ZEBRA MUSSEL (DREISSENA POLYMORPHA) PROMOTION OF CYANOBACTERIA IN LOW-NUTRIENT LAKES AND THE SUBSEQUENT PRODUCTION AND FATE OF MICROCYSTIN

M. Megan Woller-Skar

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Committee:

Rex L. Lowe, Advisor

Dawn L. Anderson
Graduate Faculty Representative

Karen V. Root

Dan M. Pavuk

George S. Bullerjahn
ABSTRACT

Rex L. Lowe, Advisor

The ability of established populations of the non-native zebra mussel (*Dreissena polymorpha*) to influence phytoplankton communities and promote *Microcystis aeruginosa*, a potentially toxic cyanobacterium, has been reported by Fahnenstiel *et al.* (1995), Vanderploeg *et al.* (2002) and others, in the Great Lakes region.

This study documents changes following zebra mussel establishment in six low-nutrient inland lake basins in northwest lower Michigan (Leelanau County). Shifts in phytoplankton communities that occurred only in basins with zebra mussels included declines in spring diatoms and chrysophytes prior to blooms of cyanobacteria. Decreases in these taxa support a competitive release hypothesis for *M. aeruginosa* dominance. It should also be noted that study basins did not experience increases in phosphorus or summer temperatures.

*M. aeruginosa* proliferation may be related to zebra mussel filtering behavior, a combination of total zebra mussels and lake morphology. Zebra mussel populations were estimated using underwater video, *M. aeruginosa* densities were quantified from surface water and lake bathymetry and basin-wide zebra mussel densities were estimated using ArcGIS. Underwater video ground-truthed using SCUBA was an effective, yet labor intensive method to estimate zebra mussel populations, and kernel interpolation provided acceptable zebra mussel density estimates basin-wide. The relationship between *M. aeruginosa* density and zebra mussel filtering capacity was not significant, however the sample size may have been an issue.
Microcystin (MC), the hepatotoxin produced by *M. aeruginosa* and other cyanobacteria, was measured before and after blooms of *M. aeruginosa* over depths and across seasons in whole water, sediment, macroinvertebrates, bivalves and fishes, using enzyme linked immunosorbent assay. A subset of sediment and *Hexagenia* spp. samples were analyzed using high performance liquid chromatography and mass spectrometry for microcystin-LR. MC was present in all components, including spring samples prior to *M. aeruginosa* blooms and exceeded the recreational and total daily intake guidelines of the World Health Organization in whole water (on at least one occasion) and in fish white muscle, for consumption by men, women and children. *Hexagenia* spp. and walleye contained the highest concentrations of MC. MC should be monitored in these lake basins because of the potential risk of chronic sub-lethal exposure to humans.
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**ZEBRA MUSSELS IN THE GREAT LAKES BASIN**

The zebra mussel (*Dreissena polymorpha*) (Pallas) was first identified in Lake St. Clair in 1988 (Herbert *et al.* 1991) and was believed to have been introduced in 1986 in ballast water from an international freighter originating in the Black, Caspian and Aral Sea region of Eurasia (Herbert *et al.* 1991; Johnson and Padilla 1996; Strayer 1999). In the two years following introduction, the zebra mussel expanded its range to include all of Lake Erie and the western basin and southern shoreline of Lake Ontario (Griffiths 1993). By 1992, smaller populations were found as far west as Duluth, Minnesota, and as far east as the St. Lawrence River at Cornwall, Ontario (Neary and Leach 1992) and by the mid-1990’s, the zebra mussel had been reported in all of the Great Lakes and the Mississippi, Ohio, Illinois and Hudson rivers (Padilla *et al.* 1996). Such dispersal occurs primarily through transient boater activity, with secondary transport by wildlife (i.e., diving ducks) and humans (i.e., bait buckets) (Carlton 1993). The expansion of zebra mussel range is also facilitated by the free swimming, planktonic larval stage and to a smaller degree postmetamorphic drifting, or passive drifting through the water using the foot and byssal thread (Griffiths 1993; Martel 1993).

The life expectancy of the zebra mussel is three to five years (Ludyanskiy *et al.* 1993), and they reach sexual maturity after approximately one year (Stanczykowska 1977; Strayer 1991). Spawning at a few months of age is possible if water temperatures remain above 12°C throughout spring and summer (Mackie 1991, Strayer 1999). Fertilization is external and gametes are shed into the water (Strayer 1999); although rare, hermaphroditism has been reported (Ludyanskiy *et al.* 1993). Females are quite fecund, releasing in excess of one million eggs in each spawning event (Sprung 1993). If eggs are fertilized, resulting veliger larvae are 80 – 220 μm in length (Strayer 1999) and require an additional 1–9 weeks to develop depending on
temperature (Martel et al. 1995). Veligers consume mainly small phytoplankton (Strayer 1999) and attach to hard surfaces using proteinaceous threads secreted from the byssus (Ludyanskiy et al. 1993).

Hard surfaces associated with industrial, municipal and hydroelectric plants support high densities of zebra mussels (Kilgour and Mackie 1993; MacIsaac 1996). Replenishment of food, removal of waste and lack of predators often result in densities leading to the biofouling of intake pipes and lines (MacIsaac 1996). Zebra mussels also readily colonize other human-made structures such as marine buoys, fishing nets and boat docks (MacIsaac 1996).

In addition to biofouling of human surfaces and structures, colonization by zebra mussels can result in mortality of native species (unionids, Gillis and Mackie 1994, Schloesser et al. 1996; dragonfly larvae, Fincke et al. 2009).
GEOGRAPHIC LOCATION

Research was conducted on six dimictic, low nutrient lake basins located in Leelanau County, Michigan (45.000 latitude, -86.001 longitude), northwest of Traverse City (Figure 1) from 1989 to 2004. Watersheds draining into these basins contain primarily deciduous forest, with the exception of South Lake Leelanau (SLL), which includes deciduous forest, agriculture and cedar swamp. Maintained boat launches for lake basins are separated by less than 20 km and transient boater activity is common.

Zebra mussels in Leelanau County, were first reported in SLL in 1996 (Keilty and Woller, 2004). Zebra mussel populations were monitored closely from introduction through establishment with artificial substrates and underwater video (Keilty and Woller, 2002 and 2004) and within six years were found in all study basins (Table 1). The population of zebra mussels was considered established three years after zebra mussel introduction, when adult individuals were found at both the inflow(s) and outflow(s) of basins. The transitional period refers to the time after introduction, but prior to establishment. Lake basins were separated into two groups to compare changes following zebra mussel establishment. Spatial control basins did not have established populations of zebra mussels during the sampling period and include Big Glen Lake (BGL), Little Glen Lake (LGL) and Lime Lake (LL). Zebra mussel effect basins were those where zebra mussel populations were established during the sampling period; such basins include Little Traverse Lake (LTL), North Lake Leelanau (NLL) and South Lake Leelanau (SLL).
PROJECT PURPOSE

The purpose of this project was to describe how the establishment of zebra mussels influenced the phytoplankton communities and production and fate of microcystin in six, low-nutrient lake basins in northern Michigan. Chapter one documents shifts in phytoplankton, including, decreases in diatoms and chrysophytes as well as increases in cyanobacteria following zebra mussel establishment; chapter two utilizes underwater video to estimate zebra mussel populations, and uses a suite of spatial statistics in an attempt to describe the relationship between zebra mussel population filtering capacity and density of Microcystis aeruginosa; and finally chapter three quantifies the production and fate of microcystin in various components of these lake basins, including water, sediment, macroinvertebrates, bivalves and fishes over depths and seasons.
CHAPTER I: INTRODUCTION

Trophic status in lakes ranges from oligotrophic systems that contain low concentrations of phosphorus, nitrate and chlorophyll-a to eutrophic systems that contain high levels of nutrients and chlorophyll-a (Wetzel and Likens 2000). The range of nutrients is paralleled by growth of phytoplankton and vascular plants, with high plant growth characteristic of eutrophic systems. As these organisms die, organic matter accumulates on the lake bottom, where it is broken down by bacteria. The bacteria can consume large amounts of oxygen thereby depleting available oxygen in the hypolimnion. Oligotrophic lake basins, with less nutrients and organic matter, tend to be saturated with oxygen from the epilimnion to the hypolimnion.

