EFFECTS OF OXYGENATION ON METALS CYCLING IN LAKES

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EFFECTS OF OXYGENATION ON METALS CYCLING IN LAKES

Abstract

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This dissertation summarizes three different evaluations of oxygenation treatments on metals in freshwater lakes conducted from 2007 to 2011. The goal of this work was to evaluate the effectiveness of hypolimnetic oxygenation in repressing methylmercury accumulation in the water column to add to the body of knowledge on how to ultimately reduce the accumulation of mercury in the aquatic food web. Methylmercury, the mercury species of concern in aquatic food webs, is formed by the methylation of inorganic mercury by anaerobic sulfate reducing bacteria. Maintaining oxic conditions should, therefore, repress anaerobic methylation of mercury.

The first evaluation covered in this dissertation is a sediment-water interface (SWI) chamber study presented in Chapter 2. There was little difference between the oxic and anoxic chambers, and after examination, it became apparent that the anoxic chambers do not have an oxic-anoxic interface (OAI), an important parameter for the dynamic of mercury methylation. To avoid these problems associated with the confining environments of chambers, the remaining assessments were conducted on full lake water columns.

In Chapter 3 and 4, a full-scale oxygenation system was evaluated at North Twin Lake, WA. The first evaluation followed a late season oxygenation test conducted after the
onset of hypolimnetic oxygenation with iron, manganese, and methylmercury already accumulated in the bottom waters. Injection of oxygen dropped iron and manganese by 70% within 8 hours of operation and dropped methylmercury 95% by the end of the two week test. Full-scale operation of oxygenation had mixed results in regards to oxygen delivery to the bottom waters. Iron, manganese, and methylmercury accumulation was repressed during times when dissolved oxygen was kept above 4 mg/L. However, considering the system was designed to deliver dissolved oxygen higher in the water column, there were many times throughout the 3 year study period where bottom dissolved oxygen dropped below 1 mg/L. Although this system was not optimal for repressing methylmercury accumulation in this application, it did demonstrate a promising treatment effect and shows potential for the optimization of future applications of hypolimnetic oxygenation for repressing methylmercury accumulation in aquatic ecosystems in the future.
TABLE OF CONTENTS

ACKNOWLEDGEMENTS...........................................................................................................iii

ABSTRACT..................................................................................................................................iv

LIST OF TABLES..........................................................................................................................x

LIST OF FIGURES........................................................................................................................xi

DEDICATION...............................................................................................................................xiii

CHAPTER

1. INTRODUCTION.....................................................................................................................1

1. OVERVIEW..............................................................................................................................1

2. MERCURY...............................................................................................................................1

3. CONCEPTUAL MERCURY MODEL.........................................................................................4

   1. Oxygenated Hypolimnion.......................................................................................................6

   2. Anoxic Hypolimnion...............................................................................................................7

4. CHAPTER 2 – CHAMBER STUDY...........................................................................................8

5. CHAPTER 3 – HYPOLIMNETIC OXYGEN TEST...................................................................8

6. CHAPTER 4 – FULL SCALE HYPOLIMNETIC OXYGENATION...........................................9

7. CHAPTER 5 – EDUCATIONAL OUTREACH.........................................................................9

8. REFERENCES..........................................................................................................................10

2. MERCURY BEHAVIOR IN OXIC VERSUS ANOXIC SEDIMENT-WATER INTERFACE CHAMBERS

1. ABSTRACT..............................................................................................................................14

2. INTRODUCTION.....................................................................................................................15

3. MATERIALS AND METHODS.............................................................................................17
3. RESPONSE OF IRON, MANGANESE, AND MERCURY IN AN ANOXIC WATER COLUMN TO SHORT-TERM HYPOLIMNETIC OXYGENATION

1. ABSTRACT...........................37
2. INTRODUCTION...........................38
3. METHODS..............................41
   1. Study Site..........................41
   2. Water Quality Monitoring.........42
   3. Water Quality and Sediment Analyses...43
4. RESULTS AND DISCUSSION.............44
   1. Dissolved Oxygen, Temperature, and Conductivity......44
   2. Iron and Manganese................48
      1. Vertical distribution prior to oxygenation........48
      2. Vertical distribution during oxygenation...........51
3. Vertical distribution post oxygenation test.............. 55

3. Total Mercury and Methylmercury............................... 56

1. Vertical distribution prior to oxygenation................. 57

2. Vertical distribution during oxygenation................. 57

3. Vertical distribution post oxygenation test............. 59

5. CONCLUSION..................................................................... 60

6. REFERENCES................................................................... 62

4. EFFECTS OF HYPOLIMNETIC OXYGENATION ON WATER COLUMN IRON, MANGANESE, AND METHYLMERCURY

1. ABSTRACT........................................................................ 74

2. INTRODUCTION................................................................... 75

3. METHODS......................................................................... 79

1. Study Site.......................................................................... 79

2. Water Quality Monitoring............................................. 80

3. Water Quality Analysis................................................... 80

4. RESULTS........................................................................... 81

1. Dissolved Oxygen.......................................................... 81

2. Iron and Manganese....................................................... 82

3. Methylmercury............................................................... 84

5. DISCUSSION..................................................................... 86

1. Inter-Annual Variation in Response of Dissolved Oxygen.... 87

2. Response of Iron and Manganese to Oxygenation......... 86

3. Response of Mercury to Oxygenation......................... 91
4. Co-Variation in Response of Dissolved Oxygen……………….. 96

6. REFERENCES………………………………………………………..99

5. TARGETED OUTREACH TO ENHANCE DIVERSITY IN UNIVERSITY-
SPONSORED TECHNOLOGY COMPETITIONS

1. ABSTRACT…………………………………………………………. 118

2. INTRODUCTION……………………………………………………. 118

3. RATIONALE………………………………………………………… 120

4. PRACTICE DESCRIPTION……………………………………… 121

5. RESULTS AND DISCUSSION…………………………………… 121

   1. High School Academic and Ethnic Characteristics………….. 121

   2. Outreach to Study High School…………………………… 123

6. CONCLUSION………………………………………………………. 126

7. REFERENCES……………………………………………………… 129

6. CONCLUSION………………………………………………………. 132
LIST OF TABLES

2.1. Deer Lake Sediment Characteristics................................................. 36
2.2. Sediment Flux Rates for Fe, Mn, SRP, and MeHg for Last 15 Days of the Incubation................................................................. 36
3.1. Iron, Manganese, Conductivity, and Sulfide in the Hypolimnion of North Twin Lake............................................................... 72
3.2. Total Mercury and Methylmercury in the Hypolimnion of North Twin Lake.................................................................................. 73
4.1. Physical Characteristics of North and South Twin Lakes...................... 104
4.2. Volume Weighted Averages in the Hypolimnion and Maximum Bottom Meter Concentrations for Iron, Manganese, and Methylmercury in North Twin Lake in Mid-October................................................. 108
4.3. Volume Weighted Averages in the Hypolimnion and Maximum Bottom meter Concentrations for Iron, Manganese, and Methylmercury in North Twin Lake in Mid-October................................................. 108
LIST OF FIGURES

1.1. Conceptual Model of Mercury Cycling ............................................. 13

2.1. Bathymetric Map of Deer Lake, WA ................................................. 34

2.2. Concentration Time Series in Chambers ........................................... 35

3.1. Bathymetric Map of North Twin Lake, WA ...................................... 69

3.2. Pre, During, and Post Test Profiles .................................................. 70

3.3. Dissolved Oxygen Isopleths during Oxygen Test .............................. 71

4.1. Bathymetric Map of North and South Twin Lakes, WA .................. 103

4.2. Dissolved Oxygen, Iron, Manganese, and Methylmercury
Isopleths for North Twin Lake .............................................................. 105

4.3. Dissolved Oxygen, Iron, Manganese, and Methylmercury
Isopleths for South Twin Lakes .............................................................. 106


4.5. Iron, Manganese, and Methylmercury in the Epilimnion,
Metalimnion, and Hypolimnion in North Twin Lake ........................... 110

4.6. Iron, Manganese, and Methylmercury in the Epilimnion,
Metalimnion, and Hypolimnion in South Twin Lake ............................ 111

4.7. Iron, Manganese, and Methylmercury Profiles in North
and South Twin Lakes on July 28\textsuperscript{th}, 2010 .......................... 112

4.8. Synoptic Profiles of Iron and Manganese in North Twin
Lake on June 22\textsuperscript{nd}, 2011 ............................................................. 113

4.9. Dissolved Oxygen versus Iron and Manganese in North
and South Twin Lakes for 2009 – 2011 ............................................. 114
4.10. Mean Volume Weighted Averages for North and South Twin Lakes from June through September for all years..........................115

4.11 Methylmercury versus Iron and Manganese for North and South Twin Lakes for 2009 – 2011..................................................116

4.12. R² for Methylmercury versus Iron and Manganese for each Individual Year in North Twin Lake..............................................117

5.1. 2008 Washington Assessment of Student Learning Pass Rates..................131
Dedication

I would like to dedicate this dissertation to my son Zander and to our child who is on the way. I hope that this accomplishment will be an inspiration to you both. Do great things with your lives and set high goals for yourselves. The opportunities available to you in this life are limitless.
CHAPTER 1. INTRODUCTION

1.1 OVERVIEW

This dissertation focuses on mercury cycling in freshwater lakes, with a particular emphasis on the effects of a hypolimnetic oxygenation lake treatment on mercury (Hg) accumulation in the water column. The dissertation is broken up into 6 chapters. Chapter 1 is a broad introduction, encompassing the background of Hg topics covered in Chapters 2 through 4. Chapter 1 also includes a conceptual Hg model that will aid the reader in interpreting the material in Chapters 2 through 4, and includes a brief introduction to each chapter. Chapter 2 is a summary of a chamber study on profundal sediments from Deer Lake, WA. Chapter 3 is a summary of an evaluation of a whole lake in-situ hypolimnetic oxygenation test conducted at North Twin Lake, WA. Chapter 4 presents three years of iron (Fe), manganese (Mn), and Hg data from North Twin Lake which was oxygenated, along with a neighboring lake, South Twin Lake, as a reference. Chapter 5 summarizes a case study of an educational outreach endeavor that I participated in as a National Science Foundation Fellow in the GK-12 program at Lake Roosevelt High School in Grand Coulee, WA. Chapter 6 is a succinct conclusion wrapping up the key points of the Chapters 2 through 4.

1.2 MERCURY

Hg is a unique pollutant that has the ability to cause problems globally, resulting in bioaccumulation in food webs not only at contaminated sites, but also in remote and seemingly pristine lakes (Cabana et al., 1994; Lathrop et al., 1991). Globally, human post-industrial activities have resulted in a three-fold increase of Hg deposition rates, 5-20% of which is
estimated to end up in aquatic ecosystems where it may accumulate in aquatic biota (Swain et al., 2007). Hg in fish tissue has been discovered in freshwater ecosystems all across the United States. Nearly one-third of US lakes have consumption advisories in place due to elevated concentrations of Hg in fish tissue, and 20 states have statewide lake fish consumption advisories (USEPA, 2004). Methylmercury (MeHg), one of the most toxic natural forms of Hg (Tchounwou et al., 2003), is the primary form of the metal that accumulates in fish, typically representing more than 98% of total Hg (THg) in fish biomass (Bloom, 1992). MeHg is typically produced in-situ, rather than through areal deposition or watershed runoff, in freshwater lakes (Eckley et al., 2005; Orihel et al., 2007). Production of MeHg is commonly observed with the onset of anoxic conditions over the sediment-water interface (SWI) (Watras, 2009).

In mesotrophic and eutrophic freshwater lakes, an anoxic layer of water commonly forms over the SWI during thermal stratification. Consumption of dissolved oxygen (DO) leads to anoxia and is related to nutrient inputs, both natural and anthropogenic, that promote both the production of organic carbon and its subsequent decomposition (Wetzel, 2001). Anoxia typically increases in area and thickness over the duration of the stratification period, sometimes encompassing the entire hypolimnion prior to a fall or spring turnover event (Horne and Goldman, 1994). Anoxic conditions result in the reduction and dissolution of Mn and Fe oxides in surficial sediments (Davison, 1993), with a corresponding release of phosphorus sorbed to the metal oxides (Niemistö et al., 2008; Welch and Jacoby, 2001; Boström et al., 1988). Anoxic lake sediments are also an important source of ammonia to overlaying water (Beutel, 2006). A number of studies have observed an increase in MeHg in bottom waters coincidental with the onset of anaerobic conditions in the hypolimnion (He et al., 2008; Eckley and Hintelmann, 2006; Herrin et al., 1998; Slotton et al., 1995; Watras et al., 1995).
The accumulation of MeHg in anoxic water is a combined effect from the methylation of inorganic Hg, and the release of previously sequestered MeHg. MeHg is likely produced by anaerobic bacteria, including sulfate reducing bacteria (SRB), which flourish in the anaerobic bottom waters and sediments of productive lakes (Gilmour et al., 1992; Benoit et al., 2003). Many studies have also shown a concurrent release of MeHg into overlying waters with the reduction of Fe and Mn oxides (Merritt and Amirbahman, 2008; Chadwick et al., 2006; Jacobs et al., 1995; Slotton et al., 1995; Gill and Bruland, 1992).

The accumulation of MeHg in anoxic bottom waters is generally the difference between in-situ MeHg production and demethylation. Watras et al. (1995) found the main source of MeHg production to the hypolimnion was from an anoxic microbial layer directly beneath the oxic-anoxic interface (OAI), whether that be in the sediment or the water column. Ancillary experiments showed a high level of sulfate reduction within the microbial layer, indicating the activity of SRB. Similarly, Mauro et al. (2002) observed high levels of MeHg formation below the OAI that coincided with high optical density in the water column, suggesting an active microbial layer. Eckley and Hintelmann (2006) evaluated seasonal MeHg dynamics in Canadian lakes. When the hypolimnion was oxygenated, the highest Hg methylation potential was observed at or just below the SWI. However, as anoxia began to develop and the OAI migrated up into the water column, so too did the location of peak methylation potential.

MeHg demethylation can occur via three mechanisms. One mechanism is Hg detoxification that is triggered by high Hg concentrations from a wide range of micro-organisms, both gram negative and gram positive, that have the mer-operon gene (Hobman et al., 2000). This demethylation ability consists of a Mer-B gene that encodes an organomercurial-lyase enzyme that cleaves the methyl group off of Hg(II). This process often includes a second gene,
the mer-A gene, that produces mercuric reductase which then reduces Hg(II) to Hg⁰, which is insoluble and rapidly leaves aquatic systems.

A second demethylation pathway is reductive demethylation, which is carried out by SRB (Baldi et al., 1993). In this process, two molecules of MeHg react with a microbially generated sulfide atom to generate a (MeHg)₂S intermediate. This intermediate breaks down into dimethylmercury and mercuric sulfide. Dimethylmercury eventually degrades to MeHg and methane. The mercuric sulfide can either be taken up by SRB, where the Hg could be methylated again, or precipitate out of solution as a solid.

The third demethylation pathway is known as oxidative demethylation, a process carried out by heterotrophic bacteria via cometabolism of MeHg as a carbon source (Marvin-Dipasquale and Oremland, 1998). Oxidative demethylation is ubiquitous in natural environments and is likely the most commonly occurring pathway in uncontaminated systems (Marvin-Dipasquale et al., 2000). One key theme to keep in mind when evaluating the Hg cycle in aquatic systems, is to consider the location of the zone of peak methylation. A conceptual model designed from the synthesis of the above fundamentals of Hg cycling in freshwater environments was developed to help interpret the results of the research in this dissertation.

1.3 CONCEPTUAL MERCURY MODEL

Lakes are complex ecosystems and holistic tools are needed to inform complicated lake management problems. Ecological models can be very useful in predicting how forcing functions (e.g., spatial and temporal extent of hypolimnetic anoxia) can affect ecosystem health (e.g., Hg levels in the aquatic food chain) (Jørgensen, 2006). Informed through our research and efforts of others, I have developed a conceptual model of Hg cycling in the profundal zone of freshwater
lakes (Figure 1). This model illustrates the fundamental interactions of the Hg cycle that are
covered in Chapters 2 through 4 of this dissertation.

The overriding hypothesis for this conceptual model is that MeHg production is restricted
to a region below the OAI where three prerequisites for methylation need to be met: (1) the
presence of biologically available ionic Hg, (2) redox conditions conducive to ensure the activity
of SRB, and (3) a “just right” balance of sulfide levels to accommodate small neutrally charged
Hg/S species (ie. HOHgSH\(^0\)), but not so high to facilitate the formation of large and negatively
charged Hg-S complexes (Benoit et al., 2003). The OAI is critical to methylation in low-sulfate
freshwater systems since it stimulates the recycling of sulfide produced by SRB back to sulfate,
thereby sustaining SRB activity and maintaining low enough sulfide levels that promote Hg
bioavailability for methylation. If sulfide levels are too high and large and negatively charged
Hg-S complexes are formed, Hg is prevented from entering the SRB cell, and therefore
methylation is inhibited (Benoit et al., 1999; Benoit et al., 1998; Compeau and Bartha, 1987).
Also of key importance are redox-sensitive metal oxides of Fe and Mn in surficial sediments.
These metals act as a sink for MeHg and ionic Hg via sorption when surficial sediments are oxic.
These metal oxides can also be a source of Hg species via desorption when surficial sediments
are anoxic and metal oxides undergo reductive dissolution (Merritt and Amirbahman, 2008;
Chadwick et al., 2006). In a typical eutrophic lake, the OAI moves vertically upwards as the
stratification season progresses and anoxia becomes more severe and expansive. Rate of vertical
migration of the redoxocline is concomitant with trophic status lakes. In very productive
systems, the entire hypolimnion (Twin Lakes, Chapters 3 through 5) can go anoxic within weeks
after the start of thermal stratification. In meso-oligotrophic lakes like Deer Lake (Chapter 2),
anoxia may occur only at the end of the stratified season and only in waters near the SWI. This
conceptual model presents two contrasting scenarios: one in which the water column is fully oxygenated, surficial sediments are oxygenated and the OAI is in the sediments, and the other where deeper waters and surficial sediment are anoxic and the OAI is poised in the water column. These two scenarios are discussed in brief below.

1.3.1 Oxygenated Hypolimnion

When the entire water column is oxygenated, the OAI resides at or below the SWI, commonly a few cm below the sediment’s surface (Figure 1A). In this case, there is no niche for SRB activity in the water column (Watras, 2009). In addition, oxidized surficial sediments retain metal oxides of Fe and Mn, as well as any ionic Hg or MeHg associated with the metal oxides or sequestered organic matter. The methylation zone resides just below the OAI in sediments where sulfur cycling between sulfate and sulfide promotes SRB activity, and where low levels of sulfide enhance bioavailability of ionic Hg through formation of uncharged Hg-sulfide complexes. MeHg builds up in pore water around the methylation zone and diffuses into overlaying water. Metal oxides in oxic surficial sediments can act as a sink to upwardly diffusing MeHg, temporarily sequestering the dissolved toxic metal. In situations where metal content of the sediment is low, MeHg could potentially diffuse out into the oxygenated overlying water (Benoit et al., 2003). In deeper sediments, sulfide accumulates and impedes bioavailability of ionic Hg through the formation of large and charged Hg sulfide complexes. In addition, with no source of sulfate, SRB activity diminishes, which may explain the low MeHg accumulation in anoxic chambers in Chapter 2, where there was not enough sulfate in chamber water to promote SRB activity.
Treatments that enhance depth of oxygen penetration into the sediment, or other oxidants such as nitrate, could potentially decrease MeHg efflux to the overlying water for a number of reasons. Increasing the diffusional distance from the methylation zone to overlying water would create a spatial distance between the site of methylation and water column. Also, increasing the potential for sorption of MeHg to metal oxides in oxic surficial sediments would act as a physical barrier to dissolved MeHg species migrating upwards. However, elevated oxygen concentrations have also been found to influence efflux in estuarine sediments by increasing the activity and density of benthic macrofauna, which can greatly affect chemical cycling at the SWI. Benoit et al. (2006) found that higher densities of macrofauna enhanced oxygen penetration into sediments, leading to a deeper and thinner methylation zone and lower levels of MeHg in estuarine sediments. Hammerschmidt and Fitzgerald (2008) found that at dissolved oxygen levels above 80% saturation, MeHg fluxes increased as a result of bioirrigation. They conclude that increasing oxygen levels could increase sediment-water effluxes of MeHg by promoting the colonization of benthic invertebrates into previously uninhabited sediments.

