Effects of Lake Oxygenation on Mercury Accumulation in Zooplankton in Twin Lakes, Washington

By

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To the Faculty of Washington State University:

The members of the Committee appointed to examine the thesis of BRANDON REED find it satisfactory and recommend that it be accepted.

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Effects of Lake Oxygenation on Mercury Accumulation in Zooplankton in Twin Lakes, Washington

ABSTRACT

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Mercury is an important global pollutant due to its mobility in the environment, high toxicity, and ability to bioaccumulate in aquatic food webs. This study examined total mercury and methylmercury in water and in zooplankton at oxygenated North Twin Lake and non-oxygenated South Twin Lake in 2009 and 2010. Hypolimnetic oxygenation is a lake treatment strategy that was hypothesized to limit the amount of methylmercury in lake water and subsequent uptake into zooplankton.

Water and zooplankton were collected using trace metal sampling and handling techniques, and are analyzed for THg and MeHg with ultrasensitive DMA-80 and the MERX-M autoanalyzers. In both 2009 and 2010, the hypolimnion of South Twin Lake was anoxic, while the oxygenation of North Twin Lake maintained dissolved oxygen levels generally above 5 mg/L. In 2009 bottom waters of South Twin Lake accumulated MeHg up to 0.5 ng/L and MeHg concentration was negatively correlated with dissolved oxygen concentration (ANOVA, $p = 0.023$). Hypolimnetic MeHg concentrations in North Twin were below 0.05 ng/L, likely as a result of oxygenation. In contrast to water column results, THg and MeHg in zooplankton from North Twin were consistently higher than in South Twin. Average zooplankton MeHg concentrations in North Twin were 63.8 and 127.9 µg/kg in 2009 and 2010, while in South Twin

they were 46.0 and 40.3 µg/kg. Potential explanations behind these perplexing results included: difference in pH and dissolved organic carbon, key water quality parameters known to control mercury accumulation in zooplankton; biodilution in the form of both zooplankton density dilution and bloom dilution; and enhancement of mercury uptake by zooplankton due to the presence of metal oxides in the water column. The first two explanations were discounted based on a review of lake water quality. The third explanation is thought to be the most plausible and suggests that the presence of metal oxides is more important than the level of MeHg in the water column in controlling mercury uptake into zooplankton. In addition, results suggest that to limit mercury uptake into zooplankton, hypolimnetic oxygenation systems must inhibit the release of metal oxides by maintaining a well-oxygenated sediment-water interface.

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1. INTRODUCTION

Mercury pollution is a global health concern (Megler et al., 2007). Once emitted into the atmosphere it can be transported anywhere on earth and deposited into remote and otherwise pristine lakes. While there are natural releases of mercury from volcanic eruptions and weathering of rocks containing mercury, the development and popularity of coal fired power plants has led to the anthropogenic release of mercury into the atmosphere (Morel et al., 1998).

Almost all mercury in the atmosphere is in the elemental form Hg(0). When elemental mercury dissolves into water droplets or sorbs onto dust particles, it can be oxidized then deposited, primarily through wet deposition, in its inorganic ionic form Hg(II) (Fig. 1.1). The deposited ionic mercury can be transformed to toxic methylmercury in the form CH_3Hg^+ by anaerobic bacteria, most notably sulfate-reducing bacteria, in anoxic aquatic environments (Benoit et al., 2003). Once produced, methylmercury is taken up by algae and other microscopic biota, and then biomagnified up the aquatic food web with concentrations increasing in successive trophic levels (water → algae → zooplankton → planktivores → piscivores) (Watras et al., 1998).

Mercury concentrations in fish are prominently in the form of methylmercury and typically account for over 95% of the total mercury (Morel et al., 1998). Biomagnification can lead to high levels of mercury in fish, even in pristine ecosystems. In humans and wildlife that eat contaminated fish, methylmercury is a potent neurotoxin that impairs reproduction and fetal development (Megler et al., 2007). Currently, half of US states have statewide fish consumption advisories with regards to mercury, and every state has at least one lake under advisory (USGS,

2009). Washington has a statewide fish consumption advisory in place for all lakes and rivers (USEPA, 2004).

Methylmercury cycling in lakes is tied to dissolved oxygen levels in bottom waters. In a recent review, Watras (2009) noted that anoxic hypolimnia can accumulate both ionic mercury and methylmercury to levels much higher than those in surface waters, but accumulation is not typically observed in oxic hypolimnia. The onset of anaerobic conditions enhances a range of complex and interrelated processes that enhance methylmercury cycling (Watras, 2009). The presence of sulfide in bottom waters can strip ionic mercury and methylmercury from settling particulate matter, as well as surficial sediments, resulting in the buildup soluble mercury-sulfide complexes in bottom waters, rather than their burial in the sediments. The reduction and dissolution of iron and manganese oxides in sediments can also lead to the associated release of bound dissolved organic carbon, to which ionic mercury and methylmercury are commonly attached. Anaerobic conditions also support the activity of anaerobes like sulfate-reducing bacteria that convert ionic mercury into methylmercury.

Zooplankton are key conduits of mercury into aquatic food webs. The use of zooplankton as indicators of eco-toxicity is widespread because of their relatively short lifespan and their position in the food chain (APHA, 1998). Zooplankton, which are typically filter feeders, take up ionic and methylmercury both from the water column and through the consumption of contaminated pray and particles (Watras et al., 1998). In contrast, consumption of contaminated prey such as zooplankton is the primary pathway for mercury uptake in fish (Hall et al., 1997). Large bodied zooplankton such as daphnia can make up a large fraction of diet of pelagic fish such as trout (Christensen and Moore, 2008). A handful of studies have linked hypolimnetic accumulation of MeHg with uptake into the aquatic food chain (Herrin et al., 1998; Slotton et al.,

1995). For example, Slotton et al. (1995) found that mercury levels in zooplankton in a small northern California reservoir impacted by historical mercury mining showed a seasonal increase in fall. They concluded that autumn mixing of anaerobic methylmercury-rich bottom waters into the lake enhanced mercury uptake into the aquatic food web. While aquatic food webs are very complex, the conventional wisdom regarding mercury uptake into these food webs is captured in the following quote by Morel et al. (1998): “the key factor determining the concentration of mercury in the biota is the methylmercury concentration in the water."

Twin Lakes, the focus of this study, are located in northeastern Washington on the Colville Indian Reservation. The lakes provide a vital fishery, as well as a tourist attraction that provides an important source of income to the tribe. It is important to note that tribal populations tend to eat fish and wildlife from their reservations, resulting in high risk of human exposure to mercury. Levels of mercury in American Indian populations can be six times higher than the general population, and diet (e.g., contaminated fish) is typically a key exposure pathway (Xue et al., 2010). Trout in Twin Lakes have experienced high levels of disease and limited growth due to poor summertime habitat. During the summer, the oxygen rich surface waters are too warm for cold water fish, and the deep cold waters do not contain enough dissolved oxygen for trout. To expand and improve habitat for trout, an oxygenation system was installed in North Twin Lake and operated in 2009 and 2010. Twin Lakes offer a unique scientific opportunity to compare the effects of oxygenation on fish habitat, health and water quality. Since only North Twin was oxygenated in 2009 and 2010, its sister lake South Twin was monitored as an anaerobic “reference” lake.

The objective of this research was to evaluate the effects of lake oxygenation on the uptake of mercury into the aquatic food web. This was done by quantifying total and

methylmercury in zooplankton in Twin Lakes during the first two years of oxygenation, 2009 and 2010. The original working hypothesis was that hypolimnetic oxygenation would result in lower mercury concentrations in zooplankton in North Twin Lake relative to South Twin Lake. This is based on the assumption that cycling of methylmercury is a function of the aerobic/anaerobic states of the sediment water interface. High oxygen should inhibit release of methylmercury which should result in less mercury in zooplankton. However, we found that methylmercury dropped dramatically, but mercury in zooplankton was higher in oxygenated North Twin compared to the anaerobic reference lake, South Twin. This thesis includes a discussion of two possible mechanisms for this observation, biodilution and metals-enhanced uptake. Our results point to the complexity of mercury cycling in lake ecosystems and the need for continued study to better elucidate these factors.

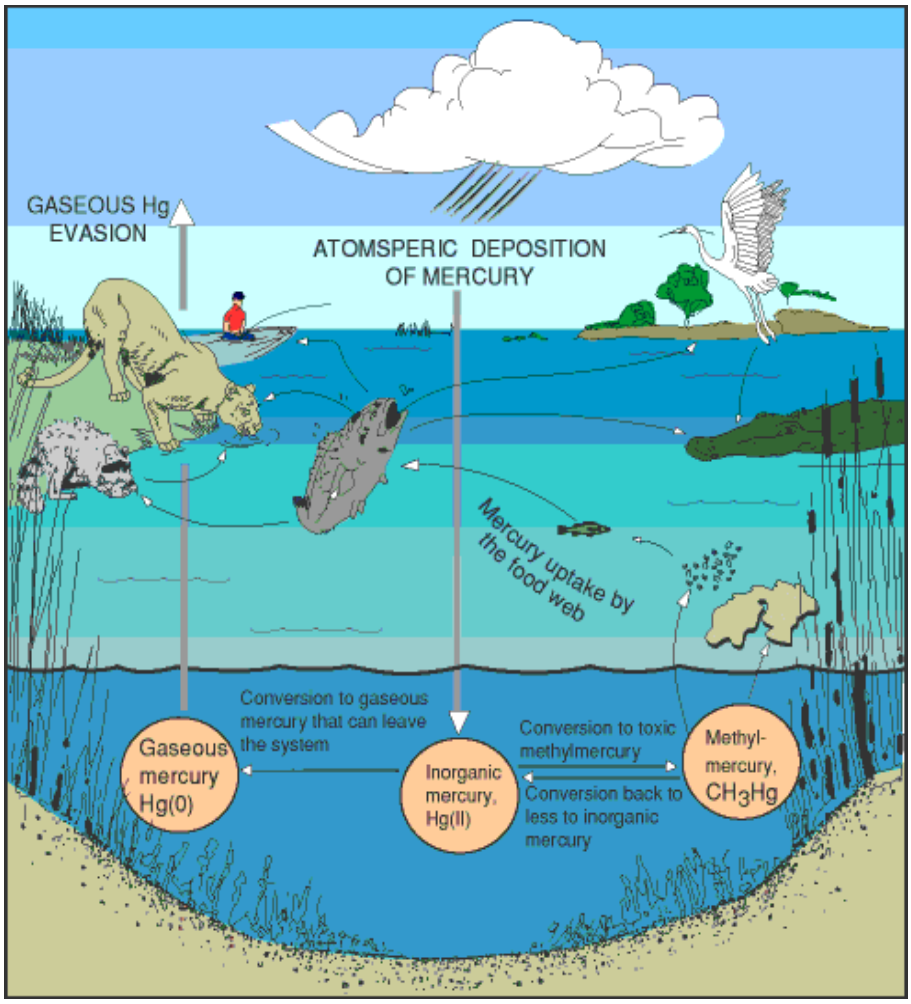

Figure 1.1. A simplified schematic of the mercury cycle in aquatic systems. Figure from the USGS.

2. STUDY SITE

North and South Twin Lakes are located on the Colville Indian Reservation near Inchelium, Washington (Fig. 2.1). The Confederated Tribes of the Colville Indian Reservation include nearly 10,000 descendants from 12 aboriginal tribes in northeastern Washington State (Confederated Tribes of the Colville Reservation, 2000). The Reservation was established in 1872, and today encompasses over 1.4 million acres (2,100 square miles), rich in timber, wildlife, fish, and water resources. The tribe has an intense cultural connection to their water and land, and wild game and fish are important sources of food for many tribal members.

The lakes are especially prized for recreational and fishing opportunities. These lakes are the only reservation waters open to non-tribal members, and are economically important to the region. The dimictic, mesotrophic lakes are similar in size with a mean depth around 10 m, a maximum depth around 15 m, and a surface area around 400 ha (Fig. 2.2). As with many lakes throughout the country, the Twin Lakes fish communities have been dramatically altered due to human activity. The lakes once supported populations of native inland redband trout (*Onchorhynchus mykiss gairdneri)* (Fig. 2.3). The fish community is now dominated by non-native species including Eastern brook trout (*Salvilinus fontinalis*), largemouth bass (*Micropterus salmoides*), and the non-game golden shiners (*Notemigonus crysoleucas*). The lakes have remained a popular fishing destination for both trout and bass, but public perception is that the trout fishery has been declining in recent decades.

Extensive field studies by Christensen and Moore (2007, 2008, 2009, and 2010) showed that the classic temperature-dissolved oxygen “squeeze” was dramatically and negatively impacting trout health. In both lakes, stable thermal stratification typically begins by late April or

early May and persists through the summer into September or October. With thermal stratification and increasing water temperatures, hypolimnetic oxygen demand results in rapid development of anoxia at the sediment-water interface. Anoxia continues to ascend in the water column, so that by late July, the entire hypolimnion is devoid of oxygen. These conditions lead to greatly reduced summer habitat availability. The summer epilimnion is too warm, and cold-water refuge afforded by the hypolimnion becomes inaccessible due to lack of oxygen, forcing trout to live within the metalimnion. Stocked rainbow and brook trout health declines throughout the summer and rainbow trout caught in Twin Lakes often display infestations of parasitic copepods, likely a result of stress and overcrowding. Estimates suggest that total mortality of stocked trout in recent years exceeds 90%.

Hypolimnetic oxygenation is the use of engineered systems to enhance dissolved oxygen levels in bottom waters of lakes and reservoirs using pure oxygen gas without disrupting stratification (Beutel and Horne, 1999). The technology has been installed in a number of lakes and reservoirs to improve water quality and enhance fish habitat. In the fall of 2008, a hypolimnetic oxygenation system was installed in North Twin Lake to improve summertime trout habitat. The system in North Twin Lake was operated during the summer and fall of 2009 and 2010 during which this study was conducted. The oxygenation system consisted of a 6,000 gallon temporary on-shore liquid oxygen storage tank and evaporator (Fig. 2.4) connected to 2,500 feet of submerged line diffuser located at the bottom of the lake. The ultimate system capacity, which accounted for diffuser induced oxygen demand, recovery from unanticipated shutdown, diffuse oxygen transfer efficiency, and an additional safety factor, was 80 standard cubic feet per minute or 4.3 metric tons per day.

The line diffuser is a simple design that spreads fine bubbles over a large area (Mobley and Brock, 1996). The system uses long lines of flexible porous hose connected to a high density polyethylene supply pipe that distributes the gas along the length of the diffuser (Fig. 2.5). Flow control orifices regulate the oxygen flow to independent lengths of porous hose to maintain a continuous flow of fine pure oxygen bubbles along the full length of the diffuser. A second buoyancy pipe can support the entire weight of the diffuser system when filled with air, thereby bringing the entire system to the surface for maintenance as needed. Concrete anchors are attached to the diffuser piping with stainless steel cable and saddle connections. The line diffuser has proven to be an economical diffuser design that transfers oxygen efficiently, minimizes temperature destratification, and minimizes sediment disruption.