Dominant phytoplankton are determined primarily by trophic status in lentic environments. Greater amounts of diatoms and green algae are common in oligotrophic basins, as they are superior competitors for phosphorus, and oligotrophic environments provide sufficient silica and nitrate. Silver and Chock (1986) demonstrated that chrysophytes can also dominate oligotrophic systems. High densities of cyanobacteria are more common in eutrophic systems, as other phytoplankton outcompete cyanobacteria for limited phosphorus in oligotrophic environments (Wetzel and Likens 2000). With eutrophication, as densities of diatoms and green algae increase, they consume available silica and nitrogen. In the absence of ample silica and nitrogen, these divisions decline. The newly accessible phosphorus and potential for nitrogen fixation allow cyanobacteria to become dominant (Wetzel and Likens 2000).

Established populations of zebra mussels alter aquatic communities through filtering behavior (Bastviken et al. 1998). Zebra mussels are efficient filter feeders, with adults capable of filtering over one liter of water per individual per day (Reeders and bij de Vaate 1990;
Stegemann 1992). The rate of filtration is a function of food size and availability (Reeders et al. 1993) however further research is necessary to rule out taste and/or toxicity selection. The gills are also responsible for respiration and ion regulation; therefore, larger quantities of water are filtered than necessary for feeding purposes alone (Padilla et al. 1996). Particles that are too large, along with some suspended sediments are sorted, enveloped in mucus and rejected as pseudofeces (Padilla et al. 1996; Vanderploeg et al. 2001). Ten Winkel and Davids (1982) reported that zebra mussels consumed mainly small phytoplankton between the sizes of 15 and 40 μm, but are capable of ingesting particles up to 750 μm in size. More recently, zebra mussels have been shown to consume a greater range of plankton including small zooplankton, bacteria and detritus (MacIsaac et al. 1991; Silverman et al. 1996; Vanderploeg et al. 2002). With established populations of zebra mussels in excess of 342,000 individuals per square meter (Leach 1993), zebra mussels have the potential to remove large amounts of seston on a lake-wide basis. In fact, declines in the chlorophyll of Saginaw Bay (Fahnenstiel et al. 1995) and of the phytoplankton biomass in Lake Erie (Leach 1993; Nichols and Hopkins 1993), occurred following zebra mussel establishment. In addition, dramatic increases in water clarity following zebra mussel establishment were reported in the Great Lakes (Holland 1993; MacIsaac 1996), inland lakes (Keilty and Woller 2002) and streams (Strayer 1991), which provide additional evidence for large-scale seston removal.

Zebra mussels promote many species of cyanobacteria by consuming and removing competitors (Fahnenstiel et al. 1995; Vanderploeg et al. 2001). In fact, zebra mussels are capable of consuming large amounts of green algae and diatoms while selectively rejecting the cyanobacterium Microcystis aeruginosa (Kutzing) Kutzing (Vanderploeg et al. 2001). In
southwest Michigan, blooms of cyanobacteria including *M. aeruginosa*, have been linked to zebra mussel occurrence (Raikow *et al.* 2004).

The increase in underwater light penetration by zebra mussels may indirectly promote blooms of *M. aeruginosa*. Since cyanobacteria in general are adapted to photosynthesize under low light conditions, compared to other phytoplankton (Mur *et al.* 1999), the amount of light may not be the limiting factor for *M. aeruginosa* that it may be for diatoms and green algae. Although *M. aeruginosa* can control its buoyancy and suspend under ideal photosynthetic conditions (Mur *et al.* 1999), an increase in water clarity could further increase photosynthetic output of *M. aeruginosa*.

In oligotrophic systems with established populations of zebra mussels, large scale removal of diatoms and green algae through zebra mussel filtering behavior may provide the inferior competitor *M. aeruginosa* with access to limited phosphorus and nitrogen, resulting in cyanobacterial blooms in oligotrophic systems. Filtering by zebra mussels might therefore directly promote *M. aeruginosa* by selective rejection of *M. aeruginosa* and, indirectly through increased light penetration and removal of competitors (diatoms and green algae). If competitive release is one mechanism by which zebra mussel populations promote *M. aeruginosa* blooms, study basins with established zebra mussels should experience a decline in diatoms and green algae, followed by an increase in *M. aeruginosa*, while total phosphorus and nitrate concentrations and water temperatures decrease or remain the same.

There were two objectives of this project. First was to quantify cyanobacterial blooms in basins with and without established populations of zebra mussels; and second was to determine if diatoms, green algae and chrysophytes were declining prior to such blooms.
CHAPTER I: METHODS

All water samples and transparency determinations were collected from the deepest locations of lake basins from April through October (1990 to 2005). Water samples were collected using a Wildco two liter vertical water sampler. Water transparency was estimated using standard limnological methods, lowering a secchi disk.

Samples for nutrient analyses were collected from the surface and one meter from the lake bottom. Subsamples of water were distributed into acid-washed and pre-acidified glass 250 ml bottles supplied by the Great Lakes Environmental Center (GLEC, Traverse City, MI). Samples from one round were collected in triplicate for quality control. Bottles were maintained on ice, or refrigerated until transport to GLEC for analyses.

Dissolved oxygen and temperature were determined using a Hydrolab, which was calibrated following the manufacturers recommendations, at one or five meter increments from the surface to the lake bottom.

Composite water samples of 50 ml were used to quantify chlorophyll-a, with equal fractions (approximately 16 ml) from the surface, secchi depth and twice the secchi depth. Samples were pre-filtered (0.45 μm), wrapped in foil and frozen upon return from the field.

Phytoplankton assemblages were determined from 870 ml whole water samples and preserved using 30 ml of formalin. Samples collected in 1993 were composites with one liter collected from the surface, the secchi depth and twice the secchi depth. From 2001 through 2004, separate samples were collected from the surface and, prior to thermal stratification, twice the secchi depth. Following thermal stratification, the deeper sample was collected from a depth of one meter above the thermocline. Samples collected in 1993 were analyzed by Dr. Jan Stevenson (Michigan State University, East Lansing, MI). Samples collected from 2001 to 2004...
were analyzed by Dr. Rex Lowe (Bowling Green State University, Bowling Green, OH). All phytoplankton were evaluated using standard phytoplankton enumeration techniques. To compare phytoplankton assemblages collected in 1993 with data collected from later years, mean densities from the surface and twice the secchi depth were calculated for data collected from 2001 to 2004.