1.3.2 Anoxic Hypolimnion

When the OAI is in the water column rather than the sediments, the optimal zone for methylation can remain between the SWI and the OAI (Figure 1B). Redox at the sediment surface decreases during anoxia, resulting in the release reduced Fe, Mn, associated ionic Hg, and MeHg into the overlying water. Released ionic Hg can increase background levels already in the water column from atmospheric deposition and watershed runoff, thereby enhancing MeHg production.
Lowered reducing conditions can lead to inhibition of Hg methylation. During anoxia bottom waters and sediments become enriched in sulfide. As described above, high levels of sulfide can lead to formation of large and charged Hg species, thereby impeding Hg bioavailability. In this case, although SRB are active, Hg is impeded from entering the cell.

1.4 CHAPTER 2 – CHAMBER STUDY

Chapter 2 of this dissertation is a summary of a SWI chamber study that was conducted to evaluate the effect of oxic versus anoxic condition on metal cycling at the SWI. The hypothesis of this study was that anoxic chambers would accumulate more MeHg than anoxic chambers, as anaerobic conditions are commonly associated with Hg(II) methylation and reducing conditions foster metal reduction and subsequent release from sediments. The chambers were composed with sediments and water collected from Deer Lake, WA, an unpolluted oligomesotrophic lake.

1.5 CHAPTER 3 – HYPOLIMNETIC OXYGEN TEST

Chapter 3 summarizes an evaluation of an oxygen test that was conducted in North Twin Lakes, WA, towards the end of the stratification season. The purpose of this study was to determine the effect oxygen addition to an anoxic hypolimnion on the accumulated Fe, Mn, and Hg. Our hypothesis was dissolved Fe and Mn would oxidize and strip out accumulated MeHg as they precipitated out of the water column. The test was conducted for a two period towards the end of the lakes summer stratification season.

1.6 CHAPTER 4 – FULL-SCALE HYPOLIMNETIC OXYGENATION
Chapter 4 encompasses four years of Fe, Mn, and MeHg monitoring at North and South Twin Lakes, with North Twin Lake undergoing hypolimnetic oxygenation during the summer stratification period for the last three years of this study. The objective of this study was to evaluate the effect of hypolimnetic oxygenation on metal cycling in lakes. We hypothesized that Fe, Mn, and MeHg accumulation would be reduced by oxygenation as compared to the pre-treatment year and the reference lake. The oxygenation system was designed to open up the hypolimnion for cold water fish habitat by delivering oxygen to the upper hypolimnion. South Twin Lake, a nearby lake of similar size and trophic status, was used as a reference lake in the evaluation of North Twin Lake’s treatment.

1.7 CHAPTER 5 - EDUCATIONAL OUTREACH

In Chapter 5 of this dissertation, I present a method for universities to promote awareness and interest in engineering is outlined as a coordination of the Washington State University’s (WSU) educational outreach program, the Culturally Relevant Engineering Applications in Mathematics (CREAM), and the WSU Imagine Tomorrow high school environmental design competition. As a CREAM fellow, I was charged with implementing culturally-relevant engineering applications in mathematics. In this particular case, the Imagine Tomorrow competition worked as the vehicle to get students interested in participating in science and engineering. This outreach effort took place at a rural high school with a high percentage of minority students and low academic performance. This paper serves as a model for future educational outreach efforts on how to effectively use state, federal, and private dollars to promote participation of underrepresented groups in the STEM fields.
1.5 REFERENCES


Figure 1. Conceptual model of mercury cycling in the profundal zone of a freshwater lake under two conditions: A. with the oxic-anoxic interface within profundal sediments, and B. with the oxic-anoxic interface in water column. See text for discussion. Hg (II) = ionic mercury; CH$_3$Hg$^+$ = methylmercury; Me(OH)-Hg (s) = Ionic mercury bound to solid metal oxides of iron and manganese; Me(OH)-CH$_3$Hg$^+$ (s) = Methylmercury bound to solid metal oxides of iron and manganese; Me(d) = dissolved metals (iron and manganese); SO$_4^{2-}$ = sulfate; HS$^-$ = sulfide; SRB = sulfate-reducing bacteria; (Hg-S)$^0$ = uncharged mercury-sulfide complexes; (Hg-S)$^-$ = charged mercury-sulfide complexes.
CHAPTER 2. MERCURY BEHAVIOR IN OXIC VERSUS ANOXIC SEDIMENT-WATER INTERFACE CHAMBERS

2.1 ABSTRACT

Methylmercury (MeHg) contamination of freshwater aquatic food webs in lakes is widespread. A key source of MeHg to freshwater lakes is in-situ production in the anoxic profundal zone. The objective of this study was to evaluate the effects of oxic versus anoxic conditions on mercury (Hg) cycling at the sediment-water interface (SWI), testing the hypothesis that anoxic conditions over freshwater lake sediment enhances efflux of MeHg from sediments, presumably through the enhanced activity of sulfate reducing bacteria (SRB) that methylate Hg. Replicate SWI chambers containing samples from Deer Lake, WA, an unpolluted, deep, oligo-mesotrophic lake, were incubated for 27 days under oxic and anoxic conditions. Overlying water was monitored for iron (Fe), manganese (Mn), and MeHg. In contrast to our hypothesis, MeHg concentrations in chamber water were higher in oxic versus anoxic chambers (~0.24 ng L\(^{-1}\) versus ~0.12 ng L\(^{-1}\)) at the end of the incubation. These results can be explained when considering sulfur cycling in surficial freshwater sediments under oxic conditions. The presence of an oxic-anoxic boundary within sediments could facilitate MeHg production and efflux from sediments by: (1) sustaining activity of SRB by providing the bacteria with a continuous recycled supply of sulfate, and (2) enhancing ionic Hg bioavailability for methylation within sediments by depressing sulfide concentrations. Results from this study will help in the development of in-lake management strategies that decrease MeHg uptake into aquatic food webs, and ultimately protect humans and wildlife from eating fish contaminated with excessive levels of Hg.
2.2 INTRODUCTION

Accumulation of mercury (Hg) in freshwater aquatic food webs is a widespread concern in the US where nearly one-third of its lakes have consumption advisories in place due to elevated concentrations of Hg in fish tissue (USEPA, 2004). Methylmercury (MeHg) is one of the most toxic natural forms of Hg (Tchounwou et al., 2003). It is also the primary form of the trace metal that accumulates in fish, typically representing more than 98% of total Hg (THg) in fish biomass (Bloom, 1992). In-situ production of MeHg, rather than areal deposition or watershed runoff, is the primary source of MeHg in freshwater lakes (Eckley et al., 2005; Orihel et al., 2007).

An increase in MeHg in bottom waters is often coincidental with onset of anaerobic conditions in the hypolimnion (He et al., 2008; Eckley and Hintelmann, 2006; Herrin et al., 1998; Slotton et al., 1995; Watras et al., 1995). In eutrophic freshwater lakes, anoxia commonly forms over the sediment-water interface (SWI) in late spring after onset of thermal stratification. The entire mass of dissolved oxygen can be consumed in a productive and anoxic hypolimnion by mid summer or early fall (Horne and Goldman, 1994). MeHg is typically produced by anaerobic bacteria, including sulfate reducing bacteria (SRB), which flourish in anaerobic bottom waters and sediments of these productive lakes (Gilmour et al., 1992; Benoit et al., 2003).

There is a specific set of prerequisites that needs to be met for inorganic Hg to be methylated by SRB. First, ionic Hg must be available for methylation in a labile form, second, reducing conditions must be low enough to activate SRB, and third, sulfide concentrations must remain low enough to avoid formation of large sulfidic complexes with Hg which would shield it from methylation by SRB (Benoit et al., 2003). This optimal methylation zone where conditions are just right is often referred to as the “Goldilocks” zone, typically located in a narrow band just
below the oxic-anoxic interface (OAI) where sulfide concentrations are beginning to accumulate but are relatively low (Benoit et al., 2003). This zone can be below the SWI when overlying water is oxygenated (Benoit et al., 2006), or up in the water column when the hypolimnion goes anoxic and the OAI moves up towards the thermocline (Watras, 1995). Anoxic conditions also result in the reduction and dissolution of iron (Fe) and manganese (Mn) oxides in surficial sediments (Davison, 1993), with a corresponding potential release of soluble reactive phosphorus (SRP) (Welch and Jacoby, 2001; Boström et al., 1988) and Hg species sorbed to the metal oxides (Merritt and Amirbahman, 2008; Bloom et al., 1999). Released phosphorus from anoxic sediments contributes to internal nutrient loading which can exacerbate lake productivity long after external loads have been eliminated (Welch and Jacoby, 2001; Beutel and Horne, 1999). MeHg released to the water column can increase the exposure of the trace metal to the food web, thereby giving rise to elevated levels in fish tissue (Watras, 2009).

The growing awareness of Hg issues in aquatic ecosystems has led to Hg targeted TMDLs requiring lake and reservoir managers to reduce the accumulation of Hg in both the water column and fish tissue (http://www.waterboards.ca.gov/sanfranciscobay/water_issues/programs/TMDLs/guadaluperivermercurytmdl.shtml). There is a critical knowledge gap between the extensive research that has gone into Hg cycling in aquatic ecosystems and treatments that can mitigate its accumulation in the aquatic food web. Our working hypothesis was that the maintenance of an oxygenated SWI should lower MeHg efflux from meso-oligotrophic sediments by maintaining an oxidized SWI.
2.3 MATERIALS AND METHODS

2.3.1 Study Site

Deer Lake is a moderately deep ($Z_{\text{mean}} = 15.9$ m; $Z_{\text{max}} = 22.9$ m; $A = 445$ ha), dimictic, oligo-mesotrophic lake located north of Spokane in eastern Washington (Figure 1). A number of anthropogenic impacts including shoreline housing development, cattle grazing, and logging activities have led to enhanced sediment and nutrient runoff within the watershed (O’Neal et al., 2001). Although a majority of these external sources have been abated, hypoxia still develops in the hypolimnion towards the end of summer stratification (Soltero et al., 1991). Deer Lake’s profundal sediments consist of fairly low organic carbon and carbonate content, approximately 15% and 5% respectively (Table 1). Low organic carbon content corresponds with Deer Lakes slow hypolimnetic oxygen consumption during stratification, with anoxia not even beginning to form until late August (Cox, 2011).

2.3.2 Chamber Collection and Incubation

Four SWI samples were collected at a depth of 21 m and incubated based on methods of Beutel et al. (2008) with a number of methodological improvements. Sediments were cohesive enough to plug an Ekman dredge and allow for collection of a minimally disturbed SWI subsample, capturing the top 6 cm, in a 1.8 L cylindrical Plexiglas chamber. In the laboratory, chambers were immediately placed in a dark, 8 °C incubator to simulate in-situ lake-bottom conditions. Four chambers were incubated in pairs for a period of 27 days under their respective oxic and anoxic conditions. Gas from compressed air and ultra pure nitrogen tanks were fed to the chambers using Teflon tubing, stimulating oxic and anoxic conditions. Bubbling resulted in a
well mixed water column with no visible sediment re-suspension in chambers. A crimped gas line occurred overnight between day 10 and 11 of the initial incubation period, which allowed a brief window of oxygen exposure to the anoxic chambers, and was immediately restored on day 11 where it continued to operate without malfunction through the end of incubation.

### 2.3.3 Water Quality Sampling and Analysis

Water samples were collected for Fe, Mn, SRP, and MeHg from chambers at regular intervals during oxic versus anoxic incubation (Figure 2). Fe and Mn samples were filtered through 0.45 µm filters and preserved with nitric acid until analysis using inductively coupled plasma mass spectrometry (ICP-MS). SRP samples were filtered through 0.45 µm filters and frozen until analysis using a Seal AutoAnalyzer 3, automated segmented flow analyzer (APHA, 2005). MeHg samples were preserved with HCl, then distilled, ethylated, purged on to carbotraps, separated by gas chromatography, pyrolyzed, and detected using cold vapor atomic fluorescence spectroscopy (Horvat et al., 1993; USEPA, 2001). To avoided potential contamination from inserting sampling tubes into chamber water, chambers were fitted with sampling ports located on the side of the chamber a few centimeters above the SWI. Prior to sampling, the sampling ports were flushed with a small volume of chamber water. Detection limits for Fe, Mn, SRP, and MeHg were 45 µg/L, 0.5 µg/L, 0.004 mg/L, 0.5 ng/L, and 0.04 ng/L, respectively.

Sediment samples were collected for particle size, carbon content, Fe, and Mn. Particle size distribution was determined by the hydrometer method (Bouyoucos, 1962). Organic matter and CaCO₃ was determined by loss on ignition using a muffle furnace (Heiri et al., 2001). Total
Fe and Mn was determined by EPA Method 6010 with a Varian 720ES ICP-OES (USEPA, 1996).

2.4 RESULTS AND DISCUSSION

2.4.1 Iron and Manganese

Initial Fe concentrations were fairly stable, just at or below 100 µg/L, for approximately the first half of the incubation. After 12 days, Fe increased to approximately 140 to 220 µg/L in oxic chambers and 170 to 270 µg/L in anoxic chambers (Figure 2). Although average values were slightly higher in the anoxic chambers throughout the incubation, Fe response in both were similar. Average flux rates for Fe during the last 15 days of the incubation were 565 and 625 µg/m²/d for oxic and anoxic chambers respectively (Table 1). Mn concentrations remained repressed in oxic chambers through the entirety of the incubation. In anoxic chambers, Mn increased early on with the onset of anoxic conditions, then decreased between day 7 and 12 due to a malfunction with gas delivery. Anoxic conditions were reestablished by day 12 and Mn steadily increased to approximately 175 µg/L in both chambers by day 27 (Figure 2). Average flux rates for Mn during the last 15 days were -7.5 and 1,090 µg/m²/d in oxic and anoxic chambers respectively (Table 2). Concentrations of Fe and Mn in chamber water were on the bottom of the range for many freshwater lakes, which typically range from 20-8,000 µg/L and 100-3,000 µg/L for Fe and Mn respectively (Stauffer, 1987; Davison and Woof, 1984; Davison et al., 1982). The top 3 cm of sediment had Fe levels of 2475 mg/kg dw and Mn levels of 30 mg/kg dw (Table 1), falling well below ranges for both Fe and Mn with ranges 20,000-30,000
mg/kg, and 600-8,000 mg/kg respectively, of values reported for other freshwater sediments (Stauffer, 1987; Davison and Woof, 1984).

There was little difference between the magnitude and response of Fe between the two sets of chambers throughout the duration of incubation. Lack of divergence between the two sets suggests that reducing conditions never reached an redox potential optimal for Fe reduction, 100-300 mV (Bohm et al., 2001), in surface sediments. A previous SWI chamber study was conducted on Deer Lake to evaluate metal cycling between oxic and anoxic conditions (Beutel et al., 2008). During this incubation, it took nearly 30 days after Mn was observed before Fe accumulation in chamber waters was observed. Considering that Deer Lake takes a majority of its summer stratification season before anoxia begins to form in bottom waters, it seems logical that the transition phase between aerobic to anaerobic surface sediments is temporally extensive. Even though oxygen was driven out of anoxic chambers by nitrogen gas, an optimal Fe reduction potential was not reach during the duration of this incubation, as evidenced by a lack of Fe accumulation in chamber waters.

An increases in Fe, as seen with the flux ~0.5 mg/m²/d in both chambers (Table 2), may be due to colloidal Fe(III), <0.45 μm, and Fe(II) migrating up from the reduced subsurface sediments in both oxic and anoxic chambers to the small well mixed water column. Elevated Fe has been observed in the oxic hypolimnion of the nearby North Twin Lake, WA during operation of a hypolimnetic oxygenation system (Beutel et al., 2011). Ongoing observations at North Twin Lake suggest that advective mixing of the bubble plume is entraining fine Fe particulate into the upper hypolimnion, resulting in a concentration profile with Fe slightly higher in the upper hypolimnetic waters but significantly lower in bottom waters in comparison to pre treatment conditions.
There was a clear difference in Mn accumulation within oxic and anoxic chambers during the last 15 days of incubation. After an initial 12 days, the steady increase of dissolved Mn in anoxic chambers was indicative of a drop in redox potential to within the optimal range of Mn reduction, $< 200-400 \text{ mV} \ (\text{Bohm et al., 2001})$, with a corresponding net average accumulation of $1 \text{ mg/m}^2/\text{d}$ in anoxic chambers, while accumulation in oxic chambers remained close to zero (Table 2). Considering that Fe did not appear to reach an optimal reduction potential, the redox was temporarily flat-lined after reaching the optimal Mn reduction range. Fe in Deer Lake sediment is 2 orders of magnitude higher than that of Mn, 2,500 versus 30 mg/kg (Table 1).

Greater concentrations of Fe in comparison to Mn would be expected in chamber water if Fe reduction was occurring at the surface sediments. In oxic chambers, Mn concentrations followed a horizontal concentration curve over the same 15 days indicating that reducing conditions remained poised above the reduction potential of Mn.

2.4.2 Soluble Reactive Phosphorus

SRP concentrations in all chambers remained low and steady, around 0.05 mg-P/L, for the first 12 days of incubation (Figure 2). After day 12, SRP began to increase in anoxic chambers, ultimately reaching 0.3 and 0.6 mg/L at the end of incubation on day 27, while remain repressed in oxic chambers. Average SRP flux rates for the last 15 days of incubation were 0.2 and 2.5 mg/m$^2$/d in oxic and anoxic chambers respectively (Table 2). Several studies have examined SRP release from anoxic sediments in North American lakes. A study evaluating nine eutrophic lakes in Alberta, Canada, found release rates ranging from 0.01 to 2.3 mg/m$^2$/d, which would put Deer Lake on the high end of SRP release (Shaw and Prepas, 1990). However, in a study of seven lakes in eastern Canada and the United States, release rates ranged from 0.1 to 8.1
mg/m²/d, placing Deer lake sediments in the mid to low range of SRP release (Nürnberg, 1987). On a broader scale, a study of 79 lakes throughout the globe, ranging from mesotrophic to hypereutrophic, found release rates that ranged from 2.0 to 27 mg/m²/d, which would put Deer Lake SRP release squarely on the low end (Nürnberg, 1988).

There is a clear divergence in the response of SRP between oxic and anoxic chambers during the last 15 days of incubation. SRP follows the same pattern observed with Mn release to chamber water, showing a slight increase from day 12 to 17, and then a greater increase between day 17 to 27. The conventional explanation for SRP release following the onset of anoxia is a concomitant release with Fe(II) after the reduction of Fe-oxides (Welch and Jacoby, 2001; Boström et al., 1988). However, reducing conditions optimal for Fe reduction were apparently not reached based on a lack of accumulation in chamber water. Mn reduction, however, was evidenced in this incubation as evidenced by its accumulation in chamber water. Some studies attribute SRP is controlled by a combined effect of both Fe and Mn oxides (Wetzel, 2001; Balistrieri et al., 1992; Förstner and Wittmann, 1981). A group of researchers observed an increased flux of SRP from reducing Mn oxides that had been recently deposited in Lake Lac Saint-Charles (Lavoi and Auclair, 2012). By using Teflon sheets to prevent interactions from pore water, the group was able to isolate freshly deposited Mn oxides and found that SRP could be co-sequestered with associated organic matter. Shahandeh et al., (2003) investigated metal oxide contributions to SRP availability in rice patty soil. Researchers found that although approximately 78% of SRP variability in their soil was controlled by Fe-oxides, an estimated 21% of SRP variability was attributed to Mn-oxide sequestration of phosphorus. In our chamber study, under mild reducing conditions, it appears that release of SRP is indeed concomitant with Mn reduction. Considering that background Fe concentrations are nearly an order of magnitude
below the range associated with many freshwater lakes, Mn may play a role in internal nutrient cycle during lengthy transitional periods between aerobic and anaerobic conditions in Deer Lake.

2.4.3 Methylmercury

MeHg was consistently low though the first 17 days of incubation, averaging about 0.09 ng/L in oxic chambers and 0.07 ng/L in anoxic chambers. Several of the early anoxic MeHg samples were lost due to an analytical malfunction and limited sample volume. At the end of the 27 day incubation, MeHg increased to approximately 0.24 and 0.13 ng/L in oxic and anoxic chambers respectively. Average MeHg flux rates for the last 15 days of incubation were 0.85 and 0.25 ng/m²/d for oxic and anoxic chambers respectively (Table 1).