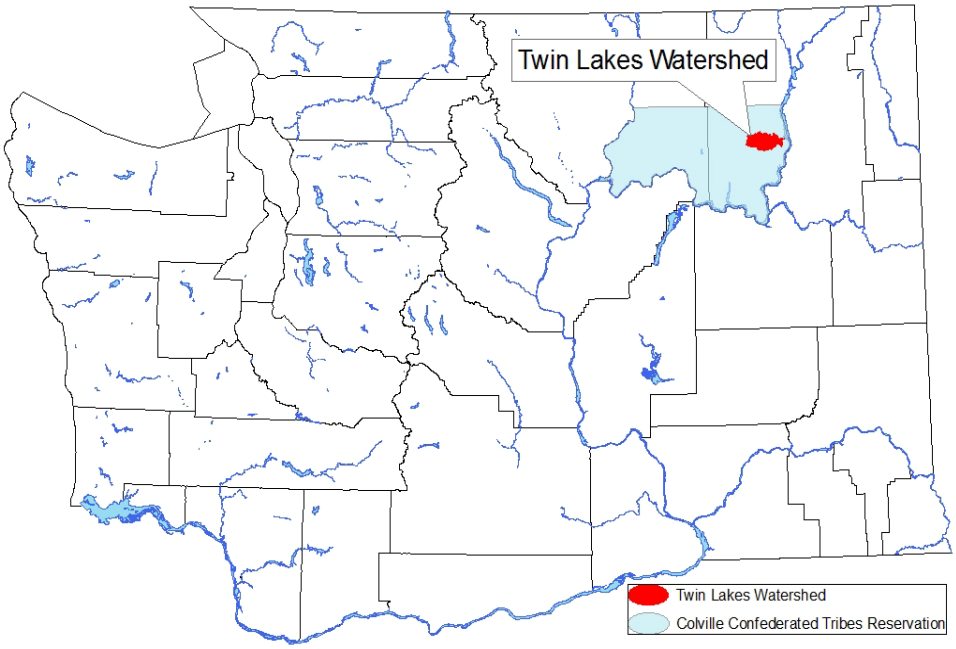

Figure 2.1. Location of the Colville Confederated Tribes Reservation and the Twin Lakes Watershed. Figure complements of Drs. Barry Moore and Dave Christensen, Washington State University.

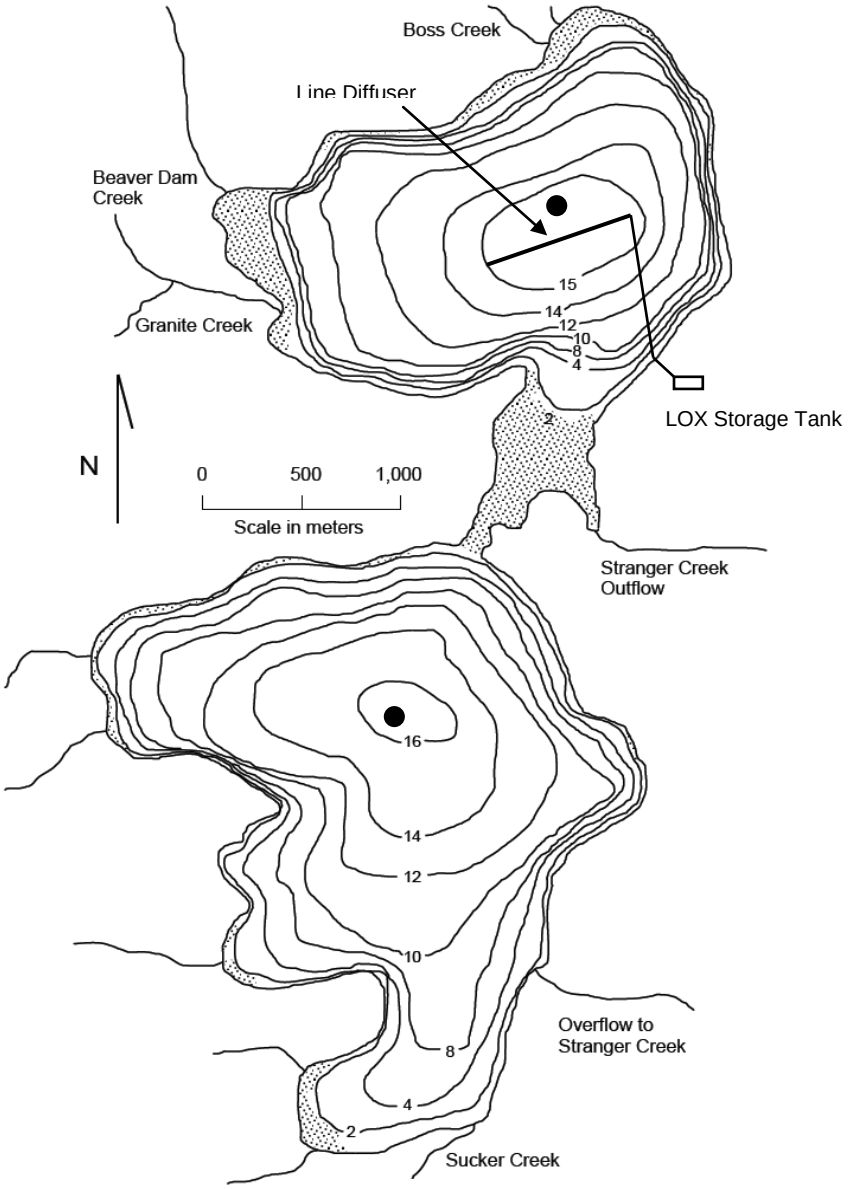

Figure 2.2. Bathymetric map of Twin Lakes showing the location of the oxygen line diffuser and liquid oxygen (LOX) storage tank in North Twin Lake. Sampling sites are shown with a dark circle. Depth contours are in meters. Modified from figure provided by Drs. Barry Moore and Dave Christensen, Washington State University.

Figure 2.3. The native inland redband trout (*Onchorhynchus mykiss gairdneri)* from Twin Lakes, Washington. Photo compliments of the Colville Indian Tribe's Department of Fish and Wildlife.

Figure 2.4. On-shore liquid oxygen storage tank and evaporator at North Twin Lake.

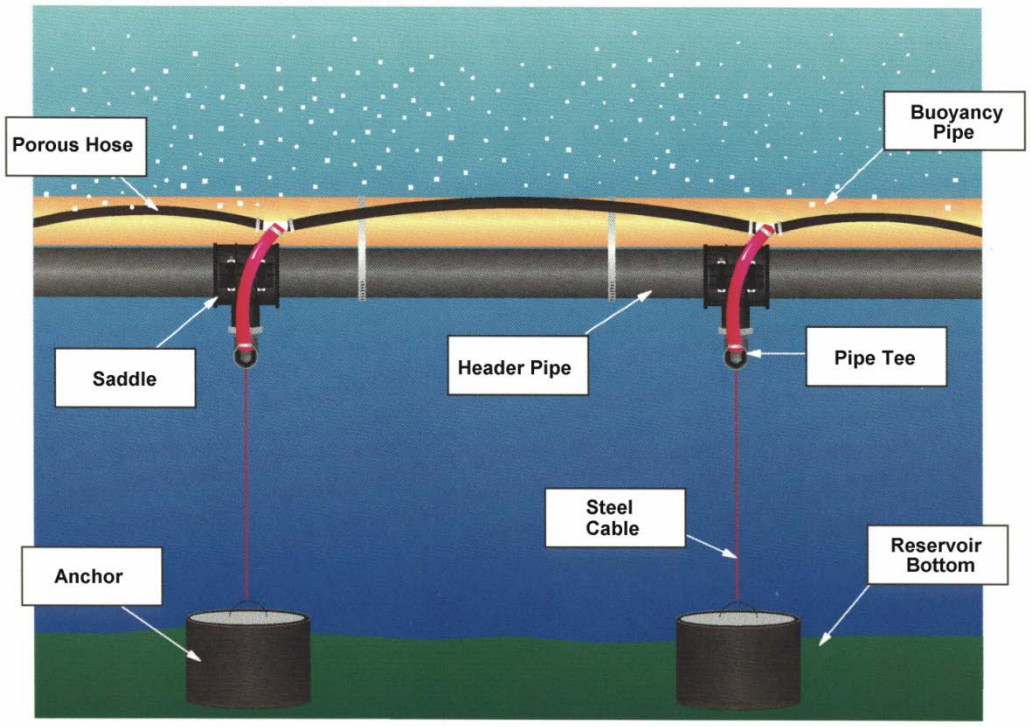

Figure 2.5. Schematic of submerged line diffuser in North Twin Lake. Complements of Mobley Engineering, Inc., Norris, Tennessee.

3. METHODS

3.1 Zooplankton Sample Collection and Mercury Analysis

Zooplankton were collected every three to four weeks from May through October in 2009 and 2010 at stations located in the deepest area of each lake (Fig. 2.2). Samples consisted of multiple full vertical tows with a 0.29-m-diameter non-metallic zooplankton net with a 73-micron mesh connected to a 250-µm-mesh dolphin bucket. The zooplankton were concentrated into acid washed glassware and stored in the dark in a chilled in a cooler. Approximately 4 to 6 tows per lake were performed during monitoring in 2009, however there was insufficient material for complete mercury analysis on some sampling dates. In the 2010 sampling season, 15 tows per lake were performed to ensure sufficient biomass for mercury analysis. Zooplankton were filtered and rinsed into size fractions (>1000 µm, >500 µm, >250 µm) in the laboratory using acid rinsed non-metallic simple sink sieves. Zooplankton greater than 1,000 µm were considered large-bodied zooplankton and were the primary focus of this study since they are a primary food source for planktivorous trout in Twin Lakes (Christensen and Moore, 2008, 2009). After separation, zooplankton were freeze dried and stored in a freezer for later mercury analysis.

Zooplankton total mercury (THg) samples were analyzed using a Direct Mercury Analyzer 80 (DMA-80), following EPA method 7473 (Mercury in solids and solutions by thermal decomposition, amalgamation and atomic absorption spectrophotometer; USEPA, 2007). The DMA-80 was calibrated using water-phase calibration standards ranging from 0.005 ng to 30 ng. Water-phase calibration standards were created by dilution of a mercury standard reference sample from the National Institute of Standards and Technology. Samples were run in triplicate, and additional samples were run if relative standard deviation was greater than 20%.

Values are presented as micrograms of mercury per kilogram dry weight of zooplankton (µg/kg). Certified reference material MESS-3, a low mercury marine sediment from the National Research Council of Canada (Ottawa, Ontario), was analyzed in tandem with each batch of samples to ensure adequate recovery. THg recovery for the MESS-3 averaged 86.7% (n = 65). Method blanks were also consistently run and blank THg levels averaged 0.06 ng (n = 87), which was around 5% of the mercury mass detected in a typical zooplankton sample. The detection limit for the DMA-80 is 0.002 ng.

For methylmercury (MeHg) analysis, zooplankton were first crushed into a powder using a mortar and pestle, and then digested in five mL of 5 M nitric acid at 65 °C for 8 hours (USGS, 2010). The digestate was analyzed with a Brooks Rand "MERX" Automated Methylmercury Analyzer following EPA method 1630 (Analysis of methylmercury in biological samples by cold vapor atomic fluorescence detection; USEPA, 2001). Values are presented as micrograms of MeHg per kilogram dry weight of zooplankton (µg/kg). The detection limit for MeHg was 0.002 ng/L. Standard quality control procedures for MeHg included duplicates (< 25% relative percent difference), matrix spikes (77-125% recovery), and method blanks. Certified reference material TORT-2, lobster biomass from the National Research Council of Canada (Ottawa, Ontario), was digested and analyzed in tandem with each batch of samples to ensure adequate ongoing recovery.

3.2 Water Column Sampling and Mercury Analysis

Methods are briefly presented here, more detailed methods will be presented in Dent, (2011). Water samples were collected every 3 to 4 weeks from around May through October in 2009 and 2010 at central deep stations in each lake. Samples were collected every two meters

with a Teflon sampler following the EPA clean hand dirty hand Technique to avoid sample contamination (USEPA, 1996). Sample bottles were acid washed and had Teflon lined caps. Great care was taken while retrieving, storing and transporting to maintain integrity of samples for trace mercury analysis. Samples were stored in the dark and chilled with sealed ice containers during transport. THg samples were preserved within 12 hours with 1% bromine monochloride (BrCl) and MeHg samples were preserved with 0.5% hydrochloric acid and stored at 4 °C. MeHg samples were shielded from photodegredation by UV light by storage in amber bottles.

THg in water samples were analyzed using a Brooks Rand MERX-T mercury auto analyzer based on EPA method 1631, cold vapor atomic fluorescence spectroscopy (CVAFS) (USEPA, 2002). MeHg water samples were analyzed using EPA Method 1630 as noted above.

3.3 Other Water Quality Sampling and Analysis

A range of supporting limnological measurements were performed concurrent with mercury sampling (Clegg et al., 2009, and Lanouette, 2010). Data presented here is preliminary in nature and based on an initial development of the raw data, particularly for zooplankton and phytoplankton densities. Methods for some key analyses are briefly described here. Temperature, pH, conductivity were measured at 1 meter intervals using a MS5 Sonde Hydrolab (Hach Corp., 2008). For zooplankton enumeration, 2 replicate samples of vertical tows each were collected, and preserved with formaldehyde. In the lab a 1-mL subsample is drawn from the sample, and added to a Sedgwick-Rafter counting cell. Zooplankton are counted using a microscope, and this process is performed in duplicate for each sample and averaged together. Zooplankton counts were then extrapolated over the volume of the tow to get density of zooplankton per cubic meter using this formula:

Algae counts were also performed. Samples were collected by pumping at top, middle and bottom depths using tubing attached to a marine utility pump. Samples were preserved with gluteraldehyde. A 1-mL subsample was drawn from the preserved sample and counted with a microscope on a Sedgwick-Rafter counting cell. Results were reported as biovolume per volume of water in units of $\mu m^3/mL$.

For this effort, iron and manganese were analyzed using inductively coupled plasma mass spectrometry (ICP-MS) at the WSU School of Earth and Environmental Sciences Laboratory (APHA, 1998). Detection limits were on the order of 50 µg/L for iron and 0.1 µg/L for manganese. Duplicate analyses were performed on every tenth sample. Percent relative standard deviation on duplicate samples averaged around 2% for both manganese and iron.

4. RESULTS

4.1 Hypolimnetic Dissolved Oxygen Content

Oxygenation had a significant impact on DO content in North Twin Lake. Volume-weighted hypolimnetic DO concentrations in North and South Twin lakes were calculated by multiplying discrete concentrations at one-meter intervals by the total volume for each interval, summing those values, then dividing the sum by the hypolimnetic volume, which was found using thermocline depths (Fig. 4.1, Fig. 4.2). North Twin had significantly higher hypolimnetic DO concentration than those in South Twin over the course of the 2009 and 2010 sampling season (paired two sample t-test for means, $p = 0.0012$). Peak DO uptake rate early the season in the South Twin hypolimnion was around 0.05 mg/L/day in 2009 and 0.1 mg/L/day in 2010. Beginning in July 2009 the entire hypolimnion of South Twin Lake was hypoxic (less than 4 mg/) around September the hypolimnion was primarily anoxic (less than 1 mg/L).

Volume-weighted hypolimnetic DO concentration in North Twin showed a slight increase in 2009 and a slow decrease in 2010 at a rate of around 0.03 mg/L/day. The lowest volume-weighted hypolimnetic DO concentrations in North Twin were 7.4 mg/L in August 2009 and 5.3 mg/L in September 2010. The low DO in 2009, which includes a dramatic dip in DO from early to late August, was the result of a short-term experimental decrease in oxygen delivery to the lake in mid August. While oxygenation in North Twin in 2009 and 2010 led to a well-oxygenated water column, as indicated by elevated volume-weighted hypolimnetic DO concentrations throughout the stratified season, DO concentrations near the sediment-water interface experienced anoxic conditions (Lanouette, 2011). North Twin had two recordings of

sediment-water hypoxia in 2009, including in August when the oxygenation system was turned down, and four recordings of sediment-water hypoxia in 2010.

4.2 Hypolimnetic Mercury Content

Mercury data for hypolimnetic waters suggest that oxygenation had an effect on decreasing mercury levels in the bottom waters of North Twin relative to South Twin. 2009 volume-weighted hypolimnetic THg concentration (Fig. 4.3) and MeHg concentration (Fig. 4.4) were estimated in the same fashion as for DO. 2010 data was not available at the time of this draft. Mean 2009 volume-weighted hypolimnetic THg concentrations in North and South Twin were fairly similar at 0.50 ng/L and 0.43 ng/L, respectfully. The data sets were not statistically significant using a paired two sample t-test for means ($p \sim 0.2$). However, if we look at the temporal trend in THg from late June to late October, North Twin shows no accumulation while South Twin shows an accumulation rate of around 0.12 ng/L/month.

