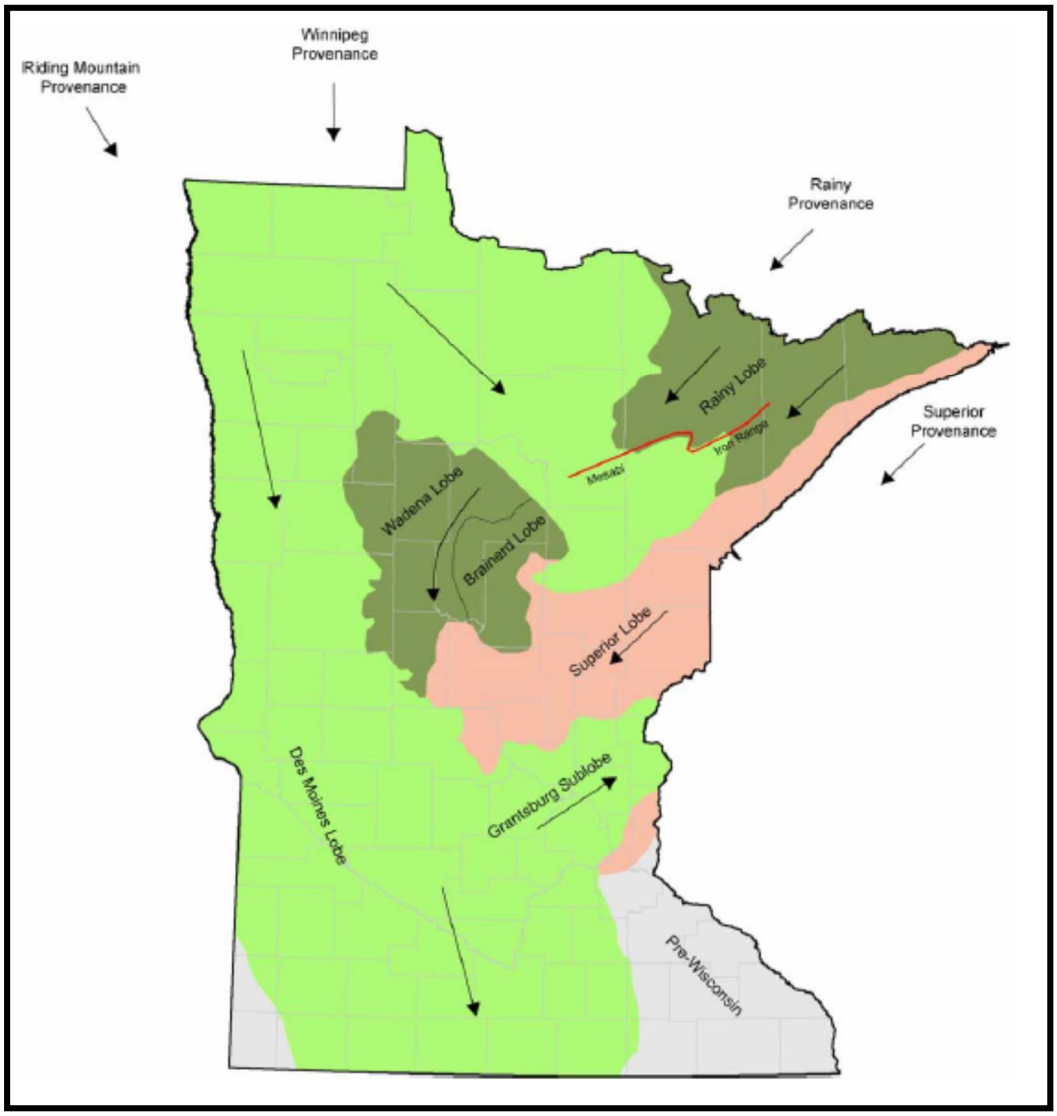

Figure 2-10 Direction of ice flow and provenance of glacial sediments in Minnesota. (Lively, 2009)

2.3 Field Methods

Sampling was done by canoe with the help of tribal officials Jeff Wark (Environmental Technician at White Earth) and Tom Howes (Resource Management Division, Environmental Manager for Natural Resources at Fond du Lac). For each lake, 4 sites were sampled and at each site, 2 water samples (one for THg and the other for MeHg) and 1 sediment sample taken. For each lake, there are 4 water samples for THg analysis, 4 water samples for MeHg, and 4 total sediment samples for both THg and MeHg analysis. Unfiltered water samples were collected 1-30 cm below the surface in 250 mL polyethylene terephthalate (PET) sterile media bottles using the Clean Hands/Dirty Hands (CH/DH) technique, and each sample was preserved in the field using 1 mL of hydrochloric acid (HCl). Sediment samples were collected from the water-sediment interface of each lake using an Ekman dredge and were stored in 125 mL PET sterile media bottles. Immediately following the sampling of each area, the water and sediment samples were placed into a cooler with ice to preserve them while transported to the University of Minnesota.

Wild rice was sampled both as unfinished rice and as finished rice, with the exception of Long Lake (finished rice was unavailable). Unfinished rice was collected using canoes and rice knocking tools, and this was done at random locations within the lake. Unfinished rice from the Fond du Lac and 1854 Treaty Areas was taken by Tom Howes to be finished. The finishing process consists of drying, parching, hulling, and

winnowing. Drying and parching remove moisture from the grain and loosen the hull, while hulling and winnowing remove the loosened hull, leaving only the dried grain.

Finished rice from Lower Rice Lake was provided by Jeff Wark when White Earth lakes were being sampled. Sampling on the White Earth Reservation was done too late in the harvest season to allow the collection of the necessary amount of unfinished rice needed to also get finished rice from Long Lake. However, Jeff Wark suggested the inclusion of Long Lake, as it is locally a popular lake for ricing. Once unfinished rice was knocked into the canoes, rice samples were stored in ziplock bags, separated by lake. The finished rice samples were also stored in ziplock bag, also separated by lake.

After collection, samples were taken to the Department of Soil, Water, and Climate at the University of Minnesota to be analyzed in Ed Nater's Mercury Analytical Laboratory. Water samples and wild rice samples (unfinished, wet rice and finished, dry rice) were stored in a refrigerator and wet sediment was stored in a freezer. The sediment was later thawed and excess lake water was drained from the 125 mL bottles. The remaining sediment was then dried for two days in an oven at 70^{o}C (Figure 2-11). Using a pair of tweezers, unfinished wild rice was split into its two components (grain and hull), which were then put into separate ziplock bags.

Figure 2-11 Image of sediment samples (drained of excess lake water) prior to being dried in an oven at 70 C. Each row from top to bottom: Lower Rice Lake, Long Lake, Perch Lake, Dead Fish Lake, Clark Lake, and Wolf Lake.

A lake core sample was taken from Perch Lake (February 20, 2015) using a Livingston Piston Corer and a ~91 cm plastic core tube. The sampling location, seen in Figure 2-12 below, exhibited 74 cm water depth (including lake ice) and was in the same area of the lake as water and sediments samples taken July 17, 2014 (Figure 2-4). Coring was done using a "clean" and "dirty" hole in the lake ice, with the "clean" hole being the actual sample location. The "dirty" hole was used for water depth analysis and for obtaining lake water to use in pressurizing the core tube. The core was overlain by 20 cm of core top water. Approximately 100 mL of unfiltered core top water was collected into sealed plastic 50 mL vials with no headspace and transported on ice. The core was then capped and transported back to the University of Minnesota upright and on ice.

The core was immediately taken to Dr. Jake Bailey's Geobiology laboratory in the Department of Earth Sciences at the University of Minnesota for further processing. The core top water sampled in the field was 0.2 μM filtered and stored in 50 mL vials at -20^{o}C. Core extrusion started 4 hours after collection. When extruding the core, sediment samples were taken from the center of the top of the core at 2.5 cm intervals for the first 10 cm, and at 5 cm intervals for the next 20 cm. The 8 sediment samples were placed in 50 mL vials and centrifuged at 3,000 $\times g$ for 10 minutes in order to extract pore water. This pore water was then filtered (0.2 μM) into 15 mL vials, and immediately frozen and stored at -20^{o}C for chemical analysis, and the remaining sediment stored at -20^{o}C for DNA extraction.

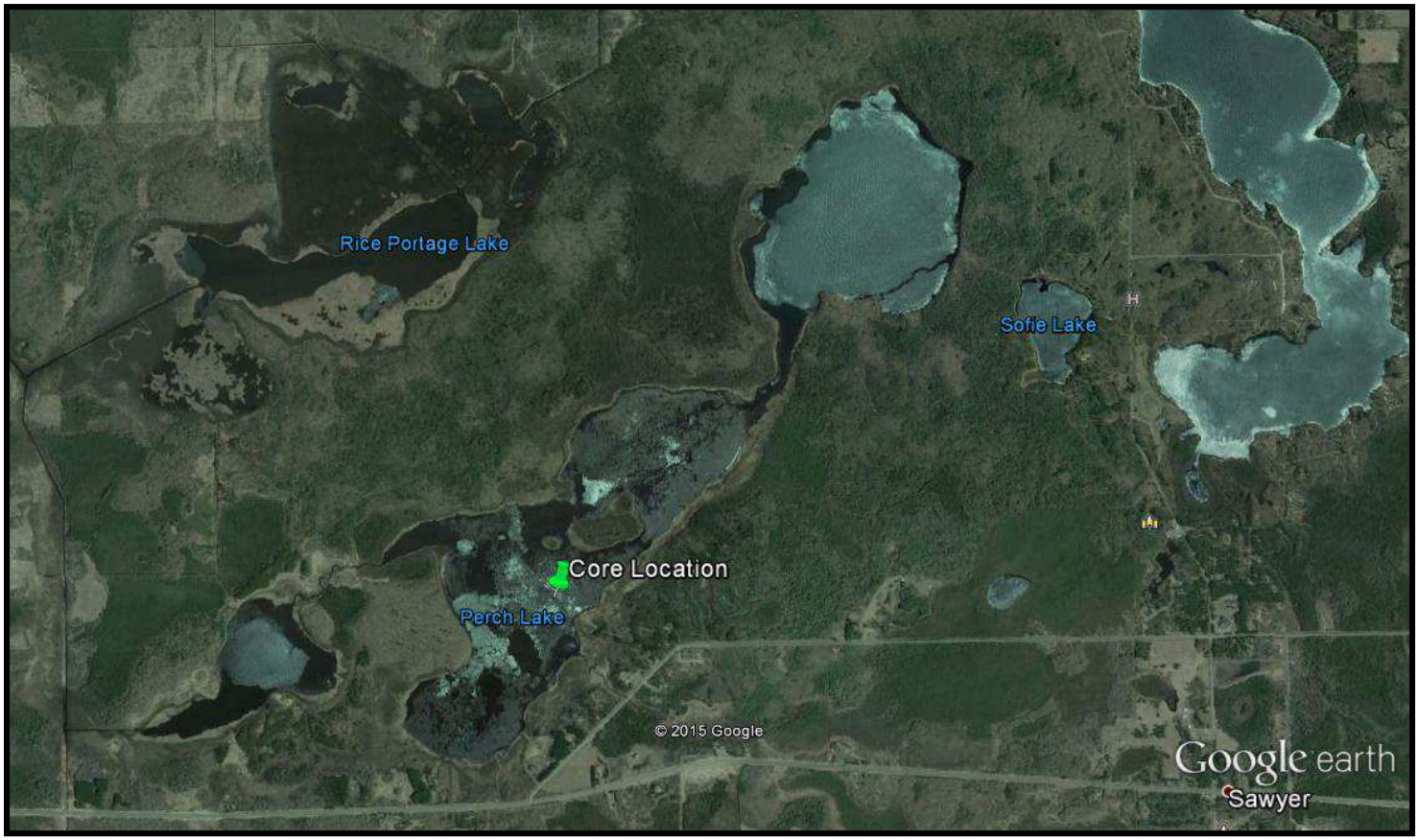

Figure 2-12 Google Earth image of the lake core sampling location within Perch Lake.

2.4 Total Hg Analysis

All samples analyzed for Total Hg content were done so in the fall of 2014: water (August 1, 5 and October 13, 2014), sediment (October 24, 2014), and rice grains and hull (November 17, 2014). Samples are digested to allow gas to flow through an atomic fluorescence spectrophotometer (CVAFS) linked to a peak integrator, which allows the peak area to be recorded. This peak area represents concentration of THg in each sample.

Prior to running the water, sediment, rice grain and rice hull samples, 0.5-100 mL of laboratory deionized H_2O was added to four flasks (bubblers) and reduced by 0.5 mL of stannous chloride ($SnCl_2$). The bubblers were purged for 21 minutes using Hg-free N_2 gas at a steady flow of ~15 psi to expedite the volatilization of the reduced Hg. Water was removed from the gas stream using soda lime traps and Hg was collected on a conditioned (standardized) gold coated glass bead trap. Sample gold traps were inserted in the analyzer train between incoming Hg-free argon gas at a steady flow of ~45 psi and a second, analytical gold trap. The sample gold traps were heated for 4 minutes at ~500ºC by a nichrome wire, which was heated by a variable transformer. Hg^0 was collected on the second, analytical gold trap and that was heated for 3 minutes. The gas flow carried the desorbed Hg^0 through the atomic fluorescence spectrophotometer (CVAFS) and this was linked to a peak integrator, which allowed the peak area to be recorded. This bubbler purge was completed each day before analysis in the lab.

After the bubblers were purged using this method and all sample gold traps were "cleaned," a standardized curve was obtained using a standard Hg solution, which consisted of 99 mL of DI water and 1 mL of THg standard in a 125 mL Teflon bottle. The standard curve consisted of 1.6 mL, 0.8 mL, 0.4 mL, 0.2 mL, 0 mL, 0.2 mL, 0.4 mL, and 0.8 mL of the standard Hg solution added to each bubbler in addition to 0.5 mL $SnCl_2$. This was done before each day's analysis and a standard curve R^2 greater than 0.995 was obtained each time. Once the purging was done and an acceptable standard curve was obtained, samples were run using the same system mentioned above. This included 24 water samples, 24 sediment samples, 6 unfinished rice grain samples, 6 rice hull samples, and 10 finished rice samples (2 finished rice samples from each of the 5 lakes, Long Lake being the excluded lake).

For Hg analysis quality control and quality assurance, particulates are filtered from the incoming air by a high efficiency particulate filter (HEPA filter). All operations with open containers were conducted under the HEPA filter. Disposable, cleanroom quality, polyvinyl chloride gloves were worn at all times during handling of samples, sample bottles, sample bags, and sample collection equipment. All gases were pre-cleaned by gold traps prior to contact with the sample. Reagents were routinely analyzed and cleaned of Hg to the extent possible. Sets of standards and bubbler blanks were analyzed several times each day. Also analyzed each day were digestion duplicates (for samples digested prior to analysis), analytical duplicates, digestion blanks, 0.4 mL Hg standard spikes, and National Institute of Standards and Testing (NIST) reference

material 2976 (SRM #2976), freeze-dried muscle tissue. The necessary duplicates, spikes, and SRM #2976 values were within the acceptable 80-120% range for analysis.

For water samples, 2 mL of bromine monochloride (BrCl) were added to each 250 mL bottle and the samples were digested overnight in an oven at 70ºC. Each water sample then received 0.2mL of Hydroxylamine hydrochloride ($HONH_2$-HCl). The BrCl oxidizes the sample and the $HONH_2$-HCl subsequently reduces it to destroy any free halogens. Water samples were added to a bubbler, in addition to 0.5 mL of $SnCl_2$, and analyzed using the system mentioned above (with amount added of each water sample ranging from 52-121 mL).

For sediment samples, dried sediment was placed into individual vials (bombs) with a range of 0.45-0.72 g per sample and 15 mL of nitric acid (HNO_3) was added to each. The bombs were then put in an oven at 70ºC overnight in order to digest. Using the THg system discussed above, 1 mL of digested sediment was added to each bubbler in addition to 0.5 mL of $SnCl_2$.

Unfinished rice grains, rice hull, and finished rice grains were placed into individual bombs and 10 mL of HNO_3 were added to each. Rice grains measured 1.02-1.18 g per sample and rice hull measured 0.20-0.37 g per sample. The bombs were then placed into an oven at 70ºC overnight in order to digest. Following digestion, each sample was added to a bubbler (in addition to 0.5 mL of $SnCl_2$) and analyzed for THg using the system mentioned above. For the rice grains, 1 mL of each digested sample was added to a bubbler, while 2 mL of digested rice hull was added to a bubbler due to

the low weight of the samples. For the digestion duplicates, the 5 finished rice samples were ran twice, resulting in 5 digestion duplicates for the wild rice THg analysis.