MeHg concentrations increased in both chambers at the end of incubation, with greater increases being observed in oxic chambers. These results are contrary to a number of studies that have shown lower levels of MeHg in sediments and/or overlaying water under oxic conditions versus anoxic conditions at the SWI in heavily polluted and/or saline sediments (Watras, 2009; Kuwabara et al., 2002; Herrin et al., 1998; Slotton et al., 1995; Henry et al., 1995). One study that was performed on ‘pristine’ freshwater sediments (i.e., low Hg), similar to the sediments evaluated in this study, found that MeHg production was decreased in chambers bubbled with air relative to chambers bubbled with nitrogen (DeLaune et al., 2004). In the DeLaune et al. (2004) study, only the top 1 cm of their surface sediments were incubated and therefore oxygen could potentially penetrate and oxidize the entire depth of their core. In our study, the sediment consisted of a 6 cm sediment core, where a 1.0 to 1.5 cm thick orange oxidized layer was observed in both aerated chambers, corresponding to the depth of oxygen penetration and oxidation.
As discussed in the introduction, a key component to Hg methylation is the presence of an OAI with an active cycle of sulfate reduction, via SRBs, and sulfide oxidation. Our oxic chambers had an OAI a few centimeters below the sediment surface. Hg methylated in this “Goldilocks” zone can potentially diffuse up to the sediment surface where it would be mixed into circulating chamber waters. This could explain the increase of MeHg observed in the oxic chambers at the end of incubation. This phenomenon was observed at North Twin Lake, another non-Hg impacted lake nearby Deer Lake, which was undergoing hypolimnetic oxygenation. MeHg increases in the upper hypolimnion were observed after a summer season of oxygenation, relative to both pre-oxygenation conditions and its hydrologically similar neighbor South Twin (Beutel et al., 2011). What appears to be happening in North Twin is that MeHg diffusing out of the sediment is drawn up the water column by advective forces of the bubble plume, which can decrease the diffusible boundary layer and induce concentration forcing from sediments to the water column. Although the OAI was not necessarily repressed into the sediments (Chapter 4), turbulence induced increases in flux has been noted in other oxygenation applications (Bryant et al., 2011; Gantzer et al., 2009).

In quiescent settings, a potentially shallow zone above the sediment could accumulate MeHg, leaving upper waters unaffected. When mixing occurs from bubble plume induced advection, any diffusing MeHg from the sediment would be quickly mixed through the water column, raising concentrations in the upper waters, and decreasing concentrations above the sediment. Lower concentrations above the sediment could generate a negative feedback by keeping the gradient sharp at the SWI, thereby promoting diffusion.

Although MeHg rose slightly in anoxic chambers, it did not increase as expected or as much as observed in oxic chambers. Unlike oxic chambers, our anoxic chambers do not have a
defined OAI, since the oxygen had been driven out by bubbled nitrogen. Lack of a sharp redox interface would prevent the needed balance between sulfate and sulfide, and therefore may limit production of MeHg in comparison to the established methylation zone beneath the sediment surface in oxic chambers. If there was not as much MeHg production in anoxic chambers as with oxic chambers, resulting MeHg accumulation would be controlled by release of previously formed and sequestered MeHg from reducing metal oxides.

Several studies have implicated the importance of Mn-oxides as a potential sink for MeHg. In Mn-rich Davis Creek Reservoir, CA, Mn-oxides were found to sequester dissolved MeHg out of the water column during fall turnover (Slotton et al., 1995; Gill and Bruland, 1992). Chadwick et al. (2006) observed a link in the fate of dissolved organic matter bound Hg species and Fe and Mn-oxides. Chadwick et al. (2006) also observed that Fe(III) persisted in anoxic waters longer than thermodynamic models predicted. This implies that sequestered Hg could remain bound to Fe in the presence of anoxia longer than predicted as well. Considering that MeHg did not accumulate in the chamber with Mn and SRP, it appears that in this system, Mn is not a significant sink for MeHg. In-situ profiles of MeHg in Deer Lake taken during late August at the onset of anoxia above the sediments show MeHg concentrations in the water column even lower what was observed in our chamber water (Cox, 2011). It would appear that anoxic conditions over these meso-oligotrophic sediments do not stimulate substantial MeHg release into overlying waters.

2.5 CONCLUSION

The results of this study showed that anoxic conditions in waters overlying un-impacted freshwater meso-oligotrophic sediments did not lead to increased efflux of MeHg, and that
effluxes were, in some cases, higher under oxic conditions which conflicts with our hypothesis. We contend that the presence of an OAI within sediments can still stimulate the activity of SRB, subsequently inducing the production of MeHg, which can then diffuse into the overlying waters. Currents generated from bubbling air above the sediment keep the water column mixed and therefore decrease the concentration at the SWI, which may lead to increased efflux of MeHg from the sediment. Similarly, in-situ aeration/oxygenation treatments have resulted in MeHg being transported into upper hypolimnetic waters by advective mixing from the bubble plume. This is not to say that anoxic conditions are preferable to oxic conditions with respect to Hg methylation and uptake into aquatic food webs. While anoxic conditions may repress MeHg production in this chamber study, it is most likely due to a lack of an OAI in the closed system. In-situ production in anoxic sediments that are not in a closed system have demonstrated a clear methylation capacity. Also, in anoxic sediments, the optimal zone of methylation can be transferred up into the water column where the OAI is poised, which cannot be easily demonstrated within a closed chamber. Several questions arise: (1) is methylation more of a threat to aquatic biota when it occurs just below oxic surficial sediments or above anoxic sediments at an OAI at or above the sediment surface; (2) what are the implications of aeration/oxygenation on Hg impacted systems that have sediment Hg concentrations many times that of Deer Lake sediments; and (3) what are the implications of using other redox poising treatments such as nitrate that avoids bubble plume mixing.

Much is known about in-lake lake management strategies that combat eutrophication and its symptoms, commonly by managing redox potential at the SWI (e.g., aeration, oxygenation, and sediment treatment with nitrate salts) or enhancing sediment sorption capacity (e.g., sediment treatment with aluminum sulfate) (Cooke et al., 2005). Since many of the same
mechanisms appear to drive MeHg cycling in lakes, further research should focus on evaluating how these conventional lake treatment strategies might mitigate or exacerbate MeHg in the profundal zone of lakes. By answering these many questions through applied research, in-lake management strategies may be developed that decrease MeHg uptake into aquatic food webs, and ultimately protect humans and wildlife from eating fish contaminated with excessive levels of Hg.
2.6 REFERENCES


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Figure 1. Bathymetric map of Deer Lake, WA.
Figure 2. Concentration time series in chamber water under oxic versus anoxic conditions for soluble iron, soluble manganese, soluble reactive phosphorus, and methylmercury.
Table 1. Deer Lake sediment characteristics.

<table>
<thead>
<tr>
<th>Texture</th>
<th>Organic Matter (%)</th>
<th>Carbonate (%)</th>
<th>Iron (mg/kg)</th>
<th>Manganese (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loam</td>
<td>14.89 ± 0.98</td>
<td>5.12 ± 0.88</td>
<td>2476 ± 443</td>
<td>32.83 ± 7.50</td>
</tr>
</tbody>
</table>

*Data represents mean and standard deviation of three samples (n=3).

Table 2. Sediment flux rates for Fe, Mn, SRP, and MeHg for last 15 days of the incubation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Oxic</th>
<th>Anoxic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe*</td>
<td>565</td>
<td>625</td>
</tr>
<tr>
<td>Mn*</td>
<td>(7.5)***</td>
<td>1090</td>
</tr>
<tr>
<td>SRP**</td>
<td>0.2</td>
<td>2.5</td>
</tr>
<tr>
<td>MeHg*</td>
<td>0.85</td>
<td>0.25</td>
</tr>
</tbody>
</table>

*Units presented in µg/m²/d

**Units presented in mg/m²/d

***Values in parenthesis represent a negative number.
CHAPTER 3. RESPONSE OF IRON, MANGANESE, AND MERCURY IN AN ANOXIC WATER COLUMN TO SHORT-TERM HYPOLIMNETIC OXYGENATION

3.1 ABSTRACT

Hypolimnetic oxygenation is a lake treatment designed to address hypolimnetic anoxia by supplying pure oxygen gas to bottom waters. The impact of a late season oxygenation test on accumulated iron (Fe), manganese (Mn), total mercury (Hg), and methylmercury (MeHg) in the hypolimnetic water column of North Twin Lake, WA, was evaluated in this study. Three novel findings of this study include: (1) accumulated Fe and Mn decreased by over 70% from the water column, within 8 hr after implementation of oxygen addition; (2) total Hg and MeHg enrichment in the hypolimnion decreased substantially after 2 weeks of oxygenation, with concentrations near the sediment-water interface (SWI) dropping 60% and 95%, respectively, from pre-oxygenation conditions; and (3) after termination of the oxygenation test, metals and Hg concentrations rebounded to pre-oxygenation levels within one month. The primary mechanism for Mn and Hg loss from the water column during the oxygenation test was most likely from sequestration via settling Fe oxides. This conclusion is based on the observations that there was rapid loss of metals from the water column, there were low concentrations sustained throughout the oxygen test of both metals and Hg, and rapid rebound of metals and Hg after the test was over. The data from this study demonstrates the sensitivity of Fe, Mn, and Hg cycling to oxygen, and highlights hypolimnetic oxygenation as a potential tool for managing hypolimnetic accumulation of both conventional (Fe, Mn) and toxic (Hg) metals.
3.2 INTRODUCTION

Hypolimnetic anoxia is a frequent problem in stratified, nutrient rich, productive lakes (Wetzel, 2001). Summer hypolimnetic oxygen consumption in thermally stratified lakes occurs as allochthonous and autochthonous organic material settle into profundal regions of lakes and are decomposed. Oxygen levels and oxidation-reduction potential at the sediment-water interface (SWI) are particularly critical in regulating the flux of reduced and oxidized substances into and out of profundal sediments (Golterman, 2001).

One of the key concerns lake managers have regarding anoxia is internal nutrient loading caused by recycling of nutrients from the SWI to the water column (Beutel and Horne, 1999). Oxygen depletion at the SWI can result in release of phosphorus into the water column through a number of mechanisms including reduction of phosphorus-rich metal oxides (Boström et al., 1988), induced activity of anaerobic microorganisms that metabolically release phosphorus under fluctuating oxygen conditions (Gächter and Meyer, 1993), mineralization of decaying organic matter (Hupfer and Lewandowski, 2008), and via enhanced solubility caused by lowering of pH in anoxic waters (Golterman, 2001). Anoxic conditions can also cause hypolimnetic enrichment of ammonia (Beutel, 2006) and methane (Addess and Effler, 1996) due to inhibition of biological oxidation of these compounds. Diffusion, entrainment, and fall turnover can subsequently transfer accumulated nutrients into the photic zone where they stimulate primary productivity, increasing organic delivery to sediments and perpetuating anoxia in bottom waters and internal nutrient loading.

Another impact of hypolimnetic anoxia is accumulation of nuisance and toxic compounds that can impede drinking water treatment and potentially harm aquatic biota. Depressed oxygen levels and oxidation-reduction potential at the SWI have a profound impact on metal cycling, as
reduced iron (Fe) and manganese (Mn) tend to accumulate in anoxic waters (Bryant et al., 2011a; Gantzer et al., 2009a). Furthermore, a number of compounds accumulate under anoxic conditions, such as ammonia and sulfide, which are extremely toxic to aquatic biota (Beutel et al., 2001). More recently, a growing body of evidence is linking hypolimnetic anoxia with mercury (Hg) enrichment in hypolimnetic waters of lakes. Anoxic bottom waters can contain inorganic Hg (Hg(II)) and toxic monomethylmercury (MeHg) at concentrations much greater than that of oxic bottom waters (Watras, 2009; Eckley and Hintelmann, 2006). Higher MeHg concentration within the water column increases exposure of the contaminant to the base of aquatic food webs (Herrin et al., 1998; Slotton et al., 1995). Biomagnification of MeHg up trophic levels of aquatic food webs can produce elevated levels of MeHg in fish tissue (> 1 mg/kg) even though levels are negligible in the water column (< 1 ng/L) (Watras, 2009).

Hg contamination in aquatic systems is a growing and significant concern throughout the world. On a global scale, rates of atmospheric Hg deposition have increased three-fold as a result of human post-industrial activities, and an estimated 5-20% of deposited Hg is delivered to aquatic ecosystems where it may accumulate in aquatic biota (Swain et al., 2007). In the U.S., three-fourths of states currently have blanket fish consumption advisories due to elevated Hg in fish tissue, and nearly half of the total U.S. lake acres and over a third of U.S. river miles are subject to Hg related consumption advisories (USEPA, 2009). MeHg, the form of Hg that is toxic to humans, is the primary species that accumulates in fish, typically representing more than 95% of total Hg in fish biomass (Tchounwou et al., 2003). In the absence of point sources, Hg typically enters a lake in the inorganic form via aerial deposition and watershed runoff. Hg(II) can complex with dissolved organic carbon (DOC), and co-precipitate with settling particles such as metal oxides of Fe and Mn (Watras, 2009; Chadwick et al., 2006). Hg(II) in an anoxic
hypolimnion can be methylated by sulfate reducing bacteria (SRB) in both anoxic profundal waters and surficial sediments (Eckley et al., 2005; Benoit et al., 2003). MeHg can also enter the water column from sediments as it is released from metal oxide complexes that become reduced after onset of anoxia (Merritt and Amirbahman, 2008; Chadwick et al., 2006).

Anoxia in bottom waters is commonly treated for concerns related to dissolved metal concentrations complicating potable water treatment (Betancourt et al., 2010; Gantzer et al., 2009a), internal nutrient loading impairing water quality or prolonging lake recovery (Lioriussen et al., 2009), ammonia and sulfide toxicity debilitating aquatic biota (Beutel, 2006; Beutel et al., 2001), and anoxia limiting cold-water fish habitat (Christensen and Moore, 2009; Beutel et al., 2001). A variety of in-lake management strategies are used to ameliorate problems associated with hypolimnetic anoxia (Cooke et al., 2005).

One strategy that has grown in application is hypolimnetic oxygenation, the use of an engineered system to enhance dissolved oxygen (DO) levels in bottom waters using pure oxygen gas (Moore and Christensen, 2009; Beutel et al., 2007; Singleton and Little, 2006; Mobley and Brock, 1995). Hypolimnetic oxygenation systems are designed to maintain adequate DO in the hypolimnion while maintaining thermal stratification, thereby significantly reducing the occurrence of anoxia.

There is growing interest and a critical knowledge gap in how oxygen affects Hg cycling in lakes, with some arguing that oxygenation could decrease Hg bioaccumulation by inhibiting MeHg accumulation in bottom waters (Beutel et al., 2008), while others contending that macrofauna recolonization of oxygenated profundal sediments could open a new pathway for Hg accumulation (Effler and Matthews, 2008).
This study was conducted in North Twin Lake, in northeastern Washington State, USA, a resort and recreational fishing lake that supports a cold water fishery which has been impaired by a temperature/DO “squeeze” due to hypolimnetic anoxia (Christensen and Moore, 2009). To ameliorate the loss of cold-water fish habitat, a hypolimnetic oxygenation line diffuser system was installed and tested in North Twin Lake in the summer of 2008 (Beutel et al., 2011). The system test ran for 17 days near the end of the typical summer thermal stratification period in late August/early September. This test provided a unique opportunity to evaluate effects of short-term oxygenation on accumulated Fe, Mn, and Hg in an anoxic hypolimnion. We hypothesized that oxygenation would cause accumulated Fe(II) and Mn(II) to oxidize and scavenge enriched Hg(II) and MeHg from the water column. While previous studies have focused on the effect of oxygen addition on hypolimnetic accumulation of nutrients and conventional metals (i.e., Fe and Mn), this study is novel in that it also evaluated the fate of Hg, a potent and widespread bioaccumulatory toxin in aquatic ecosystems. With few options to manage external inputs of Hg to lakes and reservoirs, a better understanding of in-lake processes is needed to assess whether oxygenation can be used as an innovative management approach to repress Hg uptake into lake and reservoir food webs.

3.3 METHODS

3.3.1 Study Site

North Twin lake is a moderately deep ($Z_{\text{mean}} = 9.7$ m; $Z_{\text{max}} = 15.2$ m; $A = 371$ ha), dimictic meso-eutrophic lake located on the reservation of the Colville Confederated Tribes in eastern Washington State (Figure 1). After spring melt, usually in late April, hypolimnetic
oxygen depletion occurs rapidly following development of thermal stratification in North Twin. Typically the entire hypolimnion is anoxic by mid June and remains so until fall turnover, usually in October. During the period of hypolimnetic anoxia, cold-water trout reside in a 2-m-thick zone in the metalimnion, unable to access the anoxic cold-water habitat below (Christensen and Moore, 2008). A hypolimnetic oxygenation system, consisting of a 750-m-long fine bubble (1-2 mm initial diameter) diffuser line connected to an on-shore 23-m³ liquid oxygen storage tank, was installed in the early fall of 2008 to improve the habitat for cold-water fish. This type of oxygenation system was chosen for its ease of installation in remote areas and its effectiveness in oxygenating bottom waters while preserving thermal stratification (Mobley and Brock, 1995). The oxygenation system was operated for approximately 8 hr during installation on the morning of August 28. The two-week oxygenation test formally began on the evening of August 28, with continuous operation through September 14.

### 3.3.2 Water Quality Monitoring

Extensive water quality monitoring was conducted at a 15-m-deep site located approximately 20 m perpendicular to the oxygen diffusion line (Figure 1). Water quality monitoring for DO, temperature, conductivity, total Fe, total Mn, total Hg, and MeHg was conducted on August 13, September 13, and October 20, representing conditions before, during, and after the oxygenation test. DO, temperature, and conductivity profiles were measured at 1-m vertical intervals with a MS5 Sonde Hydrolab (Hach Company, Inc.). Additional monitoring for total and soluble Fe and Mn was performed on August 28 (~8 hr into oxygenation) and September 3, and for soluble Fe and Mn on September 13. An additional DO, conductivity, and temperature profile was measured at 0.5-m vertical intervals using a Zebra-Tech D-Opto optical
DO probe (Ocean Instruments Ltd.) and a SBE-37SI (Sea-Bird Electronics, Inc.) on August 28, about 8 hr into the oxygenation test. Samples for all dates were also tested for the presence of sulfide using a serendipitous sniff test.

Fe and Mn samples were collected at 1-m intervals along a vertical profile using Tygon tubing attached to a Dayton model 1P580E pump. Samples collected for both total and soluble metals were split, with a portion 0.45-µm-filtered for soluble metals and unfiltered for total metals. All Fe and Mn samples were preserved with nitric acid (APHA, 2005). Profiles for total Hg and MeHg were sampled at 2-m vertical intervals with a Teflon Kemmerer bottle. Samples were stored in acid washed glass bottles with Teflon lined caps. Clear glass was used for total Hg and amber glass for MeHg. All Hg sampling protocol followed EPA methods 1630 and 1631 (USEPA, 2001; USEPA, 2002). Total Hg samples were preserved with bromine monochloride and MeHg samples were preserved with trace metal grade hydrochloric acid then were stored at 4°C until analysis. To assess the relative dominance of Fe versus Mn in sediments, a single sediment sample was collected for metals analysis at the deep-water site using an Ekman dredge. Dredged sediment was scooped into a polyethylene bottle and then frozen until processing.

To quantify spatial distribution of oxygen, vertical DO profiles were also measured every 0.5 m along a perpendicular transect at 40, 80, 160, 320, 480, and 640 m from each side of the diffuser using a Zebra-Tech D-Opto optical DO probe (Ocean Instruments Ltd.) on August 28 (just prior to oxygenation), August 30, September 6, and September 15.

3.3.3 Water Quality and Sediment Analyses

Fe and Mn in water samples were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) (APHA, 2005). Total Hg samples were analyzed using a Tekran 2600
cold vapor atomic fluorescence spectroscopy (CVAFS) Hg auto analyzer (USEPA, 2002). MeHg samples were analyzed at Battelle Marine Science Laboratories with EPA method 1630 modular analytical technique which involves sample distillation, ethylation, purge onto Tenax™ traps, thermal desorption, gas chromatography separation, pyrolyzation, and subsequent detection via a Tekran 2500 CVAFS (USEPA, 2001). Mean hypolimnetic water column concentrations for the 15-m-deep site for conductivity, Fe, Mn, total Hg, and MeHg were calculated by averaging values from a sampled profile. Values of % soluble and the Fe:Mn ratio were also based on mean water column concentrations. Volume-weighted concentrations were not used since the hypolimnion exhibited substantial spatial heterogeneity in DO levels, and presumably metals of interest as well, during the oxygenation test. Sediment samples were analyzed for total Fe and Mn using EPA Method 6010 with a Varian 720ES ICP-OES (USEPA, 1996).