Concentrations of total phosphorus and nitrate/nitrite were compared using regression analyses from 1990 through 2004. Water temperature, secchi transparencies and chlorophyll-a concentrations before, during and after zebra mussel establishment in the months of June, July and August were compared using repeated measures ANOVA in Minitab 14. Some analyses of chlorophyll-a excluded LGL because its shallow nature predisposes it to mixing during summer months. Additionally, twice the secchi samples for phytoplankton enumeration were not collected from 2001 to 2004 in LGL because of depth. Therefore, phytoplankton comparisons from 1993 and 2001 to 2004 were not possible in LGL. Relative abundances of phytoplankton divisions based on densities were compared from 1993, 2002 and 2003. Colonies of *M. aeruginosa* were counted as “cells” for density estimates. Shifts in phytoplankton assemblages were identified using non-metric multidimensional scaling (NMDS), analysis of similarities and dissimilarity analyses in Primer 5, by comparing data collected in 1993, 2002 and 2003. The years were chosen based on similarity and completeness of sampling regimes so as to avoid bias of seasonality.
CHAPTER I: RESULTS

Although nutrient concentrations, chlorophyll-a and secchi transparency classified all basins in the oligotrophic range, summer dissolved oxygen (DO) concentrations dropped below two mg L\(^{-1}\) in most basins, which is uncharacteristic of oligotrophy (Table 2).

Total phosphorus concentrations declined in five of the six lake basins from 1990 to 2004 (Table 3). In BGL and LTL, significant declines were measured only in the epilimnion, whereas LL, NLL and SLL experienced declines in both the epilimnion and the hypolimnion (Figures 2 & 3). From 1990 to 2004, nitrate/nitrite did not change in basins other than LTL, where nitrate/nitrite decreased in the epilimnion (Figures 4 & 5). Mean temperature in the summer months (June, July, August) did not increase following zebra mussel establishment in LTL, NLL nor SLL (Figure 6).

Prior to zebra mussel establishment, summer secchi depth remained relatively constant in all lake basins (Figures 7 & 8). Once populations of zebra mussels were established however, dramatic increases in water clarity were observed in June and July in LTL and NLL (Table 4). June and July secchi transparencies in SLL increased following zebra mussel establishment, however they were not significant once Bonferroni corrections for multiple comparisons were applied. It should also be noted that June secchi transparencies in SLL were high relative to other basins even prior to zebra mussel establishment.

Chlorophyll-a concentrations decreased from 1993 to 2004 in all study basins (Table 5). Peaks of chlorophyll-a were those that exceeded the third quartile for each basin. In the absence of zebra mussels, peaks were expected in the spring and fall following turnover, since algal growth commonly accompanies the redistribution of nutrients. In BGL and LL, peaks of chlorophyll-a occurred primarily in spring or early summer and fall (Figure 9). Chlorophyll-a
peaks in zebra mussel effect basins, followed a similar pattern until zebra mussel establishment, after which they were more likely to occur in August (Figure 10). Sampling from April through September was fairly balanced in BGL and LL, however, sampling in LTL, NLL and SLL was more frequent in August post zebra mussel establishment when all three basins are considered (Figure 11).

Foam in the form of Langmuir streaks was and continues to be common in all study lake basins. Following the establishment of zebra mussel populations, massive foaming events coupled with the occurrence of a pollen-like substance floating in the top meter of the water were observed (Figure 12). The foam was concentrated in nearshore areas, but was also detected farther out in the basin. Upon closer observation, the pollen-like substance was primarily *M. aeruginosa* (Rex Lowe, personal communication).

*M. aeruginosa* was entirely absent from surface samples in BGL and in very low (less than 400 cells/ml) densities in LGL and LL from 2001 to 2004, in contrast to zebra mussel effect basins, where annual maxima ranged from 450 to 4450 cells ml\(^{-1}\) (Figure 13). When composite samples were compared from years 1993, 2002 and 2003, the relative abundance of Cyanophytes in BGL was greater than expected compared to Bacillariophyta, Chlorophyta and Chrysophyta (Figure 14). A large of bloom of *Chroococcus minimus* (Keis. ex Lemmermann) occurred in BGL in 2002 with densities greater than 62,000 cells ml\(^{-1}\) at the surface. Although blooms of cyanobacteria were not detected in BGL in any other samples from 1993 or 2001 through 2004, this bloom was measured on three dates and over two depths in BGL. Relative abundances of phytoplankton divisions in LL were characteristic of low nutrient basins, with Bacillariophyta Chlorophyta and Chrysophyta dominance from spring through fall. Despite the influence of *C. minimus* on relative abundances, *M. aeruginosa* in composite samples was absent in BGL and
found only in very small densities (20 cells ml\(^{-1}\)) in LL. *Chroococcus* spp. and *Aphanocapsa* spp. were measured in LTL, NLL and SLL prior to zebra mussel establishment and account for Cyanophytes in 1993 (Figure 15). Similar to control basins, *M. aeruginosa* was only found in very low densities (<55 cells ml\(^{-1}\), LTL) before zebra mussel establishment, and increased in all zebra mussel effect basins in 2002 and 2003 (Figure 15).

The estimated biovolume of *M. aeruginosa* colonies was 65 um\(^3\) compared to 5 um\(^3\) for *Chroococcus* spp. and 4 um\(^3\) for *Aphanocapsa* spp. in Leelanau lake basins. Because of the large range of biovolumes and because *M. aeruginosa* “cells” were actually colonies, it may be more appropriate to examine phytoplankton dominance from a biovolume rather than density or relative abundance standpoint. Although the biovolume of *Chroococcus* spp. in BGL was quite large in 2002, other cyanobacteria were absent in this basin, and contributions were minimal in LL (Figure 16). All zebra mussel effect basins experienced biovolume increases in *M. aeruginosa* as well as *Aphanocapsa* spp. and *Chroococcus* spp. from 1993, before zebra mussel establishment, to 2002 and 2003, following zebra mussel establishment (Figure 17).

NMDS ordination of composite phytoplankton from all basins indicated that assemblages from spatial controls (BGL and LL) were similar enough to zebra mussel effect basins (LTL, NLL and SLL) to be effective comparisons (Figure 18). Phytoplankton assemblages appear to have changed seasonally (spring/early summer to late summer early fall) and from 1993 to 2002/2003 in both spatial controls and zebra mussel effect basins. These changes were more evident in LTL, NLL and SLL since fewer samples were collected in control basins, especially in spring/early summer of 2002 and 2003 (Figure 19). In fact, the small sample size likely contributes to the lack of significance comparing early seasonal changes from 1993 to 2002/2003 using ANOSIM (Table 6). Although shifts in phytoplankton assemblages appeared to have taken
place in both spatial control basins and following zebra mussel establishment, dissimilarity analyses comparing the changes from 1993 to 2002/2003 indicate that spatial control basins (BGL, LL) and zebra mussel effect basins (LTL, NLL, SLL) changed in different ways (Table 7). Basins without established populations of zebra mussels experienced decreases in early season *Cyclotella* spp. and *Synedra* spp., along with increases in three species of *Dinobryon* and penate diatoms, whereas zebra mussel establishment coincided with an increase in *Cyclotella* spp., *Aphanocapsa* spp. and *Chroococcus* spp., and decreases in other diatoms and chrysophytes (Table 7A). Spatial control basins experienced increases from 1993 to 2002/2003 late in the season in diatoms and notably *Chroococcus* spp. and *Aphanocapsa* spp. Zebra mussel effect basins contained increases in four cyanobacterial taxa (Table 7B).
CHAPTER I: DISCUSSION

Overall decreases in nutrient concentrations in all lakes since 1990 may be the result of improved land use practices by riparian landowners and lake management, or carryover from the reduction in phosphorus in synthetic detergents. It is unknown why lake basins with low available nutrients and low chlorophyll-a would result in clinograde profiles of DO. Other lakes in the region with similar nutrient, chlorophyll-a and DO concentrations, have slash deposits from timber harvesting in the early 1900’s, whose decomposition likely contributes to low summer DO (Rex Lowe, personal communication). A similar scenario may also exist for Leelanau lakes, as timber and sawdust were deposited in many underwater locations with harvesting (Leelanau Historical Society, Leland, MI, personal communication).