2009 volume-weighted hypolimnetic MeHg concentrations in North and South Twin showed a greater divergence than for THg, with mean MeHg levels of 0.033 ng/L and 0.063 ng/L, respectfully. Again, the data sets were not statistically significant using a paired two sample t-test for means ($p \sim 0.15$). However, there is a dramatic difference in the temporal tends in 2009 MeHg. Levels remained below 0.06 ng/L in North Twin. Levels in South Twin gradually increased from July until the beginning of October, and then peaked to nearly 5 times the seasonal average in late October at 0.2 ng/L. This was followed by a dramatic decrease in November once the lake turned over. 2009 South Twin volume-weighted hypolimnetic MeHg concentrations were significantly and negatively correlated with 2009 volume-weighted hypolimnetic DO concentrations (ANOVA, $p = 0.023$), suggesting a linkage between these two

variables. THg to MeHg ratios, a surrogate for the relative importance of MeHg production in aquatic systems (DiPasquale et al., 2003), was approximately 15 for North Twin and 7 for South Twin, indicating the higher importance of MeHg production in North Twin. These multiple observations suggest that hypolimnetic oxygenation was effective in repressing MeHg accumulation in North Twin Lake.

4.3 Zooplankton Mercury Content

THg in North Twin zooplankton shows a clear trend of higher concentrations relative to South Twin (Table 4.1, Fig. 4.5). 2009 THg zooplankton concentrations ranged from 46.7 to 127.4 µg/kg (mean of 95.1 µg/kg) in North Twin and from 41.9 to 113.9 µg/kg (mean of 70.9 µg/kg) in South Twin. Levels in North Twin in 2010 were 62.0 to 282.3 µg/kg (mean of 143.2 µg/kg), an approximate 50% increase compared to 2009. In contrast, South Twin experienced a slight decrease in 2010, ranging from 27.2 to 101.3 µg/kg (mean of 62.5 µg/kg). A paired two sample t-test for means for the 2009-2010 dataset confirms that North Twin zooplankton concentrations were significantly higher than South Twin ($p = 0.0012$). A general seasonal trend can be seen in both lakes with increasing concentrations early in the season followed by a drop later in the season, though the reduction appears to occur earlier in South Twin.

MeHg concentrations in zooplankton were also higher in North Twin (Fig. 4.6). 2009 and 2010 mean concentrations in North Twin were 63.8 and 127.9 µg/kg. Values for South Twin were 46.0 and 40.3 µg/kg. Note that the 2009 dataset was fairly limited (n = 3-4) due to limited availability of biomass after the THg analysis. The 2010 dataset was more comprehensive (n = 7). The ratio of MeHg to THg ranged from 0.42 to 1.0 (mean of 0.73) for North Twin and from 0.30 to 0.87 (mean of 0.62) for South Twin. Based on the 2009-2010 dataset, MeHg

concentrations in zooplankton were significantly higher in North Twin (paired two sample t-test for means; p = 0.006). The MeHg to THg ratio in the two lakes was nearly significantly different (p = 0.084).

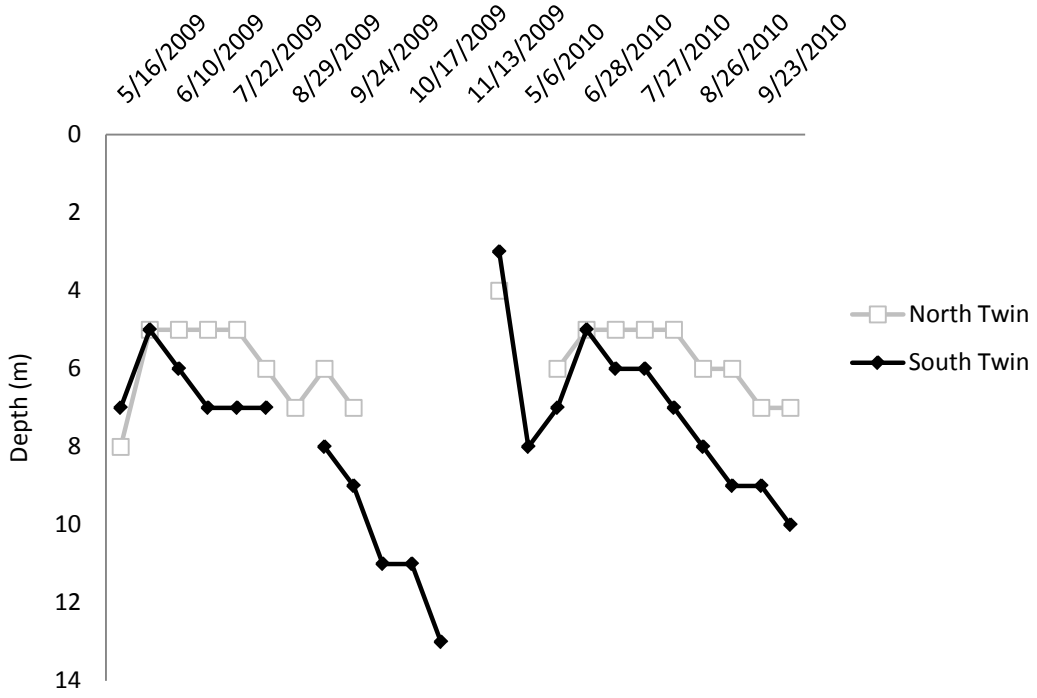

Figure 4.1. Thermocline depths for North and South Twin lakes, 2009 and 2010, a lack of a data point indicates isothermic conditions.

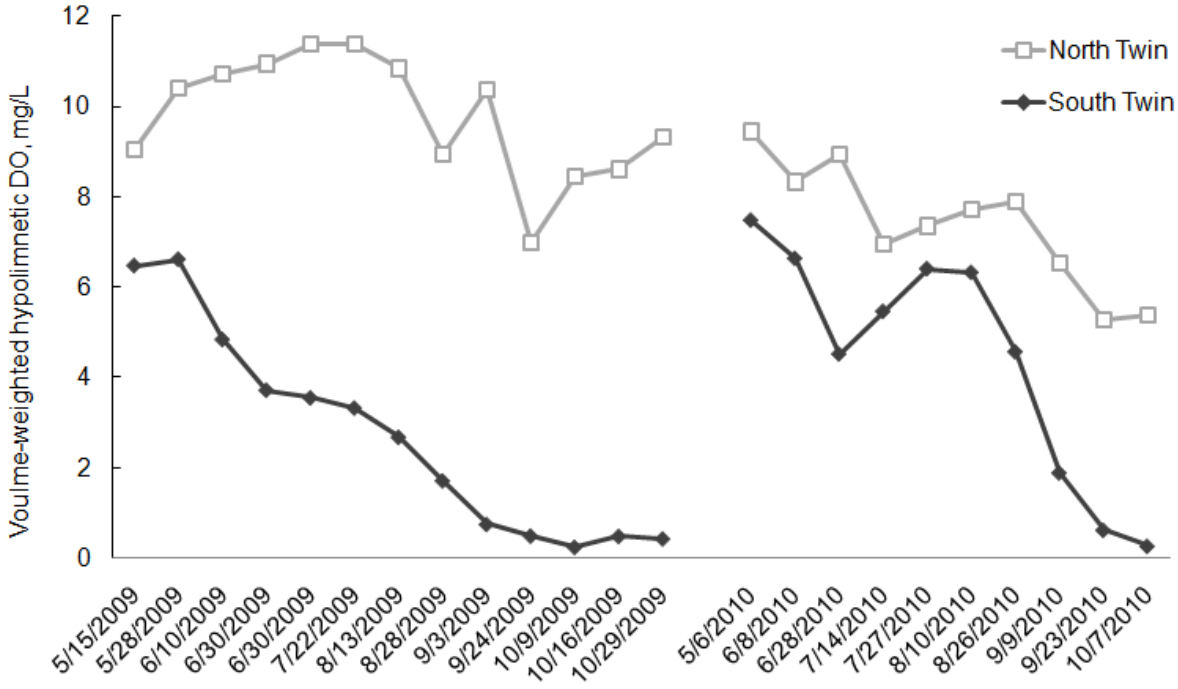

Figure 4.2. Volume-weighted hypolimnetic dissolved oxygen concentration in Twin Lakes, 2009 and 2010. Thermocline data in Fig. 4.1 was used in coordination with bathymetric data provided in the appendix to determine the volume of the hypolimnion for each sampling event.

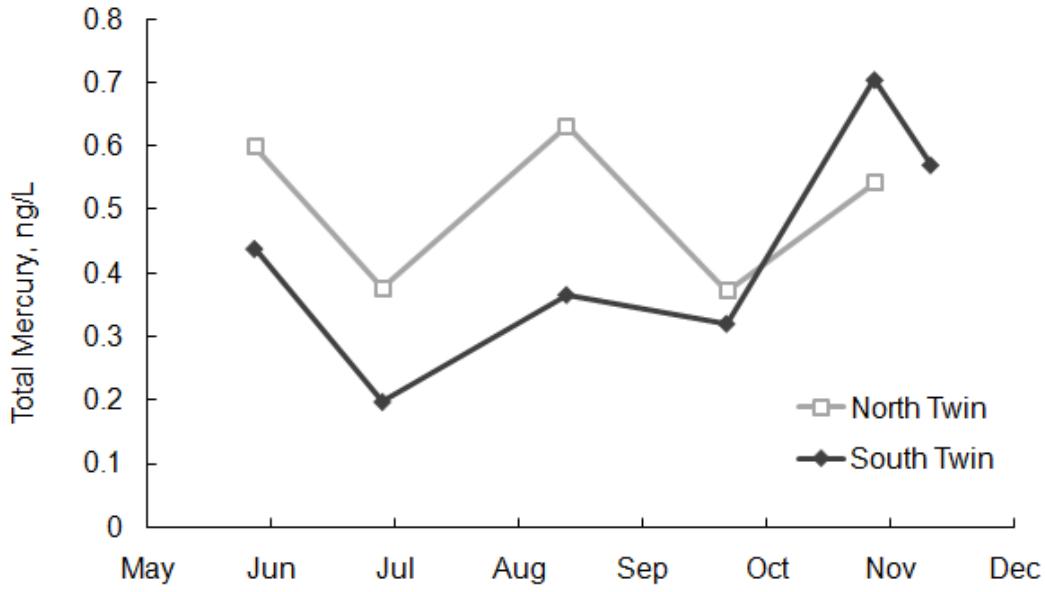

Figure 4.3. Volume-weighted hypolimnetic total mercury concentrations in Twin Lakes, 2009. Thermocline data in Fig. 4.1 was used in coordination with bathymetric data provided in the appendix to determine the volume of the hypolimnion for each sampling event.

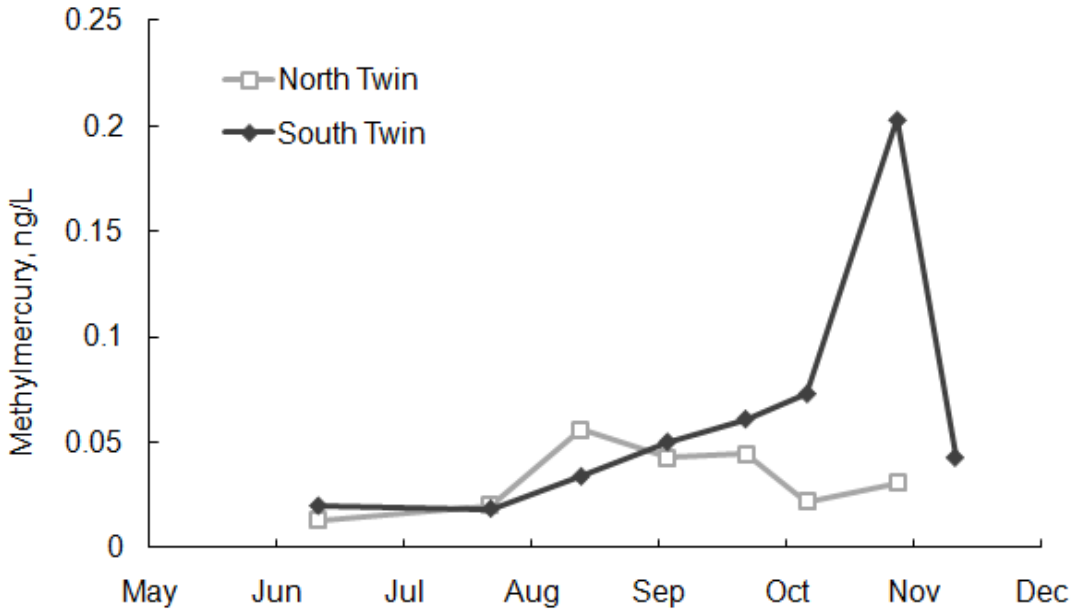

Figure 4.4. Volume-weighted hypolimnetic methylmercury concentrations in Twin Lakes, 2009. Thermocline data in Fig. 4.1 was used in coordination with bathymetric data provided in the appendix to determine the volume of the hypolimnion for each sampling event.

Table 1. Summary of mercury burdens in zooplankton in North and South Twin lakes, 2009 and 2010. THg, and MeHg, are determined using DMA-80 and MERX-M respectively and units are µg of mercury per kg of dry weight

	North Twin Lake			South Twin Lake		
Date	**THg, µg/kg**	**MeHg, µg /kg**	**MeHg:THg**	**THg, µg/kg**	**MeHg, µg /kg**	**MeHg:THg**
6/30/2009	63.5			41.9		
7/23/2009	105.4			59.5		
8/13/2009	102.2			97.5		
9/3/2009	127.4			113.9		
9/24/2009	103.4	69.5	0.67	58.0		
10/13/2009	117.2	78.2	0.67	49.2	22.3	0.45
10/29/2009	46.7	43.8	0.94	67.5	38.5	0.57
11/13/2009				79.4	64.6	0.81
12/9/2009					58.7	
2009 Average	95.1	63.8	0.76	70.9	46.0	0.61
4/22/2010	73.0			47.8		
5/7/2010	62.0	27.1	0.44	90.6	26.7	0.29
5/19/2010	84.4	35.7	0.42	27.2	19.5	0.72
6/9/2010	118.3			56.5		
6/29/2010	148.6	118.3	0.80	93.8	56.2	0.60
7/28/2010	179.8	175.0	0.97	101.3	68.8	0.68
8/11/2010		174.4			43.7	
8/27/2010	168.6	174.0	1.03	35.3	30.5	0.86
9/24/2010	282.3	190.6	0.68	58.5	36.7	0.63
10/8/2010	188.0			62.6		
11/12/2010	127.3			51.0		
2010 Average	143.2	127.9	0.72	62.5	40.3	0.63

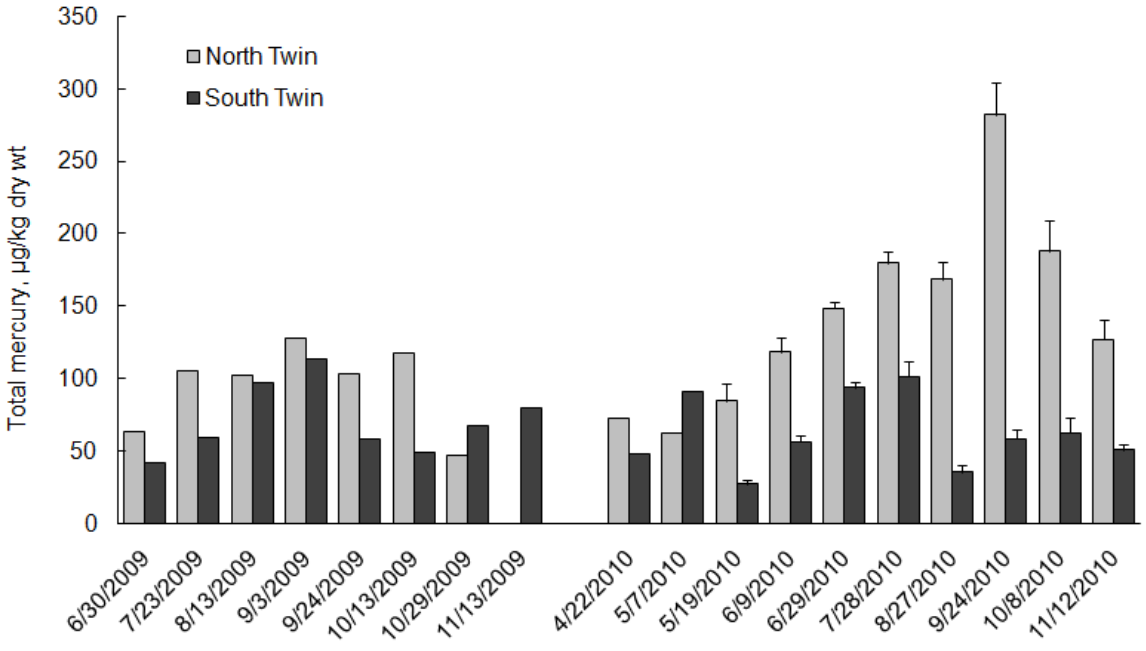

Figure 4.5. Total mercury concentrations of large-bodied zooplankton from Twin Lakes, 2009 and 2010. Error bars for 2010 data are plus one standard deviation for triplicate analyses where sufficient biomass was available for replicate analyses.