3.4 RESULTS AND DISCUSSION

3.4.1 Dissolved Oxygen, Temperature, and Conductivity

Bi-monthly monitoring of DO and temperature showed that thermal stratification started in April and that hypolimnetic anoxia was fully established by late June. DO and temperature profiles in North Twin Lake on August 13, 2008, prior to the oxygen test, showed that hypolimnetic DO was less than 1 mg/L beneath the 7 m deep thermocline (Figure 2). The rapidity and severity with which anoxia formed highlights the urgency for a solution in regards to the lake's cold-water fishery (Christensen and Moore, 2008). DO in the epilimnion was uniformly under-saturated at around 6 mg/L, corresponding with stagnant warm summer weather and lack of wind mixing. The oxygen profile showed a positive heterograde curve with a
pronounced DO increase at about 6 m, corresponding with peak algae densities in the water column (Moore, unpublished data). Mean conductivity in the hypolimnion was 67 µS/cm with a corresponding peak near the sediments of 81 µS/cm (Table 1). Conductivity in the hypolimnion was substantially higher than levels in the epilimnion (mean of 53 µS/cm). Observed enrichment of conductivity in bottom waters and peak concentrations near the sediments corresponded with observed accumulation and vertical gradient of Fe and Mn in bottom waters (Figure 2).

On August 28, an additional water quality profile was taken approximately 8 hr after oxygenation. Hypolimnetic DO was around 1.3 mg/L, approximately 0.5 mg/L higher than pre-oxygenated conditions, thus oxygen had already begun to accumulate in the hypolimnion early in the test. Hypolimnetic temperature was around 6.6 °C, a slight increase of around 0.2 °C from pre-oxygenated conditions. This increase was likely due to mixing induced between the hypolimnion and lower metalimnion, a common phenomenon observed in early stages of hypolimnetic oxygenation operations (Gantzer et al., 2009b). Compared to August 13, mean conductivity in the hypolimnion dropped to 47 µS/cm, and the peak near the sediments also dropped to 53 µS/cm (Table 1). The drop in dissolved compounds in bottom waters coincident with onset of oxygenation mimicked the substantial decrease in Fe and Mn levels in bottom waters (Table 1).

On September 13, approximately 2 weeks into the oxygenation test, DO and temperature in the hypolimnion at the deep-water site increased (Figure 2). DO levels ranged from 4 mg/L in the upper hypolimnion to 1-3 mg/L in the lower hypolimnion. Hypolimnetic temperature continued to warm to 9-10 °C, around 2 °C warmer than before oxygenation. Mean conductivity was 63 µS/cm in the hypolimnion with a peak of 70 µS/cm near the sediments. Based on mean concentrations, conductivity decreased slightly compared to pre-oxygenated conditions (67
µS/cm on August 13). This decrease in mean conductivity correlated with lower levels of Fe and Mn in the oxygenated water column of the hypolimnion.

A profile on October 20 at the deep-water site, nearly one month after oxygen was turned off, showed that the epilimnetic temperature dropped 6 °C and the thermocline declined from 7 m to 10 m, indicating that lake overturn was imminent. The remaining hypolimnion below 11 m was anoxic. Reestablishment of anoxic and metal-enriched conditions in the hypolimnion also yielded a mean hypolimnetic conductivity of 74 µS/cm and a peak of 104 µS/cm near the sediments (Table 1).

DO isopleths were developed from transects perpendicular to the line diffuser for August 28 (prior to oxygenation), August 30 (two days into oxygenation), September 7 (nine days into oxygenation), and September 15 (12 h after oxygen was turned off), to show a more detailed distribution of DO spreading from the diffuser (Figure 3). On August 28, just prior to initial input of oxygen, the hypolimnion in North Twin was completely anoxic up to the thermocline (Figure 3A). Two days into oxygenation, an oxygen plume that extended roughly 250 m horizontally to both the north and the south of the line diffuser was detectable in the upper hypolimnion, a DO distribution often referred to as a “top hat” distribution based on the hat-like shape of spreading DO (Figure 3B). Nine days into the oxygenation event, there was considerable DO migration horizontally from the bubble plume to the south, and little DO migration to the north (Figure 3C). A day after the end of the test, DO distribution was more uniform, although DO in the southern end of the hypolimnetic zone was higher than that of the northern zone (Figure 3D). Throughout the entire test, isopleths showed little to no DO 0.5 m above the SWI.

There are a number of contributing factors affecting observed DO heterogeneity during the oxygen test at North Twin. Most oxygenation systems are operated from onset of thermal
stratification and therefore maintain high DO in the water column through the stratification season. For this test, however, oxygen was turned on late in the stages of stratification with an established oxygen deficit. Commonly, it can take up to 7 days or more for a uniform flow pattern to establish within a hypolimnion after initiating an oxygenation system (Bryant et al., 2011b). A bubble plume model was used to estimate oxygen delivery rates and hypolimnetic flow dynamics during the test (Singleton et al., 2007). Model inputs included diffuser length (750 m), diffuser depth (12.7 m), oxygen flow rate 2.25 m$^3$/min, as well as initial conditions for temperature, DO, and conductivity. The model estimated 20% of the hypolimnion was circulated each day by the bubble plume. Therefore it would take an estimated 5 days to reach a dynamic flow equilibrium. This was illustrated by the clear “top hat” DO distribution observed on day 3 (Figure 3B).

Oxygen was not administered long enough to reach homogeneous oxygen levels throughout the bottom waters and overcome both established DO deficit and hypolimnetic oxygen demand during this test. The plume model predicted a hypolimnetic oxygen input rate of 4,300 kg/day over the course of the test. This would theoretically result in a final average hypolimnetic DO of 5.7 mg/L at the end of the 17 day test, assuming a hypolimnetic volume of 0.013 km$^3$ and no oxygen demand in the hypolimnion. However, measured average hypolimnetic DO was 1.9 mg/L at day 14 of the test, suggesting a total hypolimnetic oxygen demand of 2,300 kg/day. Based on the observed rate of DO accumulation, it would take approximately 30 days to reach an average hypolimnetic DO content of 5 mg/L, a level suitable for fish habitat and more likely to result in oxygen penetration into profundal sediments.
3.4.2 Iron and Manganese

3.4.2.1 Vertical distribution prior to oxygenation

Elevated Fe and Mn were measured in the hypolimnion prior to oxygenation (Figure 2). Mid August mean hypolimnetic water column concentrations were 833 µg/L for total Fe and 119 µg/L for total Mn, yielding a Fe:Mn ratio of 7.0 (Table 1). Maximum hypolimnetic concentrations above the sediments in mid August were nearly 3,000 µg/L for total Fe and approximately 250 µg/L for total Mn. These concentrations are within the lower to mid range for Fe and the lower range for Mn reported in anoxic bottom waters of freshwater lakes. Values typically range from 20-8,000 µg/L for total Fe and 100-3,000 µg/L for total Mn (Stauffer, 1987; Davison and Woof, 1984; Davison et al., 1982). Fe was also elevated relative to Mn in the sediments with concentrations of 36,000 mg/kg dw total Fe and 600 mg/Kg dw total Mn. Other studies report a range of metal levels in lake sediments between 20,000-30,000 mg/kg for total Fe and 600-8,000 mg/kg for total Mn (Stauffer, 1987; Davison and Woof, 1984), suggesting that North Twin Lake sediments are relatively high in Fe and low in Mn. An earlier investigation of North Twin Lake found elevated Fe concentrations in inlet tributaries relative to Mn and noted the predominance of Fe-rich minerals within the watershed (Juul and Hueftle, 1992).

Fe and Mn displayed contrasting vertical distributions prior to the oxygen test. In mid August a steep gradient of Fe extended from the bottom to 12 m, while the Mn gradient extended from the bottom to about 8 m (Figure 2). A number of mechanisms can explain this observation. Since oxidized Mn (Mn(IV)) is reduced at a higher oxidation-reduction potential than that of oxidized Fe (Fe (III)), dissolved Mn(II) likely diffused out of sediments first and migrated up
into the water column earlier than Fe(II). Over time, this could result in Mn diffusing higher up in the hypolimnetic water column relative to Fe. Internal recycling dynamics can also lead to spatial differentiation of Fe and Mn in the water column (Hongve, 1997; Davison, 1993). Metal oxides, either from fluvial inputs or produced internally when reduced metals are oxidized at the oxic-anoxic interface (OAI) in the water column, continuously settle down from surface waters. Fe(III) is more chemically stable than Mn(IV) and can settle deeper into the anoxic hypolimnetic water column with a relatively low oxidation-reduction potential. After having settled into deep anoxic water, Fe(III) can eventually be reduced to Fe(II) and accumulate in bottom waters. This cycle of Fe transfer back and forth across redoxocline zones is commonly referred to as the "Ferrous Wheel" (Davison, 1993). Although this cycle works in a similar way with Mn, unstable Mn-oxides can be quickly reduced to soluble reactive Mn (Mn(III)) or Mn(II) at the oxidation-reduction boundary. As a result, soluble Mn is more likely to remain within the upper water column of anoxic hypolimnia.

Another phenomenon that may account for the sharp decrease in Fe at a depth of 12 m is abiotic reduction of Mn(IV) by Fe(II). When settling Mn(IV) comes in contact with Fe(II), Mn(IV) can be reduced abiotically to Mn(II) by stripping an electron from Fe(II), resulting in freshly oxidized Fe(III) settling out of the water column. As a result, Fe(II) can be inhibited from migrating up into the water column in the presence of Mn(IV). In stable redoxclines, this electron transfer can lead to an accumulation of the intermediate species Mn(III), which can quickly be oxidized to Mn(IV) via microbial oxidation in the presence of oxygen, or abiotically reduce to Mn(II) in the presents of Fe(II) or sulfide (Dellwig et al., 2012). In lakes where the oxidation-reduction boundary is not stable, this intermediate Mn species does not have the chance to accumulate due to rapid oxidation or reduction. Mn dynamics were investigated in the
meromictic Lake Nordbytjernet in Southeastern Norway by Hongve (1997). Although the lake had Fe loading rates that were three times higher than Mn, Mn concentrations were twice as high as Fe concentrations in stagnant anoxic bottom water of the Norwegian lake. There was also an observed decrease in Mn sedimentation with depth in Lake Nordbytjernet, but an increase in Fe sedimentation with depth within the stagnant non-mixing zone. In this meromictic system, the electron transfer of Fe(II) to Mn(IV) was identified as the key mechanism responsible for enhanced Mn enrichment and Fe loss from the water column, and similar dynamics may have occurred in North Twin Lake.

Another potential loss mechanism for Fe(II) is through FeS precipitation. As sulfide concentration rise, HS\(^-\) can bind with Fe(II), forming particulate FeS (Murray, 1995). Fe bound with sulfide is no longer available for complexation, and this can lead to phosphorus enrichment in lake bottom waters (Gächter and Müller, 2003). This phenomenon was documented by Caraco et al. (1993). They examined Fe and phosphorus levels in 51 lakes and found a positive correlation between sulfate reduction and phosphorus concentrations in the water column. The solubility product (pK\(_{sp}\)) for the reaction between Fe(II) and sulfide is:

\[
pK_{sp} = -\log \left[ \frac{(Fe^{2+})(HS^-)}{(H^+)} \right] \quad (1)
\]

where 0.72 is the activity coefficient for Fe(II) (Snoeyink and Jenkins, 1980). Values of pK\(_{sp}\) for FeS range between 2.6-3.8 (Murray, 1995). This relationship can be used to assess the potential for FeS formation in the hypolimnion of North Twin. Assuming a pK\(_{sp}\) of 3.0, and typical levels for Fe(II) (3,000 µg/L) and pH (6.5), FeS would form at sulfide concentrations of around 0.3 mg/L. Sulfide was detected via a serendipitous sniff test in water samples near the bottom
observed prior to oxygenation (Table 1). North Twin’s neighbor, South Twin, also routinely exhibits strong sulfide odors in its bottom waters. A handful of sulfide analyses have been performed in South Twin, and levels were below 0.3 mg/L. Presuming North and South Twin exhibit similar sulfide concentrations, which would most likely be a conservative assumption considering that stronger sulfide odors were observed in South Twin during pre-oxygenation sampling, the formation of FeS appeared to not control Fe(II) dynamics in North Twin Lake.

3.4.2.2 Vertical distribution during oxygenation

Mean hypolimnetic total Fe and Mn concentrations ~8 hr after initiation of oxygenation decreased from 830 µg/L and 120 µg/L to 240 µg/L and 30 µg/L, respectively, with soluble fractions in the remaining metals of 58% for Fe and 100% for Mn (Table 1). Peak concentrations above the sediments also fell to 780 µg/L for total Fe and 70 µg/L for total Mn. Mean hypolimnetic total Fe continued to drop through the test, to 210 µg/L on day 7 and 170 µg/L on day 17. Soluble fractions fluctuated in the range of 30-60% of total. In two weeks of oxygenation, mean hypolimnetic total Mn ranged from 30-50 µg/L, with the soluble fraction decreasing from around 100% on day 7 to 50% on day 17. Peak concentrations of total Fe and Mn displayed a similar pattern as the mean hypolimnetic concentrations.

Both Fe and Mn responded rapidly to several hours of preliminary oxygen addition. Fe and Mn decreased by about 70% after 8 hr after initiation of oxygenation (Table 1), which corresponds to the observed drop in hypolimnetic conductivity described above. Fe(II) is very sensitive to abiotic oxidation, and quickly oxidizes when exposed to oxygen. The half life of Fe(II) in air saturated solutions at pH 7 has been reported to range from 27-380 min (Davison, 1993). Based on estimates of initial and final Fe(II) concentrations of 830 µg/L and 140 µg/L
(Table 1), respectively, and the initial 8 hr oxygenation period, an approximation of the Fe(II) half life in North Twin Lake was 190 min, which falls within range of laboratory observations.

Unlike Fe(II), abiotic oxidation of Mn(II) is extremely slow in natural waters, and this mechanism likely played a small role in observed decreases in Mn during several hr of preliminary oxygen addition during installation of the diffuser. While oxidation of Mn(II) via surface catalysts such as goethite (α-FeOOH) can be extremely rapid (Davies and Morgan, 1989), typical half lives of Mn(II) in natural waters are on the order of years (Diem and Stumm, 1984). Conversely, several studies of lake systems have reported relatively rapid removal of Mn(II) from anoxic waters upon oxygenation. Rapid loss of both Fe and Mn in water upon reaeration has been demonstrated in experimental chambers containing lake sediments and overlaying water (Beutel et al., 2008 and 2007). Considering that abiotic Mn oxidation kinetics are much slower than that of Fe, these studies concluded that the observed drop in Mn was attributed to chemolithotrophic Mn-oxidizing microorganisms that are ubiquitous in aquatic sediments (Lovley et al., 2004). Wehrli et al. (1995) made a similar conclusion in Lake Sempach, Switzerland where Mn(II) half-lives in bottom waters were only a few days. Detailed studies in Carvins Cove Reservoir, Virginia, indicated that oxygenation of the SWI promoted biotic oxidation of Mn (Bryant et al., 2011a) and enhanced the presence of the metal-reducing bacteria Geobacteraceae in surfacial sediments (Bryant et al., 2012).

The probable mechanism for Mn loss observed in the hypolimnion of North Twin during preliminary oxygen addition event was Mn(II) co-precipitation with Fe(III). This reaction is known to occur in natural waters above a pH of 6.7 (Hem, 1963), though co-precipitation is optimal above a pH of 8.0 (Handa, 1969). Hongve (1997) found evidence of Fe(III)-mediated Mn(II) removal from a meromictic lake with bottom water pH of approximately 7.5. The same
phenomenon likely accounted for part or much of the Mn loss in North Twin Lake, and may have been enhanced by a relatively high Fe:Mn ratio, around 7.0 (Table 1). Other lakes typically have lower Fe and Mn ratios within the water column, many below 1.0 (Balistrieri, 1992; Stauffer, 1987). The large quantity of oxidized Fe likely overwhelmed the relatively low quantity of Mn(II), and even with a small fraction of Fe oxides binding with Mn(II), the end result would be a substantial loss of Mn. Fe(III)-mediated Mn(II) removal is also consistent with an observed lack of particulate Mn in the water column. After initial oxygenation and Mn loss, remaining Mn was all still in the soluble form, whereas nearly half of the remaining Fe in the water column was in the particulate form (Table 1). Although results from our analysis are not able to differentiate between soluble Mn(II) and reactive Mn(III), it is likely that Mn(III) would be stripped out of the water column similarly to Mn(II) by settling Fe-oxides (Dellwig et al., 2012).

A DO mass balance calculation for initial oxygen addition during diffuser installation confirmed that oxygen delivery rates were high enough to account for the mass of Fe(II) removed through oxidation and subsequent settling. Cooke et al. (2005) summarized the stoichiometry of Fe(II) oxidation in lake waters as:

\[
Fe^{2+} + \frac{1}{4}O_2 + 2OH^- + \frac{1}{2}H_2O \rightarrow Fe(OH)_3(s)
\] (2)

Average water column hypolimnetic Fe(II) concentrations decreased from around 830 µg/L to 240 µg/L in the first 8 hr of oxygenation, assuming that the pool of hypolimnetic Fe on August 13 was nearly all in the soluble reduced form given that the hypolimnion was completely anoxic (Table 1). Considering Fe, like oxygen, likely exhibited spatial variability horizontally in the hypolimnion, we can stoichiometrically validate that enough oxygen was delivered to the
hypolimnion to oxidize Fe(II) within the portion of water that moved through the bubble plume during the 8 hr of oxygenation prior to water column sampling. The bubble plume model estimated that 20% of the hypolimnetic volume was circulated through the bubble plume each day, thus approximately 7% of the hypolimnion would have been oxygenated after 8 hr. Assuming that the oxidation and subsequent decrease in Fe occurred throughout 7% of the 0.013 km³ hypolimnion or 910,000 m³, an estimated 630 kg of Fe was potentially oxidized. Given that it takes one mole of oxygen to oxidize four moles of Fe(II), or 0.14 kg of oxygen for every 1 kg of Fe(II), it would take 90 kg of oxygen to account for the Fe(III) oxidized in the hypolimnion. The bubble plume model predicted an oxygen addition of 160 kg/hr of DO for a total of 1,280 kg after 8 hr. Considering that residual DO in the water column 8 hr into oxygenation was 1.3 mg/L, an increase from pre-oxygenation conditions of 0.8 mg/L, there was 455 kg of newly injected oxygen left in the hypolimnion after satisfying all oxygen sinks. This analysis suggests that 825 kg of oxygen had been consumed in the first 8 hr, which is well over the estimated Fe(II) demand of 90 kg. Oxygen is also typically consumed by aerobic microbial respiration as well as oxidation of other reduced species such as ammonia, sulfide, methane and organic matter, though only the reaction kinetics of sulfide approach those of Fe(II). Estimates suggest that the oxygen demand of sulfide (< 0.3 mg/L) in the bottom meter of the hypolimnion would have been less than 500 kg. This analysis confirms that sufficient oxygen was added during the initial 8 hr of the oxygenation test to account for the loss of Fe(II) via oxidation at the deep water site in North Twin.

Following initial decreases, Fe continued to decline slowly while Mn gradually increased during the remainder of the test. DO never exceeded 4 mg/L above the diffuser and remained below 0.5 mg/L near the bottom. The slow accumulation of Mn may be explained by its slow
oxidation kinetics, and by the fact that newly oxidized Mn(IV) can be reduced to Mn(II) via Fe(II) that is diffusing upwards from the sediments. After the initial decrease in Fe(II), there was no longer a large settling pool of Fe(III) to strip out Mn(II). After 17 days, the fraction of soluble Mn dropped from around 100 to 50% of total, indicating that either Mn(II) was partially sequestered into the suspended particulates of Fe(III) and organic matter or Mn oxides were forming, most likely due to biotic oxidation as described above. Accumulation of Mn(II) in mildly oxygenated waters, like that observed in North Twin Lake, has been observed in many mildly oxic lakes (Hongve 1997; Davison 1993; Davison and Woof, 1984), and reinforces the observation that Mn(II) can accumulate and persist in aerobic lake waters. The steady decrease in Fe, both in the bulk hypolimnion and peak concentrations 1 m above the sediments, suggests that oxygenation continued to influence the removal of Fe from the water column via oxidation, and also prevented reduction and re-entrainment of Fe from the sediments to the bottom waters.