2.5 MeHg Analysis

All samples were analyzed for MeHg content in fall of 2014: water (October 17, 2014), sediment (October 14, 31, 2014), and rice grains and hull (November 24, 2014). Samples are digested and distilled in a heating block. They were then run through an automated MeHg analyzer, which detects Hg by cold vapor atomic fluorescence spectrometry (CVAFS). MeHg concentrations are determined from headspace gas analysis.

The water samples were shaken and 35 mL was poured into a Teflon distillation vial, in addition to 157 µL of ammonium pyrrolidine dithiocarbamate (APDC), which is a chemical that aids in distillation and is often used for metal chelation. The distillation vials were capped and placed in a 36-vial heating block set to 125ºC. High purity N_2 gas was used during distillation at a steady flow of ~15 psi and samples were collected into glass vials (containing 5 mL DI water) until a volume of 30 mL was reached. The 24 water samples were run along with 3 distillation blanks, 3 digestion duplicates, 3 digested MeHg standard spikes of 0.1 ng/L concentration, and 3 digested MeHg standards of 0.1 ng/L concentration (ongoing precision recovery-OPRs). The necessary duplicates, spikes, and SRM #2976 values were within the acceptable 80-120% range for analysis. Once distilled, all 36 samples received 40 µL of ascorbic acid to prevent interference from any Cl^- that could have distilled over, 263 µL of acetate buffer to bring them to a pH

of 4.5, and 30 µL of sodium tetraethylborate (STEB) in order to ethylate and volatilize the methylmercury. The glass vials were then shaken and left to sit for at least 30 minutes.

Solids (sediment, wild rice grains and wild rice hull) were prepared using a slightly different method. Prior to digestion, 36 acid-clean amber vials were filled with an HCl solution and heated in an oven overnight at 70ºC. Once rinsed, each vial was filled with a solid sample: sediment (sample weights ranging 0.0973-0.3371 g), unfinished and finished wild rice grains (sample weights ranging 0.4993-0.5558 g), and wild rice hull (sample weights ranging 0.101-0.1346 g). In addition to the sample, 10 mL of 25% KOH/methanol solution was added to every vial. Samples were then placed into the oven at 90ºC for 4 hours and methanol was later added to the vials for a total volume of 20 mL.

For distillation, 30 mL of DI water was poured into each of the 36 Teflon distillation vials, in addition to 388 µL of 50% sulfuric acid (H_2SO_4), 156 µL of KCl solution, and an aliquot from a digested sample (1 mL for sediment, 2 mL for wild rice grains and hull). Distilled deionized water was used to bring each vial to a volume of 35 mL; the vials were then capped and placed into a 36-vial heating block at 135ºC. The distillation of sediment samples included 24 sediment samples, 3 distillation blanks, 3 digestion blanks, 2 digestion duplicates, 2 distillation duplicates, 3 digested MeHg standards of 0.1 ng/L concentration OPRs, 1 digested MeHg standard spike of 0.1 ng/L concentration, and 1 SRM #2976. The distillation of wild rice samples included 17 wild

rice samples (5 finished rice grain samples, 6 unfinished rice grain samples, and 6 hull samples), 5 digestion duplicates (similar to THg analysis, each was a duplicate of the 5 finished rice grain samples), 3 distillation blanks, 3 digestion blanks, 3 distillation duplicates, 3 digested MeHg standards of 0.1 ng/L concentration OPRs, 1 digested MeHg standard spike of 0.1 ng/L concentration, and 1 SRM #2976. Once each glass vial reach a volume of 30 mL, all 36 vials received 40 µL of ascorbic acid, 263 µL of acetate buffer, and 30 µL of STEB. The glass vials were then shaken and left to sit for at least 30 minutes. The necessary duplicates, spikes, and SRM #2976 values were within the acceptable 80-120% range for analysis.

For MeHg analysis quality control and quality assurance, a calibration curve was obtained at the start of each run of samples. This consisted of 6 calibration standards (concentrations of 0.05, 0.1, 0.2, 0.5, 2.0, and 4.0 ng/L) and used working standards of 10 ng/L concentration and 500 ng/L concentration. Next, 4 calibration blanks (CBs) were made in order to run 3 before the actual curve, and these were averaged to get the 0 value for the calibration curve. The fourth was run right after the calibration curve to rinse the system after the highest standard. Finally, 4 standards were made, the first being an initial precision recovery (IPR) and this was the MeHg standard of 0.1 ng/L concentration. The remaining 3 standards were ongoing precision recovery (OPR) and were also the 0.1 ng/L concentration. All 14 vials of the calibration curve were brought to 30 mL volume using DI water and each received 40 µL ascorbic acid, 225 µL of acetate buffer, and 30 µL of STEB. For analyzing samples, the IPR is run before any

CBs or the actual curve, while the OPRs are interspersed between actual samples to ensure the system continues to perform well during analysis.

Once samples were prepared, MeHg concentrations were determined from headspace gas analysis by a Tekran 2700 Methyl Mercury Analysis System with EPA method 1630. This automated MeHg analyzer detects Hg by cold vapor atomic fluorescence spectrometry (CVAFS) following capillary gas chromatography and pyrolization of ethylated Hg species. The system uses ultra-pure argon gas at a steady flow of ~45 psi.

2.6 Lake Core Analysis

The core top water sample and the 8 pore water samples were analyzed for total iron (TFe) at Jake Bailey's Geobiology Laboratory, and for anions at Rick Knurr's Analytical Geochemistry Laboratory. Included in the anion chromatography were: Fluoride (F), Acetate ($C_2H_3O_2$), Formate (HCO_2), Chloride (Cl), Nitrite (NO_2), Bromide (Br), Nitrate (NO_3), Sulfate (SO_4), Thiosulfate (S_2O_3), and Phosphate (PO_4). Sediment at the sediment-water interface and 8 sediment samples throughout the core were analyzed for microbial DNA in Jake Bailey's Microbial Laboratory.

The core top water and pore water samples were analyzed for TFe on March 11, 2015 by the Ferrozine assay (Stookey, 1970). First, the samples were thawed and 1 mL of each immediately added to 1 mL of 0.5 M hydroxylamine hydrochloride. Four of the samples were analyzed in duplicate, resulting in a total of 13 samples to be analyzed. The 13 samples were reduced in hydroxylamine HCL for one hour, and subsequently

analyzed by the ferrozine assay. The 13 samples were diluted 1:4 with deionized water, and each analyzed in duplicate at an absorbance of 562 nanometers. The remaining core top water and pore water was transferred to 15 mL vials and refrigerated at 4ºC prior to anion analysis by ion chromatography.

DNA was extracted from sediment samples using the PowerSoil DNA Isolation Kit (MoBio Laboratories), according to the manufacturer's instructions. DNA concentration was determined with a nanodrop spectrophotometer (Thermo Scientific), and 16S rRNA genes were initially amplified using primers 27F and 1492R as in Jones et al. (2015) to ensure that the DNA was free of PCR inhibitors. rRNA 'amplicon' libraries of the hypervariable V5-V6 region were generated from 7 sediment samples (the sediment-water interface sample and the bottom sample were not included, but two of the 7 samples used were duplicated to give a total of 9 samples) by submitting samples to the University of Minnesota Genomics Center automated amplicon sequencing service, described in Jones et al. (2015). *hgcA* genes were cloned from the top 0-2.5 cm core interval as in Jones et al. (*in prep*), using the primers and amplification protocol of Schaefer *et al.* (2014).

A phylogenetic tree was created that included all cloned *hgcA* genes from Perch Lake core top sediment, as well as representative *hgcA* sequences from complete microbial genomes identified by Parks *et al*. (2013). Peptide sequences were aligned with ClustalW v. 2.1 (Bioinformatics, 2007) available at the online EMBL-EBI workbench at (http://www.ebi.ac.uk/Tools/msa/clustalw2/), using the 'fast' alignment

option with default parameters. A neighbor joining tree was created using the clustalw_phylogeny tool at the EMBL-EBI workbench (http://www.ebi.ac.uk/Tools/phylogeny/clustalw2_phylogeny/). In addition to the phylogenetic tree, cloned *hgcA* genes were characterized by a BLASTX comparison against the NCBI-nr database (http://blast.ncbi.nlm.nih.gov/).

CHAPTER 3: RESULTS

3.1 Water Samples

Water and sediment samples had the most data of all samples collected, as four samples of each were analyzed per lake. For water samples, the concentration is measured in ng/L and for sediment it is measured in ng/g. The THg values for lake water were relatively consistent between the six lakes, ranging from 1.09-18.53 ng/L, and these data can be seen in Table 1. The 6.76 ng/L value for Long Lake and 18.53 ng/L value for Wolf Lake are significantly higher than the rest of the samples for those lakes, suggesting these two samples could have been contaminated at some point during collection or analysis. An analytical duplicate of the third Wolf Lake sample was run and this yielded a value of 18.56 ng/L, which suggests analytical protocol was followed and that the sample was probably contaminated by suspended sediment, as water sampled were not filtered.

The MeHg values for water samples aren't as consistent as the THg values for the six lakes, or more appropriately, the three studied areas. These data can be seen in Table 1 below and they range from 0.0243-.4000 ng/L. There is a significant trend as you move west to east across the three studied areas, as the MeHg values for lakes in each area get larger. This trend holds up for much of the data, but the 0.4000 ng/L for Wolf Lake is much higher than the other three samples for the lake, again suggesting that this sample was contaminated either during collection or analysis.

Lake Sampled	THg (ng/L)	MeHg (ng/L)
Lower Rice Lake	1.20	0.0246
Lower Rice Lake	1.27	0.0295
Lower Rice Lake	1.23	0.0567
Lower Rice Lake	2.94	0.0243
Long Lake	1.19	0.0243
Long Lake	2.02	0.0405
Long Lake	6.76	0.0355
Long Lake	1.23	0.0244
Perch Lake	3.27	0.0879
Perch Lake	1.09	0.1550
Perch Lake	2.15	0.1173
Perch Lake	1.52	0.0681
Dead Fish Lake	3.00	0.1027
Dead Fish Lake	2.53	0.0247
Dead Fish Lake	2.85	0.0405
Dead Fish Lake	3.06	0.1087
Clark Lake	1.65	0.1435
Clark Lake	2.93	0.1538
Clark Lake	2.17	0.1457
Clark Lake	2.11	0.1557
Wolf Lake	1.87	0.0914
Wolf Lake	2.70	0.4000
Wolf Lake	18.53	0.1790
Wolf Lake	2.95	0.1677

Table 1 Water samples: THg and MeHg values for the six studied lakes.

The mean THg values for water samples can be seen in Table 2 and Figure 3-1, and they range from 1.48-2.51 ng/L. Mean THg values for the six lakes were very close to one another, if the 6.56 ng/L sample from Long Lake and the 18.53 ng/L sample from Wolf Lake were omitted. Before these values were dropped, Long Lake had a mean value of 2.8 ng/L and Wolf Lake had a mean value of 6.51 ng/L. However, due to the high values likely caused by contamination, the values weren't included and this resulted in a more consistent range of numbers. The mean THg values for the White Earth Reservation lakes and the 1854 Treaty Area lakes are exceptionally consistent, but there is bigger range (0.85 ng/L) in mean values for the Fond du Lac Reservation lakes.

The mean MeHg values for water samples can be seen in Table 2 and Figure 3-2, and they range from 0.0338-0.1460 ng/L. It is easier to see the west to east trend here, as White Earth lake values are the lowest, Fond du Lac lake values are intermediate, and the 1854 Treaty Area lake values are the highest. The mean MeHg value for Wolf Lake was 0.2095 ng/L before removing the 0.4000 ng/L sample due to the possibility of contamination. Similar to the THg mean values in the White Earth Reservation lakes and 1854 Treaty Area lakes, the MeHg values are also very consistent, with the greatest range coming from the Fond du Lac lakes (0.0379 ng/L). Of the 48 total water samples and 48 total sediment samples taken from the 6 lakes, only the 3 water samples (6.76 ng/L, 18.53 ng/L and 0.4000 ng/L) were considered high enough to be outliers within their respected lakes. Therefore, only ~3% of water and sediment samples were considerably high

compared to the other samples in their respected lakes, and this low percentage simplified the decision to omit these data points from the mean values in Table 2.

Lake Sampled	THg (ng/L)	MeHg (ng/L)
Lower Rice Lake	1.66	0.0338
Long Lake	1.48	0.0312
Perch Lake	2.01	0.1071
Dead Fish Lake	2.86	0.0692
Clark Lake	2.22	0.1497
Wolf Lake	2.51	0.1460

Table 2 Water samples: THg and MeHg mean values for the six studied lakes.

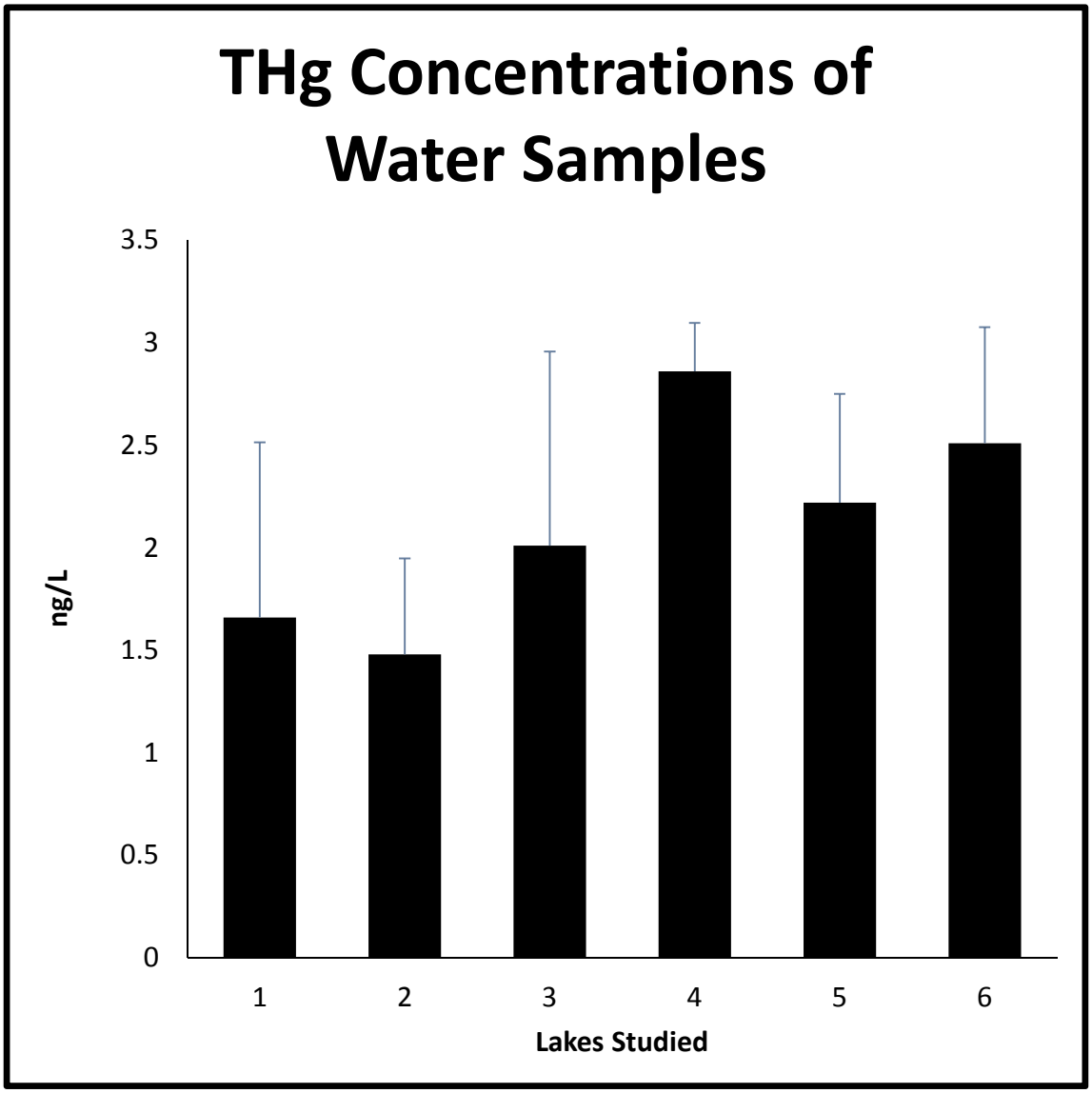

Figure 3-1 Mean THg values of water samples for the six studied lakes: Lower Rice Lake (1), Long Lake (2), Perch Lake (3), Dead Fish Lake (4), Clark Lake (5), and Wolf Lake (6). Standard deviation positive error bars.