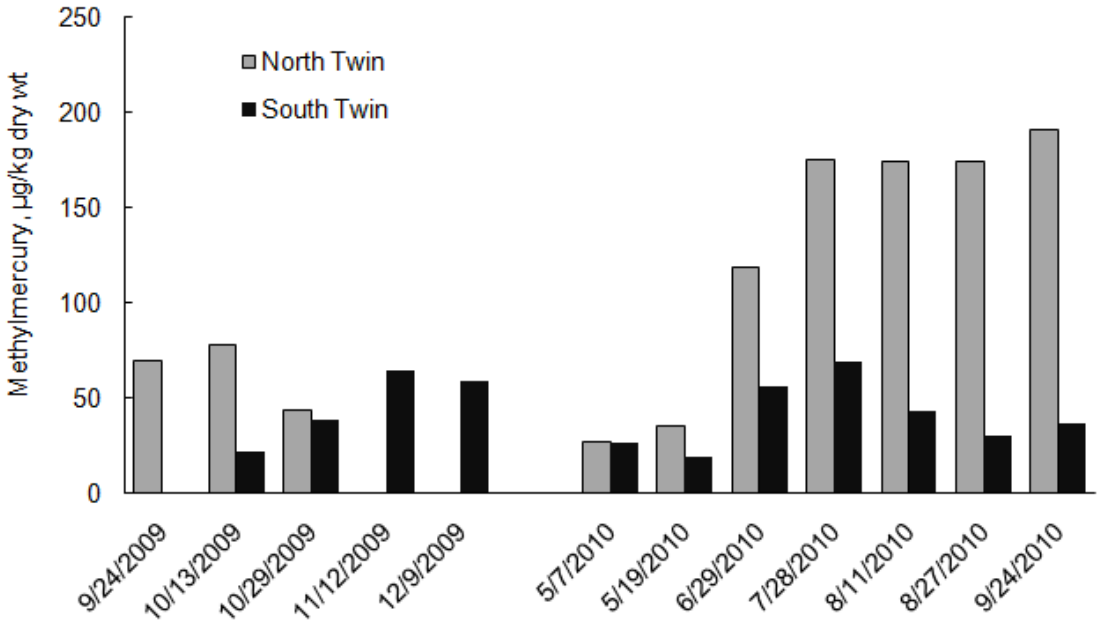

Figure 4.6. Methylmercury concentrations of large-bodied zooplankton from Twin Lakes, 2009 and 2010. There is limited data for 2009 due to a lack of sufficient zooplankton biomass for both THg and MeHg analyses.

5. DISCUSSION

5.1 Mercury Levels in Zooplankton

THg levels measured in zooplankton in 2009 and 2010 ranged from 47 to 282 µg/kg for North Twin and 27 to 114 µg/kg for South Twin, and are similar to values reported in the literature. Chen et al. (2000) studied 20 lakes in the Northeast US and reported THg ranges in zooplankton of 28 to over 7,000 µg/kg dry weight. In a comprehensive study of 20 Wisconsin lakes, THg in zooplankton ranged from 33 to 206 µg/kg dry weight (Watras et al. 1998).

Zooplankton MeHg concentrations ranged from 36 to 196 µg/kg in North Twin and 19 to 69 µg/kg in South Twin. These levels were also very similar to those reported by Watras et al. (1998) of 60 to 161 µg/kg. MeHg is the primary form of mercury to bioaccumulate in biota because of its ability to efficiently transfer and be retained up the food chain (Watras, 2009). This is reflected in our results. The average percentage of MeHg in THg zooplankton body burden for North Twin was 75% in 2009 and 73% in 2010. Values in South Twin were 61% and 63%, respectively. Again, these values were similar to the 57% average for 20 lakes studied in Watras et al. (1998).

Watras (1992) introduced the simple concept of a mercury bioconcentration factor (BF) to quantify the relationship between how much of mercury is in biota versus how much is in the water. The formula is — where C_b (µg/kg wet weight) is mercury concentration in the biota and C_w (µg/kg) is the mercury concentration in water. BF values were calculated for 2009 using mean THg and MeHg concentrations in zooplankton and hypolimnetic waters. Dry weight concentrations for zooplankton were converted to wet weight assuming 80% water content (Watras, 1992). For THg, North Twin and South Twin had similar BFs of 4.58 and 4.54,

respectively. BFs for MeHg were higher and showed a considerable difference, 5.82 for North Twin and 5.18 for South Twin. The higher BF for MeHg was expected since it has a greater potential to bioaccumulate than total mercury. The difference between BF of MeHg between North and South is strong evidence that there is an enhancement of the uptake of MeHg in North Twin. BF values for zooplankton of a remote meso-oligiotrophic lake in Wisconsin were approximately 4.8 and 6.0 for THg and MeHg, respectively (Watras 1992). These values are similar to the values calculated for Twin Lakes, and exhibited the same trend of increased BF for MeHg relative to THg. Watras et al. (1998) also presented BF values for MeHg for 15 Wisconsin lakes. Values typically ranged from 5 to 6 and again were in close agreement with this study.

One interesting observations in this study, which I did not find previously documented in the literature, was an apparent seasonal trend in the MeHg to THg ratio. In 2010 in both lakes the ratio was relatively low in May (~0.4 in North Twin; 0.3-0.7 in South Twin) and then peaked in late August (1.0 in North Twin; 0.87 in South Twin). The lower ratios in the spring may be because thermal stratification and related anoxia in bottom waters, which should stimulate MeHg production and accumulation in bottom waters and uptake into biota, has yet to occur.

5.2 Zooplankton Mercury Levels in North versus South Twin Lake

For a long time researchers have acknowledged the difference in mercury cycling in eutrophic lakes with seasonally anaerobic bottom waters and oligotrophic lakes with oxic bottom waters. In an early study somewhat similar to this one, Larson (1977) found substantial differences in mercury uptake into fish in two nearby lakes in southwestern Oregon, eutrophic Upper Squaw Lake and oligotrophic Big Squaw Lake. Both lakes exhibited summer thermal stratification, but only Upper Squaw Lake exhibited hypolimnetic anoxia. On two sampling dates

THg levels in cutthroat trout were higher in Upper Squaw versus Big Squaw (April 1975, 0.205 versus 0.090 mg/kg, August 1975, 0.149 versus 0.049 mg/kg). This was the case even though the fish from Upper Squaw were relative large, indicating they were older fish that had more time to uptake mercury. The author attributed this difference to the fact that Upper Squaw exhibited anoxia in bottom waters and that summertime trout habitat, like in Twin Lakes, was drastically limited to a warm metalimnion, though no direct mechanism was proposed. In a general review of mercury cycling in lakes Watras (2009) notes that anoxic bottom waters of eutrophic lakes tend to accumulate mercury compared to the aerobic bottom waters of oligotrophic lakes. As noted in the introduction, the mixing of MeHg-rich bottom waters into surface waters has been implicated as an important mechanism that enhances the uptake of mercury into zooplankton (Herrin et al., 1998; Slotton et al., 1995).

Based on monitoring related to this study, oxygenation repressed MeHg accumulation in bottom waters of North Twin in 2009. In contrast, the bottom waters of South Twin accumulated MeHg (Fig. 4.3). While water column mercury data for 2010 is not available, DO data shows that levels in the bottom of North Twin in 2010 were high as a result of oxygenation. Thus it is assumed that MeHg accumulation in North Twin was again repressed in 2010. In the Morel et al. (1998) review of the chemical cycle and bioaccumulation of mercury, they state that "the key factor for determining the concentration of mercury in the biota is the MeHg concentration in the water". So based on the conventional model of mercury uptake into aquatic biota, a drop in MeHg in the water column should translate to a drop in levels in biota at the base of the food web. But as demonstrated in the results, South Twin had higher concentrations in the water while North Twin zooplankton had higher concentrations in both THg and MeHg, especially in 2010.

Two fundamental parameters that have been shown to affect mercury uptake into biota in aquatic systems is dissolved organic carbon (DOC) and pH. DOC is a strong ligand for mercury and other metals, acting as a competitor for mercury with methylating microbes (Watras, 2009). Since DOC molecules are generally too large to cross cell membranes, higher DOC concentrations are generally associated with lower MeHg concentrations (Ravichandran, 2004). Once MeHg is formed, DOC facilitates MeHg solubility and transport through complexation, making MeHg more soluble in the water column (Miskimmin, 1991). Monson (1998) evaluated the effect of aquatic humus DOC on mercury concentrations in the zooplankton *Daphnia magna*. While THg concentrations in zooplankton were positively correlated with DOC concentrations in the study, MeHg concentrations were inversely correlated. So DOC appeared to depress MeHg uptake. But the correlate between DOC and MeHg uptake in aquatic biota has been inconsistent (Watras, 1998). Supporting this idea, a comprehensive study of 20 Northeastern lakes with a wide range of water quality characteristics found that DOC did not significantly correlate with mercury burdens in fish (Chen et al., 2000).

In addition to DOC, the relationship between pH and the aquatic mercury cycle has been studied extensively. Chételat et al. (2010) statistically analyzed 52 mid-latitude lakes in North America for a variety of different variables and their correlation with mercury in invertebrates; pH was the only environmentally significant factor explaining variation ($p<0.001$). Supporting this observation, a lake that was experimentally acidified from 6.1 to 4.7 had higher levels of MeHg concentrations in water, phytoplankton and zooplankton (Watras, 1992). It is thought that pH affects DOC's ability to complex with mercury. At low pH a more negatively charged DOC is less available to bind with mercury, and this may increase bacterial methylation rates (Miskimmin et al., 1992).

While DOC and pH have been proven to affect MeHg uptake into zooplankton, large amounts of uncertainty exists regarding their effect. From limited sampling events in this study, mean DOC from North and South Twin for 2010 were 5.11 and 4.15 mg/L, respectively. This compares to the Chen et al. (2000) dataset ranging from 2.9 to 12.6 mg/L. 2010 mean pH values also exhibited the same similarity, 7.1 and 7.7 for North and South respectively. Note the lakes are also in close proximity and share the same watershed. The likeness of the means of DOC and pH suggest that these variables are not substantially controlling the dramatic differences observed in mercury uptake into zooplankton in North and South Twin Lake. Below I explore two other potential mechanisms that may explain these puzzling results, biodilution and enhanced mercury uptake via metal oxide.

5.3 Biodilution and Zooplankton Mercury Uptake

Zooplankton are primarily filter feeders, and large bodied zooplankton such as *Daphnia* are especially effective and efficient filter feeders (Dodds, 2002). Zooplankton filter and feed on plankton, bacteria, organic matter, and other particles. Particles and water ingested by zooplankton contain Hg and MeHg which are accumulated in the organisms. Kainz et al. (2002) analyzed fatty acid biomarkers of zooplankton and found that they were composed of more than 60% algal fatty acids and less than 10% bacterial fatty acids. These percentages offer insight into what zooplankton eat - primarily phytoplankton. While ingestion of mercury is the main pathway for mercury accumulation in zooplankton, a number of recent studies described below present a very interesting picture of the complexity of mercury uptake by zooplankton, and how it can be affected by a range of factors including relative amounts of phytoplankton and zooplankton.

These dynamics may have some bearing on the observed patterns of mercury uptake in zooplankton in Twin Lakes.

A potential reason why THg and MeHg concentrations are higher in North Twin relative to South Twin is a phenomenon known as biodilution. Biodilution can be defined as higher biomass concentration resulting in less mercury mass per unit mass of biomass. Three different types of biodilution have been detailed in the literature: algal bloom dilution, zooplankton density dilution, and growth dilution (Chen and Folt, 2005, Watras, 2009). A negative correlation between phytoplankton density and zooplankton mercury concentration is known as bloom dilution, while a negative correlation between zooplankton density and mercury concentration of zooplankton is defined as zooplankton density dilution. Growth dilution is the concept of growth rates exceeding the rate of mercury uptake.

There have been multiple studies on the effect of eutrophication and associated increases in phytoplankton density on the assimilation of mercury in freshwater food webs (Chen et al., 2000; Kainz and Mazumder, 2005; Pickhardt et al., 2002). Pickhardt et al. (2005) looked at the effects of phytoplankton density on mercury accumulation in zooplankton in 12 artificially fertilized mesocosms. As the phytoplankton density increased with the addition of nitrogen and phosphorus, mercury accumulation as measured by mercury mass per mass of zooplankton decreased significantly. THg and MeHg concentrations in zooplankton from the mesocosm with the lowest nutrient concentration were around four times higher than in zooplankton from the mesocosm with the highest nutrient concentration. Chen et al. (2000) looked at data from 38 Northeastern lakes and found both bloom dilution and zooplankton density dilution. In the dataset of lakes, they found a negative correlation between algal density and THg and MeHg

concentrations in zooplankton. They also found that with an increase in large-body zooplankton there was a reduction in mercury burden in zooplankton and ultimately in fish.

Another concept affecting mercury accumulation rates in biota is growth dilution. Organisms in the presence of an overabundance of nutrients can grow faster than they accumulate mercury, thus lessening the mercury burden (Sunda and Huntsman, 1998). A nutrient pulse or a sudden change causing rapid growth can elicit lower mercury concentrations in biota. Another aspect of growth dilution is the effect of food quality. Karimi et al. (2006) fed high quality algae (C:P ratio ~130) and low quality algae (C:P ~1,300) spiked with MeHg to zooplankton. They determined that zooplankton fed the high quality algae grew approximately 3.5 times faster than the lower quality algae, and as a result had lower mercury burdens attributed to them.

To evaluate the possibility that biodilution accounted for the relatively lower mercury concentrations in South Twin Lake zooplankton, I looked at several different data sets of environmental variables for Twin Lakes. One possibility was that zooplankton density dilution in South Twin resulted in lower mercury burden in zooplankton. In order for this to occur, zooplankton populations in South Twin would need to be higher than North Twin, but this is not the case (Fig. 5.1). Both magnitude (generally 2,000-8,000 #/m^3) and seasonal pattern (peak density in June) of zooplankton density in 2010 appeared similar. A paired t-test for means confirmed that the difference between the two data sets were not statistically significant. To assess the potential for of algal bloom dilution, two metrics were evaluated including phytoplankton biovolume (Fig 5.2) and Secchi depth (Fig 5.3). Phytoplankton biovolume for 2010 in North and South Twin peaked in the fall and spring, which corresponds to higher nutrient availability. Biovolumes were low in the summer, likely a result of nutrient limitation

and zooplankton grazing. Secchi depth followed a related inverse pattern with comparable peak transparency in the summer during periods of low phytoplankton biovolume. A paired sample t-test for means showed that algal biovolumes and Secchi depths were not significantly different for the two lakes ($p = 0.12$ and 0.73, respectively), suggesting that bloom dilution was not a factor. In addition, there was no corresponding decrease in THg or MeHg concentrations in South Twin zooplankton during peak of algal biovolume, further undermining the role of bloom dilution. The evaluation of zooplankton and phytoplankton density does not support the contention that biodilution in South Twin Lake is responsible for the lower levels of mercury burden in South Twin zooplankton compared to North Twin zooplankton.