3.4.2.3 Vertical distribution post oxygenation test

On October 20, about a month after the oxygenation test was terminated, anoxia had completely reestablished within the hypolimnion. Mean hypolimnetic concentrations rebounded to around 640 µg/L for total Fe and 120 µg/L for total Mn, near levels observed prior to oxygenation (Table 1). Peak concentrations above the sediments were approximately half of the pre-oxygenation level for Fe (1,500 µg/L) but similar to pre-oxygenation levels for Mn (261 µg/L). Conductivity also rebounded to 105 µS/cm, a level higher than pre-oxygenation conditions (81 µS/cm). The vertical gradient of Fe reached up to 12 m, while the vertical gradient of Mn reached up to the 10-m-deep thermocline (Figure 2). A key difference between pre- and post-oxygenation concentrations of Fe and Mn was illustrated by the Fe:Mn ratio, which
decreased from 7.0 before the oxygenation test to 5.3 one month after the test. Faster recovery of Mn enrichment, as reflected by a lower Fe:Mn ratio and relatively low peak Fe concentration, is consistent with the fact that Mn(IV) reduction is more rapid upon depletion of oxygen than Fe(III) reduction (Davison, 1993).

3.4.3 Total Mercury and Methylmercury

3.4.3.1 Vertical distribution prior to oxygenation

Profiles for total Hg and MeHg on August 13, prior to oxygenation, showed a general trend of lower concentrations in the upper hypolimnion and enrichment in the bottom waters above the SWI (Figure 2, Table 1). Profiles of total Hg exhibited greater variation throughout the water column with small peaks of around 0.9 ng/L in the epilimnion and upper hypolimnion, and a concentration of 1.4 ng/L near the sediments. MeHg was uniformly 0.1 ng/L in the upper water column, but increased below 12 m to a concentration of 0.44 ng/L near the sediments. North Twin Lake has no point sources of Hg contamination and falls into the middle to low range for Hg enrichment in pristine lakes. Watras (2009) summarized Hg enrichment in nine remote freshwater lakes and reported that total Hg and MeHg levels range from 0.1-5 ng/L and 0.003-0.8 ng/L, respectively.

Sources of Hg include both freshly deposited Hg from direct atmospheric deposition, Hg in watershed runoff, and previously settled Hg in surficial sediments. A Canadian study found that, after amending the test lake with trace amounts of stable Hg isotopes, there was a higher fraction of MeHg from the spiked isotopes than the ambient Hg pool, indicating that freshly deposited Hg is particularly available for methylation (Chadwick et al., 2006). A key facilitator
of Hg enrichment in anoxic zones is sulfide, a byproduct of biological sulfate reduction, and a strong ligand for both inorganic Hg and MeHg (Watras, 2009). Sulfide can strip Hg from settling metal oxides and other seston before it settles onto sediments (Morel et al., 1998). MeHg, in addition to being scavenged from settling seston by sulfide, can also be enriched by in-situ methylation of Hg(II) by SRB within the water column and in surficial sediments (Eckley et al., 2005; Compeau and Bartha, 1985). Sediments can be a source of both Hg(II) and MeHg to the hypolimnion through reduction of oxyhydroxides of Fe(III) and Mn(IV) with subsequent diffusion of newly dissolved Hg species to the overlying water (Merritt and Amirbahman, 2008; Chadwick et al., 2006). Enrichment of both Hg and sulfide was observed in waters above sediments in North Twin prior to the oxygen test, which is consistent with observed patterns of Hg accumulation in other freshwater lakes.

3.4.3.2 Vertical distribution during oxygenation

At the end of oxygenation on September 13, both total Hg and MeHg had decreased throughout the water column (Figure 2, Table 1). Total Hg concentrations were highest at 4 m in the epilimnion, potentially due to settling Hg sequestering onto a dense pocket of algae, but lower in the hypolimnion. Hypolimnetic mean total Hg was 0.58 ng/L, 25% lower than pre-oxygenation conditions. Total Hg concentration in bottom water above the sediments was about 60% lower, at 0.6 ng/L. MeHg concentrations were also lower in the epilimnion and hypolimnion. Hypolimnetic mean MeHg concentration was 0.10 ng/L, nearly half that of pre-oxygenation conditions. MeHg concentrations in bottom water were about 95% lower, 0.02 ng/L, compared to pre-oxygenation.
Short-term introduction of oxygen into the hypolimnion decreased Hg(II) and MeHg in the hypolimnion 17 days into the oxygenation test compared to pre-oxygenation conditions. Hg that had accumulated prior to onset of oxygenation was likely stripped from the water column during oxidation of Fe(II) following initial operation of the oxygen diffuser. A number of researchers have implicated Fe and Mn oxides as a potential sink for water column Hg (Merritt and Amirbahman, 2008; Chadwick et al., 2006). Precipitation of Fe(III) and Mn(IV) in the form of metal oxides can provide a surface for adsorption of a number of metals including Hg, arsenic, copper, zinc and nickel (Davison, 1993). In Davis Creek Reservoir, freshly precipitated Mn oxides were found to strip aqueous MeHg out of the water column during fall turnover (Slotton et al., 1995; Gill and Bruland, 1992). Unlike North Twin Lake, Davis Creek Reservoir was rich in Mn and poor in Fe; after fall turnover, Mn concentrations increased 100 fold in the surface waters, whereas Fe was nearly undetectable. Gill and Bruland (1992) reported a correlation between increased Mn in surface waters and an increase in particulate bound Hg, indicating sequestration of dissolved Hg by particulate Mn (VI). Hurley et al. (1994) also found a strong correlation between Fe and Hg in four northern Wisconsin seepage lakes, where increases in Fe flux correlated positively with both Hg(II) and MeHg fluxes in hypolimnetic waters. Chadwick et al. (2006) in a subproject of the “Mercury Experiment to Assess Atmospheric Loading in Canada and the United States” (METAALICUS), found strong interactions between dissolved species of Hg and DOC, and formation of Fe(III)-DOC-Hg complexes under oxic conditions. These observations support the contention that decreases of total Hg and MeHg in Twin Lakes, similar to decreases in Mn(II), were results of Fe(II) oxidation and subsequent sorption and sedimentation of Fe(III)-DOC-Hg complexes.
3.4.3.3 Vertical distribution post oxygenation test

As with Fe and Mn, total Hg and MeHg increased rapidly after the end of oxygenation. By October 20, around one month after the test, mean hypolimnetic concentrations of total Hg increased to about 1.5 ng/L with a peak of 1.78 ng/L above the sediments, higher than pre-oxygenation conditions (Table 1). MeHg also increased to concentrations higher than pre-oxygenation conditions with mean hypolimnetic concentrations of 0.27 ng/L, with a peak above the sediments of 0.49 ng/L. The gradient of Hg accumulation in the hypolimnion was much greater near the sediment, having the bulk of re-released Hg concentrated below 13 meters. The upper hypolimnion, from the now deeper 10 m thermocline to 13 meters was less than pre-oxygenated conditions, suggesting that the large mass of MeHg released from the sediment was limited by the slow rate of diffusion in its vertical migration.

A salient observation is the speed at which both total Hg and MeHg reestablished themselves in the water column after termination of the oxygen test, and the apparent reversibility of Hg removal mechanisms. The rapid return to anoxic conditions in the hypolimnion apparently resulted in the reduction of recently deposited Fe oxides with concurrent re-release of Hg into the hypolimnion. A similar result was observed at Round Lake, MI where an addition of nitrate was used to poise the oxidation-reduction potential in the bottom waters (Austin et. al., 2011). Nitrate addition initially repressed MeHg enrichment, but MeHg concentrations rapidly escalated to levels higher than pre-treatment conditions after nitrate was consumed. Elevated summer MeHg concentrations in the hypolimnion are particularly significant due to potential for mixing into the surface waters at fall turnover. Studies have shown direct relationships between elevated MeHg in the bottom waters and a fall mixing event increasing both surface water MeHg and Hg body burdens of zooplankton and young-of-year.
Therefore, to achieve the goal of repressing Hg uptake in biota, it is critical that applications of hypolimnetic oxygen repress Hg enrichment in the hypolimnetic waters up to the fall turnover event, thereby avoiding exposure of biota to the contaminant.

3.5 CONCLUSION

Hypolimnetic oxygenation is a control technology that is increasingly employed to maintain oxic conditions in productive lakes and reservoirs for water quality and fish habitat enhancement. At North Twin Lake, a late season short-term oxygenation test provided a unique opportunity to evaluate oxygen addition on biogeochemical cycling of Fe, Mn, and Hg. Addition of oxygen removed Fe, Mn, and Hg from the water column and repressing its accumulation for the duration of the test. Results suggested that the key removal mechanism for Fe, Mn and Hg from the water column was Fe oxidation and subsequent sedimentation.

This is one of the few studies to document rapid loss of hypolimnetic Fe as a result of engineered oxygen addition to a lake. In addition, although several studies have implicated the importance of metal oxides and oxidation-reduction potential in Hg cycling, this is one of the first studies to show loss of Hg from the hypolimnetic water column coincident with engineered oxygenation addition. Key supporting conclusions include:

1. Loss of Fe from the anoxic lake water column was extremely rapid. Near the diffuser, nearly three quarters of Fe was lost within 8 hr of initial oxygenation. The rate of Fe loss, quantified as an in-lake Fe half-life of 190 min, was comparable with laboratory studies of Fe(III) oxidation.
2. The mass of oxygen added was sufficient to account for Fe oxidation. Estimated oxygen consumed in oxidation of accumulated Fe(II) to Fe(III) was less than 10% of overall oxygen delivered to the oxygenated water column, leaving a substantial amount of oxygen available for other potential oxygen sinks (e.g., Mn(II), ammonia, methane, sulfide, organic matter).

3. Fe oxidation can be an important sink for Mn. Mn was also rapidly removed from the water column near the diffuser during initial oxygenation. Since Mn(II) does not undergo rapid abiotic oxidation like Fe(II), the likely removal mechanism was sequestration of Mn(II) via settling Fe(III).

4. Fe oxidation can be an important sink for Hg. Enriched MeHg was dramatically reduced in the bottom waters of North Twin following initiation of an oxygen diffuser. Based on previous investigations of metal oxide interactions with Hg(II) and MeHg, the likely loss mechanism was formation of Fe-oxides and subsequent stripping of Hg from the water column.

5. Loss of Fe, Mn, and Hg from the water column was reversible when anoxic conditions were reestablished. This supports the contention that Hg was indeed trapped in surficial sediments. This implies that for an oxygenation system to inhibit Hg enrichment of bottom waters in lakes rich in Fe and Mn, they should be engineered and operated to maintain a well oxygenated SWI capable of sequestering metal oxides and any associated Hg in surficial sediments up until the point of turnover.
3.6 REFERENCES


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Figure 1. Bathymetric map of North Twin Lake showing the placement of the oxygen storage tank and diffuser line and the deep-water sampling site (star). Contours are in m.
Figure 2. Profiles of dissolved oxygen and temperature (top), total Fe and total Mn (middle), and total Hg and MeHg (bottom). Profiles were collected prior to oxygenation on August 13, 2008, at the end of the oxygenation test on September 13, 2008, and one month after the end of the oxygenation test on October 20, 2008.
Figure 3. Dissolved oxygen isopleths running from north (right) to south (left) on August 28 (A), August 30 (B), September 7 (C), and September 15 (D). Yellow dot is location of line diffuser.
Table 1. Iron, Manganese, Conductivity, and Sulfide in the Hypolimnion of North Twin Lake.

<table>
<thead>
<tr>
<th>Oxygenation Timeline</th>
<th>Aug 13</th>
<th>Aug 28</th>
<th>Sep 3</th>
<th>Sep 13</th>
<th>Oct 20</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 days before</td>
<td>Day 1</td>
<td>Day 7</td>
<td>Day 17</td>
<td>35 days after</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hypolimnentic Water Column</th>
<th>Fe (µg/L (% dissolved))</th>
<th>Mn (µg/L (% dissolved))</th>
<th>Fe: Mn</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Metals</td>
<td>833 243 (58) 208 (28) 173 (58) 642</td>
<td>119 32 (100) 36 (94) 49 (49) 122</td>
<td>7.0 7.9 5.8 3.5 5.3</td>
</tr>
<tr>
<td>Mean Conductivity, µS/cm</td>
<td>67 47 ND 63 74</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fe:Mn</td>
<td>7.0 7.9 5.8 3.5 5.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bottom Water (1 m above sediments)</th>
<th>Fe (µg/L (% dissolved))</th>
<th>Mn (µg/L (% dissolved))</th>
<th>Conductivity, µS/cm</th>
<th>Sulfide Presence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metals, µg/L (% dissolved)</td>
<td>2,882 782 (34) 381 (20) 344 (88) 1519</td>
<td>250 71 (99) 85 (58) 135 (52) 261</td>
<td>81 53 ND 70 104</td>
<td>Y N N N Y</td>
</tr>
<tr>
<td>Conductivity, µS/cm</td>
<td>81 53 ND 70 104</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfide Presence</td>
<td>Y N N N Y</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

aData for day 1 represent 8 hours after oxygenation began.
Table 2. Total Mercury and Methylmercury in the Hypolimnion of North Twin Lake.

<table>
<thead>
<tr>
<th>Oxygenation</th>
<th>13-Aug</th>
<th>13-Sep</th>
<th>20-Oct</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>15 days before</td>
<td>Day 17</td>
<td>35 days after</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hypolimnetic Water Column</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean concentration, ng/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>THg</td>
<td>0.77</td>
<td>0.58</td>
<td>1.14</td>
</tr>
<tr>
<td>MeHg</td>
<td>0.20</td>
<td>0.10</td>
<td>0.27</td>
</tr>
<tr>
<td>MeHg:THg</td>
<td>0.26</td>
<td>0.17</td>
<td>0.24</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bottom Water (1-2 m above sediment)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Concentration, ng/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>THg</td>
<td>1.43</td>
<td>0.60</td>
<td>1.78</td>
</tr>
<tr>
<td>MeHg</td>
<td>0.44</td>
<td>0.02</td>
<td>0.49</td>
</tr>
</tbody>
</table>
CHAPTER 4. EFFECTS OF HYPOLIMNETIC OXYGENATION ON WATER COLUMN IRON, MANGANESE, AND METHYLMERCURY

4.1 ABSTRACT

This chapter presents the full-scale treatment and evaluation of a hypolimnetic oxygenation system, which was introduced in chapter 3, and its impact on iron (Fe), manganese (Mn), and methylmercury (MeHg) in the water column of North Twin Lake, WA. The system was operated from June to October in 2009, 2010, and 2011. North Twin Lake’s neighbor, South Twin Lake of similar size and shape, was used as a reference. The oxygenation system was designed to increase the dissolved oxygen (DO) in the bulk hypolimnion to restore the cold water fish habitat in the system. During oxygenation, Fe and Mn accumulation correlated with bottom water DO in the treated lake for all three years, with $R^2$ values of 0.5 and 0.6 ($P < 0.001$). There were extended periods of anoxia above the sediments during treatment years, in particular in 2010 and 2011, that resulted in higher Fe and similar Mn accumulation in North Twin Lake as compared to the untreated South Twin Lake. MeHg accumulation in North Twin was lower than South Twin Lake with some significance in 2009 ($P = 0.024$), but were fairly similar in 2010 ($P = 0.12$). Late season averages of MeHg volume weighted averages were highest when DO dropped to near zero in the bottom waters. MeHg accumulation was also tightly correlated to Fe and Mn accumulation, with an $R^2$ of 0.7 ($P < 0.001$) for both metals. Correlation between MeHg and metals grew stronger each treatment year, which suggests that dissolved organic carbon, a strong ligand for MeHg, was becoming less of a controlling factor, potentially due to mineralization from the oxygen treatment. There is clear evidence that oxygen influences the cycle of Fe, Mn, and MeHg in North Twin Lake, however, the design and operation of the
oxygenation system may need to be optimized to increase the DO delivery to the water overlying the sediments.

### 4.2 INTRODUCTION

Many monomictic and dimictic freshwater lakes with elevated productivity can develop oxygen depletion, anoxia, in hypolimnetic waters. This hypolimnetic anoxia is commonly caused by an increased flux of allochthonous and autochthonous organic material that settles into profundal regions of lakes and is decomposed both in the water column and at the sediment-water interface (SWI) (Hupfer and Lewandowski, 2008).

Elevated productivity is initially enhanced by an increase in external nutrient loading from the watershed, but over time as organic matter builds up a legacy oxygen demand at the SWI, internal nutrient loading can become a significant source of nutrients to the water column as well (Beutel and Horne, 1999). Phosphorus can be released to hypolimnetic waters as the stores of decomposing organic matter become mineralized and oxygen is consumed through several mechanisms. One such mechanism is through lowered redox conditions activating anaerobic microorganisms which metabolically release phosphorus (Gächter and Meyer, 1993). Another redox sensitive mechanism involves the reduction of phosphorus-rich metal oxides releasing phosphorus during dissolution (Welch and Jacoby, 2001; Boström et al., 1998). Finally, decreasing redox condition lowers pH in anoxic lake waters, increasing phosphorus solubility and subsequent dissolution (Golterman, 2001). During fall and spring turnovers, accumulated nutrients in the hypolimnion are mixed throughout the water column. Increasing surface water nutrient concentrations, where temperatures are warmer and the sunlight is more intense, induces primary productivity. These mechanisms of internal nutrient loading prolong lake recovery, since
loading can continue long after external nutrient sources have been abated (Lioriussen et al., 2009).

There are several concerns with hypolimnetic anoxia other than internal nutrient loading that incentivize management and treatment of these systems. One primary concern is loss of habitat for fish and other aquatic biota, as anoxic waters are not accessible to oxygen dependent organisms (Davis, 1975; Heinis and Crommentuijn, 1992; Christensen and Moore, 2009). Another concern is hypoxia at the SWI which decreases the redox potential, thus enabling both abiotic and biotic reduction of oxidized iron (Fe) and manganese (Mn) solids (Davison, 1993), which increases dissolved Fe and Mn in the water column that interfere with the purification process of potable water (Betancourt et al., 2010; Gantzer et al., 2009a). A third concern relates to the increased toxicity to aquatic biota from accumulating reduced compounds such as sulfide and ammonia (Beutel, 2006; Beutel et al., 2001).

A relatively new issue for lake managers is the accumulation of methylmercury (MeHg) in both the water column and aquatic biota. Globally, atmospheric mercury (Hg) deposition has increased three-fold since the industrial revolution (Swain et al., 2007), and in the U.S., about half of all lakes acres and a third of river miles have fish consumption advisories due to elevated Hg in fish tissue (USEPA, 2009). The reason MeHg is the Hg species of such concern is that it is the primary species that accumulates in fish. Typically, MeHg represents more than 95% of total Hg in fish biomass, which enables it to build up to levels toxic in humans (Tchounwou et al., 2003). Biomagnification of MeHg through food webs can lead small changes in water column concentrations (<1 ng/L) resulting in orders of magnitude increases in fish tissue (>1 mg/kg) (Watras, 2009). Historically, attempts to address Hg issues targeted reductions in atmospheric deposition by regulating discharge of Hg at point sources. However, areas around the U.S. that
are identified with very high levels of Hg within their watersheds and water bodies have resulted in issuances of water body and water shed specific total daily maximum load (TMDL) regulations (http://water.epa.gov/lawsregs/lawsguidance/cwa/tmdl/mercury.cfm). For example, the Guadalupe River Watershed near San Jose, CA currently has a TMDL that requires fish within the watershed to be under 0.5 ppm and 1.0 ppm for fish 5 to 15 cm long and fish 15 to 35 cm long, respectively (http://www.epa.gov/waters/tmdldocs/38859_guad%20tmdl.pdf). Hg at these sites is at such high levels that decreasing Hg deposition alone would do little to rectify the problem within a reasonable time frame.