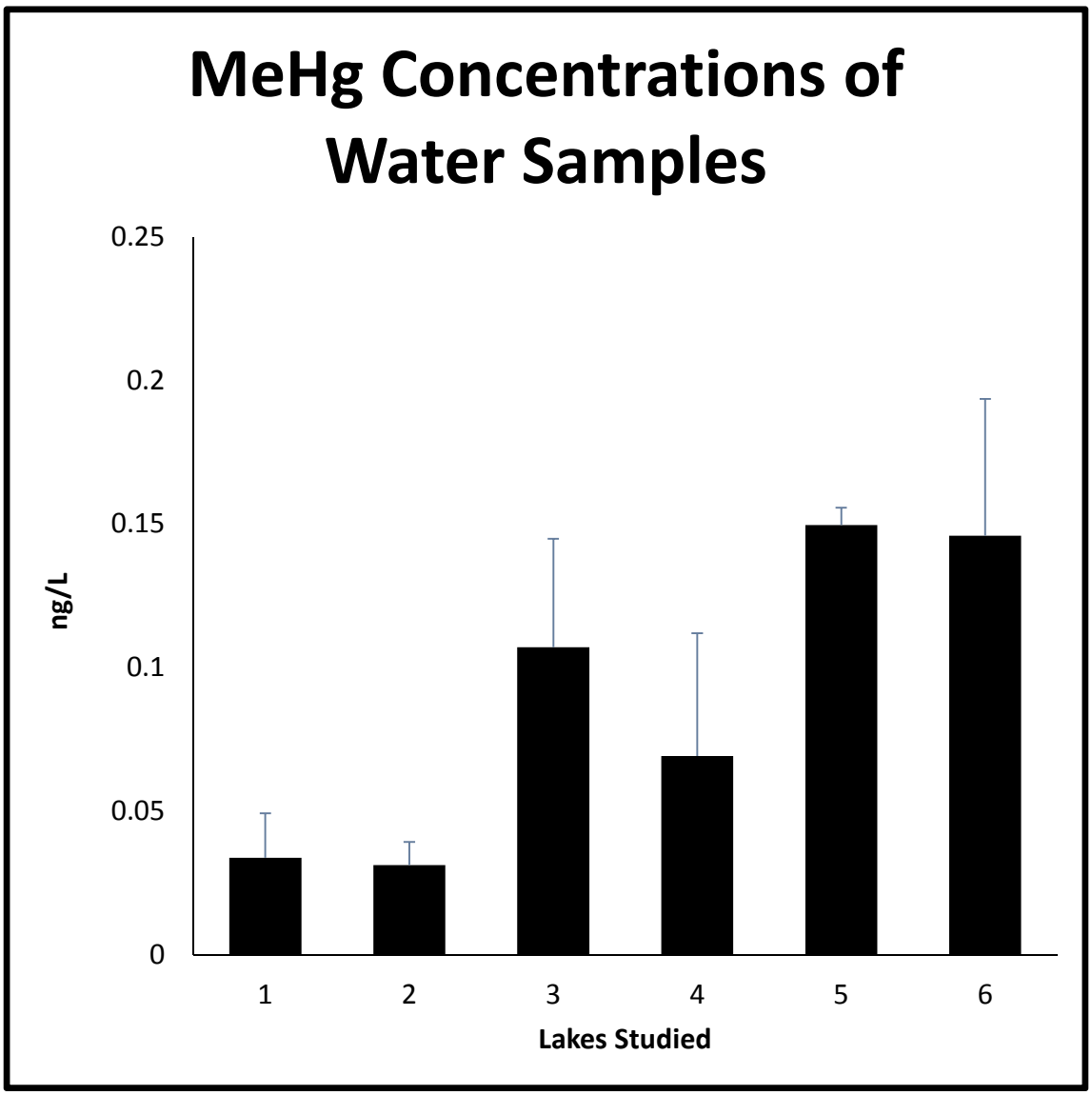

Figure 3-2 Mean MeHg values of water samples for the six studied lakes: Lower Rice Lake (1), Long Lake (2), Perch Lake (3), Dead Fish Lake (4), Clark Lake (5), and Wolf Lake (6). Standard deviation positive error bars.

3.2 Sediment Samples

The THg values for sediment samples vary significantly when looking at the three studied areas, and the data range from 9.19-226.0 ng/g. Within each lake and each area, the numbers are consistent and this can be seen in Table 3. The MeHg values for lake sediment across the six lakes is slightly more consistent than that of THg values for the sediment, and these can also be seen in Table 3. These numbers are consistent within each lake and each area, and they range from 0.2846-1.951 ng/g

Lake Sampled	THg (ng/g)	MeHg (ng/g)
Lower Rice Lake	27.55	0.3658
Lower Rice Lake	52.29	1.233
Lower Rice Lake	49.26	0.8462
Lower Rice Lake	36.53	0.7595
Long Lake	14.79	0.7691
Long Lake	15.41	0.4984
Long Lake	15.6	0.4598
Long Lake	9.19	0.2846
Perch Lake	127.89	1.166
Perch Lake	147.91	1.107
Perch Lake	138.06	0.9502
Perch Lake	58.37	1.051
Dead Fish Lake	183.51	1.951
Dead Fish Lake	171.25	1.077
Dead Fish Lake	187.71	1.371
Dead Fish Lake	183.99	1.457
Clark Lake	142.7	0.956
Clark Lake	168.32	0.8126
Clark Lake	182.49	0.6806
Clark Lake	176.56	0.6741
Wolf Lake	190.49	1.643
Wolf Lake	175.53	0.7231
Wolf Lake	243.5	1.153
Wolf Lake	225.98	0.8128

Table 3 Sediment samples: THg and MeHg values for the six studied lakes.

The mean THg values for sediment can be seen in Table 4 and Figure 3-3, and these data range from 13.75-208.9 ng/g. The trend is easy to see in mean values, as White Earth lake sediment is exceptionally low when compared to Fond du Lac and 1854 Treaty Area lake sediment. The mean MeHg values for sediment, which can be seen in Table 4 and Figure 3-4 below, range from 0.5030-1.464 ng/g. The mean values for MeHg across the three areas are slightly more consistent than THg values. However, the trend seen from lake to lake remains the same for both THg and MeHg, and this can be seen when comparing Figure 3-3 to Figure 3-4. The lowest value comes from Long Lake, but this value isn't very far from the values seen in Lower Rice Lake and Clark Lake. No THg or MeHg lake sediment samples were small or large enough in comparison to the rest of the data to be considered an outlier.

Lake Sampled	THg (ng/g)	MeHg (ng/g)
Lower Rice Lake	41.41	0.8011
Long Lake	13.75	0.503
Perch Lake	118.1	1.069
Dead Fish Lake	181.6	1.464
Clark Lake	167.5	0.7808
Wolf Lake	208.9	1.083

Table 4 Sediment samples: THg and MeHg mean values for the six studied lakes.

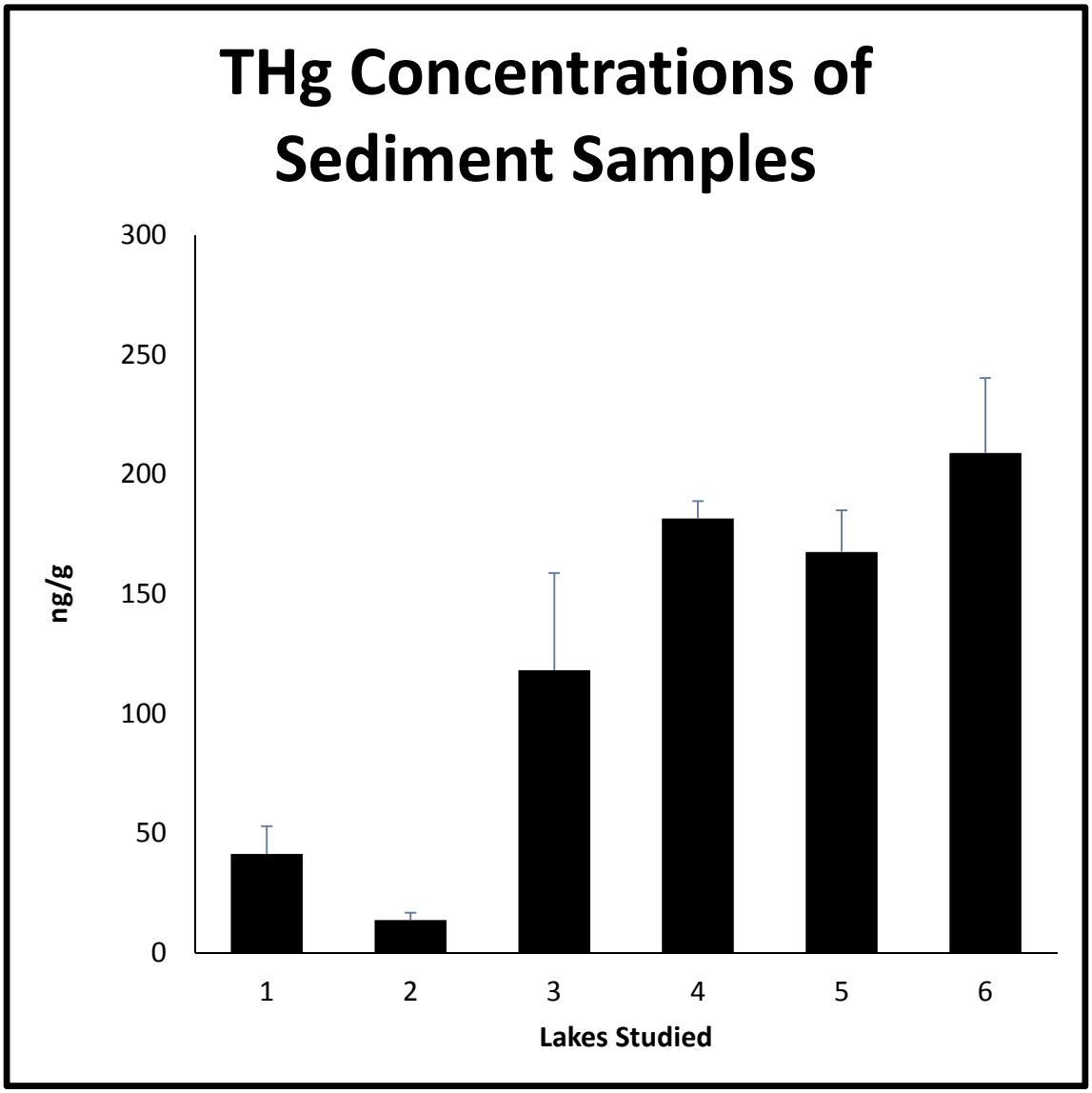

Figure 3-3 Mean THg values of sediment samples for the six studied lakes: Lower Rice Lake (1), Long Lake (2), Perch Lake (3), Dead Fish Lake (4), Clark Lake (5), and Wolf Lake (6). Standard deviation positive error bars.

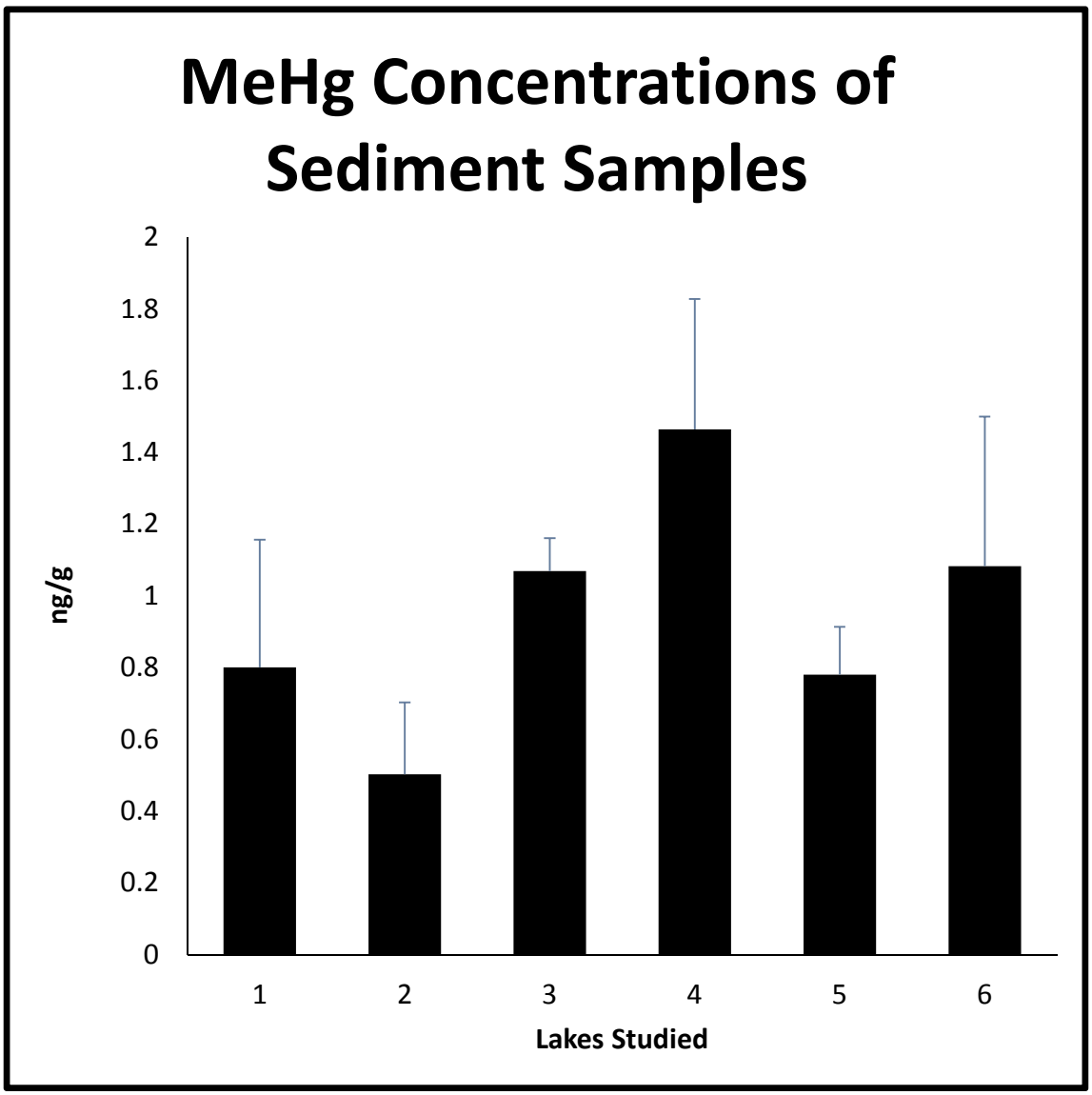

Figure 3-4 Mean MeHg values of sediment samples for the six studied lakes: Lower Rice Lake (1), Long Lake (2), Perch Lake (3), Dead Fish Lake (4), Clark Lake (5), and Wolf Lake (6). Standard deviation positive error bars.

3.3 Wild Rice Grain Samples

The finished rice grains provide the most important numbers in this study, as it is the finished product that is ultimately consumed. Due to the importance of finished rice, two samples from each lake (with the exception of Long Lake) were analyzed and both the THg and MeHg values found were very close to one another. The range for THg values is 1.25-3.41 ng/g and the range for MeHg values is 0.2139-0.6984 ng/g, and this can be seen in Table 5. The exceptional consistency within lakes allows the focus of finished rice values to be on the mean values. The mean THg values for finished rice range from 1.38-3.28 ng/g, with Clark Lake exhibiting the lowest value and Perch Lake exhibiting the highest. The mean MeHg values for finished rice range from 0.227-0.638 ng/g, with Clark Lake and Perch Lake again exhibiting the low and high values, respectively. These data can be seen in Table 6 and Figure 3-5.