Conroy et al. (2005) demonstrated that established populations of zebra mussels can shift nutrients from the pelagic to the benthic zone via fecal and pseudofecal deposition. As a result, declines in epilimnetic nutrient concentrations, and increases in hypolimnetic concentrations were expected in basins with established populations of zebra mussels. General trends in nutrient concentrations over time were similar in all basins, and not related to the presence or timing of zebra mussel establishment. Since Leelanau lake basins have low summer hypolimnetic oxygen concentrations, zebra mussel colonization is unlikely at depths greater than the mean summer thermocline (Mackie et al. 1989). A shift in nutrients may occur in the littoral zones that support zebra mussel populations. If that is the case, data collected from the epilimnion and hypolimnion from the deepest location per basin may not reflect these nutrient changes in the littoral zone.

Initial concentrations of nutrients indicate all study lake basins are similar to oligotrophic systems. Decreased or constant concentrations of phosphorus and nitrate over time indicate that
eutrophication was not responsible for dominance of *M. aeruginosa*. In addition, when comparing years before and after zebra mussel establishment, water temperatures did not differ and thus were not responsible for increases in *M. aeruginosa*.

As water temperatures increase in spring and early summer, zebra mussel populations increase filtering behavior. Study basins historically experience blooms of diatoms at that time, and such densities are a likely food source for zebra mussel populations. Prior to zebra mussel establishment, June secchi water transparencies were relatively low in LTL and NLL, as would be expected with high densities of diatoms. Following zebra mussel establishment, secchi transparencies in June and July began to increase in LTL and NLL. Dramatic increases in water clarity was one of the first consequences reported for zebra mussels (Holland 1993; Fahnenstiel *et al.* 1995; and Heath *et al.* 1995) with increased transparency indicative of the removal of seston by growing zebra mussel populations (Fahnenstiel *et al.* 1995). In these basins, the increase in secchi transparencies in June and July may reflect removal of diatoms. This trend was not as clear in SLL however, even after zebra mussel establishment. There were fewer samples collected in SLL in June. The smaller sample size may have been an issue, since statistical comparisons pre and post zebra mussel establishment were significant prior to corrections for multiple comparisons.

The decrease in chlorophyll-a in all basins from 1993 to 2004, was not surprising giving the decrease in nutrients during that time period. Because of the overall decline in chlorophyll-a, it follows that fewer peaks, values greater than the third quartile, would occur in later years. Seasonal changes in chlorophyll-a were also not surprising since these lake basins are dimictic. In BGL and LL, peaks of chlorophyll-a were more frequent in spring and fall, and likely associated with the redistribution of nutrients of lake turnover. It appears that in basins with
established populations of zebra mussels, peaks of chlorophyll-a shift from April and September to August. Although there was an increase in sampling effort in August post zebra mussel establishment, it does not follow that a higher number of samples translate to more frequent peaks of chlorophyll-a. The occurrence of massive foaming events with surface blooms of *M. aeruginosa* were dramatic indicators that lakes with zebra mussels appeared to be changing in ways that lakes without zebra mussels were not. Comparisons of composite samples in 1993, 2002 and 2003 however, did not necessarily support the competitive release hypothesis for cyanobacterial dominance in low nutrient basins. A bloom of *Chroococcus* spp. occurred in BGL in September and October of 2002. In addition, cyanobacteria in the three zebra mussel effected basins were more dominant than expected in 1993, years before zebra mussels were introduced. In the five years of complete phytoplankton sampling, dominance by cyanobacteria in basins without established zebra mussel populations has not been recorded again, making the 2002 bloom in BGL difficult to explain. Although relative abundances of cyanobacteria were common in 1993, *M. aeruginosa* was not measured in the two basins of Lake Leelanau and was extremely low (less than 100 cells/ml) in LTL. In all lake basins, only three cyanobacterial taxa, *Aphanocapsa* spp., *Chroococcus* spp., and *M. aeruginosa*, were recorded in 1993, 2002 and 2003. The cellular biovolume of *M. aeruginosa* is much larger than either *Aphanocapsa* spp. or *Chroococcus* spp. (65 um³ compared to 4 um³ and 5 um³, respectively). Therefore a comparison of cyanobacterial biovolume by taxa in lake basins, over time and pre and post zebra mussel establishment might be more appropriate. Only composite samples were collected in 1993, and in order to compare pre (1993) and post (2002, 2003) zebra mussel establishment years, it was necessary to use composite samples. This is important since *M. aeruginosa* densities were
greatest at the water surface, whereas densities of *Aphanocapsa* spp. and *Chroococcus* spp. were similar at the surface and twice the secchi depth. Composite densities of *M. aeruginosa* thus appear to be less dominant in comparison.

Seasonal changes in the phytoplankton communities were expected in all basins, and support previous discussions of shifts in secchi transparency and chlorophyll-a. NMDS plots also indicate changes based on season and from 1993 to 2002/2003, in all basins. Shifts in phytoplankton communities over a nine year time period, although not surprising, neither support nor refute the competitive release hypothesis. The ways in which these lake basins, and treatment groups, have changed are of greatest interest. When comparing the communities in 1993 to 2002/2003 early in the season (May through July 15th), increases in diatoms and chrysophytes were largely responsible for the shift in phytoplankton. However, when zebra mussels were established by 2002/2003, changes in phytoplankton were largely the result of declining diatoms and chrysophytes, and increasing cyanobacteria. A decrease in the diatom, *Synedra* spp., occurred in both groups and therefore is unlikely to be related to zebra mussel establishment. It is possible that the general decline in early season diatoms and chrysophytes in LTL, NLL and SLL, is related to zebra mussel filtering capacity. *Aphanocapa* spp. and *Chroococcus* spp., were present early in the season in all three basins, when mean water temperatures ranged from 13.79 °C [NLL] to 14.42 °C [LTL] and 15.28 °C [SLL]. The presence of cyanobacterial taxa at such low water temperatures was surprising since Robarts and Zohary (1987) and Konopka and Brock (1978) both reported an optimal growth of 25 °C for the cyanobacteria *Aphanizomenon, Anabaena* and *Microcystis*. It is possible that *Aphanocapsa* spp. and *Chroococcus* spp. have lower growth optima than 25 °C and that growth can occur, at slower rates at temperatures less than 16 °C, or that other environmental parameters such as increased
light availability play a role in their occurrence early in the season. Late in the season (July 16th through October 15th), changes in spatial control basins were mainly the result of increases in diatoms, chrysophytes and the cyanobacteria, *Chroococcus* spp. and *Aphanocapsa* spp. Increases in four cyanobacterial taxa and the diatom, *Cyclotella* spp., had the greatest influence on phytoplankton shifts in zebra mussel effect basins. It is unknown why *Aphanocapsa* spp. and *Chroococcus* spp. increased in all basins from 1993 through 2002/2003, but it is not the result of zebra mussel establishment. Although increases in *Cyclotella* spp. were observed in basins with and without zebra mussels, different species may be driving those increases. Increases in the benthic diatom, *Cyclotella ocellata* Pant., following zebra mussel establishment in low nutrient systems has been observed in other regional lakes (Rex Lowe personal communication). Since zebra mussels may increase nutrient concentrations near established populations, growth of benthic species such as *C. ocellata* or *Merismopedia* spp. may be more common in zebra mussel effect basins. However, additional research is necessary and would require taxonomic identification of species and quantification of nutrients in the presence and absence of established populations of zebra mussels.
CHAPTER I: CONCLUSIONS