Formation and accumulation of MeHg in bottom waters are both tightly linked to hypolimnetic anoxia, where lowered redox conditions enhance in-situ MeHg production, MeHg flux from the sediment, and MeHg retention in the water column. MeHg enrichment has been observed in anoxic hypolimnia to concentrations 100 times greater than during oxic periods (Watras, 2009; Eckley and Hintelmann, 2006). Anaerobic sulfate reducing bacteria (SRB) have been implicated as being the primary microbe of concern in methylating inorganic Hg (Hg(II)) to MeHg in anoxic environments (Benoit et al., 2003). SRB are activated once the redox potential drops to an energy level optimal for sulfate reduction. Methylation is facilitated by a metabolic mechanism within the SRB at low sulfide concentrations (Gilmour et al., 1992). Hypolimnietic enrichment of MeHg can also be enhance by release of previously methylated MeHg from sediments. As oxyhydroxides of Fe(III) and Mn(IV) are reduced to soluble Fe(II) and Mn(IV), previously methylated and sequested MeHg can be released to the overlying water. The eventual accumulation of sulfide in the anaerobic hypolimnia then aids in the sequestration and enrichment of MeHg within the water column, as sulfide has a very high affinity for Hg species (Watras, 2009). As with nutrients, mixing induced by fall and spring turnover events can lead to
an enrichment of MeHg in the upper productive waters, thereby enhancing the exposure of MeHg to the base of the food web (Slotton et al., 1995).

Hypolimnetic oxygenation is a treatment technology that has been growing in use as a tool to ameliorate hypolimnetic anoxia. The treatment involves dissolving pure oxygen gas into an anoxic hypolimnia (Moore and Christensen, 2009; Beutel et al., 2007; Singleton and Little, 2006; Mobley and Brock, 1995). Lake aeration is a similar technology that has been used for the last 60 years, where compressed air is pumped down to the bottom waters, inducing mixing of the entire water column. In contrast, hypolimnetic oxygenation maintains thermal stratification due to its lower gas flow rate, as it has five times the oxygen content of air, and therefore only needs a fifth of the volume of gas to achieve the same results. Considering this treatment technology works to prevent hypolimnetic anoxia and prevents mixing between epilimnetic and hypolimnetic waters, there is growing interest in evaluating hypolimnetic oxygenation’s effect on the Hg cycle.

In this study, we evaluate the response Fe, Mn, and MeHg to three years of hypolimnetic oxygenation. In 2008, an oxygen diffuser line was installed in North Twin Lake, WA, with operation beginning in 2009. The incentive for installing this system was to open up cold water fish habitat that had been impacted by hypolimnetic anoxia (Beutel et al., 2011). This particular oxygenation system was selected for its ease of installation in remote areas and its effectiveness in oxygenating bottom waters while preserving thermal stratification (Mobley and Brock, 1995). Water quality data for metals and Hg have been collected since 2008 to monitor the impact on the Hg cycle. A neighboring lake, similar in size, shape, and function, was also monitored at the same time as a reference lake. Our hypotheses states that in the treated lake, in comparison to the pretreatment year and the reference lake: (1) Fe and Mn will remain out of the water column
during oxygenation; and (2) MeHg enrichment above the sediment will be reduced in the bottom waters of the treated lake.

4.3 METHODS

4.3.1 Study Site

North and South Twin Lakes are moderately deep, dimictic meso-eutrophic lakes located on the reservation of the Colville Confederated Tribes in eastern Washington State (Figure 1). The two lakes are connected by a shallow channel and have relatively similar area (3.71 and 4.13 km$^2$), mean depth (9.7 and 10.4 m), and max depth (15.2 and 17.4 m). The most striking difference between the two lakes are that North Twins watershed is roughly 5 times larger than South Twin’s, 73 versus 14 km$^2$, and North Twin’s hydraulic residence time is 3 times larger, 9.4 versus 2.7 years, compared to South Twin (Table 1). After spring melt in both lakes, usually in late April and early May, oxygen depletion intensifies above the SWI following development of thermal stratification. By mid to late June, the entire coldwater hypolimnion is hypoxic to anoxic and remains so until fall turnover, usually in October. Twin Lakes is a put and take trout fishery and during the period of hypolimnetic anoxia, cold-water trout are limited to a 2-m-thick zone at the metalimnion, unable to access the anoxic cold-water habitat below (Christensen and Moore, 2008). In an effort to open up the coldwater hypolimnion for fish habitat, a hypolimnetic oxygenation diffuser system was installed. The diffuser system consisted of an on-shore 23-m$^3$ liquid oxygen storage tank feeding a 750-m-long fine bubble (1-2 mm diameter) diffuser line and installed in the early fall of 2008 to improve the habitat for cold-water fish. The system was
turned on each year between mid to late May and with average oxygen delivery ranging from 25 to 40 standard cubic feet per minute (SCFM) (Figure 4).

4.3.2 Water Quality Monitoring

Water quality samples and data were collected 1 to 2 times per month from 2008 to 2011 through the summer stratification seasons. All data collection and water samples were collected at a deep water station in each lake on the same day, approximately 15 m in North Twin and 16 m in South Twin. Water quality monitoring for DO and temperature profiles were measured every m with a MS5 Sonde Hydrolab (Hach Company, Inc.). Water samples were collected at each site for Fe and Mn at 1 m intervals vertically down the water column using Tygon tubing attached to a Dayton model 1P580E pump. All Fe and Mn samples were preserved with nitric acid (APHA, 2005). Water samples were also collected for MeHg at every 1 to 2 m with a Teflon Kemmerer bottle (Wildco, Inc.). Samples were stored in acid washed amber glass bottles with Teflon lined caps preserved with trace metal grade hydrochloric acid and stored at 4°C until analysis following EPA methods 1630 (USEPA, 2001).

4.3.3 Water Quality Analysis

Fe and Mn in water samples were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) (APHA, 2005). MeHg samples were analyzed with EPA method 1630 analytical technique which involves sample distillation, ethylation, purge onto Tenax™ traps, thermal desorption, gas chromatography separation, pyrolyzation, and subsequent detection via cold vapor atomic fluorescence spectroscopy (USEPA, 2001).
4.4 RESULTS

4.4.1 Dissolved Oxygen

Bi-monthly to monthly monitoring showed that in 2008, both North and South Twin Lakes had near complete anoxia in their hypolimnions below 8 meters (Figures 2 and 3). Both lakes also show signs of warm stagnant weather, as their epilimions are both below saturation through July and August, at which time the coming fall weather appears to have induced some epilimnetic mixing, as evidence by the >8 mg/L epilimnetic DO observed in early September (Figure 2). An increase in hypolimnetic DO was observed in North Twin which corresponds with a several week test run of the oxygenation system (Chapter 3). South Twin’s hypolimnion remained anoxic through mid October.

In 2009, the first year the hypolimnetic oxygenation system was operated in North Twin Lake, there was a stark difference between hypolimnetic oxygen between North Twin and South Twin. Oxygen was turned on in mid May through mid October, with delivery rates ranging from 25 to 35 SCFM. DO remained elevated in North Twin’s hypolimnion, greater than 6 mg/L above 1 meter from the sediment until September, when the bottom 3 m dropped to between 2 – 4 mg/L prior to turnover. South Twin, conversely, showed little variation from 2008, with the entirety of its hypolimnion remaining anoxic through the summer stratification season.

The second year of oxygenation, 2010, showed more depressed DO conditions in the deeper waters of North Twin as compared to 2009, but was still markedly higher than the pretreatment year in 2008. Oxygen was turned on in mid May and operated through mid October at a delivery rate ranging from 25 to 40 SCFM. From July on, DO below 10 m was lower than 6 mg/L, with the bottom 2 m below 2 mg/L. Unlike in 2009, virtually no portion of North Twins
hypolimnion was above 8 mg/L from July to October. South Twin again showed very little variation from the previous two years, remaining anoxic in its hypolimnion throughout the stratification season.

In year three of oxygenation, 2011, DO in North Twin’s hypolimnion was elevated higher than it was in 2010, but not as elevated as it was in 2009. Oxygen was turned on a little later in 2011, starting the first of June and running through mid October at a delivery rate around 35 SCFM. Anoxia began to take hold prior to the June start of the oxygenation, dropping under 6 mg/L below a depth of 10 m. DO was elevated between 6 and 8 mg/L through the whole hypolimnion in July, but then dropped again below 6 mg/L in the bottom 4 to 6 meters for the rest of the season. As with 2010, all of North Twin’s hypolimnion remained below 8 mg/L.

4.4.2 Iron and Manganese

Accumulation of Fe was fairly similar in August of 2008 between the two lakes, with a hypolimnetic volume weighted average (VWA) in both of approximately 300 ug/L, with bottom water concentration peaks over 1,500 ug/L in the bottom 2 m of each lake (Figure 2, 3, 5, and 6). This first data represents the best pretreatment comparison between the two lakes, as an oxygenation test introduced oxygen to the hypolimnion of North Twin for the first two weeks of September and subsequently altered the water conditions to a non-pretreatment state (Chapter 3). Monitoring showed a rapid decrease in metals with the addition of oxygen in an already anoxic hypolimnion. Fe concentrations dropped to 100 µg/L in North Twin in September right at the end of the oxygen test, and then increased slightly to above 200 µg/L a month after the completion of the test (Figure 5). Mn, like Fe, dropped to a peak concentration of 150 µg/L
North Twin in September with a corresponding hypolimnetic VWA of 30 µg/L, and then increased slightly to above 40 µg/L as the lake turned over and the lake was completely mixed.

Fe and Mn accumulation in the hypolimnion of North Twin Lake varied between the three years of hypolimnetic oxygenation. In 2009, both Fe and Mn accumulated very little until August, with hypolimnetic VWA’s remaining between 300 to 600 µg/L for Fe and 50 to 100 µg/L for Mn (Figure 5). However although the peak values 1 m off of the sediment were lower in 2009 (870 and 180 µg/L for Fe and Mn) than 2008 (1,520 and 260 µg/L for Fe and Mn) the hypolimnetic VWA’s were higher in 2009 than in 2008, 330 versus 243 µg/L for Fe and 63 versus 44 µg/L for Mn (Table 2). Fe and Mn accumulation was observed through all the years of treatment, however, the bulk of metals accumulation in 2009 was limited to August through October, intensified in June for 2010 and 2011. Hypolimnetic VWA at the end of the season were lowest in 2009, 330 and 63 µg/L for Fe and Mn, highest in 2010, 1,100, and 175 µg/L for Fe and Mn, and in between the two in 2011, 800 and 102 µg/L for Fe and Mn.

The epilimnion and the metalimnion were nearly the same and consistently lower in metals in both lakes for the majority of the seasons. However, there were a few occasions when an increase in Fe was observed in the metalimnion of both lakes. In North Twin there were noted events of metalimnetic accumulation that corresponded with events of increased hypolimnetic accumulation in 2010 and 2011 (Figure 5). In South Twin, metalimnetic accumulation of Fe was observed towards the end of the stratification season. By the end of October to early November North Twin achieved turnover, resulting in moderate increases in the epilimnion and metalimnion (Figure 5).

During the four year evaluation, South Twin never reached a full turnover event by the last sampling event of the season, which took place on the first or second week in November.
Therefore the corresponding increase in its epilimnion and metalimnion following spring turnover were not observed or recorded.

Both Fe and Mn accumulation steadily intensified from 2008 to 2011 in South Twin Lake (Figure 3). Isopleths for both metals showed a greater mass building up in the bottom waters, accumulating earlier in the season and migrating further up the water column in the Fall in 2010 and 2011 as compared to 2008 and 2009. Average October Fe VWAs for 2008 and 2009, 350 µg/L, are lower than the average for 2010 and 2011, at 2,215 µg/L (Table 3). There was also an observed increase in Mn VWAs, 223 and 488 µg/L, for the first two years and the last two years respectively. Vertical migrations of Fe also increased in 2010 and 2011, from migrating 2 to 3 m off of the sediment floor in 2008 and 2009, to migrating up 5 and 6 m up from the sediments in 2010 and 2011 (Figure 3). A similar observation was noted with Mn migrating 4 to 6 m up from the sediment in 2008 and 2009 and up to 5 to 7 m above the sediment in 2010 and 2011.

4.4.3 Methylmercury

In 2008, prior to the oxygen test, MeHg accumulation appeared to occur earlier and migrate higher up into the water column in North Twin Lake relative to South Twin Lake. Although South Twin Lake was not sampled in June or July as North Twin Lake was in 2008, South’s accumulation in August was less severe and closer to the sediment than that of North Twin Lake. As with Fe and Mn, MeHg was affected by the oxygen test in September (Chapter 3). Although the oxygen test interrupted the natural cycle of MeHg accumulation, October hypolimnetic MeHg VWAs were similar in North Twin and South Twin Lakes, 0.10 and 0.08 ng/L respectively (Table 2).
MeHg accumulation in the hypolimnion of North Twin Lake, similar to Fe and Mn, varied between the three years of hypolimnetic oxygenation. In 2009, MeHg did not seem to accumulate until August, remaining around 0.02 ng/L through the hypolimnion (Figure 2). There was a slight increase in MeHg accumulation from August to October of 2009, similar to that observed with Fe and Mn. However, October hypolimnetic VWAs are down 70% from 2008, 0.10 to 0.03 ng/L, and nearly an order of magnitude lower in peak values above the sediment from 2008 to 2009 of 0.43 to 0.07 ng/L (Table 2).

MeHg accumulation intensified in 2010 and 2011. In 2010, there was a period in June and in September when MeHg accumulation intensified, where MeHg VWAs increased nearly 0.1 ng/L and then dropped down shortly thereafter. Hypolimnetic VWA’s of MeHg at the end of the season were lowest in 2009, 0.026 ng/L, highest in 2010, 0.076 ng/L, and in between the two in 2011, 0.051 ng/L, the same trend observed with Fe and Mn. There were slight increases in MeHg in the epilimnion and the metalimnion in North Twin that occurred in conjunction with Fall turnover (Figure 5).

South Twin was fairly consistent from year to year in regards to MeHg accumulation in the bottom hypolimnetic waters, with an exception of 2009. October hypolimnetic VWAs ranged from 0.082 to 0.088 ng/L in 2008, 2010, and 2011, whereas the October VWA in 2009 was nearly three times higher at 0.226 ng/L. Although greater accumulation was observed in 2009, peak bottom water values in 2009, 0.46 ng/L, were lower than both 2008, 0.52 ng/L, and 2010, 0.59 ng/L (Table 3). MeHg accumulation accelerates toward the end of the stratification season in all four years prior to fall turnover.
4.5 DISCUSSION

4.5.1 Inter-Annual Variation in Response of Dissolved Oxygen

Higher DO delivery did not necessarily correspond with higher DO mass in North Twin Lakes. An evaluation of DO distribution vertically through the water column shows anoxic pockets forming over the sediment most significantly in 2010, least significantly in 2009, and in between in 2010 (Figure 2). Conversely, oxygen delivery was highest in 2010, lowest in 2009, and in between in 2011 (Figure 4). Higher oxygen flow rates resulted in lower DO above the sediment. The system in North Twin Lake was designed to deliver oxygen below the thermocline to restore fish habitat, which was designed to deliver up to 100 SCFM at an 85% oxygen transfer efficiency (Correspondence with Mark Mobley of Mobley Engineering). Delivery to the upper hypolimnion, in general diffuser design, is optimized by high oxygen flow rates per foot of diffuser line. Higher oxygen flow results in a stronger upwelling plume and therefore transports oxygen higher up from the diffuser (Singleton and Little, 2006). Diffuser lines designed to deliver oxygen to the sediment have longer lines per SCFM of oxygen, which may explain why in 2009 a flow of 25 to 35 SCFM resulted higher DO above the sediment than in 2010 with flows reaching up to 45 SCFM for a majority of the season.

4.5.2 Response of Iron and Manganese to Oxygenation

Fe in North and South Twin Lakes were not significantly different from each other, although South Twin was slightly higher, during pretreatment conditions in August of 2008 (P = 0.62, n = 3; Table 4) with hypolimnetic VWA’s between 200 and 300 µg/L (Figures 5 and 6), and fall within the lower range of values typically reported from literature, 20-8,000 µg/L for
total Fe (Stauffer, 1987; Davison and Woof, 1984; Davison et al., 1982). Mn for the two lakes during pretreatment years was also not significantly different (P = 0.21 and n = 3; Table 4) but averaged lower VWA’s at 50 µg/L in North Twin Lake and higher VWA’s at 150 µg/L in South Twin Lake falling at or below the range reported in literature 100-3,000 µg/L for total Mn (Stauffer, 1987; Davison and Woof, 1984; Davison et al., 1982). The untreated South Twin Lake has a natural variability in Fe accumulation between years, with nearly an order of magnitude increase from 300 to 2,500 µg/L from October 2008 to October 2010. Conversely, Mn was fairly stable over the 4 year period ranging between 240 to 300 µg/L (Table 3). This variability in Fe appears to be independent of oxygen conditions, as hypoxia formed and persisted from early June until early November in all four years (Figure 3). North Twin Lake’s Fe and Mn accumulation ranged from 250 and 50 µg/L for Fe and Mn in October of 2008 to a max of 100 and 175 µg/L for Fe and Mn in October of 2010, however, this was most likely artificially low due to a two week oxygenation test that occurred in North Twin in early September.

In Chapter 3, an evaluation of post oxygenation in North Twin Lakes shows a recovery of Fe and Mn concentrations similar to pretreatment conditions; however, considering the trends in accumulation in South Twin Lakes throughout the three years (Figure 6), it is likely that accumulation would have been even higher in North Twin Lake if the oxygenation event had not occurred and Fe and Mn flux had continued unabated. A previous evaluation of Twin Lakes showed that the two lakes are fed by tributaries that are rich in Fe and poor in Mn (Juul and Hueftle, 1992), and therefore the lack in Mn variability in the lake may be due to minimal presence of the metal. Like South Twin Lake, North Twin Lake also accumulated the greatest amount of Fe and Mn at the end of the 2010 season, with 1100 and 300 µg/L for Fe and Mn, respectively.
Although North Twin Lake has lower VWAs and near sediment maximum concentrations of Fe and Mn relative to South Twin, North Twin Lake’s mid season Fe VWAs for the three treatment years are significantly greater than those of South Twin Lake ($P = 0.015$ to 0.03 and $n=4$; Table 4). Mn was less significantly different ($P = 0.03$ to 0.09 and $n=3$; Table 4) but remained slightly elevated in South Twin Lake relative to North Twin Lake. Fe and Mn accumulated in North Twin Lake from June to October in all 3 treatment years, many times with North Twin Lake’s Fe VWAs exceeding those of South Twin, most notably in 2010 (Figures 5 and 6). Although there were similar VWA’s between the treated lake and the untreated lake, North Twin Lake had elevated Fe and Mn in the upper hypolimnetic waters and lower bottom waters relative to South Twin Lake (Figures 2, 3, and 7). For example, at the end of July in 2010, Fe and Mn at 8 m were 1,000 and 100 µg/L in North Twin Lake and 100 and 50 µg/L in South Twin Lake. Conversely, at 1 m above the sediment on that same date, Fe and Mn were 1,000 and 150 in North Twin Lake and 3,000 and 300 µg/L in South Twin Lake (Figure 7). Even though South Twin Lake has markedly higher Fe concentrations above the sediments, higher concentration in the large volume of the upper hypolimnion in North Twin Lake brings its VWA for Fe above that of South Twin Lake’s, at 750 versus 700 µg/L. Essentially, similar and sometimes more Fe and Mn accumulated in the treated versus untreated hypolimnia.

Metals entering the hypolimnetic waters of North Twin Lake are homogenized and mixed evenly throughout the water column during the oxygen treatment, unlike South Twin which has a steep concentration gradient of Fe and Mn in its bottom 4 to 6 meters. Throughout the 3 treatment years in North Twin Lake, metals were dispersed up to the thermocline, following a similar pattern to DO distribution from the diffuser (Figure 2). An evaluation of the bubble plume showed that advection induced by rising bubbles will circulate the volume of the hypolimnion.
through the upwelling plume every five days, generating sizable current through the water column (Chapter 3). This mixing is transporting Fe and Mn into the upper hypolimnion where it normally does not reach in quiescent conditions, as seen with South Twin (Figure 3).

A synoptic evaluation in North Twin Lake one month into oxygenation in 2011 shows how thorough horizontal metals transport is in the upper hypolimnetic waters (Figure 8). A perpendicular transect was sampled 300 and 600 m from to the diffuser line during the synoptic event, showing near identical profiles to the deep water site, only decreasing slightly in the upper waters of the 600 m site (200 m from the shore). Considering that the hypolimnetic VWA of North Twin Lake is similar to that of the untreated South Twin Lake suggests that oxygenation is not repressing release of metals from North Twin’s sediments, but instead of accumulating to high concentrations above the sediment, Fe and Mn are being mixed throughout the hypolimnion.