Lake Sampled	THg (ng/g)	MeHg (ng/g)
Lower Rice Lake	1.54	0.233
Lower Rice Lake	1.75	0.2483
Long Lake	-	-
Long Lake	-	-
Perch Lake	3.14	0.6984
Perch Lake	3.41	0.5779
Dead Fish Lake	1.66	0.4077
Dead Fish Lake	1.85	0.3632
Clark Lake	1.25	0.2402
Clark Lake	1.52	0.2139
Wolf Lake	2.2	0.3619
Wolf Lake	2.43	0.4336

Table 5 Finished rice samples: THg and MeHg values for the six studied lakes.

Lake Sampled	THg (ng/g)	MeHg (ng/g)
Lower Rice Lake	1.64	0.241
Long Lake	-	-
Perch Lake	3.28	0.638
Dead Fish Lake	1.75	0.385
Clark Lake	1.38	0.227
Wolf Lake	2.31	0.398

Table 6 Finished rice samples: THg and MeHg mean values for the six studied lakes.

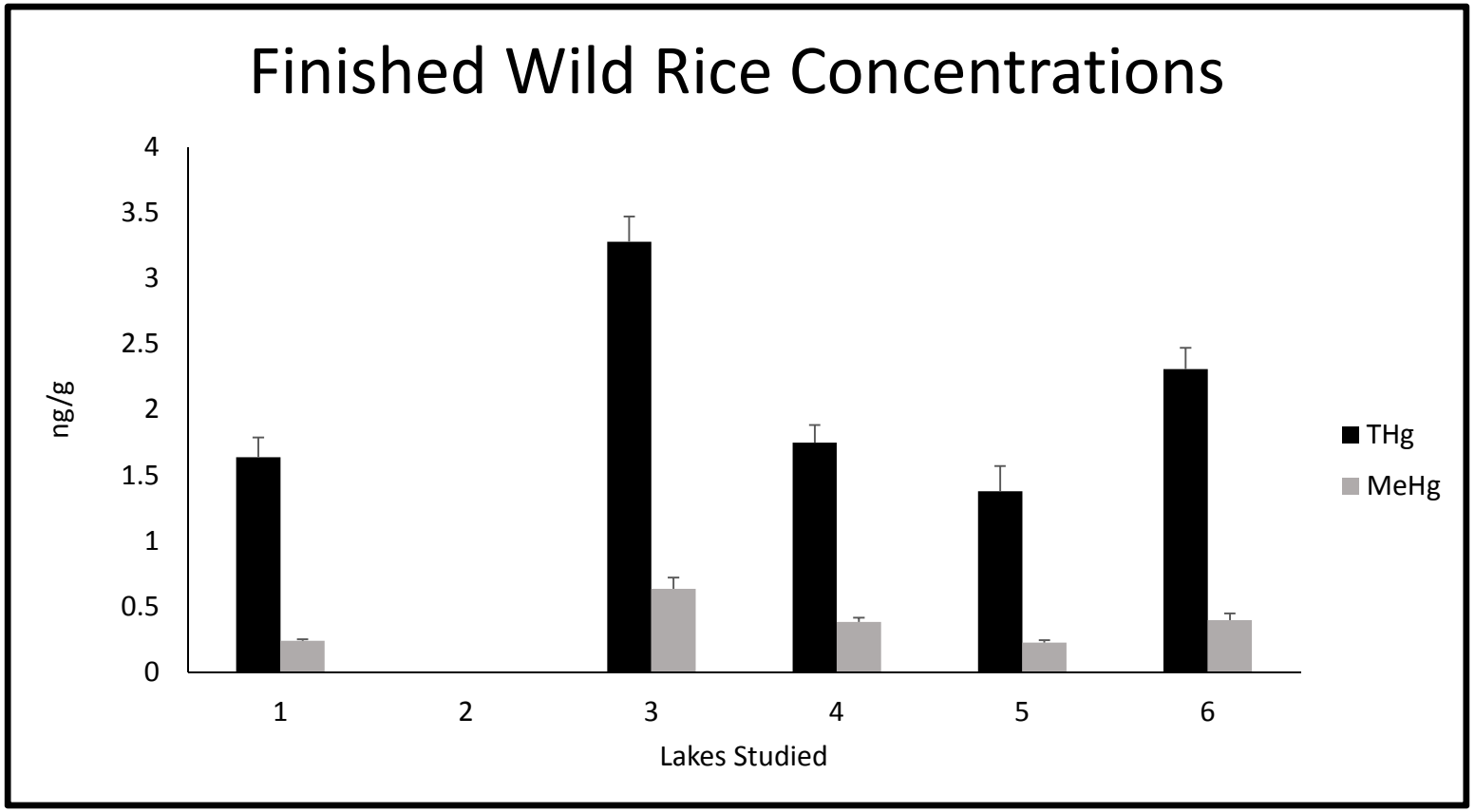

Figure 3-5 Mean THg and MeHg concentrations of finished wild rice samples for the six studied lakes: Lower Rice Lake (1), Long Lake (2), Perch Lake (3), Dead Fish Lake (4), Clark Lake (5), and Wolf Lake (6). Standard deviation positive error bars.

Unfinished wild rice grains provide a more thorough look at all six lakes, as Long Lake is included in these data. Unlike finished rice samples, the unfinished rice was analyzed once per lake, therefore there is no distinction between real values and mean values, and also no standard deviation error bars from multiple samples included in the bar graphs. The THg values for the unfinished rice range from 1.32-3.98 ng/g, with Lower Rice Lake exhibiting the lowest values and Long Lake exhibiting the highest value. The THg values for the six lakes are consistent from lake to lake, with the possible exception of Long Lake, which has a value 1.32 ng/g higher than the second highest value, that of Wolf Lake. MeHg values for unfinished rice range from 0.346-1.45 ng/g, with Clark Lake exhibiting the lowest value and Long Lake once again exhibiting the highest value. The MeHg values are also consistent from lake to lake, again with the possible exception of Long Lake, which has a value 0.796 ng/g higher than the second highest value, belonging to Dead Fish Lake. These data can be seen in Table 7 and Figure 3-6.

Lake Sampled	THg (ng/g)	MeHg (ng/g)
Lower Rice Lake	1.32	0.478
Long Lake	3.98	1.45
Perch Lake	2.47	0.389
Dead Fish Lake	2.57	0.654
Clark Lake	1.86	0.346
Wolf Lake	2.66	0.541

Table 7 Unfinished rice samples: THg and MeHg values for the six studied lakes.

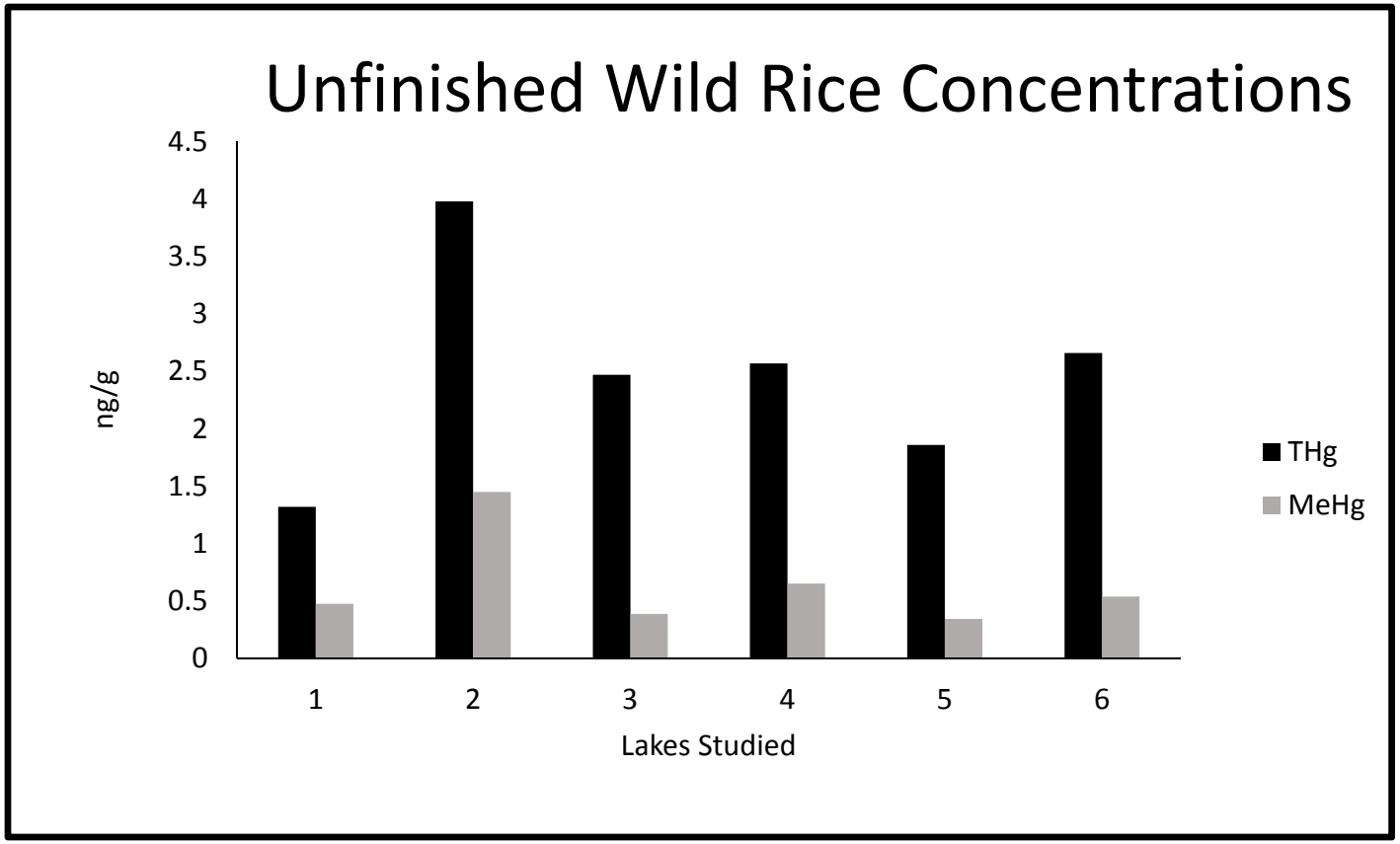

Figure 3-6 THg and MeHg concentrations of unfinished wild rice samples for the six studied lakes: Lower Rice Lake (1), Long Lake (2), Perch Lake (3), Dead Fish Lake (4), Clark Lake (5), and Wolf Lake (6).

Comparing the finished rice grains with the unfinished rice grains provides mixed results. For THg, the finished rice values are higher than unfinished rice values for Lower Rice Lake and Perch Lake, but they are lower for Dead Fish Lake, Clark Lake, and Wolf Lake. For MeHg, the finished rice values are higher than unfinished rice values for only Perch Lake, while they are lower for Lower Rice Lake, Dead Fish Lake, Clark Lake, and Wolf Lake. Of course, the data for Long Lake is incomplete, making a comparison between finished and unfinished rice difficult for the lake. However, the THg and MeHg for both finished and unfinished rice are consistent, as they have a range of 1.32-3.98 ng/g and 0.241-1.45 ng/g, respectively. The THg values can be compared in Table 8 and Figure 3-7, and the MeHg values can be compared in Table 9 and Figure 3-8.

Lake Sampled	THg (ng/g)	THg (ng/g)
Lower Rice Lake	1.64	1.32
Long Lake	-	3.98
Perch Lake	3.28	2.47
Dead Fish Lake	1.75	2.57
Clark Lake	1.38	1.86
Wolf Lake	2.31	2.66

Table 8 THg values for the six studied lakes, finished rice (left) and unfinished rice (right).

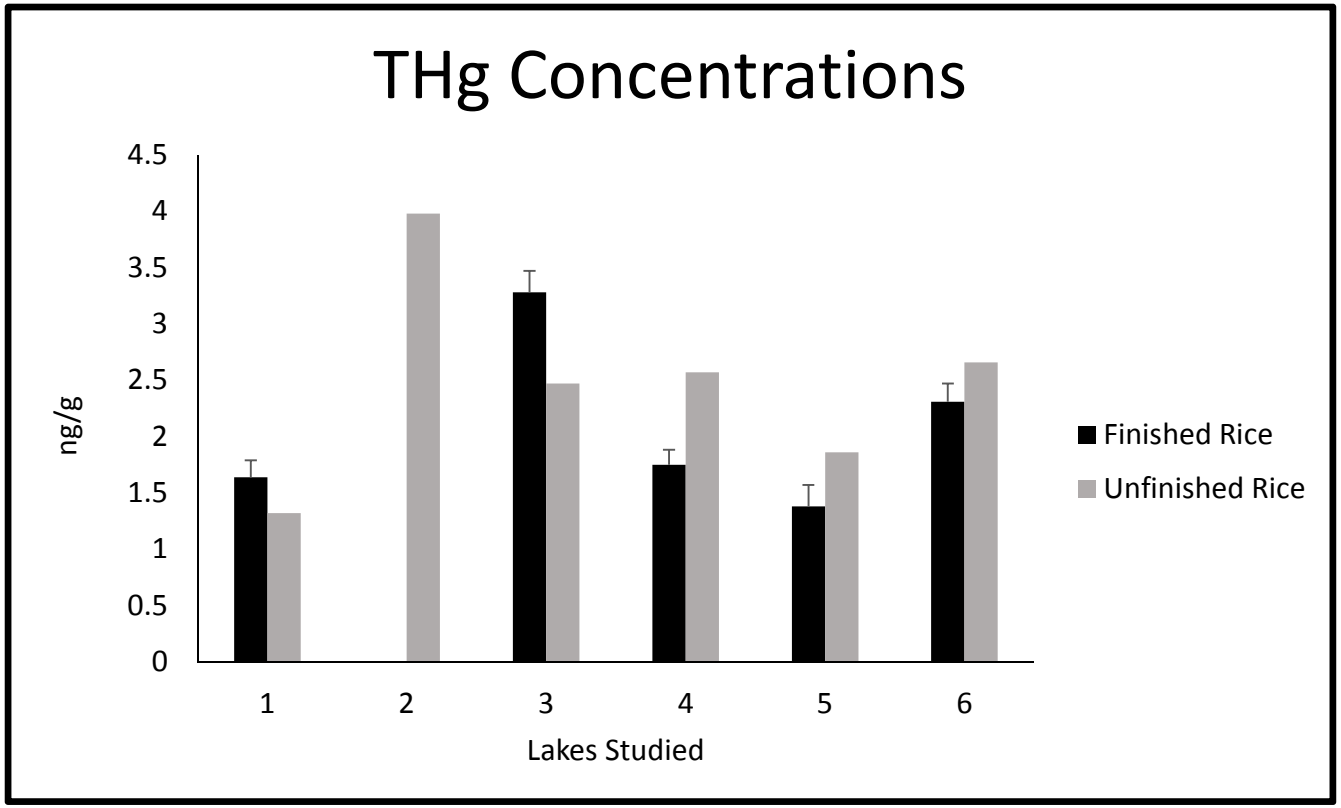

Figure 3-7 THg concentrations of finished wild rice samples and unfinished wild rice samples for the six studied lakes: Lower Rice Lake (1), Long Lake (2), Perch Lake (3), Dead Fish Lake (4), Clark Lake (5), and Wolf Lake (6). Standard deviation positive error bars.

Lake Sampled	MeHg (ng/g)	MeHg (ng/g)
Lower Rice Lake	0.241	0.478
Long Lake	-	1.45
Perch Lake	0.638	0.389
Dead Fish Lake	0.385	0.654
Clark Lake	0.227	0.346
Wolf Lake	0.398	0.541

Table 9 MeHg values for the six studied lakes, finished rice (left) and unfinished rice (right).