Blooms of *M. aeruginosa* are commonly associated with eutrophic lake basins. Their occurrence in Leelanau County lakes is not the result of increased phosphorus or temperature. The data from this study support the potential for promotion of *M. aeruginosa* by zebra mussels via competitive release. This hypothesis is supported by increased water transparency/seston removal in lake basins with zebra mussels, at times which coincided with greatest diatom growth; and by results of dissimilarity analyses that indicated similar basins without zebra mussels, experienced increases in most diatoms and chrysophytes, whereas basins with established zebra mussels experienced decreases in almost all diatoms and chrysophytes. Phytoplankton communities of spatial control basins should continue to be monitored as zebra mussels become established in those basins, to determine if the changes recorded in LTL, NLL and SLL occur.
CHAPTE II: INTRODUCTION

Zebra mussel densities reached 342,000 per square meter in Lake Erie (Leach 1993) and over 10,000 attached per unionid bivalve in Saginaw Bay (Herbert et al. 1991). High densities of zebra mussels have also been found on softer substrates, macrophytes and other native fauna and flora (Coakley et al. 2002, Burlakova et al. 2006). The availability of suitable substrate influenced zebra mussel colonization patterns in studies conducted by Yu and Culver (1999) and Stanczykowska (1977). Zebra mussel colonization is also believed to be influenced by hypoxia/anoxia in hypolimnetic waters (Yu and Culver 1999, McMahon 1996, Walz 1973), as zebra mussels rarely colonize depths deeper than the mean summer thermocline in stratified lakes (Mackie et al. 1989).

Zebra mussels disperse primarily through human vectors, specifically transient boater activity (Carlton 1993). Dressenid mussels are unique freshwater bivalves, containing free swimming veliger larvae that also contribute to dispersal (Griffiths 1993). Veliger larvae are primarily planktonic, and highly susceptible to hydrologic currents (Schloesser et al. 1998) and prevailing weather patterns (Martel et al. 1994).

Reeders et al. (1993) estimated that adult zebra mussels filter approximately one liter of water per day, consuming diverse seston, including detritus and phytoplankton (Ten Winkel and Davids 1982). At such rates, established populations of zebra mussels can remove large amounts of seston from the water column, altering aquatic communities (Bastviken et al. 1998) and decreasing phytoplankton biomass (Leach 1993, Nichols and Hopkins 1993). The removal of competing species by zebra mussels, contributes to the promotion of Microcystis aeruginosa (Kutzing) Kutzing (Vanderploeg et al. 2001, Fahnenstiel et al. 1995) in inland lakes (Raikow et

Since zebra mussel populations are found primarily in the littoral zone, they mainly filter epilimnetic water, which in some lake basins can be filtered repeatedly (based on wind and water flow, or proximity to mussels). The more water that is filtered, the greater the chance for competing phytoplankton and seston to be removed and water transparency to increase. Therefore, lakes with vast littoral zones and thus zebra mussel habitat relative to their overall volumes (high surface area [SA] to volume [V] ratios) may experience widespread effects of zebra mussel filtering, if they occur. On the contrary, lake basins that contain relatively little zebra mussel habitat compared to basin volume (low SA:V), may be less likely to experience large scale, basin-wide effects due to zebra mussel colonization and filtrations.

Zebra mussels invaded lakes in Leelanau County (Michigan) in the mid to late 1990's. All of these lake basins contain nutrient concentrations consistent with oligotrophy; however, summer dissolved oxygen can dip below 2 ug L\(^{-1}\). As zebra mussels became established in these lake basins, *M. aeruginosa* became the dominant late summer phytoplankton. The purpose of this project was to determine if zebra mussel populations in Leelanau lakes could be accurately estimated using underwater video, if such populations could be predicted based on characteristics of basin morphology and/or interpolation, and finally if zebra mussel population filtering capacities were related to maximum densities of *M. aeruginosa*. Highest densities of zebra mussels were expected near boat launches and tributaries, at depths less than the summer thermocline, and because of the prevailing winds from the west, on west facing slopes.
CHAPTER II: METHODS

Discrete bathymetric data, hydrology and location of boat launches were provided by the Michigan Department of Natural Resources (MDNR) Center for Geographic Information. Discrete bathymetry was used to estimate continuous bathymetric data using Topogrid in ArcInfo. Slope and aspect of each basin were generated from continuous bathymetry and Euclidean distance was estimated from in/outflows and maintained boat launches in ArcGIS.

Stations were selected randomly in each lake basin at depths between 0.5 and 10 meters, approximately 200 m apart beginning at maintained boat launches and continuing around the entire perimeter. At each station, global positioning coordinates and depth were documented. In Little Traverse Lake (LTL), North Lake Leelanau (NLL) and South Lake Leelanau (SLL), videos were recorded on Fuji Hi-8mm digital tapes using a Seaviewer™ underwater video system coupled with a Sony® digital camera recorder. Videos were recorded in Big Glen Lake (BGL), Little Glen Lake (LGL) and Lime Lake (LL) directly by the Sony® digital camera recorder in an Ikelite housing mounted on a customized aluminum frame. All video was collected in June, July and August in NLL and SLL (2002), in LTL (2003) and in BGL, LGL and LL (2004). The field of view for the Seaviewer™ system was 387 cm² in LTL and 453 cm² in NLL and SLL. The field of view was 13.76 cm² when the Sony® digital camera was used in BGL, LGL and LL. In all cases, the video camera was lowered to the lake bottom long enough to capture a clear image. The camera was raised approximately 0.5 m and moved a step towards the stern of the boat and returned to the lake bottom. This was repeated five to ten times at each station. Videos were projected onto a 2,800 cm² dry erase board or still images were printed and all visible zebra mussels were circled and counted. Ground truthing of zebra mussel counts was conducted at 66 sampling locations by SCUBA. At these sites, video was recorded prior to
SCUBA collection of all bottom material. Zebra mussels were immediately separated from this material and counted. In total, zebra mussels were counted in 201 images from BGL, 336 images from LGL, 397 images from LL, 401 images from LTL, 403 images from NLL and 563 images from SLL.

Dissolved oxygen and temperature were determined using a Hydrolab, which was calibrated following the manufacturers recommendations, at one or five meter increments from the surface to the lake bottom once a month in each lake basin. The mean summer thermocline was used to delineate potential zebra mussel habitat in each lake basin using ArcGIS. In addition, bathymetric data were used to calculate epilimnetic volume per basin by summing frustum or strata volumes (Wetzel and Likens 2000).

Zebra mussel densities were estimated from under water video in all lake basins. In basins with established populations of zebra mussels, densities were estimated across entire lake basins via multiple regression and by interpolating individuals counted by video using a kernel function in ArcGIS. Independent variables for multiple regression included depth, slope, aspect, distance from in/outflows and distance from maintained boat launches. The total number of zebra mussels counted per station were normalized based on the video area of lake bottom per station. The search radius for kernel density was based on the maximum distance between two sample sites locations in each lake basin. The relationship between zebra mussel density and the independent variables listed above was analyzed using Minitab 14.