In another effort to assess the effects of zooplankton density on zooplankton mercury content, the total mass of THg and MeHg contained in the entire large-bodied zooplankton population was estimated. This unique metric was calculated by multiplying the following factors: zooplankton mercury concentration (µg/kg), average zooplankton weight (µg/#), zooplankton population density (#/m^3), and lake volume (m^3). The calculation yielded grams of total mass of THg and MeHg in zooplankton (Fig. 5.4 and 5.5). If the working hypothesis of this study was valid, that lake oxygenation led to a decrease in water column MeHg and a resulting decrease in zooplankton MeHg, the total mass of MeHg in the zooplankton in North Twin should be lower than South Twin. Again, this was not the case. Total mass of THg in zooplankton in 2010 in North Twin ranged from around 0.2 to 0.9 g, while levels in South Twin ranged from around 0.05 to 0.6 g. For MeHg, total mass ranged from around 0.1 to 0.5 g in North Twin and 0.05 to 0.2 g in South Twin Lake. A paired sample t-test for means showed that both THg ($p = 0.005$) and MeHg ($p = 0.02$) were significantly higher in North Twin, the exact opposite of what was hypothesized to occur. MeHg zooplankton mass in 2010 in both North and South Twin

shared the same distinct seasonal trend of increasing early in the season before decreasing in the late summer. Presuming that South Twin accumulated MeHg in bottom waters as it did in 2009 (Fig. 4.3), it is surprising that MeHg mass did not continue to increase. Perhaps anoxia in bottom waters excludes zooplankton form accessing high MeHg waters. In contrast, the expansion of habitat provided by hypolimnetic oxygenation may have allowed zooplankton in North Twin to access deeper waters with low MeHg levels, resulting in greater MeHg uptake. Further data analysis is underway by others examining the impacts of oxygenation on zooplankton vertical migration, and these results should shed some light on the potential for zooplankton migration to partly explain the observed high THg and MeHg levels in North Twin zooplankton.

5.4 Enhanced Uptake of Mercury due to Metal Oxides

Another potential explanation for the higher mercury levels in zooplankton from North Twin Lake is that the continual presence of iron and manganese oxides in the water column enhanced mercury uptake. A surprising aspect of this project was that oxygen addition in North Twin Lake did not repress metals release from sediments. Such repression is typically observed after oxygen addition to the bottom of lakes and reservoirs (Beutel and Horne, 1999). For example, a recent study by Gantzer et al. (2009) documented a greater than 95% decrease in iron and manganese in a raw water reservoir in Virginia oxygenated with the same line diffuser system used in Twin Lakes. In contrast, iron and manganese levels in North Twin Lake remained elevated after oxygen addition (Fig. 5.6). During oxygenation in 2009, levels in North Twin were around 100 µg/L for manganese and 500 µg/L for iron in the upper hypolimnetic water column. Near the sediment water interface levels increased to around 500 µg/L for manganese and 4,000 µg/L for iron. Similar results were observed for 2010. In non-oxygenated South Twin, levels

were roughly the same, though iron in particular was only observed on the lower hypolimnion.

Since the water column in North Twin was oxygenated with DO levels above 4-5 mg/L, a large fraction of the iron and manganese in the hypolimnion was likely in the form of metal oxides. These precipitates have a strong affinity to sorb metals including mercury (Davison, 1993). This phenomenon was observed in North Twin Lake in the fall of 2008 during a test of the line diffuser system (Dent, in preparation). After only a day after oxygenation of the anoxic hypolimnion, iron and MeHg levels in bottom waters plummeted. A few weeks after the system was turned off, the hypolimnion had re-accumulated iron and MeHg to levels observed before the oxygen test. The explanation for this observation could be the reversible and redox-mediated sorption of MeHg to iron oxides. Hurley et al. (1994) also found a correlation with between the settling of iron and MeHg in a study of Wisconsin seepage lakes, and this supports the observation during the North Twin Lakes oxygen test.

Since zooplankton are filter feeders and are known to ingest small particulates (Burns, 1968), the ingestion of mercury-rich metal oxides could result in enhanced mercury uptake into zooplankton. An additional intriguing mechanism could be the conversion of inorganic mercury taken up via metal oxide ingestion to MeHg within the zooplankton (Kainz et al., 2002; Tsui and Wang, 2004). These researchers hypothesize that microfolds in zooplankton gastrointestinal tracts could provide anaerobic habitat for sulfate-reducing bacteria that produce MeHg from ingested inorganic mercury.

A few limnological studies have suggested linkage between metal oxides and mercury uptake by zooplankton. Slotton et al. (1995) monitored seasonal cycles of THg in zooplankton in Davis Creek Reservoir, a small eutrophic reservoir in California impacted by historical mercury mining. They found that mercury levels in zooplankton increased dramatically at fall overturn,

from around 100-200 µg/kg, levels similar to those observed in this study, to peak levels of 300-500 µg/kg just after fall destratification. A related study also tracked MeHg and manganese in the water column (Gill and Bruland, 1992). That study found that MeHg accumulated in anoxic bottom waters along with elevated levels of dissolved manganese. Manganese levels in anoxic bottom waters were around 500 µg/L, while iron levels were two orders of magnitude lower. This contrasts with North Twin which had waters enriched with iron relative to manganese. This difference may be because Davis Creek is a reservoir with flooded terrestrial soils while North Twin is a natural lake with sediments that are fairly rich in iron (Dent, personal correspondence). Dissolved MeHg levels in anoxic bottom waters were around 20 ng/L. This is two orders of magnitude higher than the levels observed in Twin Lakes and reflects the mercury impacted status of the reservoir. These extreme levels of MeHg in the water column also likely account for the relatively high levels of THg in the zooplankton in Davis Creek Reservoir. Upon overturn much of the mercury was associated with newly oxidized manganese. Slotton et al. (1995) suggested that this may have facilitated MeHg uptake into zooplankton during the overturn episode. In Davis Creek Reservoir, the zooplankton likely ingested the fine, mercury-rich metal oxide particulates at overturn and accumulated higher levels of mercury.

Another interesting study the evaluated the impact of anoxia and lake overturn on mercury uptake into zooplankton was performed by Herrin et al. (1998). This study looked at seasonal patterns of total and MeHg in zooplankton and fine particulates. As with the Slotton et al. (1995) study, this study reported an accumulation of MeHg in anoxic bottom waters, though at less than 0.5 ng/L, these levels were more comparable to those observed in Twin Lakes. Herrin et al. (1998) also found that mercury levels in zooplankton peaked to around 200 µg/kg at lake turnover, with roughly 50-80% of this being in the MeHg form. This study also evaluated

mercury levels of particulates in the edible size fraction for zooplankton, which included algae and inorganic particles such as iron and manganese oxides. They found that levels tripled during erosion of the mercury-rich hypolimnion and fall destratification. They concluded that the enrichment of edible particulates with mercury as a result of lake overturn fueled a subsequent increase in zooplankton mercury. The Herrin et al. (1998) study points out the important role that edible particulates, whether they be organic or inorganic, play in transferring mercury from the water column and into zooplankton.

Based on the current literature regarding seasonal mercury uptake into zooplankton, the presence of metal oxides in North Twin Lake may have been a key mechanism that resulted in relatively high levels of mercury uptake into zooplankton compared to non-oxygenated South Twin Lake. As noted earlier, oxygen did repress MeHg accumulation in bottom waters in North Twin Lake, yet zooplankton had higher levels of mercury relative to South Twin Lake. If this idea is correct, it suggests that the presence of metal oxides is more important than the level of MeHg in the water column in controlling mercury uptake into zooplankton.

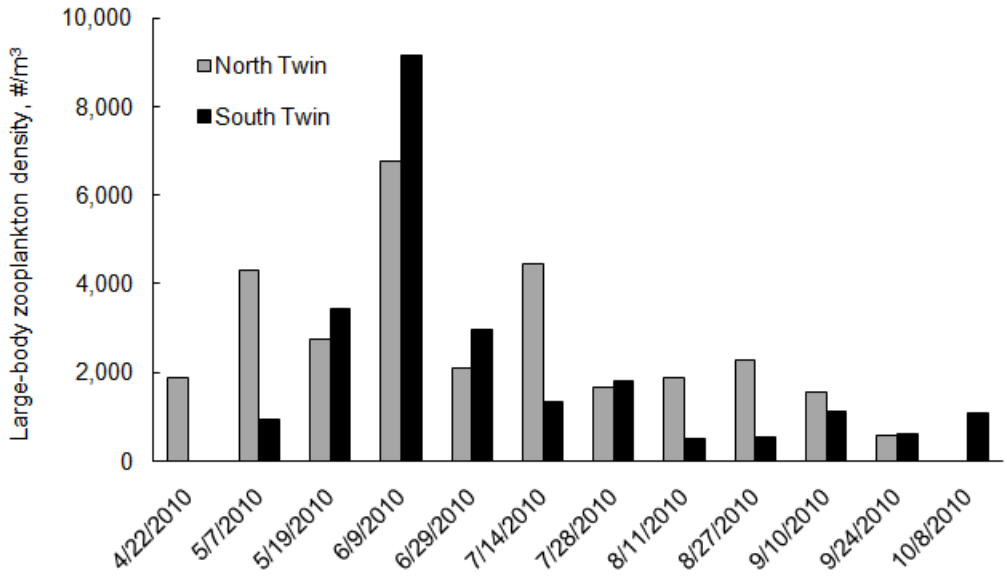

Figure 5.1. Large-bodied zooplankton densities in Twin Lakes, 2010. Densities are expressed in number of zooplankton per m^3. Data is preliminary.

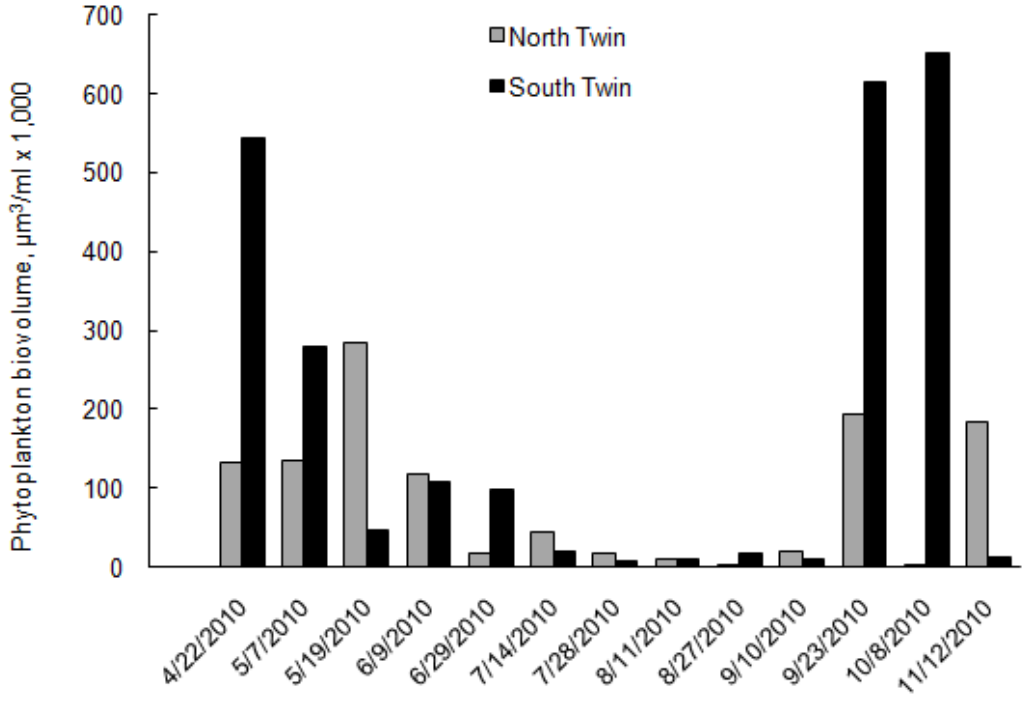

Figure 5.2. Phytoplankton densities in surface waters of Twin Lakes, 2010. Densities are expressed in phytoplankton biovolume µm^3 per ml x 1,000. Data is preliminary.

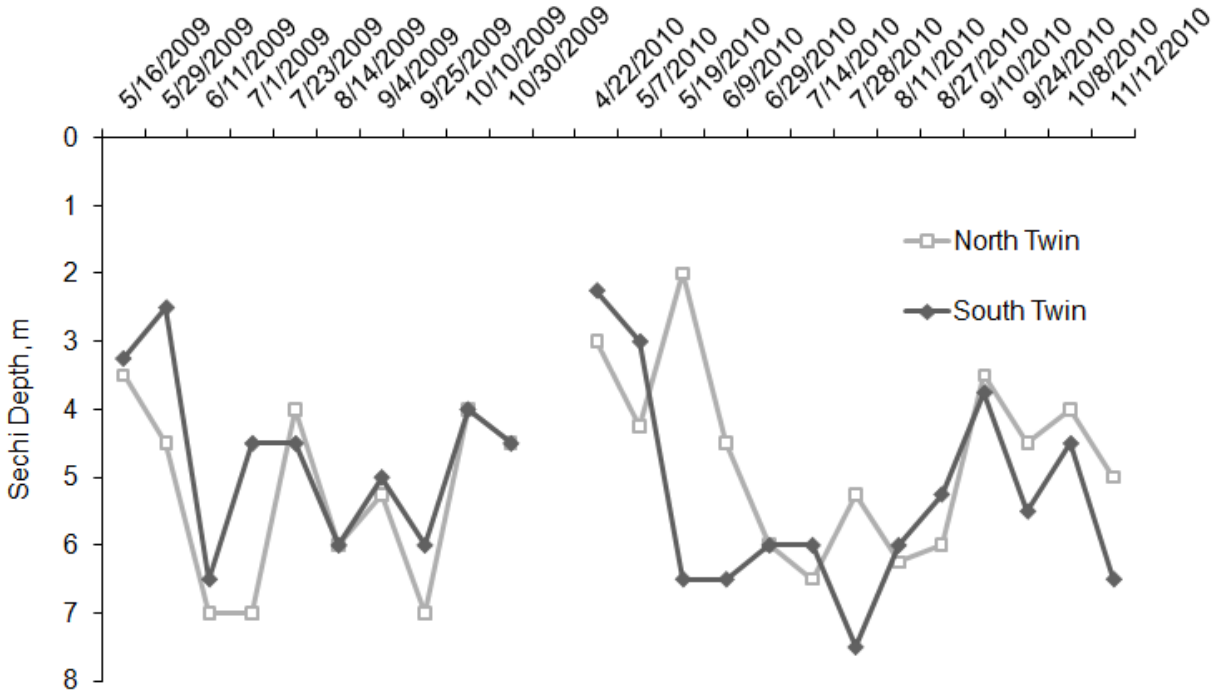

Figure 5.3. Secchi depths in Twin Lakes, 2009 and 2010.