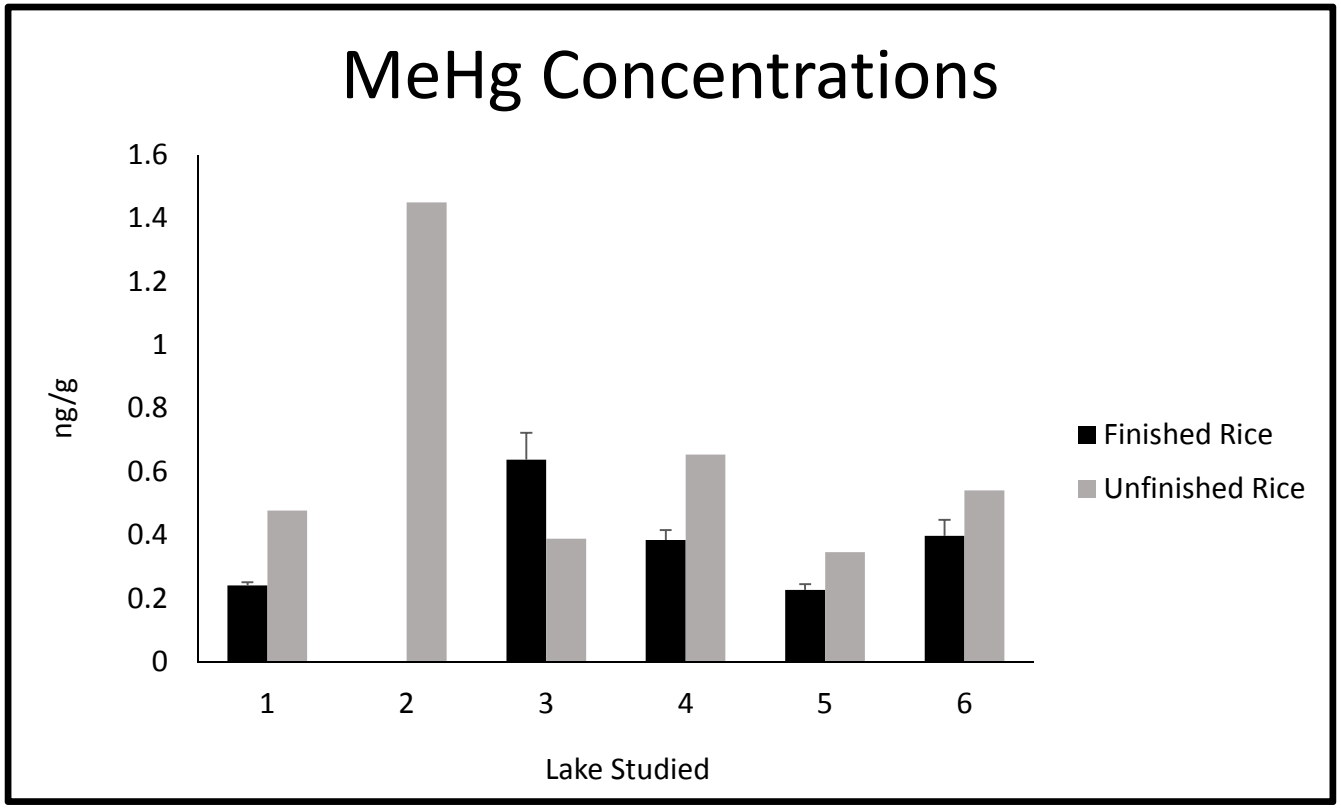

Figure 3-8 MeHg concentrations of finished wild rice samples and unfinished wild rice samples for the six studied lakes: Lower Rice Lake (1), Long Lake (2), Perch Lake (3), Dead Fish Lake (4), Clark Lake (5), and Wolf Lake (6). Standard deviation positive error bars.

3.4 Wild Rice Hull Samples

The THg values for the wild rice hull samples varied, as they ranged from 1.86-8.05 ng/g, with Perch Lake exhibiting the lowest value and Long Lake exhibiting the highest. The MeHg values for the hull samples were more consistent, ranging from 0.841-2.01 ng/g, with Dead Fish Lake exhibiting the lowest value and Long Lake again exhibiting the highest value. The THg values can be seen in comparison to the unfinished wild rice grains in Table 10 and Figure 3-9 below, while the MeHg comparison can be seen in Table 11 and Figure 3-10 below.

When compared to the THg values of unfinished wild rice grains, the wild rice hull values are considerably higher across the six lakes. However, Perch Lake is the one exception, as hull values here show a slight decrease from the grain. For the MeHg comparison, all six lakes have a hull value that increases in comparison to their respective rice grain values. Unlike the THg values, however, the increase seen in MeHg values when comparing hull to grain is not very significant.

Lake Sampled	THg (ng/g)	THg (ng/g)
Lower Rice Lake	1.32	5.27
Long Lake	3.98	8.05
Perch Lake	2.47	1.86
Dead Fish Lake	2.57	4.96
Clark Lake	1.86	6.68
Wolf Lake	2.66	4.16

Table 10 THg values for wild rice grains (left) and hull (right) for the six studied lakes.

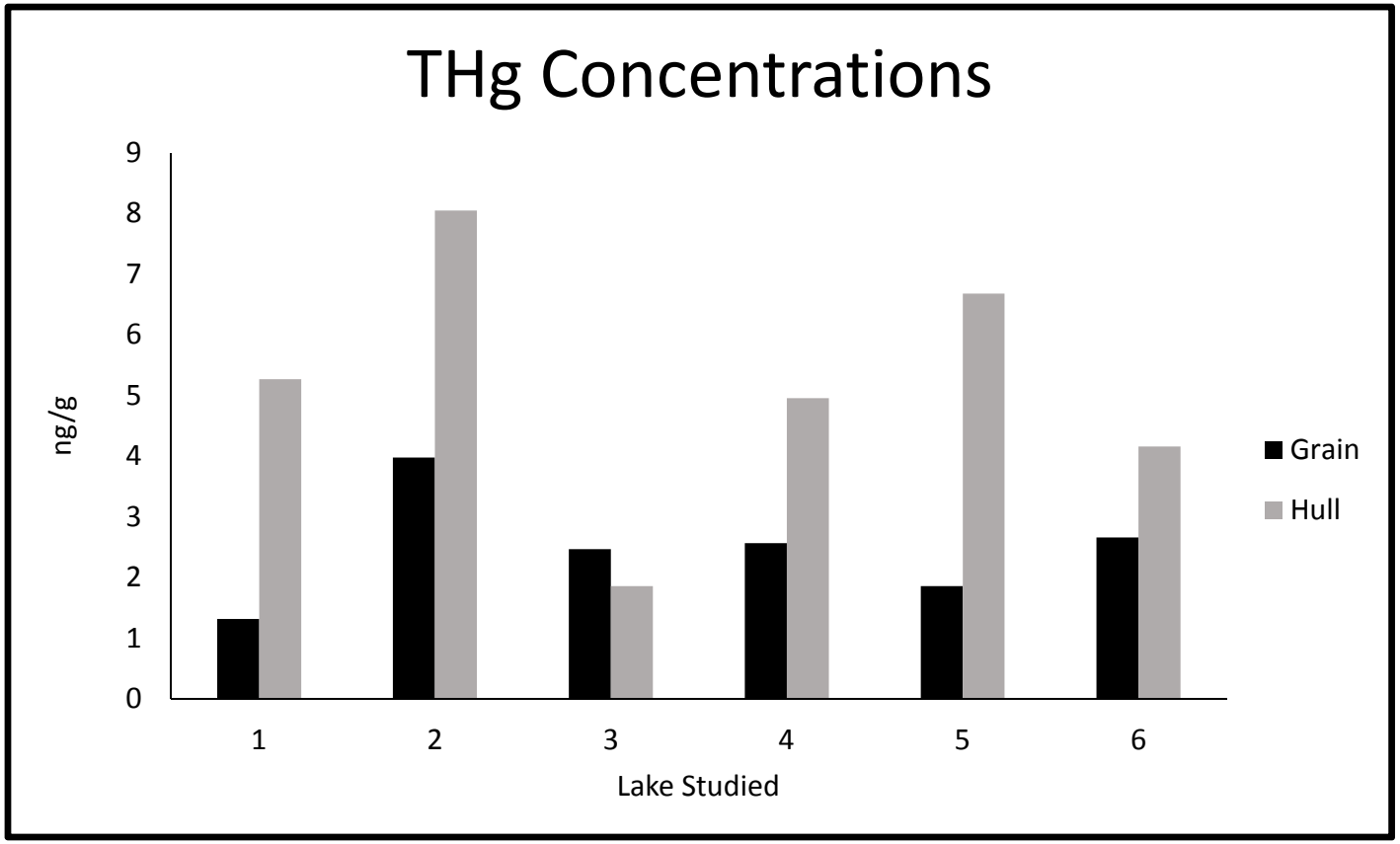

Figure 3-9 THg concentrations of unfinished wild rice grains and wild rice hull for the six studied lakes: Lower Rice Lake (1), Long Lake (2), Perch Lake (3), Dead Fish Lake (4), Clark Lake (5), and Wolf Lake (6).

Lake Sampled	MeHg (ng/g)	MeHg (ng/g)
Lower Rice Lake	0.478	0.998
Long Lake	1.45	2.01
Perch Lake	0.389	1.00
Dead Fish Lake	0.654	0.841
Clark Lake	0.346	1.08
Wolf Lake	0.541	1.26

Table 11 MeHg values for wild rice grains (left) and hull (right) for the six studied lakes.

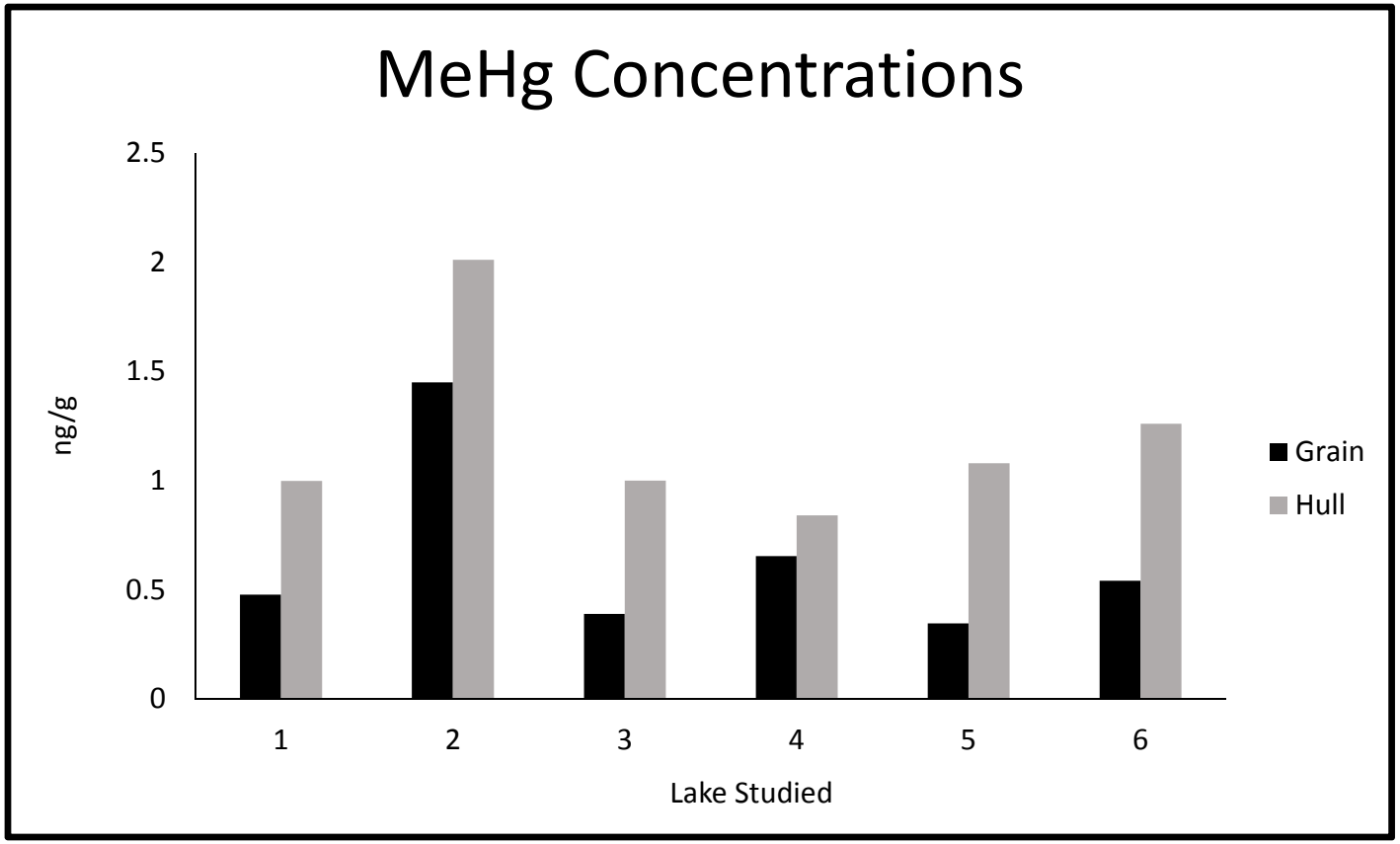

Figure 3-10 MeHg concentrations of unfinished wild rice grains and wild rice hull for the six studied lakes: Lower Rice Lake (1), Long Lake (2), Perch Lake (3), Dead Fish Lake (4), Clark Lake (5), and Wolf Lake (6).

3.5 Lake Core Samples

The 9 sediment samples used for RNA gene analysis are seen below in Figure 3-11 and the focus is on *Methanomicrobia*, *Firmicutes* and *Deltaproteobacteria*. Of the three, *Deltaproteobacteria* represents the highest relative abundance in all samples and *Firmicutes* represent the lowest. The breakdown of *Methanomicrobia* and *Deltaproteobacteria* can be seen below in Figure 3-12 and Figure 3-13, respectively. The *Methanosaeta* genus make up 61% of the *Methanomicrobia* in all 9 samples, *Methanoregula* make up 25 %, and the other 14% includes other *Methanomicrobia*, the majority of which include *Methanosarcina*, *Methanolinea*, and unclassified *Methanomicrobiales*. Of the *Deltaproteobacteria* in the 9 samples, the *Sva0485* order make up 36%, *Desulfuromonadales* make up 18%, *Syntrophobacterales* make up 15 %, unclassified *Deltaproteobacteria* make up 11%, and *Myxococcales* and 'other' *Deltaproteobacteria* each make up 10%. Of the other *Deltaproteobacteria*, *Desulfarculales*, *Syntrophorhabdaceae*, *Desulfobacterales*, and *GR-WP33-30* are the most abundant.

The clades of *Deltaproteobacteria* seen in Figure 3-14 are those abundant in sulfate and iron reducers, and each bar is representative of the combined percent in all 9 samples in order to show which order is the most abundant. The most abundant order is *Desulfuromonadales*, and the family breakdown consists of unclassified *Desulfuromonadales*, *21f08*, 'other' *Desulfuromonodales*, *LS4-241*, and *Geobacteraceae*.

The most abundant family in the *Desulfuromonadales* order is the unclassified *Desulfuromonadales*, which makes up 57% of *Desulfuromonadales*, and the least abundant is *Geobacteraceae*, which makes up 5% of *Desulfuromonadales*. Also included is *Desulfurobacterales*, *Desulfarculales*, and *Desulfovibrionales*. The *Desulfurobacterales* family breakdown includes *Desulfobacteraceae*, *Desulfobulbaceae*, and unclassified *Desulfurobacterales*. *Desulfarculales* is the second most abundant order seen in Figure 3-14 below, and the *Desulfovibrionales*, which is sparsely represented, contains *Desulfovibrio*.

The content of the sediment samples (0-2.5 cm depth) used for *hgcA* gene analysis can be seen in Table 12. Included is the taxonomy of nearest relatives (*Geobacter*-like, *Methanomicrobia*, and unknown), the number of clones, and inferred physiology (iron reducer, methanogen, and unknown). The taxonomy is dominated by 20 *Geobacter*-like *Deltaproteobacteria*, in addition to 8 *Methanomicrobia*, and 6 unknowns. This information can be seen in greater detail in Figure 3-15. This phylogenetic tree shows how the 34 sequences in this study fit amongst a background of *Geobacter*-like *Deltaproteobacteria*, sulfate-reducing *Deltaproteobacteria*, *Firmicutes*, *Methanomicrobia*, and an unknown group. Unlike the data shown in Table 12, the data in Figure 3-16 consists of 20 *Geobacter*-like sequences, 8 Methanomicrobia, 5 unknowns, and 1 Firmicutes.