Since individual, adult zebra mussels were assumed to filter one liter of water per day (Reeders et al. 1993), the filtering capacity of the zebra mussel population was quantified as the total number of zebra mussels divided by the volume of the epilimnion or the number of times a day the zebra mussel population filters the epilimnion.
Phytoplankton assemblages were determined from 870 ml whole water samples collected using a Wildco 2 L vertical water sampler, preserved with 30 mL formalin and stored in glass jars. A sample was collected at a depth of approximately 0.5 m - 1 m and a depth of twice the observed secchi depth prior to thermal stratification. After thermal stratification, the deeper sample was collected from a depth of 1 m above the thermocline. In BGL and LGL, one sample was collected in June, July, August and September. In LL, one sample was collected in May, June and August, with two samples in September. In LTL, NLL and SLL, one sample was collected in April, May, June, July, September and October, and three samples in August. Attempts were made to sample lake basins before, during and after cyanobacterial blooms. Samples were delivered to Dr. Rex Lowe (Bowling Green State University, Bowling Green, OH) where they were evaluated using standard phytoplankton enumeration techniques. Maximum *M. aeruginosa* densities were compared with zebra mussel population filtering capacities from each basin using regression analyses in Minitab 14.
CHAPTER II: RESULTS

The morphology of BGL, LGL and LL is quite variable. BGL is a kettle lake that is relatively deep (Figure 20), whereas LGL is the most shallow study basin, with a maximum depth of less than four meters (Figure 21). LL contains two deep sub-basins approximately 18 meters in depth and one very shallow sandbar less than 2 meters (Figure 22).

The deepest location in LTL is just west of the center of the lake (Figure 23), and the slope is lowest on the south side of the basin (Figure 24). The aspect is primarily north facing with some south southeast facing slopes (Figure 24). There are two main tributaries, with water flowing into LTL from LL via Shetland Creek, and emptying into Lake Michigan (Good Harbor Bay) via Shalda Creek (Figure 25). There is one boat launch maintained by MDNR relatively close to the northwest corner of the basin (Figure 25).

NLL is approximately 40 meters in depth, with the deepest location in the northern portion of the basin (Figures 26 and 27). Near the south end, approaching "the narrows" is a large shallow area less than five meters in depth. In this area, the slope is the most gradual for the basin (Figure 28). The aspect of NLL is representative of all directions (Figure 29). Numerous tributaries flow into NLL, with the largest flow entering through the narrows from SLL and exiting via the Leland River to Lake Michigan (Figure 30). There are two maintained boat launches, with most boat traffic entering via the narrows, and fewer boaters entering NLL on the north end of the basin (Figure 31).

The deepest location in SLL is approximately 18 meters deep (Figures 32 and 33), and the greatest slopes are located on the east and west sides of the basin (Figure 34). The aspect varies, with the most prominent directions being east and west (Figure 35). There are four major tributaries that empty into SLL, with water flowing north towards Central Lake Leelanau (CLL),
the narrows and NLL (Figure 36). There are two boat launches maintained by MDNR, one near the northwest corner, and one on the east side of the lake (Figure 37). In addition, boaters enter SLL heading south from CLL.

High densities (greater than 50% coverage of the lake bottom per image) of macrophytes including the genera *Chara*, *Myriophyllum* and *Potamogeton*, were present in six of the 66 locations where video was recorded and SCUBA collection took place, and made zebra mussel counts very difficult. Of the remaining samples, the mean difference between the actual zebra mussel counts (SCUBA) and the video zebra mussel counts was only 5.6 individual zebra mussels (standard error 10.2).

In BGL, LGL and LL, zebra mussel densities were not estimated using multiple regression or kernel density because densities in these basins were very low (Table 8) and not normally distributed. Under water video was collected from BGL at 23 locations (Figure 20) and zebra mussels were absent at all locations. Similarly video was collected from LGL at 40 locations (Figure 21) and from LL at 48 locations (Figure 22), and zebra mussels were absent at 17 and 25 locations respectively. Underwater video indicated that zebra mussel densities were greatest in NLL followed by LTL and SLL. Interpolation of these densities basin-wide was difficult. Although significant models were generated using regression analyses, they were not robust and varied greatly from basin to basin (Table 9). In LTL, zebra mussel density estimates increased with slope and therefore were estimated to be greatest along the drop-off on the north shore of the basin (Figure 38). Aspect was the most influential parameter in NLL (Figure 39), with high estimates of zebra mussels on the west and south ends of the basin (Figure 39). Zebra mussel density estimates in SLL were greatest at locations near tributaries and at shallow depths (Figure 40).
Interpolation using the kernel method yielded zebra mussel density estimates per square meter, per square kilometer. Since individual zebra mussels were counted within a view frame and the number of view frames varied slightly from site to site, video counts must be described on a per area basis (individuals per square meter). A kernel function estimated the magnitude per unit area of zebra mussel densities over the entire extent of the point values, using the video counts. Zebra mussel density estimates using the kernel method are logical and fit with expectations of zebra mussel establishment based on the literature. Although the units are not the most meaningful, the results of the kernel density technique provided some insight into zebra mussel distributions in LTL, NLL and SLL. Kernel interpolation estimated that zebra mussel densities were greatest near the outflow on the west end of LTL (Figure 41), in the north end of NLL (Figure 42) and near the boat launch on the east side of SLL (Figure 43). When kernel estimates were compared with original zebra mussel densities from video, these models described the data more accurately than regression analyses in LTL and NLL (Table 10, Figure 44).

The mean thermocline in BGL, LL, LTL, NLL and SLL was approximately three meters (Table 9). It was not determined for LGL, since LGL is very shallow (approximately 4 m) and is regularly polymictic in the summer with high winds. LGL was saturated with oxygen to the bottom (2004) and zebra mussel habitat was therefore determined to be the entire basin. The volume of the epilimnion was the greatest in BGL and the smallest in LTL; the area of zebra mussel habitat, or lake bottom less than or equal to the mean summer thermocline, was greatest in LGL and lowest in LTL and LL. The total number of zebra mussels reflects the amount of suitable habitat and the measured zebra mussel density from video recordings. Although lake
basins with greater zebra mussel population filtering capacities did contain the greater concentrations of *M. aeruginosa*, this relationship was not significant (Figure 20).
CHAPTER II: DISCUSSION

The morphology of Leelanau lake basins was expected to influence zebra mussel density, and size of overall zebra mussel populations. Basins with vast bottom areas less than or equal to the mean summer thermocline, or those with gentle slopes were hypothesized to provide more habitat and thus larger zebra mussel populations. By these criteria, the kettle lake BGL does not provide as much habitat for zebra mussels when compared to other Leelanau basins. Because of its shallow nature, the entire LGL basin in contrast, may provide suitable habitat and be colonized by zebra mussels to its full extent, in the future. The characteristics of suitable zebra mussel habitat include many more factors beyond dissolved oxygen and slope. The type (Marsden and Lansky 2000), or availability of substrate (Yu and Culver 1999), temperature (Matthews and McMahon 1999, Walz 1973) and sunlight (Marsden and Lansky 2000) have been demonstrated to influence zebra mussel distribution. It is likely that substrate type/availability and sunlight are correlated with depth in study basins, but since these data were not collected as part of this study, their influence on zebra mussel distribution is unknown.