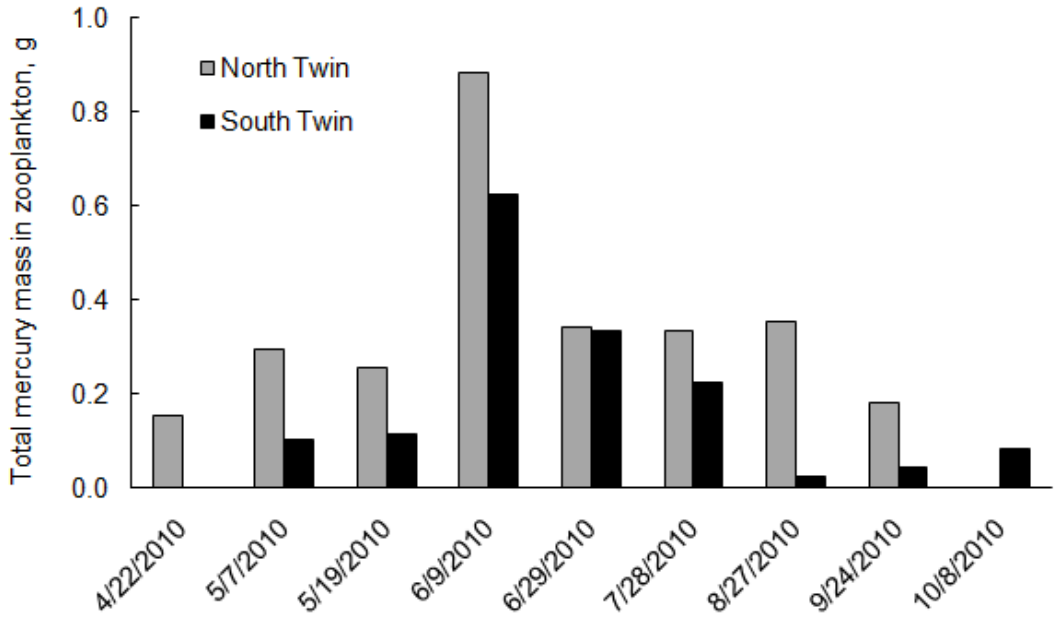

Figure 5.4. Total mercury mass contained in the zooplankton population in Twin Lakes, 2010

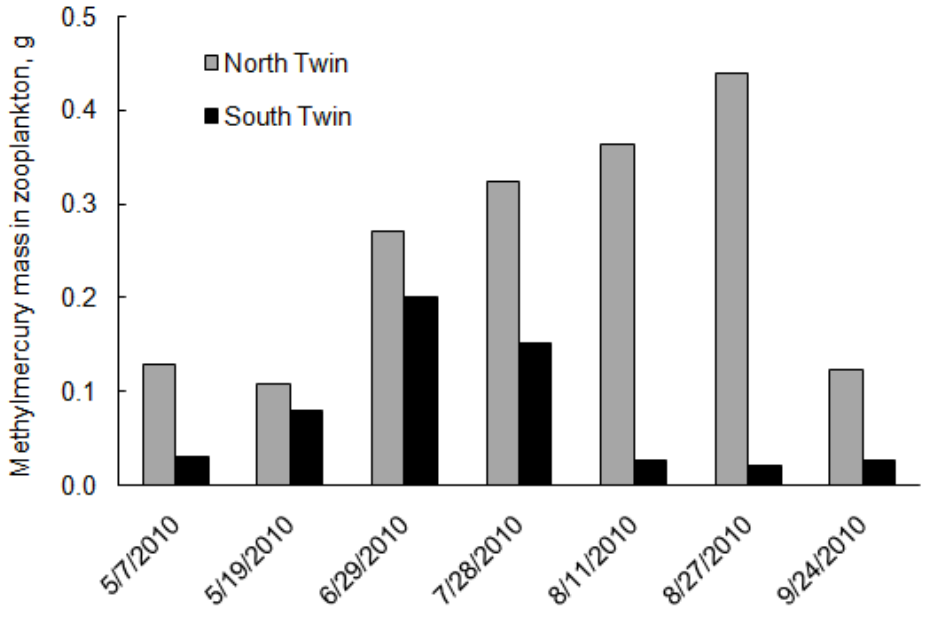

Figure 5.5. Methylmercury mass contained in zooplankton population in Twin Lakes, 2010.

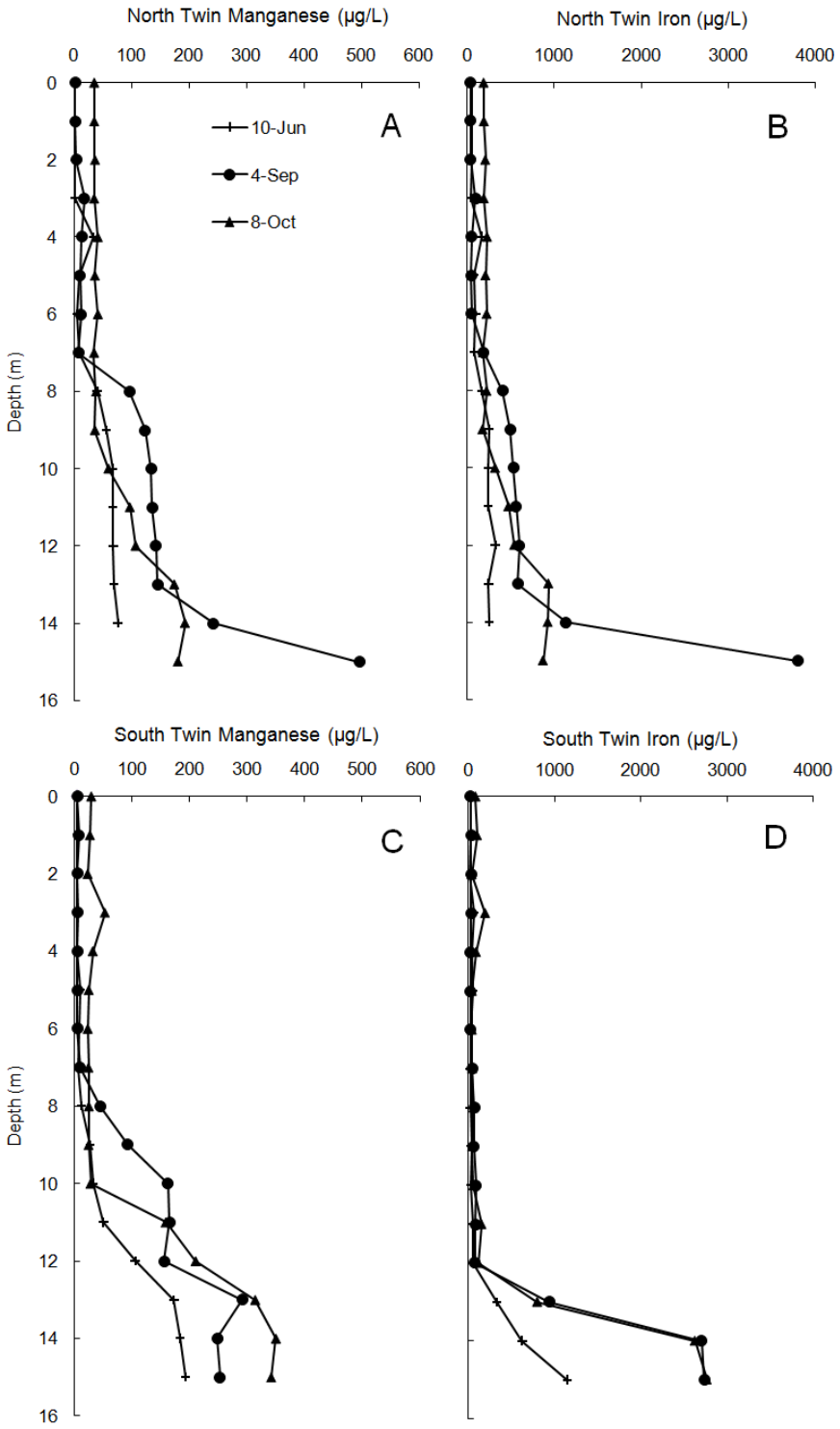

Figure 5.6. Iron and manganese profiles in Twin Lakes. A. North Twin manganese; B. North Twin iron; C. South Twin iron; and D. South Twin iron, 2009

6. CONCLUSION

Hypolimnetic oxygenation of North Twin Lake lowered MeHg concentrations in the hypolimnetic water column as predicted. We can attribute this to elevated dissolved oxygen concentrations suppressing a range process that enhance MeHg mobility and production in bottom waters, including methylation by sulfate-reducing bacteria. While MeHg concentrations in the hypolimnion decreased, THg showed little response to the hypolimnetic treatment, with North Twin levels only slightly above those in South Twin. The most compelling finding of this study is also the most difficult to understand. THg and MeHg concentrations in large-bodied zooplankton did not respond to lower water column MeHg concentrations in North Twin.

With many variables and multiple pathways for zooplankton to accumulate mercury, it is unknown why this occurred, but possible mechanisms that may enhance uptake of mercury were explored. Several types of biodilution were investigated. Zooplankton density and algal bloom dilution were ruled out since there was no significant difference between zooplankton and phytoplankton density in North and South Twin lakes. With regards to growth dilution, it is not known how oxygenation effects algal nutrient composition and related zooplankton growth rates. If it were to decrease algal C:P ratio and induce higher zooplankton growth rates, as illustrated in Pickhardt et al. (2005), then this could be a plausible explanation. But oxygenation generally decreases internal nutrient loading, thus the C:P ratio in North Twin would be expected to increase.

The conjecture that showed the most promise was the enhancement of mercury uptake by metal oxides. Metal oxides in the bottom waters of North Twin Lake, which were unexpectedly present all summer and fall, may have sorbed mercury and then been ingested by zooplankton,

resulting in elevated mercury uptake. If this idea is correct, it suggests that the presence of metal oxides is more important than the level of MeHg in the water column in controlling mercury uptake into zooplankton. If true, this observation also has important implications of lake oxygenation. Systems must be sized and engineered in such a way as to inhibit iron and manganese release form sediments by maintaining a well-oxygenated sediment-water interface. Further research is necessary to understand how metal oxides affect mercury uptake into zooplankton. A first start could be a laboratory bioassay experiment in which mercury uptake into zooplankton is measured in water that contains MeHg with and without metal oxides. In addition, future field work at Twin Lakes should include sampling for total MeHg and dissolved MeHg to evaluate if water column MeHg is associated with fine particulates that could be ingested by zooplankton.

One of the strength if this study, the side by side study of North and Twin Lakes, was also a bit of a limitation. While North Twin Lake showed an increase in zooplankton MeHg relative to South Twin, MeHg trends in North Twin Zooplankton before the 2009 hypolimnetic oxygenation are not known. The lakes are similar and it is assumed that the differences in hydrology, biogeochemistry, and mercury loading between the two lakes is negligible. Thankfully this limitation is a short-term one. After collecting two years of data for non-oxygenated South Twin, an oxygenation system was installed in this basin in fall 2010 and start-up is set for summer 2011. Continued sampling of South Twin will offer a more conclusive before and after study of the effects of lake oxygenation on mercury uptake in zooplankton.

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APPENDIX

Total Mercury Analysis DMA-80

First Run	**Hg [ng]**	**R [µg/kg]**	**Weight [g]**
blank	1.947441459	1.947441459	1
nt 042210 zoo >243	0.689300358	37.05915833	0.0186
nt 042210 zoo >500	1.133992076	36.46276855	0.031099999
nt 042210 zoo > 1000	1.394925237	73.03273773	0.019099999
blank	0.218296364	0.218296364	1
mess 3	5.549000263	112.1010132	0.0495
blank	2.723473549	2.723473549	1
st 042210 zoo >243	0.88712424	47.43980026	0.0187
st 042210 zoo >500	0.607736349	59.00352859	0.0103
blank	0.481320441	0.481320441	1
mess 3	5.103563309	106.1031876	0.048099998

Second Run	**Hg [ng]**	**R [µg/kg]**	**Weight [g]**
blank	1E-05	1E-05	1
mess3	2.254884243	101.5713654	0.0222
mess3	2.015029192	103.8674927	0.019400001
mess3	2.406787395	101.5522156	0.023700001
blank	0.016408831	0.016408831	1
st 042210 zoo >1000	0.754556179	47.7567215	0.015799999
nt 050710 zoo >250	0.607736349	38.70932388	0.015699999
nt 050710 zoo >500	1.622133255	58.35011673	0.027799999
nt 050710 zoo >1000	1.54293251	61.96516418	0.024900001
blank	0.251888633	0.251888633	1
mess 3	2.256936073	107.9873734	0.0209
st 050710 zoo >250	0.858568907	32.3988266	0.0265
st 050710 zoo >500	1.755285859	72.5324707	0.0242
st 050710 zoo >1000	1.5587672	90.62600708	0.017200001
blank	0.102063797	0.102063797	1
mess 3	2.306184769	112.4968262	0.020500001

Total Mercury Analysis DMA-80

Third Run	**Hg [ng]**	**R [µg/kg]**	**Weight [g]**
blank	0.059237551	0.059237551	1
mess 3	4.6435709	103.1904678	0.045000002
mess 3	2.437598944	102.4201279	0.023800001
mess 3	1.095218539	104.3065262	0.0105
blank	0.040882699	0.040882699	1
nt 051910 zoo >243	0.432386607	39.66849518	0.0109
nt 051910 zoo >500	1.117147803	83.36923981	0.0134
nt 051910 zoo >500	1.305462718	80.08973694	0.0163
nt 051910 zoo >500	0.966679156	77.95800018	0.0124
nt 051910 zoo >1000	0.991159976	86.18782043	0.0115
nt 051910 zoo >1000	1.119706631	95.70142365	0.0117
nt 051910 zoo >1000	1.076303124	71.27835846	0.0151
blank	0.167318776	0.167318776	1
mess 3	1.44025588	109.9431992	0.0131
blank	0.0143693	0.0143693	1
st 051910 zoo >243	0.381413907	27.05063248	0.0141
st 051910 zoo >500	0.617931545	46.81299591	0.0132
st 051910 zoo >500	0.593463182	43.31848526	0.0137
st 051910 zoo >500	0.734163165	53.20022964	0.0138
st 051910 zoo >1000	0.825935781	53.28617859	0.0155
st 051910 zoo >1000	1.250337481	73.11915588	0.017100001
st 051910 zoo >1000	1.865551472	128.6587219	0.0145
blank	0.513943315	0.513943315	1
mess 3	2.078545094	152.8342133	0.0136

Total Mercury Analysis DMA-80

Fourth Run	**Hg [ng]**	**R [µg/kg]**	**Weight [g]**
blank	0.138770282	0.138770282	1
mess	2.423219204	145.977066	0.0166
mess	1.381020904	107.0558853	0.0129
mess	2.031418085	113.4870453	0.017899999
blank	0.057198141	0.057198141	1
nt 060910 zoo >250	0.411997527	63.3842392	0.0065
nt 060910 zoo >500	1.268711448	96.84819794	0.0131
nt 060910 zoo >1000	1.385105491	116.3954239	0.0119
nt 060910 zoo >1000	1.244213104	129.605545	0.0096
nt 060910 zoo >1000	1.340176105	108.9574127	0.0123
blank	0.427801609	0.427801609	1
mess	1.454105735	106.1391068	0.0137
blank	0.134691864	0.134691864	1
st 060910 >250	0.57715112	59.50011826	0.0097
st 060910 zoo >500	0.538410604	56.67480087	0.0095
st 060910 zoo >500	0.54912442	56.61076736	0.0097
st 060910 zoo >500	0.528215826	57.41476059	0.0092
st 060910 zoo >1000	0.61181438	59.39945221	0.0103
st 060910 zoo >1000	0.622009695	51.83414078	0.012
st 060910 zoo >1000	0.767563105	58.1487236	0.0132
blank	0.017706711	0.017706711	1
mess	2.585595369	109.0968552	0.023700001