Sulfate and total iron concentrations in the Perch Lake core porewater samples are shown in Figure 3-16. The sulfate concentration peaks near 0.20 ppm in the shallow

portion of the core but levels off below detection (<0.01 ppm) with depth. However, the total iron concentration begins around 7.0 ppm and increases to around 20.0 ppm with depth. For total iron, the average of all duplicated samples was taken and used in this plot, resulting in 9 (and not 13) plot points.

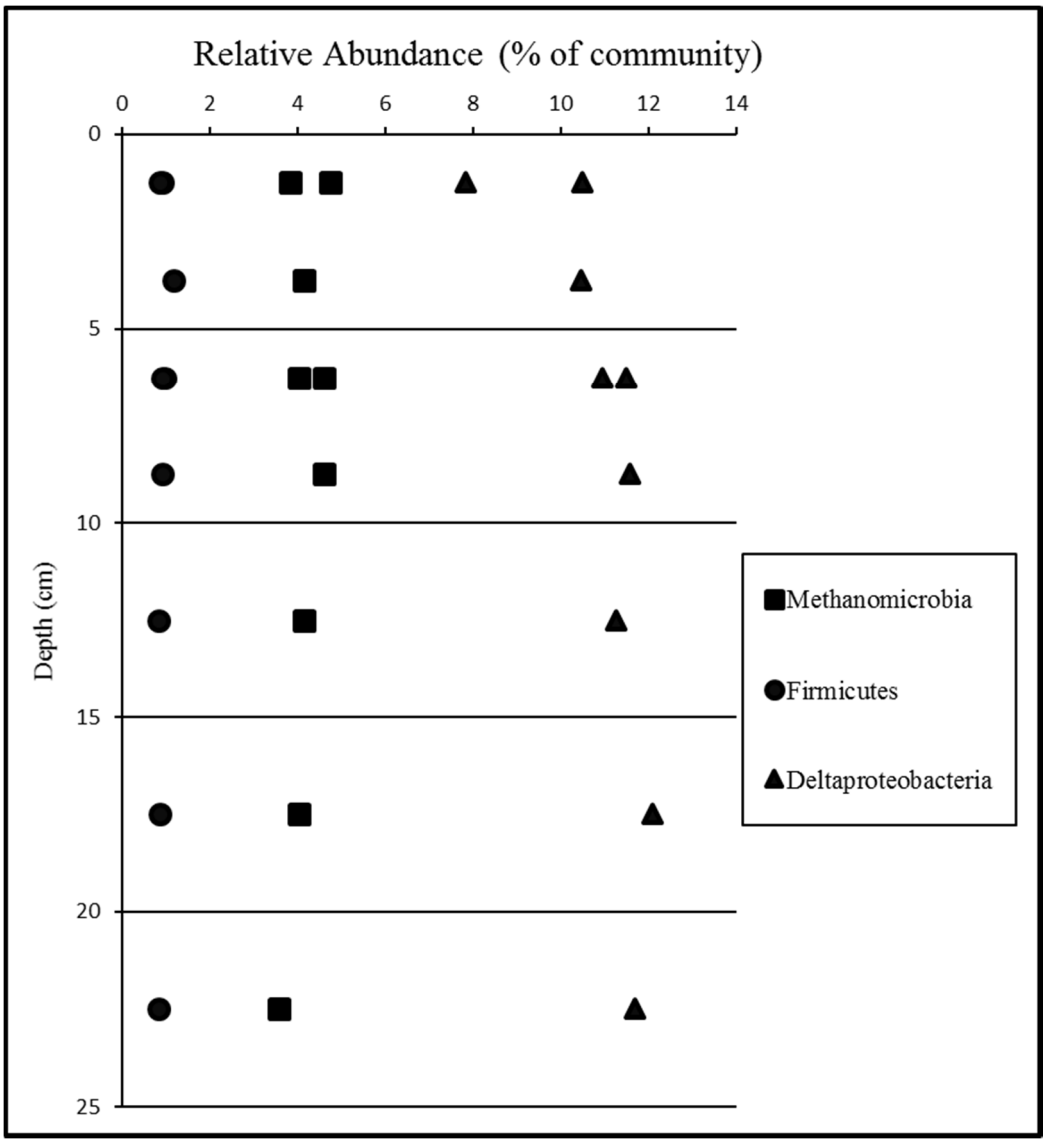

Figure 3-11 Relative abundance of Methanomicrobia, Firmicutes, and Deltaproteobacteria in 9 sediment samples from the Perch Lake core.

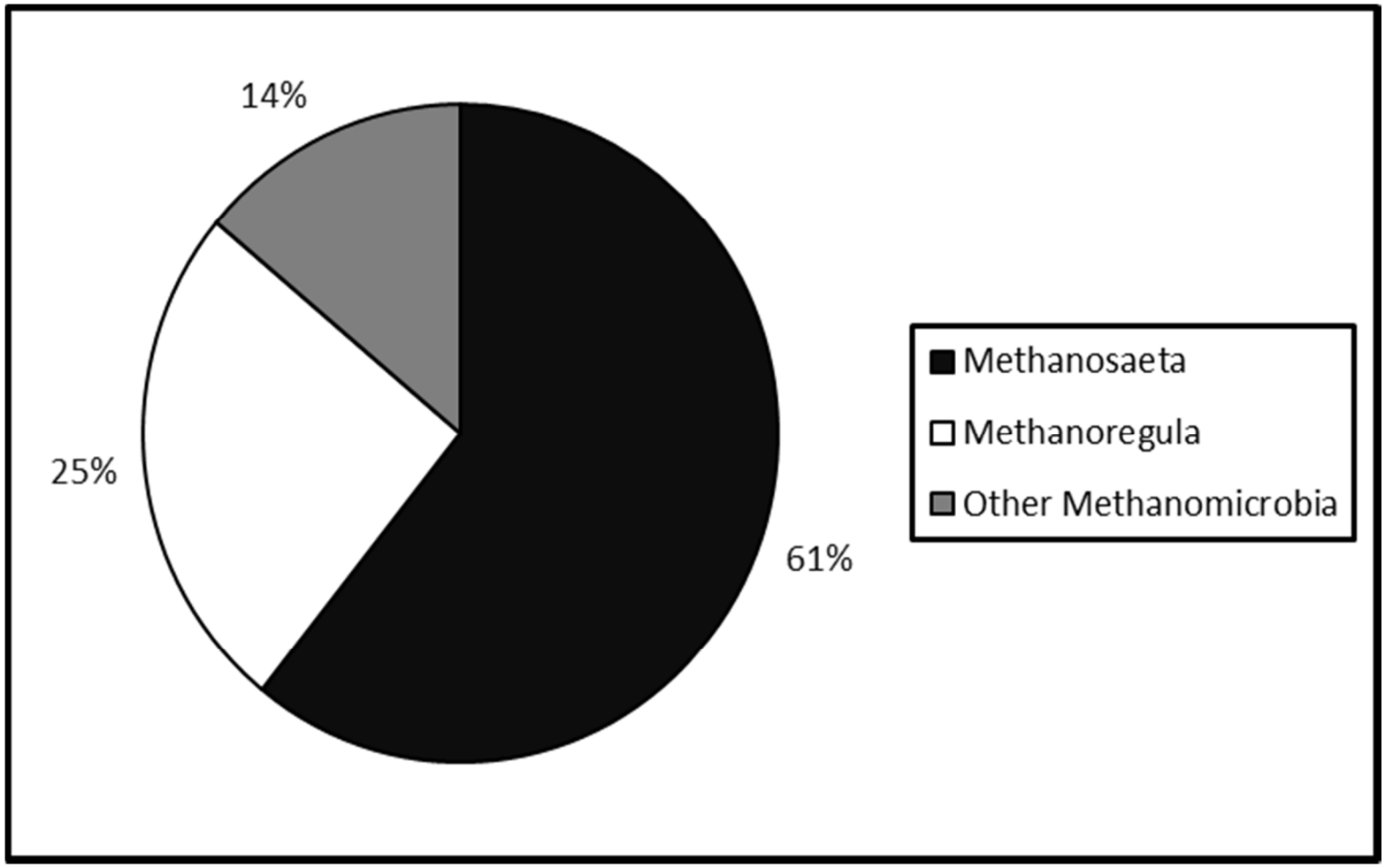

Figure 3-12 Makeup of the Methanomicrobia family in 9 sediment samples from the Perch Lake core.

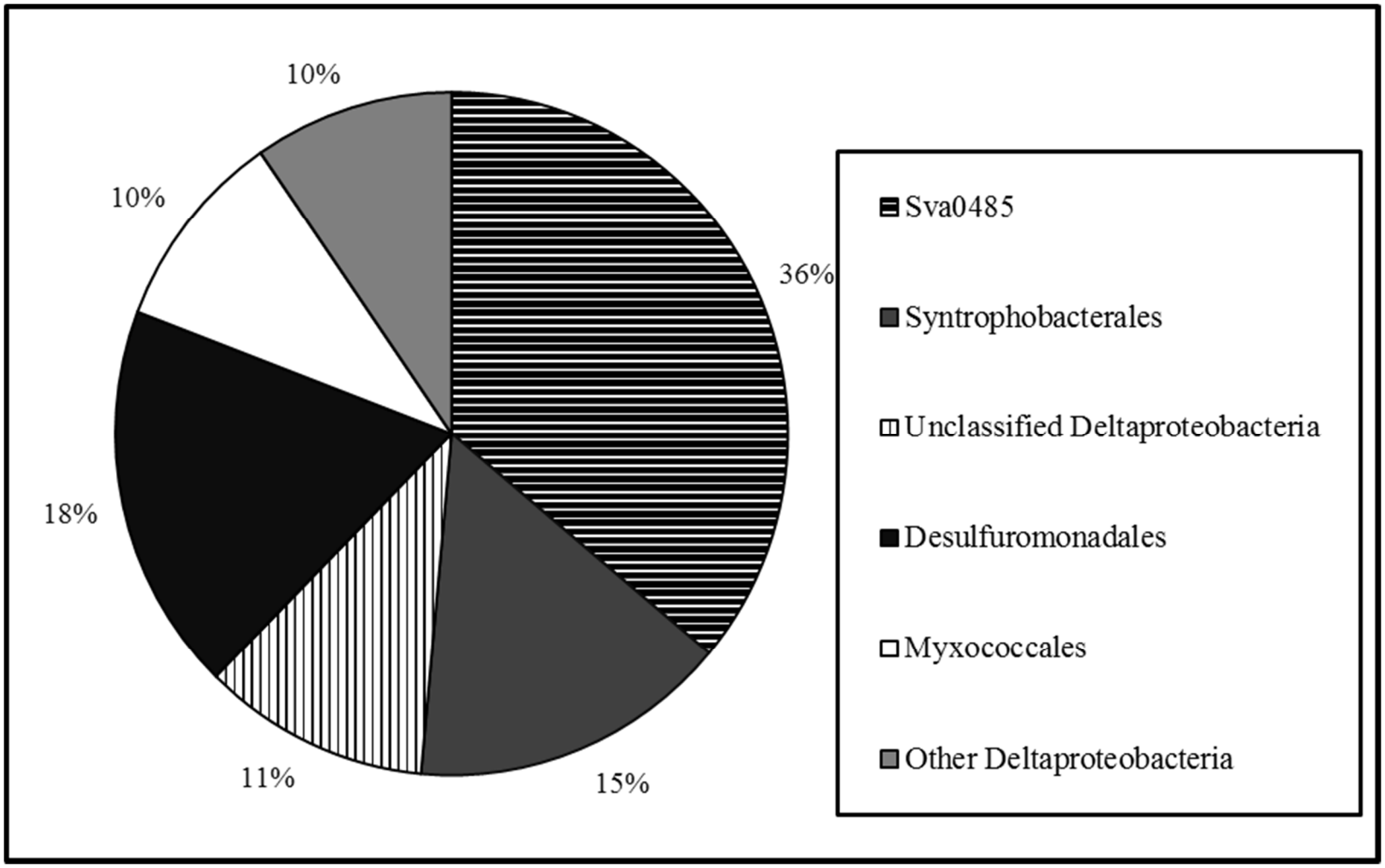

Figure 3-13 Makeup of the Deltaproteobacteria order in 9 sediment samples from the Perch Lake core.

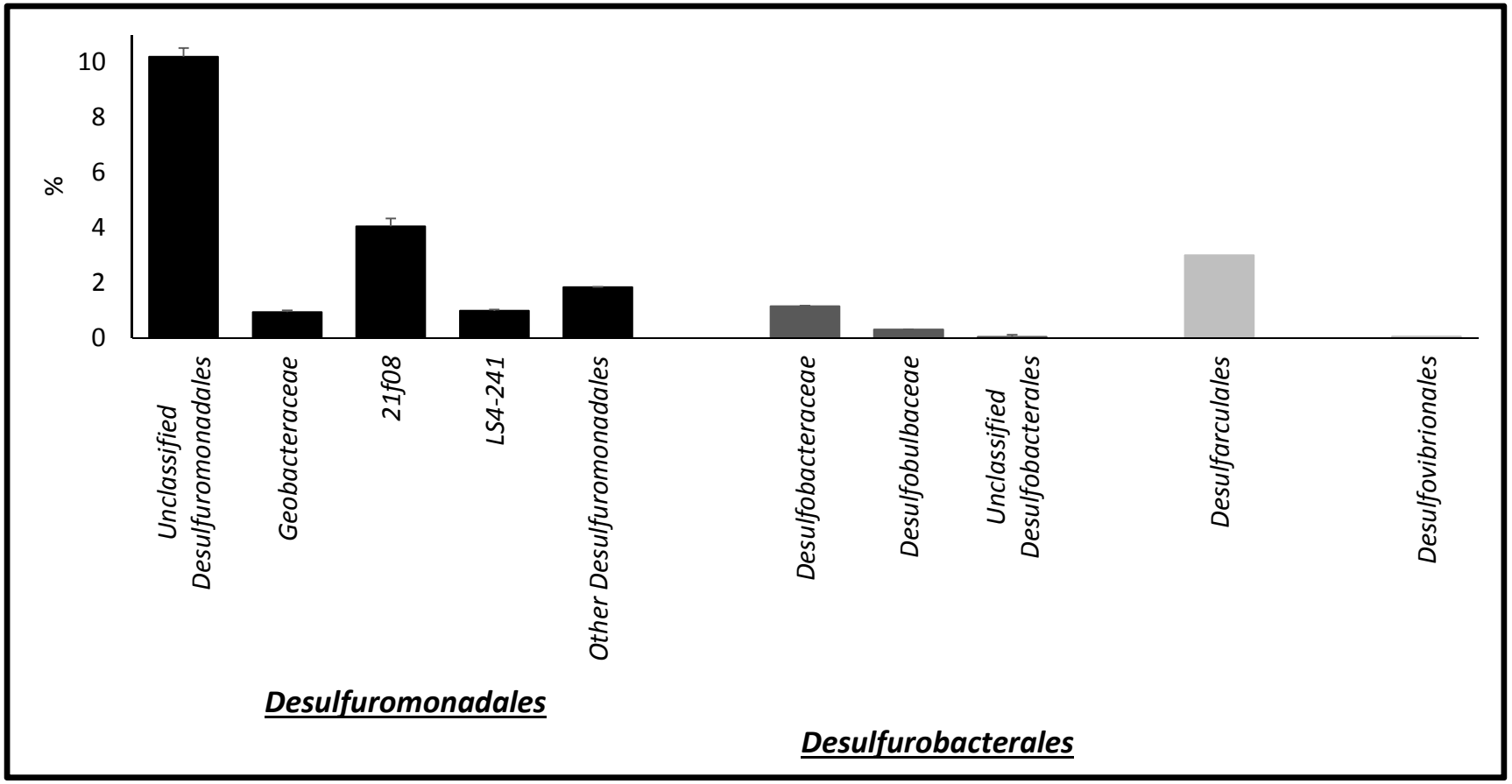

Figure 3-14 Order makeup of Deltaproteobacteria clade with abundant sulfate and iron reducers in all 9 samples, including family grouping for Desulfuromonadales and Desulfurobacterales. Standard deviation positive error bars.

Taxonomy of nearest relatives	No. of clones	Inferred physiology
Geobacter-like	20	Iron Reducer
Methanomicrobia	8	Methanogen
Unknown	6	Unknown

Table 12 Inferred physiology from top sediment in the Perch Lake core based on blastx analysis and phylogenetic placement.