Underwater video provided acceptable estimates of zebra mussel density in point locations. Attempts to predict zebra mussel distribution on larger spatial scales, based on video estimates were difficult to interpret. It is possible that the patchy distribution of zebra mussel populations is on a scale smaller than the sampling would capture. Variograms describing zebra mussel densities in each basin indicated spatial autocorrelation was very low (data not included), which was surprising for such a colonial species. In addition, models generated via multiple regression, although significant, were not robust and did not identify morphological features that could help explain zebra mussel density in Leelanau basins, nor beyond. The accuracy of these models is also suspect as not only do they explain a small fraction of the data (Table 9), but they
regularly conflict with studies of zebra mussel distributions. For example, highest densities of zebra mussels in LTL were not projected at locations with hard, textured substrates as reported by Marsden and Lansky (2000), Mackie et al. (1989) McMahon et al. (1996) and others. Predictions of zebra mussel density using the kernel technique were easier to explain. For example, the highest densities of zebra mussels in LTL were identified in the west end of the basin. This area is close to the boat launch maintained by MDNR and the outflow to Lake Michigan, and contains more rocky substrate. Highest zebra mussel densities in NLL and SLL were also estimated near the boat launch and in areas with high densities of substrate (unionids). Kernel interpolation of zebra mussel densities were more accurate than those generated using multiple regression in LTL and NLL. Unfortunately numeric values generated using the kernel technique were complicated because of the sampling methodologies. Since these data required some standardization, (using zebra mussel densities as opposed to raw counts), the resulting kernel estimates of individuals per square meter per square kilometer were difficult to interpret. In addition, changing the search radius for the kernel function would influence the results to a certain extent, and may indicate different patterns of zebra mussel distribution.

Basin morphology may also indirectly influence densities of *M. aeruginosa*. Warm water environments, ideally above 25° C (Robarts and Zohary 1987), contribute to the greatest blooms of *M. aeruginosa*. Water temperatures vary from year to year and from basin to basin. Shallow basins like LGL warm earlier in the summer and therefore may experience earlier blooms of *M. aeruginosa* relative to deeper basins, and may experience more days per year with water temperatures in excess of 25 ° C.

The estimate of zebra mussel population filtering capacity makes many assumptions. First, as previously discussed, it assumes the only factors influencing zebra mussel habitat are
depth and dissolved oxygen. Second, it is very dependent on the accuracy of the underwater video estimate. Although underwater video estimates were considered acceptable, the scale of sampling varied among all study basins to varying degrees. Zebra mussels were expected to be in greatest densities near the boat launches, mouths of tributaries, on west facing slopes and at depths less than or equal to the mean summer thermocline in each basin. To an extent these expectations, coupled with variable basin morphologies support the sampling regime. Unfortunately, since it appears that this regime failed to capture the spatial autocorrelation of zebra mussel populations in LTL, NLL and SLL, it is likely that scale influenced zebra mussel density estimates. The comparison between \textit{M. aeruginosa} densities and zebra mussel population filtering capacities was likely influenced to some extent by differences in scale from one lake basin to another, along with the small sample size of six lake basins.
CHAPTER II: CONCLUSIONS

It is unfortunate that the sampling approach limited the comparison of zebra mussel density estimates from one basin to another, and from more meaningful comparisons of zebra mussel filtering and *M. aeruginosa* densities. Future estimates of zebra mussel density should consider both expected demographics based on the literature and the potential influence of scale. Since this study was extremely labor intensive, it would be wise to collect preliminary data regarding spatial distribution of zebra mussels prior to undertaking such a large project in the future.

The results of this project do not contradict the hypothesis that zebra mussel population filtering capacities may be related to *M. aeruginosa* densities, but additional research is necessary to address the influence of scale and sample size.
CHAPTER III: INTRODUCTION

Microcystin (MC) is a cyclic peptide hepatotoxin produced by Microcystis aeruginosa (Kutzing) Kutzing and other cyanobacteria (Hyenstrand et al. 2003). There are over 75 variants of MC with LR, YR and RR being some of the most common. LR is the most toxic variant, and it can be present in cyanobacteria as free and covalently bound forms (Sivonen and Jones 1999; Fischer and Dietrich 2000).

Skin irritations, vomiting, cancer of the liver and death, have been documented worldwide in humans, pets and livestock following MC exposure (Sivonen and Jones 1999; Kuiper-Goodman et al. 1999). Human consumption and recreational exposure guidelines based on MC-LR, were determined by The World Health Organization (WHO); the drinking water limit is 1 ug L\(^{-1}\), and the recreational guidelines are 2 - 4 ppb for a low risk of side effects, and 20 ppb for a medium risk. Leelanau County is a very popular tourist destination, with over 1,000,000 visitors during the summer months, when *M. aeruginosa* blooms occur. Many of these visitors take advantage of the recreational opportunities that the inland lakes provide. Although study basins are not used by humans for drinking water, humans are likely to come into contact with high concentrations of MC through recreational exposure to whole water and foam and through consumption of fish white muscle, especially from walleye and whitefish.

MC also poses a threat to ecosystem health through bioaccumulation in aquatic food webs. Bioaccumulation of MC has been confirmed in crustaceans, mussels, zooplankton and fish (Mohamed 2001; Vasconcelos 1995; Kotak et al. 1996; Falconer et al. 1992). MC is water soluble and therefore unable to penetrate lipid membranes of animals directly, consequently toxin uptake must occur through membrane transports, making the liver the target organ in mammals (Sivonen and Jones 1999).
In the presence of full sunlight and water soluble pigments, greater than 90% breakdown of free, un-bound MC was reported by Sivonen and Jones (1999) in approximately two weeks. Rapala et al. (1994) observed free MC-LR broken down by heterotrophic bacteria in less than 30 days. In contrast, cell bound MC degrades very slowly (Sivonen and Jones 1999). In aquatic systems, most MC remains in cyanobacterial cells until lysis (Sivonen and Jones 1999).

In the absence of cyanobacterial blooms, it is unlikely that organisms will either accumulate or retain MC. For example, in as little as two hours, 80% depuration of MC can occur in black tiger prawns (Penaeus monodon) Fabricius (Kankaapaa et al. 2005) and in four to six weeks in zebra mussels (Pires et al. 2004). Prior to depuration however, MC may be available to consumers and predators, especially higher vertebrates such as walleye and whitefish.

As water temperatures begin to cool, typically in fall, cells of M. aeruginosa become carbohydrate heavy and begin to sink (Mur et al. 1999). Some cells die and with cell lysis, MC is released into the whole water, while cells reaching the lake bottom intact, may over-winter in the sediment (Sivonen and Jones 1999, Ihle et al. 2005). Dying cells of M. aeruginosa or cells that never reach full suspension back into the water column may also become associated with the sediment through re-sedimentation. These cells and their toxins may become available to benthic species during and after active blooms.

The influence of depth on sediment MC concentrations may depend on the time of year. During a M. aeruginosa bloom, the greatest densities will likely be found in near-shore areas, since M. aeruginosa cells are most common in shallow water (Ozawa et al. 2005). As a result, these surface scums are highly susceptible to winds. As M. aeruginosa blooms age, and cells begin to die and sink, it is logical that dead cells, and free MC will accumulate in shallow
sediments. Numerous studies indicate however, that the highest concentrations of overwintering *M. aeruginosa* (from fall to spring) are associated with deepest sediments in lake basins (Brunberg and Blomqvist 2002, Latour and Giraudet 2004, Verspagen *et al.* 2005). Current may therefore play a role in the redistribution of MC in deeper regions of lake basins, especially in fall through winter.