Metals release from North Twin Lake’s sediments was linked to oxygenation decreases in the hypolimnion. There is a correlation between the increase in hypolimnetic VWAs of Fe and Mn release with decrease in the bottom 1 m of oxygen, with \( R^2 \) values of 0.5 and 0.6, both with a \( P < 0.001 \) (Figure 9). During the years of oxygenation, 2009 through 2011, VWA’s for the bottom 6 m averaged from June through September showed an inverse relationship between metals and DO (Figure 10). This data suggests that oxygenation has some effect on metals release from the sediment in North Twin Lake. However, it appears there are times when the system has not oxygenated the bottom sediments sufficiently to prevent efflux of metals into the overlying water. Any metals that do diffuse from the sediment in North Twin Lake get mixed throughout the hypolimnetic waters and are therefore dispersed more evenly from what is observed in South Twin Lake.
Persistence of anoxic bottom waters appears to be a rare phenomena in the technologies application and it is difficult to find similar studies with this situation to make comparisons. In a review of nine hypolimnetic oxygenation applications, all systems maintained oxygen levels above 3 to 4 mg/L in the hypolimnion (Beutel and Horne, 1999). Accumulation of Mn(II) in mildly oxygenated waters, like that observed in North Twin Lake, has been observed in many mildly oxic lakes (Hongve 1997; Davison 1993; Davison and Woof, 1984), and reinforces the observation that Mn(II) can accumulate and persist in aerobic lake waters. Mn cycling was investigated in the meromictic Lake Nordbytjernet in Southeastern Norway (Hongve, 1997). In this meromictic system, Mn persisted in mildly oxic waters above the oxycline. It was determined that electron transfer of Fe(II) to Mn(IV) was the mechanism responsible for enhanced Mn enrichment and Fe loss from the water column, and similar may aid in the persistence of Mn in the oxygenated portion of North Twin Lake.

Mn dynamics were evaluated at a drinking water reservoir, Carvins Cove, VA, being treated with hypolimnetic oxygenation as a drinking water pretreatment to prevent accumulation of metals in the source water (Bryant et al., 2011). Using a sediment lander, researchers were able to evaluate the Mn cycling above, at, and below the sediment surface. Hypolimnetic oxygenation was observed here to increase the Mn flux coefficient at the SWI. They determined that turbulence induced by the bubble plume eroded the diffusible boundary layer, thereby increasing the concentration driving force out of the sediment, a mechanism that may be responsible for the metals accumulation in North Twin Lake. The system at Carvins Cove, however, maintained high SWI DO concentrations, which the researchers hypothesized induced an active biogeochemical cycling zone above the sediments that that prevented migration of Mn into the bulk hypolimnion.
Here in the Twin Lakes study, anoxia was forming during oxygenation, and once doing so, Fe and Mn were able to quickly migrate into the water column. Gantzer et al. (2009a) also evaluated Fe and Mn in Carvins Cove during oxygenation. During an event where oxygen was turned off, soluble Mn quickly migrated from the sediments into the bulk hypolimnetic waters. After the oxygen had been re-initiated and the DO was re-established at the SWI, Mn that had migrated up into the waters persisted until turnover.

Mn(II), the soluble form of the metal, can be quickly oxidized by chemolithotrophic Mn-oxidizing microorganisms that are ubiquitous in aquatic sediments (Lovley et al., 2004). However, typical half lives of Mn(II) abiotic oxidation in natural waters are on the order of years (Diem and Stumm, 1984). The biologically rich benthic region that Bryant et al. (2011) referred may have been able to convert Mn(II) to Mn(IV) biotically, but once the soluble metal had the opportunity to migrate up to into the water column where abiotic chemistry is more likely to dominate, Mn(II) was able to persist in the water column until turnover.

In North Twin, the oxygen diffuser was designed to deliver oxygen to the upper hypolimnion, and there were times in the three years of observation that the water above the sediments were able to go anoxic. Considering the system was running and generating turbulence water column during periods where anoxic water overlaid sediments, it seems logical that metals would migrate up from the sediment into overlying waters and get mixed into the bulk hypolimnion via that bubble plume advection.

4.5.3 Response of Mercury to Oxygenation

MeHg in North Twin Lake was slightly, but not significantly ($P = 0.76$ and $n = 3$; Table 4), higher than South Twin Lake during pretreatment conditions in August of 2008 (Figures 2
and 3), with hypolimnetic VWAs of 0.06 and 0.05 ng/L respectively (Figures 5 and 6). Both lakes falling within the lower to middle range of MeHg values typically reported from literature of 0.003 to 0.8 ng/L (Watras, 2009). Similar to Fe and Mn, MeHg accumulation fluctuated considerably from year to year in the untreated South Twin Lake, with a low in October of 2011 at 0.08 ng/L to a high in October of 2009 of 0.226 ng/L (Table 3). However, unlike Fe and Mn which accumulated more each year from 2008 to 2011, MeHg accumulation decreased from 2008 to 2011 (Figure 6).

Serendipitous sniff tests confirmed sulfides presence throughout the hypoxic period from July to October in South Twin Lake, and periodic sulfide analysis showed bottom water sulfide concentrations of approximately 300 µg/L. No sulfide was detected at any time in North Twin during oxygenation. Sulfide can help or hurt Hg methylation. Studies have shown that Sulfide concentrations around 300 µg/L can induce methylation in the presence of SRB (Benoit et al., 1998). This lower sulfide concentration can interact with inorganic Hg and form small uncharged Hg-S complexes that gain access to the SRB cell, and subsequent induce methylation. High sulfide concentrations, > 300 µg/L, can form large and negatively charged complexes that have difficulty making their way into the cell, thereby inhibiting methylation (Benoit et al., 2003). Although variability in MeHg in South Twin Lake appears to be independent of oxygen conditions, it is possible that SRB activity was enhanced enough to generate methylation inhibiting amounts of sulfide.

North Twin Lake’s late season MeHg accumulation ranged from a low of 0.026 ng/L in October of 2009 to a max of 0.10 ng/L in October of 2008. Unlike with Fe and Mn, the end of season hypolimnetic VWAs for MeHg were all lower than the pretreatment year. The low pretreatment VWA may in and of itself been artificially low in October of 2008 due to a two
week oxygenation test that was conducted in September of 2008 (Chapter 2). During the three
treatment years, North Twin Lake accumulated the greatest amount of MeHg in 2010 based on
mid-season averages between June and September, similar to that observed with Fe and Mn
(Figure 10).

MeHg accumulation in the hypolimnion of North Twin Lake during the three years of
oxygenation were generally higher than the untreated South Twin Lake. The one exception is for
2009 when average hypolimnetic VWAs were lower in North Twin Lake (P = 0.024, n = 4;
Table 4) as compared to 2010 with much less significant difference in MeHg mass between the
two lakes (P = 0.12, n = 4; Table 4). End of season hypolimnetic VWA’s in North Twin Lake
were slightly lower than South Twin Lake in 2010 and 2011, approximately 15 to 40 % lower,
and nearly an order of magnitude lower in 2009 (Tables 2 and 3).

The trend in MeHg accumulation between North and South Twin Lakes correspond to the
reciprocal trend seen in mid season DO and MeHg averaged from June to September, where
higher MeHg accumulation in the water column correspond to lower DO above the sediment
(Figure 10). For the three treatment years, mid season DO and MeHg are essentially a mirror
image of each other in North Twin Lake. This is in contrast with results from South Twin Lake,
where no reciprocal relationship with Fe, Mn, or MeHg and DO over the four year period was
observed. Peak values above the sediment in North Twin Lake were down 75 to 85 % as
compared to peak values in South Twin Lake. Considering the large variation in metals between
years in South Twin Lake, making direct VWA comparisons between the lakes is speculative.
However, comparisons of trends between the metals and DO concentrations in North Twin Lake
may be the best indicator of treatment effects. The most MeHg accumulation in North Twin Lake
was observed in 2010, which also corresponds to the lowest DO observed in bottom waters in the
three treatment years (Figure 10). The least MeHg accumulation was observed in 2009, which also corresponded with the highest DO in bottom waters. Results for 2011 fell in between 2009 and 2010 for both MeHg accumulation and bottom water DO.

Outside Chapters 2 and 3 of this dissertation, there is not a great deal of studies evaluating the effect of hypolimnetic oxygenation on MeHg accumulation in the water column. There is however an interesting case study that evaluated the addition of nitrate as an oxidant to repress accumulation of MeHg in the hypolimnion. Onondaga Lake, NY, is a system that has undergone severe Hg contamination from the effluent of several chlor-alkali plants where 75,000 kg of Hg had been deposited from 1947 to 1988 (Todorova et al., 2009). The lake is culturally eutrophic and typically the hypolimnion goes anoxic after stratification in June, remaining that way until fall turnover in October. A unique characteristic of the system is that the local wastewater treatment plant, where year round wastewater nitrification has increased the nitrate load to the surface waters of Onondaga, has increased the systems nitrate budget 2 fold, starting in 2004. Spring mixing of Onondaga’s water column homogenizes oxygen and nitrate throughout the water column, and after onset of stratification, the hypolimnion is cut off from being recharged with more oxygen or nitrate until fall turnover. After stratification, the oxygen in the hypolimnion is quickly consumed by the combined water and sediment oxygen demand. Considering that nitrate is reduced at a redox potential slightly less than oxygen but greater than that of Fe or Mn, the consumption of nitrate then follows, keeping metals oxidized in the sediments.

In Onondaga, following consumption of oxygen and nitrate, MeHg accumulates in the hypolimnion. MeHg release in Onondaga was determined to be from both release of previously deposited MeHg bound on metal oxides that were able undergo reduction and formation of MeHg
from Hg(II) by SRB. After several years of increased nitrate load to the hypolimnia during summer stratification, the metabolic dominance of SRB became less and less each year, subsequently resulting in lessening MeHg accumulation in the hypolimnion each year. It is believed the observed decrease is a result of both a domination of surface sediment metabolism of nitrate reducing bacteria as well as an oxidative mineralization of organic matter in the surface sediments.

A similar study was conducted at Round Lake, MI, where an anoxic hypolimnion was treated with addition of a liquid calcium nitrate slurry pumped to the bottom sediments (Austin et al., 2011). The addition was conducted after establishment of oxic conditions where MeHg accumulation in the hypolimnion was already occurring. The plug of nitrate quickly elevated the redox potential of the bottom waters and a subsequent decrease in accumulated MeHg was observed, most likely due to sequestration from freshly oxidized metals as observed in Chapter 2 and the prevention of further MeHg releases. The theory of using oxidizing agents, either oxygen or nitrate, to repress Hg methylation and subsequent hypolimnetic MeHg accumulation is sound. However, if the SWI is allowed to form a pocket of anoxia over it, MeHg will have an opportunity to migrate into the water column. The example of Round Lake also illustrates the importance of maintaining oxidized condition up to fall turnover to prevent accumulation in the bottom waters that could potentially get mixed into the surface waters.

Although there is more of an observable reduction of MeHg accumulation in the bulk hypolimnion in North Twin Lake during treatment years compared to Fe and Mn, there is still an observed increase in MeHg in the upper hypolimnetic waters. A look at the vertical distribution of MeHg in North and South Twin Lake in Late July of 2010 shows nearly a 75% decrease in accumulation above the sediment in North Twin Lake (Figure 7). However, at the same time
North Twin Lake’s upper hypolimnion has nearly three times as much MeHg as South Twin Lake, resulting in a nearly identical hypolimnetic VWA. Isopleths of North Twin Lake (Figure 2) in the three treatment years shows MeHg accumulating the most in the upper hypolimnetic waters in 2010, corresponding to the year of highest oxygen input and lowest DO (Figure 4). Based on the bubble plume model described in Chapter 2, upward advection is increased when the intensity of the bubble plume increases (Singleton et al., 2007). As described above, increased upward advection of the bubble plume results in delivery of oxygen higher up in the water column. Further, the combination of low oxygen at the SWI, which fostered Fe, Mn, and MeHg release from the sediment, and increased mixing induced by the oxygenation system created an ideal transport mechanism for Fe, Mn, and MeHg to the upper hypolimnetic waters.

4.5.4 Co-Variation in Response of Dissolved Oxygen

Correlation of MeHg accumulation with accumulation of Fe and Mn in the hypolimnion of North Twin Lake grew stronger each year of oxygenation. Fe and Mn had similar and strong correlations with MeHg accumulation as they did with bottom DO, with $R^2$ values of 0.7 for both (P < 0.001 for both) over the three treatment years (Figure 11). However, on a year to year evaluation, the correlation grew stronger between the two metals and MeHg. $R^2$ values from the correlation of Fe and MeHg were 0.58, 0.80, and 0.93 for 2009, 2010, and 2011, respectively, and the values for Mn and MeHg were 0.49, 0.75, and 0.95 for 2009, 2010, and 2011 respectively. In the absence of sulfide, Hg species can have a strong affinity towards dissolved organic carbon (Benoit et al., 2003), which makes it a particularly important ligand in surface waters. Sulfide is one of the strongest ligands for inorganic Hg and MeHg, and can strip the trace metal from DOC or other oxy-hydroxide metal interactions (Watras, 2009).
sulfide, laboratory studies have shown that DOC binds much more readily than metal oxides, unless in a low organic environment (Miller and Mason, 2001). However, there are field studies that have observed the importance of an indirect controlling influence metals have on DOC transport and therefore an indirect effect on Hg transport.

Lake 658 in Ontario, Canada, had its waters and watershed amended with stable Hg isotopes to evaluate the fate and transport of Hg in the aquatic system (Chadwick et al., 2006). In their evaluation, filterable Fe and Mn strongly correlated with DOC, and Hg(II) strongly correlated with DOC, indicating the two parameters have significant influence with the inorganic trace element. MeHg, conversely, did not show a strong relationship with DOC, presumably due to the influence and presence of sulfide above the sediment.

It would follow that sulfide influence in bottom waters and surface sediments would decrease during oxygenation, and based on the studies described above, the Hg fate and transport would be then linked to that of organic carbon. Why then would we see a significantly growing correlation between Fe and Mn accumulation? In a study evaluating a several year oxygenation treatment of Spring Hollow and Carbon’s Cove, VA, a long-term reduction in SOD was determined to be the result of hypolimnetic oxygenation (Gantzer et al., 2009b). Based on the lack of other contributing factors it was concluded that reduced oxygen demand was result of elevated oxygen conditions at the SWI by enhancing more complete organic carbon decomposition.

Enhanced decomposition of organic carbon in North Twin Lake’s surface sediments may explain why there is a tightening between the cycles of MeHg and Fe/Mn. Interactions of Fe and Mn with MeHg have been observed in several studies. Mn oxide sequestration of MeHg in the water column following a fall mixing event has been observed in several reservoirs (Slotten et
al., 1995; Gill and Bruland, 1992). In four different Wisconsin seepage lakes, MeHg flux from the sediment was strongly correlated with the flux of Fe within their perspective hypolimnions (Hurley et al., 1994). It is no surprise that there is a link between MeHg and the cycle of Fe and Mn, however, this link may be strengthened in the presence of an oxygen treatment as organic carbon in the surface sediments are oxidatively mineralized.
5.7 REFERENCES


Davison, W., Woof, C., Rigg, E. 1982. The dynamics of iron and manganese in a seasonally anoxic lake; direct measurement of fluxes using sediment traps. Limnology and Oceanography. 27(6), 987-1003.


Lovley, D., Holmes, D., Nevin, K. 2004. Dissimilatory Fe(III) and Mn(IV) reduction. Advances in Microbial Physiology. 49, 219-286.


Figure 1. Bathymetric map of North (top of figure) and South (bottom of figure) Twin Lakes.
Table 1. Physical characteristics of North and South Twin Lakes.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>North Twin Lake</th>
<th>South Twin Lake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area, km$^2$</td>
<td>3.71</td>
<td>4.13</td>
</tr>
<tr>
<td>Depth, m (max, average)</td>
<td>15.2, 9.7</td>
<td>17.4, 10.4</td>
</tr>
<tr>
<td>Watershed, km$^2$</td>
<td>72.8</td>
<td>14.0</td>
</tr>
<tr>
<td>Shoreline Length, km</td>
<td>25.7</td>
<td>26.7</td>
</tr>
<tr>
<td>Elevation, m</td>
<td>784</td>
<td>784</td>
</tr>
<tr>
<td>Hydraulic Residence Time, yr</td>
<td>2.7</td>
<td>9.4</td>
</tr>
</tbody>
</table>
Figure 2. Isopleths of North Twin showing dissolved oxygen (mg/L), iron (µg/L), manganese (µg/L), and methylmercury (ng/L) concentrations vertically through the water column from June to October of 2008, 2009, 2010, and 2011.
Figure 3. Isopleths of South Twin showing dissolved oxygen (mg/L), iron (µg/L), manganese (µg/L), and methylmercury (ng/L) concentrations vertically through the water column from June to October of 2008, 2009, 2010, and 2011.
Figure 4. Volume Weighted Averages of dissolved oxygen in North Twin with corresponding average delivery of oxygen flow to the hypolimnetic oxygenation system, from May to October of 2009, 2010, and 2011.
Table 2. Volume weighted averages in the hypolimnion and maximum bottom meter concentrations for iron, manganese, and methylmercury in North Twin Lake in mid-October.

<table>
<thead>
<tr>
<th>North Twin</th>
<th>Fe* VWA (Max)</th>
<th>Mn* VWA (Max)</th>
<th>MeHg** VWA (Max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>243 (1520)</td>
<td>44 (260)</td>
<td>0.10 (0.43)</td>
</tr>
<tr>
<td>2009</td>
<td>330 (870)</td>
<td>63 (180)</td>
<td>0.026 (0.068)</td>
</tr>
<tr>
<td>2010</td>
<td>1100 (400)</td>
<td>175 (434)</td>
<td>0.076 (0.093)</td>
</tr>
<tr>
<td>2011</td>
<td>800 (1500)</td>
<td>102 (212)</td>
<td>0.051 (0.11)</td>
</tr>
</tbody>
</table>

*µg/L, **ng/L

Table 3. Volume weighted averages in the hypolimnion and maximum bottom meter concentrations for iron, manganese, and methylmercury in South Twin Lake in mid-October.

<table>
<thead>
<tr>
<th>South Twin</th>
<th>Fe* VWA (Max)</th>
<th>Mn* VWA (Max)</th>
<th>MeHg** VWA (Max)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>295 (3700)</td>
<td>239 (385)</td>
<td>0.084 (0.52)</td>
</tr>
<tr>
<td>2009</td>
<td>408 (2770)</td>
<td>207 (341)</td>
<td>0.226 (0.46)</td>
</tr>
<tr>
<td>2010</td>
<td>2570 (5450)</td>
<td>398 (384)</td>
<td>0.088 (0.59)</td>
</tr>
<tr>
<td>2011</td>
<td>1860 (7450)</td>
<td>577 (558)</td>
<td>0.082 (0.39)</td>
</tr>
</tbody>
</table>

*µg/L, **ng/L
Table 4. Probability values from t-tests, paired two sample for means, comparing North Twin Lake and South Twin Lake hypolimnetic volume weighted averages of iron, manganese, and methylmercury from July to September during 2008, 2009, 2010, and 2011.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>2008*</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>0.62</td>
<td>0.015</td>
<td>0.031</td>
<td>0.030</td>
</tr>
<tr>
<td>Mn</td>
<td>0.21*</td>
<td>0.092</td>
<td>0.036</td>
<td>0.028</td>
</tr>
<tr>
<td>MeHg</td>
<td>0.76</td>
<td>0.024</td>
<td>0.12</td>
<td>NA**</td>
</tr>
</tbody>
</table>

*n = 3, all others are n = 4.