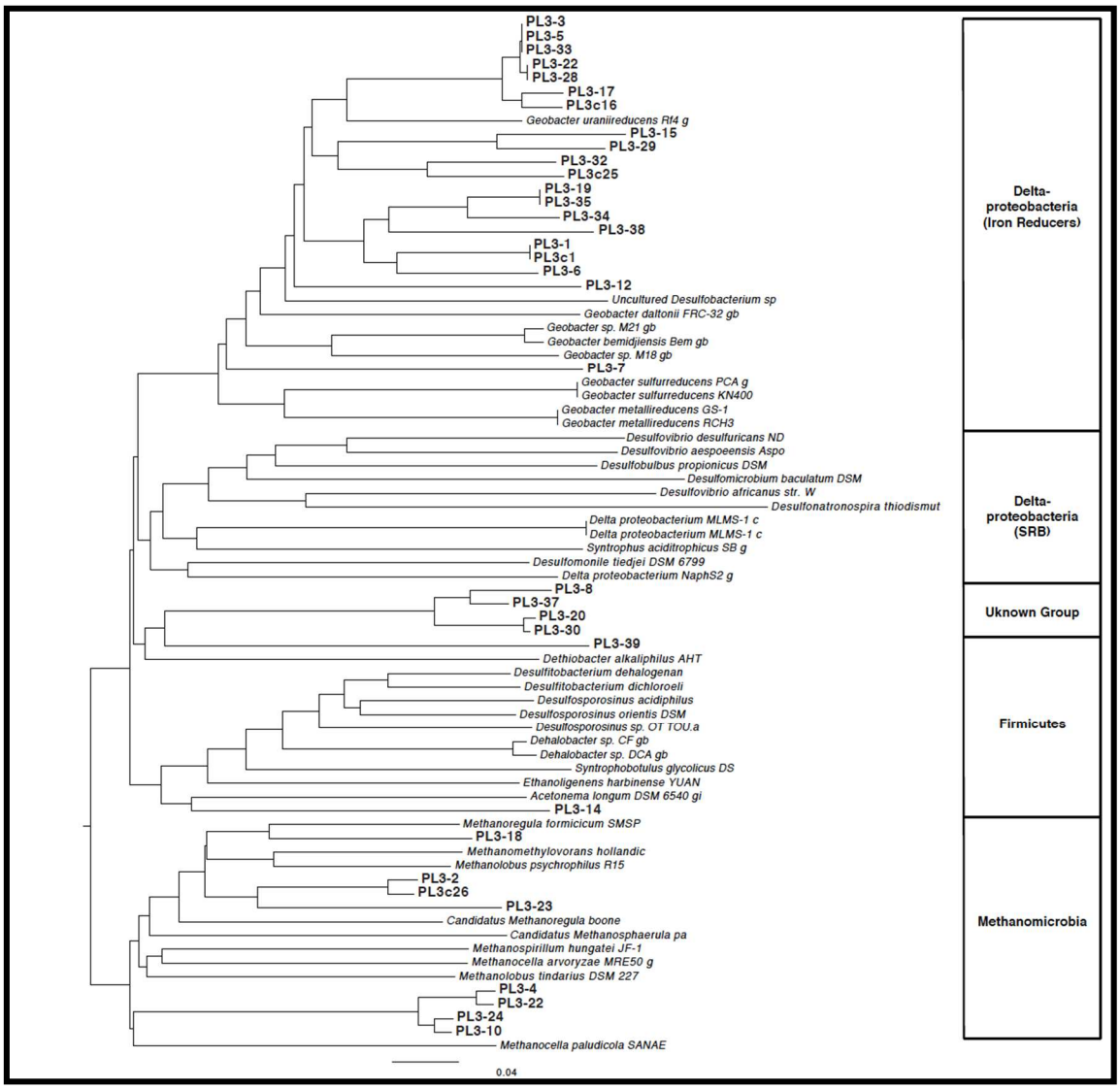

Figure 3-15 Phylogenetic Tree including hgcA sequences from the top sediment of the Perch Lake core. (Schaefer, 2014)

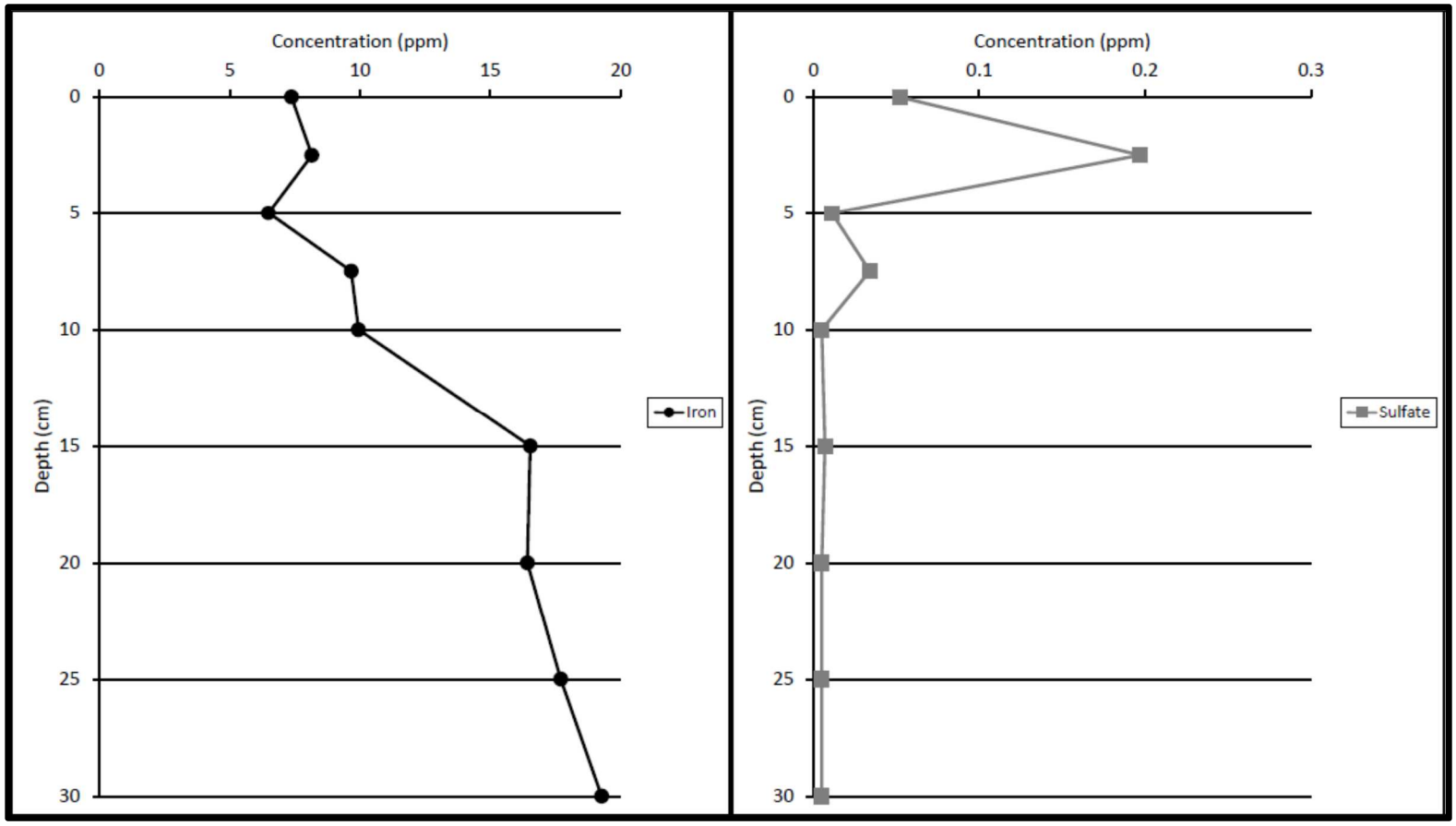

Figure 3-16 Total iron and sulfate concentration (ppm) with depth (cm) from the porewater of the Perch Lake core.

CHAPTER 4: DISCUSSION

4.1 Water Samples

The surface water samples analyzed in this study fit well with previous studies (Engstrom, 1994 and Monson, 2008), as the range of THg values (1.48-2.86 ng/L) show consistency across the state of Minnesota. The major source of Hg^0 is atmospheric deposition and it is likely that such deposition is uniform in an area of the size sampled in this study (Engstrom, 1994). In 2008, the U.S. Environmental Protection Agency (EPA) conducted a survey of the nation's lakes in order to find out which lakes were in good, poor, or fair condition for trophic state, ecological health, and recreation. Of the 909 lakes studied, 50 Minnesota lakes were sampled and analyzed for THg and MeHg (Monson, 2008). One sample per lake was taken from the middle of each lake in July and August of 2007 using the Clean Hands/Dirty Hands technique, and two samples were taken for two of the lakes.

The lake areas in the 2008 EPA study (Monson, 2008) ranged from 11-15,958 acres, compared to a range of 49-2,040 acres in this study, seen in detail in Table 13 below. The range for THg in the 2008 EPA study was 0.162-5.242 ng/L with a mean of 0.875 ng/L. Therefore, the range of 1.48-2.86 ng/L is well within the EPA's study of Minnesota lake THg concentrations, which can be seen in Table 13 and Figure 4-1 below. THg concentrations from the 2008 EPA study show a trend of 0.5-2.0 ng/L concentrations across much of northern Minnesota, with those greater than 2.0 ng/L being

found in northeastern Minnesota. The values found in the three areas of this study fit well with these data points. Furthermore, the maximum value of 5.242 ng/L found in the 2008 EPA study supports the decision to remove the likely contaminated samples of 6.76 ng/L and 18.53 ng/L in this study, although the former is still within the Minnesota water quality standard for Hg, 6.9 ng/L.

The MeHg concentrations found for surface water samples in this study also fit well with trends observed in previous studies (Monson, 2008). The lakes sampled on the White Earth Reservations show a lower mean value for MeHg concentration in surface water when compared to the lakes sampled on the Fond du Lac Reservation and the 1854 Treaty Area, as the lowest value is ~20% of the highest value. This trend is likely due to the different sediments found in each wetland area studied. White Earth lies on more mineral-rich sediment associated with the Des Moines Lobe and Wadena Lobe, and the organic-rich sediments associated with the Superior Lobe and Rainy Lobe found near Fond du Lac and the 1854 Treaty Area would likely contain more heavy metals, such as Hg. Since the MeHg in the surface water is likely coming from lake sediments, the improved water quality in White Earth lakes (compared to organic-rich eastern lakes) is represented by this difference in the composition of lake sediment. Difference in water quality can be observed in the sediment samples above (Figure 2-11), as these sediment samples contain some lake water.

The range of values (0.0312-0.1497 ng/L) for MeHg concentrations in this study fit well within the values found in the EPA's 2008 study for MeHg, which were 0.006-

0.522 ng/L with a mean of 0.093 ng/L. As seen in Figure 4-2, many of the EPA report data points for MeHg concentrations near White Earth are less than 0.10 ng/L and many of those in northeastern Minnesota are in the range of 0.10-0.30 ng/L. These trends fit well with the values found across the three areas in this study, as White Earth lakes contained less than 0.10 ng/L and Fond du Lac, 1854 Treaty Area lakes contained 0.10-0.30 ng/L, with the exception of Dead Fish Lake (0.0692 ng/L).

Study	Lake Area (acres)	THg (ng/L)	MeHg (ng/L)
2008 EPA Study (Mean)	822	0.875	0.093
This Study (Mean)	579	2.123	0.089
2008 EPA Study (Range)	11-15,958	0.162-5.242	0.006-0.522
This Study (Range)	49-2,040	1.480-2.860	0.031-0.150

Table 13 Mean and range values for lake area, THg concentrations, and MeHg concentrations for the 2008 EPA study and this study. (Monson, 2008)

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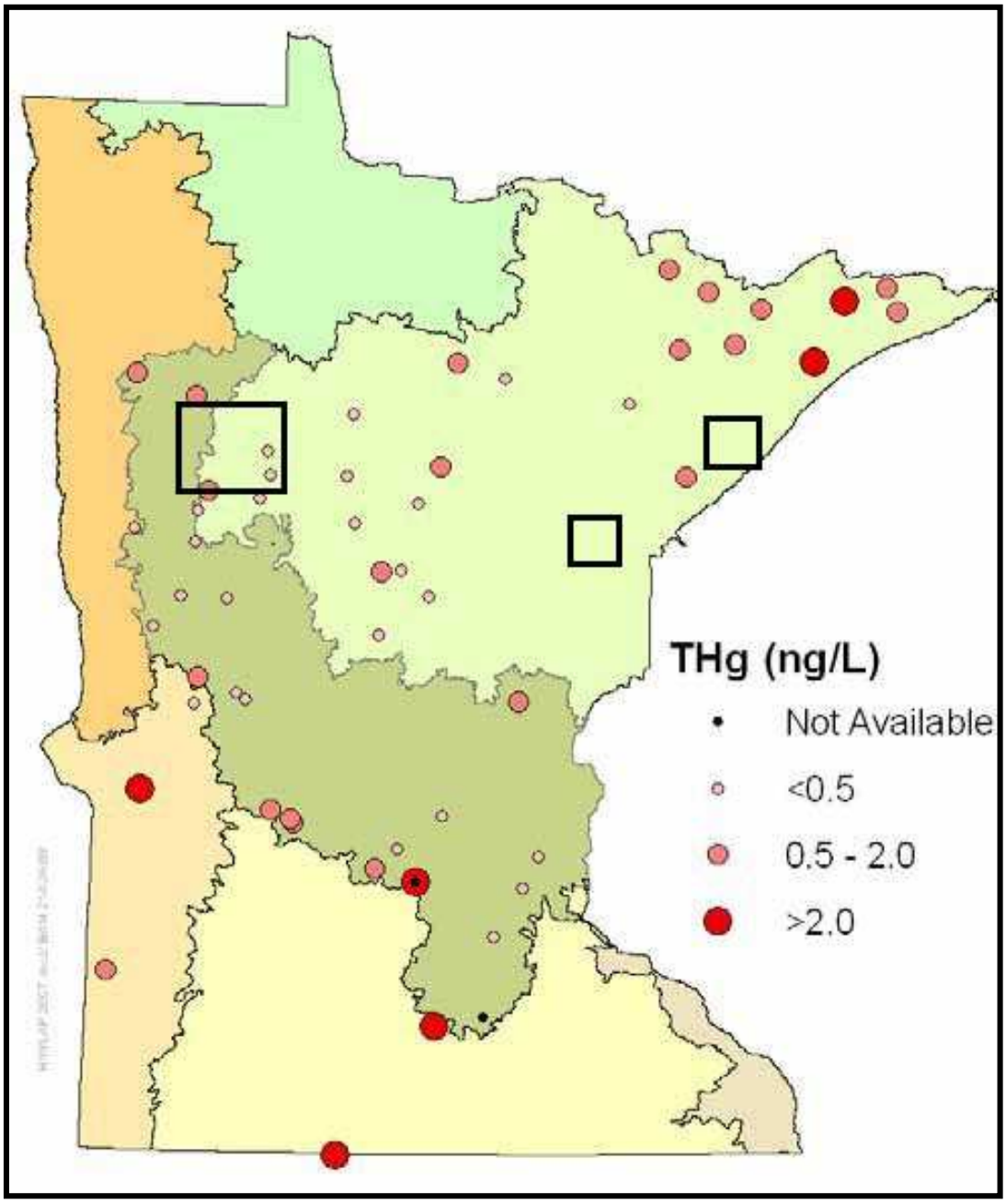

Figure 4-1 Distribution of THg concentrations from the 2008 EPA study with overlying ecoregions. Black squares represent estimations for the White Earth Reservation (left), Fond du Lac Reservation (middle) and the1854 Treaty Area sample sites (right). (Monson, 2008)