Total Mercury Analysis DMA-80

Fifth Run	**Hg [ng]**	**R [µg/kg]**	**Weight [g]**
blank	0.06127695	0.06127695	1
mess	1.849177718	128.4151154	0.0144
mess	1.986352205	109.7432251	0.018100001
mess	1.798019409	116.754509	0.0154
blank	0.051079899	0.051079899	1
nt 062910 zoo >250	1.168687582	88.53694153	0.0132
nt 062910 zoo >500	2.248729706	195.5417175	0.0115
nt 062910 zoo >500	2.246096134	190.3471375	0.0118
nt 062910 zoo >500	2.318499565	222.9326477	0.0104
nt 062910 zoo >1000	2.203602076	242.1540833	0.0091
nt 062910 zoo >1000	1.82871294	132.5154266	0.0138
nt 062910 zoo >1000	2.060102463	149.2827911	0.0138
blank	0.146927088	0.146927088	1
mess	1.683007836	120.2148514	0.014
blank	0.03068536	0.03068536	1
st 062910 zoo >250	0.69949621	55.95970154	0.0125
st 062910 zoo >500	1.591469884	78.39753723	0.020300001
st 062910 zoo >500	1.021762848	76.2509613	0.0134
st 062910 zoo >500	1.170728683	79.64141083	0.0147
st 062910 zoo >1000	1.162564635	90.12129211	0.0129
st 062910 zoo >1000	1.323839903	93.22816467	0.0142
st 062910 zoo >1000	1.214177608	97.91755676	0.0124
blank	0.117547177	0.117547177	1
mess	1.616000175	127.2441101	0.0127

Total Mercury Analysis DMA-80

Sixth Run	**Hg [ng]**	**R [µg/kg]**	**Weight [g]**
blank	0.195441723	0.195441723	1
nt 72810 zoop >1000	1.961326361	188.5890656	0.0104
nt 72810 zoop >1000	1.797994971	176.2740326	0.0102
nt 72810 zoop >1000	1.745743513	174.5743561	0.01
mess 3	3.102360964	111.1957397	0.027899999
blank	1E-05	1E-05	1
nt 72810 zoop >500	0.805444956	157.9303894	0.0051
nt 72810 zoop >500	0.96172303	192.3446045	0.005
nt 72810 zoop >500	0.971463799	202.3883057	0.0048
mess 3	2.75455451	109.7432098	0.0251
blank	1E-05	1E-05	1
mess 3	3.588589907	138.555603	0.025900001
blank	1.245343328	1.245343328	1
st 72810 zoop >1000	1.25494647	105.457695	0.0119
st 72810 zoop >1000 error	2.41725111	109.3778763	0.0221
st 72810 zoop >1000	1.290944695	89.03067017	0.0145
mess 3	2.8544662	112.8247528	0.0253
blank	3.485192776	3.485192776	1
st 72810 zoop >500	0.812000871	104.1026764	0.0078
st 72810 zoop >500	0.644585252	117.197319	0.0055
st 72810 zoop >500	0.580914557	69.15649414	0.0084
mess 3	4.393685341	171.6283417	0.025599999
blank	0.109455563	0.109455563	1
mess 3	2.962790966	113.9535065	0.026000001

Total Mercury Analysis DMA-80

Seventh Run	**Hg [ng]**	**R [µg/kg]**	**Weight [g]**
blank	0.850838125	0.850838125	1
mess	1.080717325	102.9254608	0.0105
blank	0.166760296	0.166760296	1
nt 92410 zoop >1000	0.643477023	257.3908081	0.0025
nt 92410 zoop >1000	0.957827389	290.2507324	0.0033
nt 92410 zoop >1000	0.598248899	299.1244812	0.002
blank	0.068484843	0.068484843	1
mess	0.98705399	99.7024231	0.0099
blank	0.098003879	0.098003879	1
nt 92410 zoop >500	0.687203646	214.7511444	0.0032
nt 92410 zoop >500	0.766625404	333.3153992	0.0023
nt 92410 zoop >500	0.539215684	269.6078491	0.002
blank	0.419826031	0.419826031	1
mess	1.326783895	127.5753708	0.0104
blank	0.354497463	0.354497463	1
blank	0.564292073	0.564292073	1
mess	1.32562983	106.9056396	0.0124
blank	0.094187371	0.094187371	1
mess	0.573940694	86.96070862	0.0066
blank	1E-05	1E-05	1
st 92410 zoop >1000	0.075109802	31.29575157	0.0024
st 92410 zoop >1000	0.165938571	50.2844162	0.0033
st 92410 zoop >1000	0.090371139	29.15198135	0.0031
blank	1E-05	1E-05	1
mess	0.350657761	36.91134262	0.0095
blank	1E-05	1E-05	1
st 92410 zoop >500	0.088463172	34.02429581	0.0026
st 92410 zoop >500	0.066456176	23.7343502	0.0028
st 92410 zoop >500	0.069388181	25.69932747	0.0027
blank	1E-05	1E-05	1
mess	0.476902306	80.8309021	0.0059

Total Mercury Analysis DMA-80

Eighth Run	**Hg [ng]**	**R [µg/kg]**	**Weight [g]**
blank	1E-05	1E-05	1
mess	0.813941777	79.79821777	0.0102
blank	1E-05	1E-05	1
nt 10810 zoop >500	1.174592495	192.5561523	0.0061
nt 10810 zoop >500	0.975360751	168.1656494	0.0058
nt 10810 zoop >500	0.845010281	150.8946991	0.0056
blank	1E-05	1E-05	1
mess	3.104245663	86.95365906	0.035700001
blank	1E-05	1E-05	1
nt 10810 zoop >1000	2.218818665	207.3662415	0.0107
nt 10810 zoop >1000	1.886757493	190.5815735	0.0099
nt 10810 zoop >1000	1.61206913	166.1927032	0.0097
blank	1E-05	1E-05	1
mess	3.173326969	87.90379333	0.0361
blank	1E-05	1E-05	1
blank	0.035074718	0.035074718	1
mess	1.755370855	90.95186615	0.019300001
blank	1E-05	1E-05	1
mess	2.028531075	85.23239899	0.023800001
blank	1E-05	1E-05	1
st 10810 zoop >1000	0.664747238	54.04449081	0.0123
st 10810 zoop >1000	0.756788373	68.17913055	0.0111
st 10810 zoop >1000	0.386363626	43.90495682	0.0088
blank	1E-05	1E-05	1
blank	1E-05	1E-05	1
mess	1.168719172	83.47994232	0.014
blank	1E-05	1E-05	1
st 10810 zoop >500	0.057947289	26.33967781	0.0022
st 10810 zoop >500	0.00958355	2.738157272	0.0035
st 10810 zoop >500	0.061760601	24.70423889	0.0025
blank	1E-05	1E-05	1
blank	1E-05	1E-05	1
mess	1.549596548	163.1154327	0.0095

Total Mercury Analysis DMA-80

Ninth Run	**Hg [ng]**	**R [µg/kg]**	**Weight [g]**
blank	1E-05	1E-05	1
mess	1.884120584	100.2191849	0.0188
blank	1E-05	1E-05	1
nt 82710 zoop >1000	1.482335567	156.0353241	0.0095
nt 82710 zoop >1000	1.332784653	180.1060333	0.0074
nt 82710 zoop >1000	1.764534831	169.6668091	0.0104
blank	1E-05	1E-05	1
mess	1.359050274	87.11860657	0.0156
blank	1E-05	1E-05	1
nt 82710 zoop >500	1.029074907	127.0462799	0.0081
nt 82710 zoop >500	0.931740105	109.6164856	0.0085
nt 82710 zoop >500	0.633812368	99.03318787	0.0064
blank	1E-05	1E-05	1
mess	1.485321999	85.36333466	0.0174
blank	1E-05	1E-05	1
nt 82710 zoop >250	0.14956075	29.32563972	0.0051
nt 82710 zoop >250	0.153382286	28.40412903	0.0054
nt 82710 zoop >250	0.105637997	28.55081177	0.0037
blank	1E-05	1E-05	1
mess	1.493004084	87.31018829	0.017100001
blank	1E-05	1E-05	1
mess	2.384656191	86.40058899	0.0276
blank	1E-05	1E-05	1
st 82710 zoop >1000	0.408290088	40.82901001	0.01
st 82710 zoop >1000	0.392913759	32.47220993	0.0121
st 82710 zoop >1000	0.308443993	32.4677887	0.0095
blank	1E-05	1E-05	1
mess	1.17187655	82.52651978	0.0142
blank	1E-05	1E-05	1
st 82710 zoop >500	0.227970749	36.76947784	0.0062
st 82710 zoop >500	0.164848879	28.42222023	0.0058
st 82710 zoop >500	0.134278104	25.33549118	0.0053
blank	1E-05	1E-05	1
mess	1.691953897	86.3241806	0.0196

Total Mercury Analysis DMA-80

Tenth Run	**Hg [ng]**	**R [µg/kg]**	**Weight [g]**
blank	0.113273472	0.113273472	1
blank	1.388561487	1.388561487	1
111210 nt zoo >1000	1.485112309	111.6625824	0.0133
111210 nt zoo >1000	1.100257039	137.532135	0.008
111210 nt zoo >1000	1.154039264	132.6481934	0.0087
blank	1E-05	1E-05	1
mess	3.18730855	103.8211288	0.0307
blank	1E-05	1E-05	1
111210 nt zoo >500	1.118237138	157.4981842	0.0071
111210 nt zoo >500	0.870270252	161.1611633	0.0054
111210 nt zoo >500	1.044742227	155.9316864	0.0067
blank	1E-05	1E-05	1
mess	1.627395511	87.9673233	0.0185
blank	1E-05	1E-05	1
111210 nt zoo >250	0.098003879	75.38759613	0.0013
111210 nt zoo >250	0.151471466	116.51651	0.0013
blank	0.00078892	0.00078892	1
mess	1.666336894	90.07226563	0.0185
blank	1E-05	1E-05	1
mess	1.908512831	87.14670563	0.0219
blank	0.00180363	0.00180363	1
111210 st zoo >1000	0.546929896	55.24544525	0.0099
111210 st zoo >1000	0.606763005	48.54104233	0.0125
111210 st zoo >1000	0.527647078	49.31281281	0.0107
blank	1E-05	1E-05	1
mess	3.209183216	90.65489197	0.035399999
blank	1E-05	1E-05	1
111210 st zoo >500	0.221387729	42.57456207	0.0052
111210 st zoo >500	0.153382286	29.49659348	0.0052
111210 st zoo >500	0.296206832	44.87982178	0.0066
blank	1E-05	1E-05	1
mess	1.494078994	90.00476074	0.0166
blank	1E-05	1E-05	1
111210 st zoo >250	1E-05	0.033333339	0.0003
111210 st zoo >250	1E-05	0.0125	0.0008
blank	1E-05	1E-05	1
mess	2.344338894	98.08950043	0.0239

Total Mercury Analysis DMA-80

11th Run	**Hg [ng]**	**R [µg/kg]**	**Weight [g]**
blank	1E-05	1E-05	1
mess	1.541707635	85.17722321	0.018100001
blank	1E-05	1E-05	1
st 051910 zoop >1000	0.482395411	29.77749443	0.0162
st 051910 zoop >1000	0.306526184	23.76172066	0.0129
st 051910 zoop >1000	0.496166348	28.19127083	0.0176
blank	1E-05	1E-05	1
mess	1.544336915	83.93135071	0.0184
blank	1E-05	1E-05	1
nt 62910 >1000 zoop	1.801086068	147.6300049	0.0122
nt 62910 >1000 zoop	2.087594032	144.971817	0.0144
nt 62910 >1000 zoop	1.793231368	153.2676392	0.0117
blank	1E-05	1E-05	1
mess	2.261815071	89.39980316	0.0253
blank	1E-05	1E-05	1
st 72810 >500 zoop	0.133597851	30.36314774	0.0044
st 72810 >500 zoop	0.029358501	13.34477329	0.0022
st 72810 >500 zoop	0.00078892	0.525944531	0.0015
blank	1E-05	1E-05	1
mess	2.126478672	87.87101746	0.0242
blank	1E-05	1E-05	1
st 111210 >500 zoop	0.686028242	92.70652008	0.0074
st 111210 >500 zoop	0.600238264	95.27591705	0.0063
st 111210 >500 zoop	0.528841078	62.21660233	0.0085
blank	0.217479914	0.217479914	1
mess	2.228850365	98.18724823	0.022700001

Total Mercury Analysis DMA-80

12th Run	**Hg [ng]**	**R [µg/kg]**	**Weight [g]**
blank	0.154298872	0.154298872	1
mess	3.870285273	90.21643829	0.0429
blank	0.021738069	0.021738069	1
st 092410 >1000	0.199267402	51.09420776	0.0039
st 092410 >1000	0.171730384	63.60385132	0.0027
st 092410 >1000	0.255973846	60.94615555	0.0042
blank	1E-05	1E-05	1
mess	2.777848721	104.8244781	0.0265
blank	0.130458325	0.130458325	1
nt 092410 zoo >500	0.709256291	186.6464081	0.0038
nt 092410 zoo >500	0.494886458	197.9545898	0.0025
blank	0.00927708	0.00927708	1
mess	3.159749031	91.32222748	0.034600001
blank	0.02745327	0.02745327	1
st 100810 zoop >1000	0.250947535	67.8236618	0.0037
st 100810 zoop >1000	0.224142507	50.94147873	0.0044
st 100810 zoop >1000	0.275853068	68.96327209	0.004
blank	0.0084056	0.0084056	1
mess	2.363691807	96.87261963	0.0244

Analysis 1

Calibration QA/QC

Analysis Type: MeHg

Method Number: 1630

Analyst Name: Brandon Reed

Bias and Precision

Type	Name/ID	Final Result	Units	Spike Units	Source Result	% REC	% REC Limit	Notes
Matrix Spike	NT12	0.98002	ng/L	1	0.069	91.09	71-125	accept
	STT	0.91602	ng/L	1	0.017	89.93	71-125	accept
	ST2	0.93522	ng/L	1	0.011	92.47	71-125	accept
	ST10	0.88712	ng/L	1	0.019	86.85	71-125	accept
Ongoing Precision and Recovery	OPR		ng/L	50		94.36	77-123	accept
	OPR		ng/L	50		96.25	77-123	accept
	OPR		ng/L	50		93.47	77-123	accept
	OPR		ng/L	50		99.57	77-123	accept

Calibration

QA Sample Type	Name/ID	Analyzed Result	Units	Spike Level	% REC	% REC Limit	Notes
Calibration	0.5	0.4667	pg	0.5	93.33	75-125	accept
	1	0.9636	pg	1	96.36	75-125	accept
	2	2.1164	pg	2	105.82	75-125	accept
	10	9.9290	pg	10	99.29	75-125	accept
	50	47.4308	pg	50	94.86	75-125	accept
	250	257.5720	pg	250	103.03	75-125	accept
	1000	1094.2944	pg	1000	109.43	75-125	accept
Calibration Factor		0.000012	pg/PA				accept

Blank Summary

QA Sample Type	Name/ID	Analyzed Result	Units	Criteria	Notes
Calibration/Bubbler Blank	CB1	0.028465	pg	< 50	accept
	CB2	0.168181	pg	< 50	accept
	CB3	0.027615	pg	< 50	accept
Average		0.074754	pg	< 25	accept
Method Blank A	MB1	0.200771	pg	< 0.5	accept
	MB2	0.321869	pg	< 0.5	accept
Average		0.261320	ng/L		
Method Blank B	MB3	0.168963	pg	< 0.7	accept
	MB4	0.012392	pg	< 0.7	accept
Average		0.090678	ng/L		

Analysis 1
Samples

Run	Trap	Name/ID	Final Result	Notes
52	A	CR ST101309	26.741584	accept
53	B	CR ST101309	25.599108	accept
54	C	CR ST101309	27.037967	accept
58	A	CR ST111209	57.085228	accept
59	B	CR ST111209	59.014995	accept
60	C	CR ST111209	59.901418	accept
20	B	NT12	0.069117	accept
22	A	NT13	0.099913	accept
23	B	NT14	0.065920	accept
25	A	NT14.5	0.074406	accept
24	C	NT14DUPE	0.066995	accept
40	A	ST10	0.018640	accept
34	A	ST2	0.010523	accept
36	C	ST4	0.009402	accept
37	A	ST6	0.052290	accept
38	B	ST6DUPE	0.009615	accept
39	C	ST8	0.012870	accept
26	B	STT	0.016766	accept
61	A	TU	131.270040	accept
62	B	TU	143.094480	accept
63	C	TU	137.800810	accept
49	A	UN ST101309	27.296839	accept
50	B	UN ST101309	26.606973	accept
51	C	UN ST101309	28.292814	accept
55	A	UN ST111209	49.649673	accept
56	B	UN ST111209	51.952734	accept
57	C	UN ST111209	53.525976	accept
46	A	UTORT	124.455010	accept
47	B	UTORT	128.493470	accept
48	C	UTORT	129.694320	accept