**Too few dates to compute.
Figure 5. Volume weighted averages for the epilimnion, metalimnion, and hypolimnion of North Twin Lake in the summer stratification season from 2008 to 2011.
Figure 6. Volume weighted averages for the epilimnion, metalimnion, and hypolimnion of North Twin Lake in the summer stratification season from 2008 to 2011.
Figure 7. Fe, Mn, and MeHg profiles for North (top) and South (bottom) Twin showing distribution vertically through water column on July 28th, 2010.
Figure 8. Synoptic profiles of Fe and Mn in North Twin on June 22\textsuperscript{nd}, 2011. The Deep Site is the standard monitoring site for North Twin and the 300 m and 600 m profiles were sampled on a transect directly north of the Deep Site at their labeled distances.
Figure 9. Dissolved oxygen in bottom meter of North and South Twin from 2009 until 2011 plotted against hypolimnetic volume weighted iron and manganese.
Figure 10. Mean volume weighted averages of the bottom 6 meters in both North and South Twin from June through September of 2008, 2009, 2010, and 2011. Error bars represent the standard deviation of the fluctuations in the 4 month time frame.
Figure 11. Scatter plot showing correlation between MeHg and both Fe and Mn, with corresponding R² and P values for 2009 through 2011.
Figure 12. Year specific $R^2$ coefficients for methylmercury versus iron and manganese for 2009 through 2011.
CHAPTER 5. TARGETED OUTREACH TO ENHANCE DIVERSITY IN UNIVERSITY-SPONSORED TECHNOLOGY COMPETITIONS

5.1 ABSTRACT

This chapter describes my outreach experience as a graduate fellow who participated in Washington State University's efforts to promote interest in engineering and sustainability by underrepresented high school students. I mentored students from a rural high school as they developed projects related to sustainable energy for the university's 2008 Imagine Tomorrow competition. Outreach strategies focused on fostering student ownership, using formative assessment, and promoting student preparation. In contrast to other winning high schools, the study high school exhibited low academic achievement and high ethnic diversity. Yet, the high school won the grand prize. Inspired, the school successfully competed again in 2009 and 2010 with teams that included American Indian students, a target of future outreach efforts. The experience shows that outreach to appropriate schools, in combination with university-sponsored technology competitions, can promote underrepresented student participation in engineering.

5.2 INTRODUCTION

Like many institutions, Washington State University (WSU) recognizes the necessity to increase interest and participation in engineering by underrepresented groups including women and minorities. To partly address this need, the WSU College of Engineering and Architecture implemented Imagine Tomorrow. The program is a three-day competition that challenges high school students to research innovative technologies, designs, or changes in human behavior related to alternate energy sources that contribute to a more sustainable future. Teams are judged
by academic and professional volunteers according to a judging rubric that evaluates project objectives, creativity, inquiry, thoroughness, and presentation. The competition has been held annually since 2008 and attracts hundreds of students from over two dozen high schools throughout the state. The competition, which is financed by a number of individuals, foundations, utilities and private companies, awards over $100,000 in cash prizes to winning students and schools.

A second WSU effort aimed at increasing interest and minority participation in engineering education is the Culturally Relevant Engineering Applications in Mathematics (CREAM) program. The program was started in 2006 by the WSU Engineering Education Center with funding from the National Science Foundation’s (NSF) GK-12 program. The objectives of the three-year program were to improve the mathematical skills and to instill an appreciation of engineering among traditional and underrepresented high school students. CREAM fellows implemented culturally-relevant engineering applications in mathematics, in collaboration with teachers, at middle and high schools in eastern Washington. The fellows received a 12-month, $30,000 stipend and were trained in instructional methods with WSU faculty specializing in engineering education.

This Chapter briefly describes the outreach experience and successful outreach strategies of my work as a CREAM fellow who mentored high school students from a rural high school in eastern Washington participating in the 2008 Imagine Tomorrow competition. The outreach site was a public high school located on the border of an Indian reservation in rural eastern Washington. The high school served approximately 300 students spanning grades 9-12. The student population was roughly two-thirds American Indian and one-third white. About half of students were enrolled in a free or reduced-priced meals state program. Pass rates for the
Washington Assessment of Student Learning (WASL) standardized test in 2008 for reading and writing were in the 10-20\textsuperscript{th} percentile of Washington State high schools; math and science WASL pass rates were in the 20-25\textsuperscript{th} percentile.

**5.3 RATIONALE**

The need for greater participation in science, technology, engineering, and mathematics (STEM) education by underrepresented groups is well recognized. With the accelerating intensity at which modern civilization is degrading the environment, the need is especially great for engineers trained in environmental sustainability and stewardship (Tansel, 2008; Kennard, 1995). The enhanced participation of underrepresented groups is particularly vital to satisfy the future demand for engineers in the US (Duderstadt, 2008). However, enrollment in engineering degree programs in the US is at a 20-year low, and minority students comprise only 12\% of engineering undergraduates (Frehill et al., 2008). Recognizing these needs, the federal government has made enhancing diversity of participation in STEM fields a national priority through programs including "Educate to Innovate", a $260 million public-private investment program to enhance student achievement over the next decade, and the NSF's Graduate STEM Fellows in K-12 Education (GK-12) Program, a $10-15 million per year program that promotes interaction between graduate students and K-12 STEM teachers and students. One method for universities to promote awareness and interest in engineering is to host high school technical competitions like Imagine Tomorrow at WSU. However, we speculate that these programs attract higher achieving and/or lower diversity high schools. If this is the case, targeted outreach to underrepresented high schools is needed for these competitions to promote both awareness and diversity in engineering and STEM fields.
5.4 PRACTICE DESCRIPTION

During the 2007-2008 academic year, I mentored students that competed in Imagine Tomorrow. I performed interviews with students and staff and kept a project journal, which was the source of quotes in the subsequent discussion. Three Imagine Tomorrow teams initially developed with a total of 12 students. Two of the students were American Indian and six were women. Students worked on their projects during lunch breaks and after school under my guidance, where I spent two to three days per week at the school. Two teams ultimately competed. One team was made up of two male foreign exchange students from Asia who designed a self-sustaining greenhouse that sequestered carbon dioxide emitted from greenhouse plants while chemically producing heat to warm the greenhouse. This team won a special award sponsored by the WSU College of Agriculture for sustainable agriculture. A second team included three white students, two of whom were female. They designed a modular sustainable energy system that could be customized to remote settings based on available solar, wind, and hydropower sources, as well as an electrolyzer that made hydrogen gas from electricity and a fuel cell that made electricity from hydrogen gas. This team won the grand prize. The study high school successfully completed and won prizes in 2009 and 2010 with only minor external mentoring from WSU personnel.

5.5 RESULTS AND DISCUSSION

5.5.1 High School Academic and Ethnic Characteristics

To gain a better understanding of the relative academic strength and ethnic diversity of schools competing in the 2008 Imagine Tomorrow competition, we evaluated standardized test
scores and ethnicity data for non-participating, participating and winning high schools in the 2008 Imagine Tomorrow competition. The database used to analyze academic strength was the 2008 WASL standardized test scores for 10th-graders attending Washington State high schools (WSOSPI, 2010). The study high school showed very low performance on the WASL (Figure 1). Math and science WASL pass rates were 23.5% and 22.2%, respectively. The pass rates for the study high school ranked in the bottom 25th percentile of schools participating in the competition. The pass rate for high schools competing in Imagine Tomorrow (n = 33) was 51.4 ± 17.2% (average plus/minus standard deviation) for math and 41.8 ± 16.8% for science. Average pass rates for high schools winning first, second, or third place prizes in one of four competition categories were slightly higher, 53.4 ± 17.2% for math and 45.0 ± 19.7% for science (n = 9). The state's average WASL pass rate for non-competing high schools was 41.6 ± 20.7% (n = 352) for math and 33.4 ± 19.4% (n = 335) for science. WASL pass rates for competing and winning high schools were significantly different from non-competing high schools (two-sample t-test, p < 0.05).

The Washington State high school database (WSOSPI, 2010) was also used to evaluate ethnic diversity. The study high school's proportion of non-white (American Indian/Alaska Natives, Asians, African Americans, and Hispanics) students was 63.9%, which were comprised mainly of American Indians (Figure 1). This percentage was in the upper one-tenth of schools participating in the competition. Competing and winning high schools had a non-white student population of 27.9 ± 20.6% (n = 33) and 25.1 ± 17.4% (n = 9), respectively. Non-competing high schools had a non-white student population of 30.9 ± 23.9% (n = 352). While the average proportion of non-white students was slightly lower in competing and winning high schools compared to non-competing high schools, these differences were not statistically significant.
However, within the group of winning teams, the study high school was an extreme outliner with respect to student diversity. When it was removed from the group of winning high schools, the non-white proportion of the student population for winning high schools dropped to 20.2 ± 10.2% \((n = 8)\), which was significantly (two-sample t-test, \(p < 0.05\)) different from non-competing high schools.

Results show that the study high school was unique relative to other competing and non-competing high schools in regards to academic achievement and student diversity. In addition, the competition attracted teams from academically stronger high schools with relatively low ethnic diversity. These findings are significant because they suggest that low performing and low diversity high schools are less likely to participate in university-sponsored technology competitions without targeted outreach, such as the program presented in this study. This proposition is corroborated by observations by the fellow regarding initial resistance to participating in the competition by students and teachers at the study high school.

### 5.6 Outreach to Study High School

A key element of this successful outreach effort was promoting student ownership of their Imagine Tomorrow projects. Encouraging students to take responsibility for the learning process is an important mechanism to enhance student motivation and active engagement (O'Neill, 2010). In turn, active student engagement is more likely to promote feelings of empowerment in students that can lead to action. As Kentish (1995) points out, promoting student engagement through ownership is especially important in the field of environmental sustainability, where action is needed in the face of overwhelming and seemingly intractable problems related to overpopulation and environmental degradation.
A prerequisite for developing ownership at the study high school was overcoming the students' and teachers' initial resistance to participating in the Imagine Tomorrow competition. Students expressed concerns of embarrassment from certain failure, and teachers worried about time taken away from the WASL-driven course curriculum. Only after two months of sustained outreach, did students and teachers warm to the idea of competing in Imagine Tomorrow under his mentorship. In addition, the substantial prize money offered by the competition helped to promote student interest. Students talked about buying cars and electric guitars with their potential winnings, while teachers mused over funding more elaborate projects for the following year's competition. Even the community seemed to respect the financial component of the competition. Following the high school's 2009 Imagine Tomorrow win, an article from a local newspaper opened with, "They were broke when they went, but came back with $10,000. Now that's a success story."

As students began to express greater interest in the competition, I attempted to promote student ownership of the sustainability projects. The mentoring approach was modeled partly on the practice known as formative assessment, which can play a significant role in increasing student ownership (Brookhart et al., 2009). Assessment was an iterative process consisting of three interrelated components, which were implemented weekly: (1) clearly articulating the learning goal to students, which was to successfully design and build an alternative energy generator that was both innovative and functional; (2) providing feedback and promoting self-evaluation by students regarding their efforts toward achieving the learning goal; and (3) providing advice and support to students on how to move closer to the learning goal. In addition, the format of the outreach effort included additional factors known to encourage student ownership. I did not act in the capacity of a regular teacher, or central authority figure.
Therefore, my presence and mentoring support likely provided the students with a non-intimidating environment in which to cultivate greater personal ownership of the project (O'Neill, 2010). Also, the action-based, participatory nature of developing a project for the sustainability competition likely encouraged students to take greater responsibility for their own learning (Kentish, 1995).

One illustrative example of personal ownership was a student who initiated a fruitful correspondence with a senior researcher regarding a novel wind energy technology. She initially contacted the researcher via e-mail asking for guidance on how to build a wind belt, but got little more than a "good luck." The student persisted. In a follow-up e-mail, she sent the researcher photos of her prototype and asked specific questions. As the student noted, "The second e-mail showed that he was more impressed. After seeing that we were serious and working hard on this, he was more willing to give us tips and suggestions as to how to get the wind belt working better and more effectively." This student now has a well-paying job at a regional technology firm and plans to attend college in the future.

Recognizing that the students were insecure about their competence to participate in such a high-profile academic competition, outreach strategies also stressed preparedness and practice. At least twice a week for two months prior to the competition, a high school teacher and I asked students casual questions about their projects based on the competition's judging rubric, which had been made available on the Imagine Tomorrow web site. After a while, students also started quizzing each other based on the judging criteria. In this way, the students gained confidence in presenting their technology and in articulating their views in a non-threatening environment. One week before the competition, Dr. Marc Beutel played the role of a mock judge. The two teams formally presented their projects and the professor asked questions informed by the judging
rubric. At the end of the practice session, the teachers and participating students assessed and critiqued each team's performance. After the formal practice session with the professor, students were visibly shaken and lacked self-confidence. One student noted, “I don’t think he liked our project at all.” The teams used this experience to redouble their preparation efforts in the short time remaining before the competition.

Outreach efforts reached fruition when one of the study high school's teams won the grand prize at the 2008 Imagine Tomorrow competition. At the competition, the students’ enthusiasm and ownership of their project, their homemade energy devices, and their fluid answers during questioning impressed the judges. As a result of their experience, students expressed a heightened interest in science and engineering. One student commented, "I didn’t realize I liked engineering topics before. It wasn’t something I thought about." Another student said, "This experience gave me an actual reason to go to college, I didn’t realize I was interested in science." Students also became less intimidated by the university setting commenting, "The competition and the interacting with the judges made professors look like real live people," and, "The trip made college look real, not just something you hear about in some far off place." The study high school teachers were also transformed by the experience. One teacher noted, “I was apprehensive at first, but you have me convinced, this is a working model.” Another teacher stated, “This is the first time in about eight years that there has been a positive story in the local newspaper about our school.”

5.7 CONCLUSION

The outreach effort at a rural high school with low academic performance documented in this paper emphasized participatory learning using the concepts of student ownership and
formative assessment. In this particular case, the underrepresented high school's team was extremely successful in the 2008 Imagine Tomorrow academic competition, winning the grand prize. An analysis of the relative academic and ethnic characteristics of high schools competing in Imagine Tomorrow, and reflections from participating high school students and staff at the study school, suggest that the study high school would not have participated in the competition without the targeted outreach effort described in this paper. Thus, targeted outreach to such high schools is critical to leveraging the full potential of university-sponsored technical competitions aimed at promoting engineering awareness. For the students, teachers, and community of the study high school, the feeling of success associated with winning the competition resulted in a more positive view of academic achievement in science and engineering. It is understood that success alone cannot be the sole motivator for future teams, as there are no guarantees when it comes to competitions. The richness of the experience in building their energy generating devises and interacting with college professors and other professionals at the competition, built a considerable level of enthusiasm within the students that was evident long before they were informed of their impressive victory.

Finally, even though the outreach effort occurred at a high school with a large American Indian population, no American Indians participated in the 2008 Imagine Tomorrow competition. However, participation of American Indian students from the study high school subsequently occurred in the 2009 and 2010 competitions. The 2009 competition in particular incorporated cultural themes related to fisheries and tribal identity into the design of a method to manufacture biodiesel using waste fish from a tribal fish farm. Building off these efforts, the authors, a prominent engineering company, and the Indian Tribe are taking preliminary steps to promote American Indian student involvement in future Imagine Tomorrow competitions.
Stakeholders envision a WSU Graduate Fellowship, similar to that described in this paper, in which an engineering graduate student mentors a team of American Indian students through the Imagine Tomorrow competition. This effort will be partly modeled on the community-based approach presented by Poupart et al. (2009) with an emphasis on building and maintaining fruitful relationships between the Tribe, the university, and the engineering company (Blinn et al., 2006).
5.8 REFERENCES


Figure 1. 2008 Washington Assessment of Student Learning (WASL) pass rates and non-white student population (in percent) in Washington high schools. From left to right: (1) the study high school; (2) high schools competing in the 2008 Imagine Tomorrow competition; (3) high schools winning the competition; and (4) high schools not competing in competition. Pass rates for competing and winning high schools were significantly different than values for non-competing high schools (two-sample t-test; $p < 0.05$).
CHAPTER 6. CONCLUSION

This dissertation summarized three studies that evaluate the impact of hypolimnetic oxygenation on cycling of iron (Fe), manganese (Mn), and mercury (Hg) in freshwater lakes. Hg cycling in aquatic systems is a very complex topic, and it is difficult to predict accurately the response of Hg cycling to various treatments. Many lakes with very low levels of Hg in their sediments and waters still have Hg levels in fish tissue that are higher than the consumption guideline. That is why it is important to evaluate Hg cycling and its response to treatments from many angles. Chapters 2, 3, and 4 of this dissertation present three different approaches to evaluating Hg cycling in lakes.

In Chapter 2, sediment-water interface (SWI) chambers were used to examine Fe, Mn, and Hg cycling at the SWI of freshwater lake sediments. Sediments collected from Deer Lake, WA, were incubated for 27 days, one set bubbled with air (oxic chamber) and one set bubbled with nitrogen (anoxic chamber). After incubation analytical results showed that oxic chambers accumulated more, but not significantly more, methylmercury (MeHg) than the anoxic chamber, a result counter to our hypothesis that the anoxic chamber would accumulate more MeHg. Potential mechanisms for this phenomena include: (1) the presence of an oxic-anoxic interface oxic-anoxic interface (OAI) below the surface sediments that is an optimal methylation zone in the oxic chamber, and the lack of an OAI within the anoxic chamber; and (2) turbulence generated from bubbling gas in the chamber can increase flux of dissolved metals from sediments.

In Chapter 3, in-situ hypolimnetic oxygenation of an anoxic water column was monitored to determine the response of accumulated Fe, Mn, and Hg. A hypolimnetic oxygenation line diffuser was tested in North Twin Lake, WA, late in the season, well after the formation of
hypolimnetic anoxia. Fe and Mn were quickly removed from the water column within 8 hours of the system initiation, dropping approximately 70% of what had previously accumulated in the water column. Considering that the abiotic oxidation kinetics of Fe are much greater than Mn, hours versus years, the loss was most likely due to Fe(II) oxidation and subsequent sequestration of soluble Mn(II) by Fe-oxides. By the end of the two week oxidation test, MeHg values were reduced in the bottom waters by 95%. Based on documented observations of MeHg and Hg(II) sequestration by metal oxides, it was concluded that the most likely removal mechanism of MeHg was sequestration via Fe-oxides. Once the oxygen system was turned off, Fe, Mn, and MeHg quickly, within one month, returned to pre-treatment concentrations above the sediment.

Finally, Chapter 4 presented a four year evaluation of a full-scale summer season hypolimnetic oxygenations effect on the cycle of Fe, Mn, and Hg relative to a nearby untreated lake. The evaluation looked at one pre-treatment year and three treatment years. The oxygenation system was designed to deliver oxygen to the upper hypolimnetic waters in order to restore fish habitat. As a result there were times during operation when pockets of anoxia formed above the sediment. During these times of low oxygen above the SWI, increases in Fe, Mn, and MeHg were observed. However, unlike the untreated reference lake that accumulated high concentrations above the sediment, North Twin Lake had a very homogenous profile within the hypolimnion of all three metals. It appears that the mixing induced by the bubble plume was able to transfer Fe, Mn, and MeHg higher up the water column than they are able to passively diffuse up in the quiescent reference lake. Although we did observe some negative feedback of having anoxic sediments while running an oxygen diffuser, there were events when higher DO was maintained in the bottom waters that resulted in reductions in metals accumulation. MeHg cycling also became more tightly correlated to Fe and Mn cycling each of the three years, with
R² values approaching 1.0 in year three. An increase in correlation between MeHg and the redox cycling of Fe and Mn suggest that lake treatments that target poising the redox at the SWI could become more effective at controlling Hg over time. These observations demonstrated that if designed and operated to keep DO concentrations elevated above the sediments, oxygenation has the potential to be an effective control on sediment release of Fe, Mn, and MeHg.

These three studies show promise for the potential of hypolimnetic oxygenation as a treatment for abating Hg issues in freshwater lakes. One key theme that repeats itself in this work is the importance of controlling the position of the OAI. In order to keep the methylation zone out of the water column and down beneath the SWI, high DO must be maintained above the sediments. During the full-scale hypolimnetic oxygenation, we saw the lowest MeHg accumulation in 2009, the year that DO levels in the bottom waters were highest. Another important theme with controlling Hg cycling with lake oxygenation is to make sure the treatment is continued until the turnover event. As demonstrated in Chapter 3, once the treatment system was deactivated Fe, Mn, and MeHg accumulated to pretreatment levels within one month, and when turnover occurs, those metals would have been mixed up into the surface waters. The studies presented in this dissertation show that oxygen addition to the hypolimnetic waters of freshwater lakes can prevent the accumulation of MeHg when DO in increased above the sediment surface, if designed to optimize DO delivery to the SWI. Continued monitoring of ongoing and new oxygenation systems is vital for optimizing and designing this treatment method to specifically target MeHg accumulation.