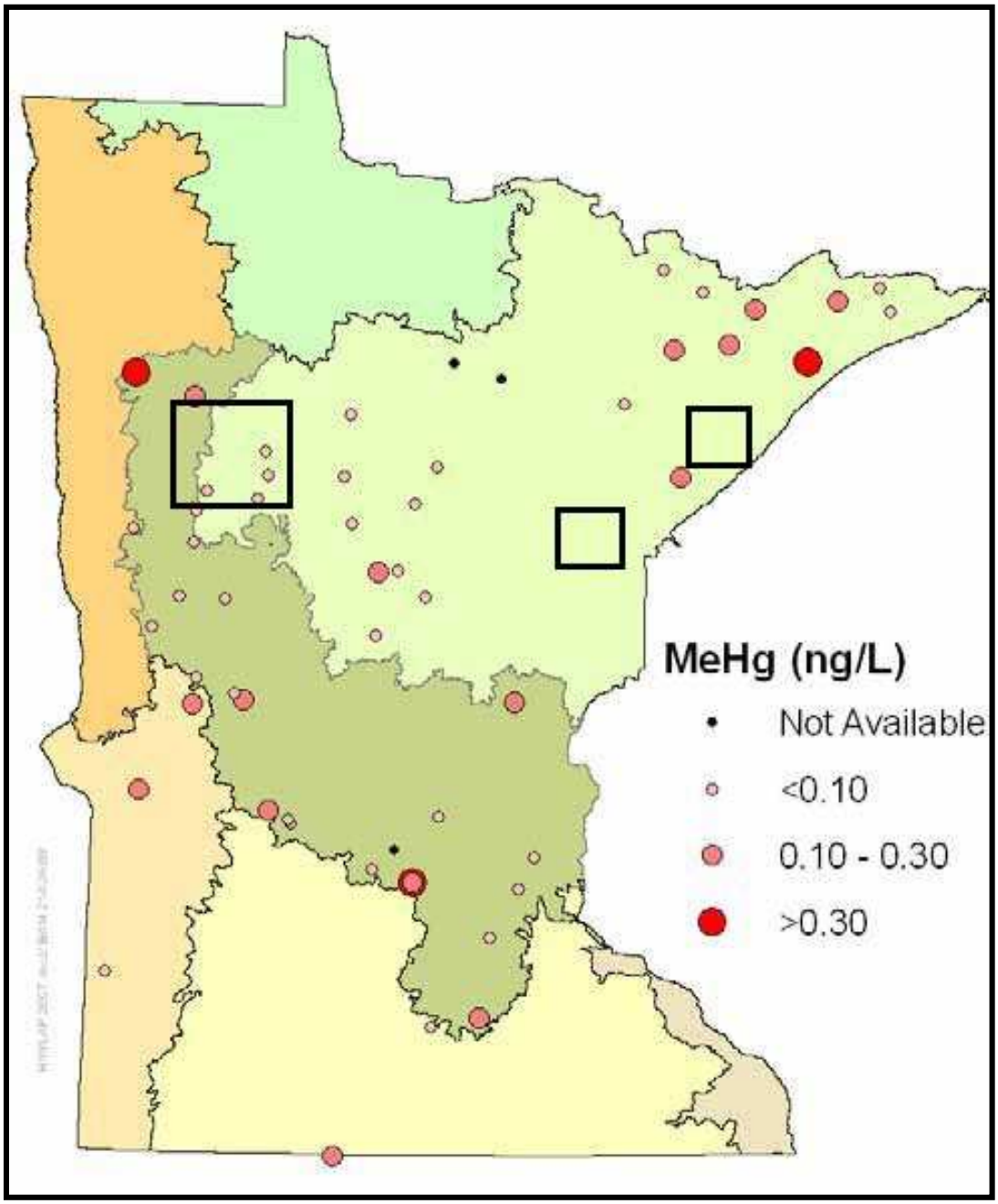

Figure 4-2 Distribution of MeHg concentrations from the 2008 EPA study with overlying ecoregions. Black squares represent estimations for the White Earth Reservation (left), Fond du Lac Reservation (middle) and the1854 Treaty Area sample sites (right). (Monson, 2008)

4.2 Sediment Samples

Similar to water samples, the concentrations of THg and MeHg in lake sediment samples showed that trends fit well with a previous study (Engstrom, 1994). The 1994 study on lake sediment conducted by Engstrom, *et al.* included lakes in western and northeastern Minnesota. The THg values for surface sediment in western Minnesota lakes (Mountain and Cedar) were found to be 63 and 101 ng/g and for 4 eastern lakes (Kjostad, Meander, Dunnigan, and Thrush), the range was 198-370 ng/g. These values agree with the THg values found in this study, as the western lakes contain less THg in the sediment and this can be due to several things, including dilution by high sediment inputs, particularly carbonates. Also, Hg has a high affinity for organic matter and this explains the high THg values seen in lake sediments associated with organic-rich till. Less organic matter in the White Earth lake sediments allows for THg to more easily escape into the water and vaporize back into the atmosphere, whereas organic-rich lake sediments associated with Fond du Lac and 1854 Treaty Area lakes tend to trap the THg. Difference in lake sediment can be seen in Figure 2-11, shown in Chapter 2. The White Earth lakes contain grey, sandy sediments and the eastern lakes contain organic-rich, dark sediments. (Engstrom, 1994)

The concentrations of MeHg in sediments across the study areas in this investigation follow a similar trend as THg concentrations, although the range is less extreme. Since not all inorganic Hg is available for methylation, the bacteria and archaea

in the lake sediment are likely limited by THg availability and this explains the consistency between the lakes, despite the drastic differences in THg concentrations. The low amount of MeHg in the sediment is likely due to the fact that methylators are competing with demethylators, thus a balance is provided in the overall amount of MeHg released to surrounding sediments. All rice lakes contain high levels of organic matter, which contributes to anoxia, and provides a food source for methylating bacteria and archaea. Despite the amount of organic matter in rice lakes, the THg content of lake sediment doesn't appear to have an extreme effect on the MeHg content in the sediment, therefore the amount of MeHg ultimately taken up by the rice plants is limited. Because the western lakes have much lower carbon content in their sediment, this may limit microbial activity and methylation.

4.3 Wild Rice Grain and Hull Samples

The wild rice samples in this study provided data that was more complex to interpret in comparison to water and sediment samples, as there is a dearth of published Hg studies involving wild rice. It is difficult to compare wild rice plants to white rice plants out right, as they differ in species and the potential pollution of their associated environments. While the THg and MeHg values for the wild rice grains, both finished and unfinished, were consistently low among the lakes, the question of bioaccumulation needed to be answered. A chronic exposure formula was used to determine potential toxicity, and this involved multiplying the concentration of Hg in the rice grain by the total amount of rice eaten on a daily basis. This value is then divided by the mass of the individual consuming the rice, and the equation can be seen below. For concentration, it

is important to use the highest THg and MeHg values found in this study, and any concentrations below these will present lower risk. For finished rice, the highest concentrations happen to be from Perch Lake: 3.28 ng/ for THg and 0.638 ng/g for MeHg. For the amount of rice eaten and the mass of the individual, it is important to use the mass of a small to average person who is eating large amounts of rice per day. For example, if a 70 kg individual consumes 0.35 kg (approximately 6 cups cooked) of rice per day, the daily intake value will be 1.71e-05 mg/kg for THg and 3.49e-06 mg/kg for MeHg.

$$(concentration) \times (mass\ consumed) \div (mass\ of\ individual)$$

Integrated Risk Information System (IRIS) is maintained by the U.S. EPA and includes Toxnet Database reference doses (RfDs) for THg and MeHg daily intake and they are 3.00e-04 mg/kg and 1.00e-04 mg/kg, repectively (National Institue of Health). If the daily intake values calculated are greater than these references doses, toxicity is a concern. However, when dividing the daily intake values of 1.71e-05 mg/kg for THg and 3.49e-06 mg/kg for MeHg by these reference doses, values of 5.7e-02 for THg and 3.49e-02 for MeHg are obtained. In summary, the highest values found in this study make up only 5.7% and 3.5% of the reference doses for THg and MeHg, respectively. Such low values indicate that finished wild rice from northern Minnesota poses a negligible risk for human health. While the finished rice is the product eaten, the unfinished rice concentrations for Long Lake are higher than the values used in this calculation.

However, these slightly higher values will not increase the low percentages obtained by much and would therefore not be a concern.

The low values (5.7e-02 for THg and 3.49e-02 for MeHg) obtained using the chronic exposure assessment and IRIS Toxnet references doses suggests that there is a very low risk for harmful bioaccumulation of THg and MeHg through the consumption of wild rice, even when considering a low-mass individual eating a large amount of wild rice per day. In fact, the values are low enough to eliminate any concern of bioaccumulation by consumption of wild rice when added to consumption of fish, as wild rice consumption will not greatly worsen Hg uptake in this scenario. The low risk assessment was confirmed in a January 2015 meeting with members of the Minnesota Department of Natural Resources, Minnesota Pollution Control Agency, and the Minnesota Department of Health. It was determined that the highest THg and MeHg values found in wild rice in this study posed extremely low risk for harmful bioaccumulation.

In analyzing the hull of the wild rice plant, it is clear that the hull contained more THg and MeHg than the grain. The rice grains being compared to the hull are the unfinished grains, although THg and MeHg in finished and unfinished rice grains are similar. This trend is seen in every lake, with the exception of THg for Perch Lake, which shows slightly higher values in the grain than in the hull. For THg, the source is likely adsorption of Hg from the atmosphere and any contamination during collection, as the hull protects the rice grain from the rice knockers, the canoe floor, and any other

contact involved with the harvesting of wild rice. However, MeHg concentrations in the hull are greater than that in the grain across all six studied lakes, and this likely represents preferential accumulation of MeHg in the hulls of this particular species of rice plant. Fortunately, the hull further protects the grain by taking up a greater amount of MeHg and the hull is eventually discarded in the finishing process.

4.4 Lake Core Sample

The archaea and bacteria found within the Perch Lake core is consistent with that found in previous studies of wetlands (Liu, 2014 and Schaefer, 2014). *Deltaproteobacteria* in this study are more abundant than *Methanomicrobia* and *Firmicutes*, similar to the 2014 Schaefer *et al.* study of the *hgcA* gene in wetland soils from Florida and Sweden and to the 2014 Liu *et al.* study of wetland soils from the Wanshan area in southwest China. The *hgcA* sequences from environmental samples cluster into six groups: *Firmicutes*, sulfur reducers, iron reducers, *Syntrophs*, *Chloroflexi*, and *Methanomicrobia* (Liu, 2014). In Perch Lake core sediments, F*irmicutes* have very low relative abundance in the core sediment. Previous studies showed low abundance results for *Chloroflexi* (Liu, 2014 and Schaefer, 2014). Therefore, this study focuses on *Methanomicrobia* and *Deltaproteobacteria*, more specifically methanogens, sulfate reducers, and iron reducers.

Previous studies suggest the sulfur reducing *Deltaproteobacteria* genera *Desulfococcus*, *Desulfobulbus*, and *Desulfovibrio* contain the *hgcA* gene and can therefore methylate mercury (Liu, 2014 and Schaefer, 2014). These genera occur in the

Desulfobacterales order, which represents about 1.6% of the *Deltaproteobacteria*. In contrast, *Desulfuromonodales*, which contains iron redcuing *Geobacter*, makes up 18% of the *Deltaproteobacteria,* although most of the sequences in that group are classified as 'unknown *Desulfuromonadales*' and only 5% are in the family Geobacteraceae (Figure 3-14). However, high concentrations of dissolved iron in the sediment porewaters indicates that iron reducers are active in these sediments. Also, Schaefer *et al.* 2014 suggests *Methanoregula* methylates mercury, and this species accounted for 25% of *Methanomicrobia* found in this study.

Given the relatively low amount of sulfate reducers present in the *Deltaprotebacteria* phlyum, the methylation of mercury in this specific core may be a result of iron reducers and methanogens. In fact, this is supported by the geochemical data, as there is a significant amount of total iron present in the porewater and little to no sulfate. The dominance of iron reducers and methanogens is also supported by the *hgcA* gene analysis data obtained from the top soil of the core, as 20 of the 34 sequences analyzed are inferred to be iron reducers and 8 were determined to be methanogens. However, primer bias could result in some misidentification of methylating bacteria and archaea.

It is likely some of the remaining 6 unknown sequences are sulfate reducers, but this data is ultimately dominated by iron reducers and methanogens. In fact, the BLASTX hits in the NCBI-nr database included a mix of sulfate reducers, iron reducers, and methanogens (in addition to one that looks to belong to the firmicutes chade, as seen

in Figure 3-15) for the 6 unkown sequences. However, these were deemed "unknown" in Table 12 due to a low (<60%) BLASTX match from the NCBI database, which is consistent with their phylogenetic placement (Figure 3-15) and this somewhat represents the challenge of inferring physiology. It is important to remember that the microbial aspect of this study is limited to a single core from just one of the six lakes included in the rest of this study and in no way represents all wild rice lakes. Nevertheless, the microbial analysis provides further insight into wetland environments and which types of microbes could be responsible for methylating mercury.

The low amount of Hg uptake by wild rice plants in Minnesota in this study suggests there is no concern for toxicity to humans. However, more research can be done to better understand these rice paddy ecosystems. Methylating bacteria and archaea continue to be studied and it is vital to better understand the role they play in the environment. Also, the way in which wild rice plants uptake substrates, including heavy metals, can be studied. Pollution may be a major player in the amount of Hg in rice plants but plant species and the presence of methylators also play a role that can be studied and better understood. Human health risks remain a concern when it comes to MeHg in the environment and learning more about environments that support rice and fish may help to lower these risks in the future.

CHAPTER 5: SUMMARY

Mercury is a naturally occurring element and it can be converted into the toxic organic form of methylmercury by microbes. Industrialization has increased the atmospheric input of Hg and this has resulted in more deposition in the environment. Wetland environments contain sulfur-reducing bacteria and even enhance microbial activity due to a high amount of organic matter and plant decay. These environments include rice lakes, and rice can be an integral part of many peoples' diets. MeHg is a potent neurotoxin and a recognized health concern. The most common dietary sources of Hg for humans are from the consumption of fish. Recent studies in Asia have also shown that consumption of white rice grown on Hg-contaminated sites is also a concern. Introducing too much MeHg to the human body can lead to chromosomal damage, and individuals at highest risk are pregnant women and children. This study compared Hg concentrations in wild rice plants growing in rice lakes that sit on different glacial tills in Minnesota in order to identify any geological influences on uptake and to assess any risk in bioaccumulation of MeHg through the consumption of wild rice.

Wild rice is found in abundance in northern Minnesota. Six lakes were studied across northern Minnesota, from calcareous-rich glacial till settings in northwestern Minnesota to organic-rich till settings in northeastern Minnesota. The hypothesis was that lakes in northwestern Minnesota would naturally contain less inorganic Hg due to sandy, mineral-rich till and this would mean less MeHg in sediment and in turn less

MeHg taken up by rice plants in northwestern Minnesota, in comparison to northeastern Minnesota. Surface water, surface sediment, and wild rice samples were taken from two lakes located on the White Earth Reservation, two lakes on the Fond du Lac Reservation, and two lakes in the 1854 Treaty Area. All samples were analyzed for THg and MeHg at the Department of Soil, Water, and Climate at the University of Minnesota.

The water samples showed a consistency in THg concentrations, likely due to steady atmospheric deposition across the state. For sediment samples, the THg concentrations ranged from extremely low for White Earth lakes to significantly larger for Fond du Lac and 1854 Treaty Area lakes. This is a good representation of different ecoregions in the state, as the mineral-rich lake sediment in the west would have lower inorganic Hg values than organic-rich lake sediment in the eastern part of the state. The MeHg concentrations for water samples were lower in the western part of the state, and this is different from the much more consistent MeHg concentrations in the sediment across the state. Since the surface water MeHg concentrations are likely coming from lake sediment, lower amounts of organic matter in western lake sediment is likely responsible for the low values seen in western surface water. The MeHg concentrations in sediment are likely low due to a balance in methylating and demethylating bacteria. Also, not all inorganic Hg is available for methylation, which would explain the consistency seen in MeHg values when compared to the accompanying, varying THg values for the lakes.

The largest THg and MeHg values obtained for finished rice in this study were used in a chronic exposure formula, in addition to a large amount of rice eaten per day by an individual of average mass. This resulted in low values for THg and MeHg concentrations when compared to the IRIS Toxnet RfDs. A January 2015 meeting with members of the Minnesota Department of Natural Resources, Minnesota Pollution Control Agency, and the Minnesota Department of Health confirmed this finding. It was determined that the highest THg and MeHg values found in wild rice in this study posed extremely low risk for harmful bioaccumulation. Furthermore, relatively uniform MeHg values in both sediment and wild rice across the state suggests that geology doesn't significantly influence the amount of THg or MeHg ultimately taken up by rice grains in Minnesota, despite the drastic difference in THg in sediments across the state.

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