Analysis 2	Analysis Type:	MeHg	
Calibration QA/QC	Method Number:	1630	
	Analyst Name:	Brandon Reed	

Bias and Precision							
Type	Name/ID	Final Result	Units	Spike Units	% REC	% REC Limit	Notes
Ongoing Precision and Recovery	OPR		ng/L	50	96.763739	77-123	accept
	OPR		ng/L	50	80.182706	77-123	accept
Calibration QA Sample Type	Name/ID	Analyzed Result	Units	Spike Level	% REC	% REC Limit	Notes
Calibration	0.5	0.50016787	pg	0.5	100.03357	75-125	accept
	1	0.94833335	pg	1	94.833335	75-125	accept
	2	2.1361094	pg	2	106.80547	75-125	accept
	10	10.031712	pg	10	100.31712	75-125	accept
	50	46.624017	pg	50	93.248035	75-125	accept
	250	255.73088	pg	250	102.29235	75-125	accept
	1000	1038.7076	pg	1000	103.87076	75-125	accept
Calibration Factor		1.29693E-05	pg/PA				accept
Calibration Date		39154					
Blank Summary QA Sample Type	Name/ID	Analyzed Result	Units			Criteria	Notes
Calibration/Bubbler Blank	CB1	0.13574917	pg			< 50	accept
	CB2	0.046922755	pg			< 50	accept
	CB3	0.18381321	pg			< 50	accept
Average		0.12216171	pg			< 25	accept

Analysis 2
Samples

Run	Trap	Name/ID	Final Result	Notes
18	C	METHOD BLANK	0	accept
19	A	METHOD BLANK	0	accept
20	B	METHOD BLANK	0	accept
24	C	NT 10/13/09	68.18853	accept
25	A	NT 10/13/09	84.207856	accept
26	B	NT 10/13/09	82.282475	accept
30	C	NT 10/29/09	45.02117	accept
31	A	NT 10/29/09	39.706651	accept
32	B	NT 10/29/09	46.657863	accept
36	C	NT 9/24/09	64.269567	accept
37	A	NT 9/24/09	75.467845	accept
38	B	NT 9/24/09	68.837053	accept
33	C	NT SPIKE 10/29/09	101.0959	accept
34	A	NT SPIKE 10/29/09	101.25944	accept
35	B	NT SPIKE 10/29/09	89.849407	accept
27	C	ST 10/13/09	22.954832	accept
28	A	ST 10/13/09	22.560094	accept
29	B	ST 10/13/09	21.520882	accept
40	A	ST 10/29/09	38.687874	accept
41	B	ST 10/29/09	39.361983	accept
42	C	ST DUPE 10/29/09	45.473765	accept
43	A	ST DUPE 10/29/09	45.336539	accept
44	B	ST DUPE 10/29/09	45.136361	accept
39	C	ST10/29/09	37.490999	accept
21	C	TORT 3/13/11	125.73713	accept
22	A	TORT 3/13/11	116.73487	accept
23	B	TORT 3/13/11	135.03667	accept
45	C	TORT SMALL	144.02955	accept
46	A	TORT SMALL	140.55888	accept
47	B	TORT SMALL	141.46913	accept

Analysis 3	Analysis Type:	MeHg	
Calibration QA/QC	Method Number:	1630	
	Analyst Name:	Brandon Reed	

Bias and Precision								
Type	Name/ID	Final Result	Units	Spike Units	Source Result	% REC	% REC Limit	Notes
Matrix Spike Duplicate	ST 72810 A	130.77981	ng/L	50	69.000128	123.55936	71-125	accept
	ST 72810 A	131.17699	ng/L	50	69.000128	124.35373	71-125	accept
Ongoing Precision and Recovery	OPR		ng/L	50		97.679898	77-123	accept
	OPR		ng/L	50		104.34473	77-123	accept
	OPR		ng/L	50		86.964809	77-123	accept
	OPR		ng/L	50		84.560391	77-123	accept
	OPR		ng/L	50		98.134036	77-123	accept
Calibration								
QA Sample Type	Name/ID	Analyzed Result	Units	Spike Level		% REC	% REC Limit	Notes
Calibration	0.5	0.57344654	pg	0.5		114.68931	75-125	accept
	1	0.86772827	pg	1		86.772827	75-125	accept
	2	2.1039838	pg	2		105.19919	75-125	accept
	10	9.4435141	pg	10		94.435141	75-125	accept
	50	54.954312	pg	50		109.90862	75-125	accept
	250	220.09488	pg	250		88.037953	75-125	accept
	1000	1086.4621	pg	1000		108.64621	75-125	accept
Calibration Factor		1.65998E-05	pg/PA					accept
Calibration Date		39154						
Blank Summary								
QA Sample Type	Name/ID	Analyzed Result	Units				Criteria	Notes
Calibration/Bubbler Blank	CB1	0	pg				< 50	accept
	CB2	0	pg				< 50	accept
	CB3	0.064423927	pg				< 50	accept
Average		0.021474642	pg				< 25	accept
Method Blank B	METH B	2.1260615	pg				< 0.7	accept
	METH B	0.70111581	pg				< 0.7	accept
	METH B	0.14160205	pg				< 0.7	accept
Average		0.98959312	ng/L					

Analysis 3
Samples

Run	Trap	Name/ID	Final Result	Notes
22	A	31910 TORT 2	187.82719	accept
23	B	31910 TORT 2	180.38388	accept
21	C	31910 TORT2	190.97826	accept
24	C	NT 72810	174.57495	accept
25	A	NT 72810	174.00244	accept
26	B	NT 72810	176.33404	accept
37	A	NT 81110	175.94375	accept
38	B	NT 81110	172.30077	accept
39	C	NT 81110	174.98719	accept
47	B	NT 82710	162.30575	accept
48	C	NT 82710	170.42983	accept
49	A	NT 82710	167.67919	accept
50	B	NT 82710 DUPE	179.64651	accept
51	C	NT 82710 DUPE	182.21704	accept
52	A	NT 82710 DUPE	181.67979	accept
63	C	NT 92410	191.73312	accept
64	A	NT 92410	194.86828	accept
65	B	NT 92410	185.18597	accept
32	B	ST 72810	68.430058	accept
33	C	ST 72810	69.022475	accept
31	A	ST 72810 A	69.000128	accept
40	A	ST 81110	42.268964	accept
41	B	ST 81110	43.417876	accept
42	C	ST 81110	45.420341	accept
55	A	ST 82710	30.185845	accept
53	B	ST 82710	30.392224	accept
54	C	ST 82710 A	30.957333	accept
66	C	ST 92410	37.667672	accept
67	A	ST 92410	36.720072	accept
68	B	ST 92410	35.567633	accept

Analysis 4		Analysis Type:	MeHg
Calibration QA/QC		Method Number:	1630
		Analyst Name:	Brandon Reed

Bias and Precision							
Type	Name/ID	Final Result	Units	Spike Units	% REC	% REC Limit	Notes
Ongoing Precision and Recovery	OPR		ng/L	50	107.56847	77-123	accept
	OPR		ng/L	50	21.416305	77-123	reject
Calibration QA Sample Type	Name/ID	Analyzed Result	Units	Spike Level	% REC	% REC Limit	Notes
Calibration	0.5	0.41473237	pg	0.5	82.946474	75-125	accept
	1	0.87563414	pg	1	87.563414	75-125	accept
	2	1.9927068	pg	2	99.635338	75-125	accept
	10	11.034118	pg	10	110.34118	75-125	accept
	50	55.499472	pg	50	110.99894	75-125	accept
	250	171.39811	pg	250	68.559246	75-125	reject
	1000	1188.3198	pg	1000	118.83198	75-125	accept
Calibration Factor		1.313E-05	pg/PA				accept
Calibration Date		39154					
Blank Summary QA Sample Type	Name/ID	Analyzed Result	Units			Criteria	Notes
Calibration/Bubbler Blank	CB1	0.00014443	pg			< 50	accept
	CB2	0.028636491	pg			< 50	accept
	CB3	0.57803434	pg			< 50	accept
Average		0.20227175	pg			< 25	accept
Method Blank B		0.40382136	pg			< 0.7	accept
		0.0392674	pg			< 0.7	accept
		-0.1131323	pg			< 0.7	accept
Average		0.10998549	ng/L				

Analysis 4
Samples

Run	Trap	Name/ID	Final Result	Notes
24	C	NT 51910	16.54	accept
25	A	NT 51910	38.41	accept
26	B	NT 51910	36.69	accept
30	C	NT 62910	36.19	accept
31	A	NT 62910	111.9	accept
32	B	NT 62910	105	accept
27	C	ST 51910	7.427	accept
28	A	ST 51910	18.68	accept
29	B	ST 51910	17.48	accept
33	C	ST 62910	15	accept
34	A	ST 62910	54.27	accept
35	B	ST 62910	48.79	accept
21	C	TORT	70.17	accept
22	A	TORT	145.8	accept
23	B	TORT	137.7	accept

Analysis 5	Analysis Type:	MeHg
Calibration QA/QC	Method Number:	1630
	Analyst Name:	Brandon Reed

Bias and Precision							
Type	Name/ID	Final Result	Units	Spike Units	% REC	% REC Limit	Notes
Matrix Spike	STT	1.1052074	ng/L	1	109.29567	71-125	accept
	ST8	1.0738719	ng/L	1	105.5979	71-125	accept
	ST 11/12/09 A	103.06845	ng/L	50	79.149415	71-125	accept
Matrix Spike Duplicate	ST 11/12/09 A	108.20889	ng/L	50	89.430296	71-125	accept
	ST 11/12/09 A	106.87917	ng/L	50	86.770859	71-125	accept
Ongoing Precision and Recovery	OPR		ng/L	50	88.862842	77-123	accept
	OPR		ng/L	50	93.562143	77-123	accept
	OPR		ng/L	50	88.813076	77-123	accept
	OPR		ng/L	50	97.180811	77-123	accept
Calibration							
QA Sample Type	Name/ID	Analyzed Result	Units	Spike Level	% REC	% REC Limit	Notes
Calibration	0.5	0.25793287	pg	0.5	51.586573	75-125	reject
	1	0.88298462	pg	1	88.298462	75-125	accept
	2	2.0018881	pg	2	100.0944	75-125	accept
	10	11.17158	pg	10	111.7158	75-125	accept
	50	48.468186	pg	50	96.936372	75-125	accept
	250	251.11593	pg	250	100.44637	75-125	accept
	1000	1,056.94	pg	1000	105.69356	75-125	accept
Calibration Factor		1.38164E-05	pg/PA				accept
Calibration Date		3/14/2011					
Blank Summary							
QA Sample Type	Name/ID	Analyzed Result	Units			Criteria	Notes
Calibration/Bubbler Blank	CB1	0.19685538	pg			< 50	accept
	CB2	0.13639503	pg			< 50	accept
	CB3	0.18471081	pg			< 50	accept
Average		0.17265374	pg			< 25	accept
Method Blank A	MB1	0.18560427	pg			< 0.5	accept
	MB2	0.30220046	pg			< 0.5	accept
Average		0.24390236	ng/L				
Method Blank B	MB	0.98970213	pg			< 0.7	accept
	MB	-0.17265374	pg			< 0.7	accept
	MB	-0.17265374	pg			< 0.7	accept
Average		0.21479822	ng/L				

Analysis 5
Samples

Run	Trap	Name/ID	Final Result	Notes
51	C	NT 5/7/10	27.642048	accept
52	A	NT 5/7/10	26.813294	accept
53	B	NT 5/7/10	26.457984	accept
54	C	NT 5/7/10 DUPE	27.453347	accept
55	A	NT 5/7/10 DUPE	27.384173	accept
56	B	NT 5/7/10 DUPE	26.766042	accept
42	C	ST 11/12/09	0.0027059	accept
43	A	ST 11/12/09	65.701924	accept
41	B	ST 11/12/09 A	63.493742	accept
57	C	ST 5/7/10	26.829839	accept
58	A	ST 5/7/10	26.887236	accept
59	B	ST 5/7/10	26.497914	accept
38	B	ST 9/24/09	114.4567	accept
39	C	ST 9/24/09	98.063198	accept
40	A	ST 9/24/09	97.066833	accept
22	A	ST2	0.0064569	accept
23	B	ST4	0.0063953	accept
24	C	ST4DUPE	0.0076123	accept
25	A	ST6	0.0115583	accept
26	B	ST8	0.0178929	accept
20	B	STT	0.0122508	accept
35	B	TORT 3/16	152.30865	accept
36	C	TORT 3/16	157.65591	accept
37	A	TORT 3/16	158.8145	accept

Thermoclines (m)		
	North Twin	**South Twin**
5/16/2009	8	7
5/28/2009	5	5
6/10/2009	5	6
6/29/2009	5	7
7/22/2009	5	7
8/12/2009	6	7
8/29/2009	7	X
9/3/2009	6	8
9/24/2009	7	9
10/8/2009	Isothermic	11
10/17/2009	Isothermic	11
10/29/2009	Isothermic	13
4/21/2010	4	3
5/6/2010	x	8
6/8/2010	6	7
6/28/2010	5	5
7/13/2010	5	6
7/27/2010	5	6
8/10/2010	5	7
8/26/2010	6	8
9/9/2010	6	9
9/23/2010	7	9
10/7/2010	7	10

North Twin Volume Data				
Depth (m)	*Surface Area of Plane (m2)*	*Volume Below Plane (m3)*	*Depth Interval m*	*Volume per 1 m strata (m3)*
0	3,155,645	32,371,943	0-1	3,102,651
1	3,034,020	29,269,292	1-2	2,951,090
2	2,880,569	26,318,202	2-3	2,816,424
3	2,760,818	23,501,778	3-4	2,711,328
4	2,669,160	20,790,450	4-5	2,624,315
5	2,584,196	18,166,135	5-6	2,540,368
6	2,501,738	15,625,767	6-7	2,458,214
7	2,417,153	13,167,553	7-8	2,366,080
8	2,313,680	10,801,472	8-9	2,246,577
9	2,179,936	8,554,896	9-10	2,108,602
10	2,035,861	6,446,294	10-11	1,945,290
11	1,845,931	4,501,004	11-12	1,707,657
12	1,552,675	2,793,348	12-13	1,367,812
13	1,168,329	1,425,536	13-14	962,655
14	741,659	462,881	14-15	449,626
15	122,012	13,254	15-bottom	13,254

South Twin Volume Data				
Depth (m)	*Surface Area of Plane (m2)*	*Volume Below Plane (m3)*	*Depth Interval m*	*Volume per 1 m strata (m3)*
0	3,867,057	35,380,989	0-1	3,791,448
1	3,690,662	31,589,540	1-2	3,571,962
2	3,460,610	28,017,578	2-3	3,352,845
3	3,256,649	24,664,733	3-4	3,164,162
4	3,073,674	21,500,571	4-5	2,984,095
5	2,911,015	18,516,476	5-6	2,832,795
6	2,759,608	15,683,680	6-7	2,678,264
7	2,593,865	13,005,417	7-8	2,478,316
8	2,360,168	10,527,100	8-9	2,252,584
9	2,145,566	8,274,516	9-10	2,041,121
10	1,940,189	6,233,396	10-11	1,833,617
11	1,722,523	4,399,779	11-12	1,585,412
12	1,431,149	2,814,366	12-13	1,230,703
13	1,002,119	1,583,663	13-14	805,197
14	635,091	778,466	14-15	495,272
15	352,156	283,194	15-16	229,548
16	123,478	53,646	16-bottom	53,646