It was hypothesized that samples (sediment, macroinvertebrates, bivalves, fish, water, foam) collected from basins with blooms of *M. aeruginosa* in 2004, would contain seasonal increases of MC related to *M. aeruginosa* densities. The purpose of this study was to quantify potential accumulation of MC in the food web before and after blooms of *M. aeruginosa*. 
CHAPTER III: METHODS

In 2004 sediment, macroinvertebrates and bivalves were collected along transects in lakes with and without established populations of zebra mussels, in spring (May-June) and repeated in fall (September-October). Lakes with established populations of zebra mussels by 2004, or zebra mussel effect basins, included Little Traverse Lake (LTL), North Lake Leelanau (NLL) and South Lake Leelanau (SLL). Lakes with zebra mussels not yet established by 2004, or spatial control basins, included Big Glen Lake (BGL), Little Glen Lake (LGL) and Lime Lake (LL). Transects sites were selected based on depth at approximately 1.5 m - 3 m intervals and included the deepest location of each basin. Depth and global positioning coordinates were recorded for each site using a sounder and a Garmin E-trex Legend GPS unit.

At each transect site, a Wildco Hand Core sediment sampler (5 cm in diameter and 8 cm in length) was used to hand collect sediment samples by SCUBA. The top 2 cm of each core was extracted and transferred into two separate 50 ml amber glass vials using a spatula. Zebra mussels and unionids, when available, were also hand collected by SCUBA and rinsed with lake water to remove sediment and detritus. Macroinvertebrates were collected using an Ekman dredge and were emptied into a glass pan. Both bivalves and macroinvertebrates samples were stored in 3.8 L plastic bags. Macroinvertebrates were subsequently picked from lake bottom material and identified. Individuals were rinsed with distilled water, blotted dry with paper towel and measured in length. Individuals were grouped with like taxa per site, wrapped in foil and frozen.

The Michigan Department of Natural Resources (MDNR, supervised by Todd Kalish, Fisheries Management Biologist, Central Lake Michigan Management Unit) collected fish in NLL and SLL. Fish from NLL were collected with Great Lakes Gill nets randomly placed
throughout the lake in 20-30 meters of water. In SLL, fish were collected using three standard
trap nets placed in three randomly selected locations in water depths ranging from 1.5 - 4.5 m.
Nets were set into position on May 19th and September 21st, 2004 and lifted the following day.
Fish were placed in coolers over ice and transported to the lab at the Water Studies Institute,
Northwestern Michigan College (Traverse City, MI). Individual fish were rinsed with tap water,
weighed and measured in length. Fish were dissected and liver and samples of white muscle
tissue were blotted dry, wrapped in foil and frozen.

Whole water was collected using a Wildco 5 L vertical water sampler, simultaneously for
microcystin analyses and for phytoplankton enumeration, from the deepest locations of all study
basins, including LL, beginning in May through October (2004) at a depth of 1 m. In BGL, LGL
and LL, samples were collected a minimum of every month. In LTL, NLL and SLL, samples
were collected every month along with weekly samples throughout July and August and
biweekly in September, in an attempt to effectively sample cyanobacterial blooms. Whole water
samples of 20 ml were frozen in 50 ml amber glass jars for MC-eq analyses.

All samples were analyzed for microcystin equivalents (MC-eq) by Mike Grant
(Analytical Chemist, University of Michigan Biological Station, Pellston, MI) using enzyme
linked immune-sorbent assay (ELISA). This technique is commonly used and quantifies the
most common variants of microcystin, -LR, -LA, -YR, -RR and nodularin to different degrees.
Critiques of ELISA in the literature vary. Metcalf et al. (2000) reported that MC concentrations
measured with ELISA can include false positives as a result of immuno-crossreactivity, whereas
Kankaapaa et al. (2005) indicated that ELISA performed well. A subset of lake sediment and
Hexagenia spp. samples were analyzed using both ELISA and high performance liquid
chromatography and mass spectrometry (HPLC-MS) by Rick Rediske (Grand Valley State
University, Muskegon, MI). This technique quantified the most toxic variant of microcystin, MC-LR, and by using both methodologies, a comparison of MC-eq and MC-LR was permitted.
CHAPTER III: RESULTS

In BGL, LGL and LL, the concentrations of both *M. aeruginosa* and whole water MC-eq were relatively low in 2004 (Figure 46). A greater number of measurements of *M. aeruginosa* and MC-eq were collected in LTL, NLL and SLL. In LTL, *M. aeruginosa* densities varied greatly during 2004 whereas whole water MC-eq concentrations followed a bimodal distribution with peaks in early August and early September (Figure 47). Although MC-eq concentrations in LTL exceeded the WHO guideline for drinking water of one ppb on two occasions, this guideline is based on MC-LR. Therefore MC-eq concentrations in LTL may not have reached hazardous concentrations. *M. aeruginosa* densities and concentrations of MC-eq measured in whole water from NLL were similar to spatial control basins. In SLL, *M. aeruginosa* was recorded on only one date. MC-eq in SLL whole water, was measured on all sampling dates other than when *M. aeruginosa* was identified. Whole water MC-eq in NLL and SLL was well below WHO guidelines during the entire summer of 2004. For all basins, the mean concentration of whole water MC-eq was positively related to the density of mean *M. aeruginosa* (Figure 48). Mean values of *M. aeruginosa* and MC-eq were greatest in LTL (Table 12). *M. aeruginosa* densities in all other basins were similar; however, zebra mussel effect basins had higher MC-eq production (Table 12). A small number of samples were collected in LTL, NLL, SLL to compare *M. aeruginosa* density, whole water MC-eq (total), MC-eq in filtered water (free or dissolved fraction) and filters (cell bound fraction). Results varied from basin to basin, with primarily dissolved MC-eq recorded in LTL, and cell bound MC-eq in NLL and SLL.

Mean sediment concentrations of MC-eq were greatest in LTL, followed by SLL, NLL, LGL and BGL (Figure 50). Generally, MC-eq concentrations increased with depth (Table 13). This trend was not significant in spring samples from spatial control basins (BGL and LGL);
these basins did not contain high enough MC-eq concentrations prior to the presence of cyanobacteria in 2004. The relationship was significant in both spring and fall in zebra mussel effect basins, with maximum concentrations of MC-eq in sediment an order of magnitude greater than determined for spatial control basins (Figures 51 and 52).

MC-eq measured in macroinvertebrates followed a similar pattern from one taxon to another. Macroinvertebrates collected from basins with established populations of zebra mussels, tended to have greater mean concentrations of MC-eq. In addition, samples collected in the fall tended to have greater mean concentrations of MC-eq. This was the case for amphipods (Figure 53), chironomids (Figure 53), crayfish (Figure 54), *Hexagenia* spp. (Figure 55) and oligochaetes (Figure 55). In many cases, the associated standard error was very large, however this is understandable since the samples were collected across depths, and depth was a significant factor when determining sediment MC-eq. In fact, for amphipods and chironomids, concentrations of MC-eq increased with increasing depths for fall samples (Table 14, Figure 56). MC-eq in other taxa were not influenced by depth; however, sample size was an issue.

Very few unionids were present along transects. Mean unionid MC-eq was greatest in LGL, however only one individual was collected in LTL, and one in SLL, with both of these occurring in the spring (Figure 57). When comparing unionids and zebra mussels collected from LGL, these taxa have similar MC-eq concentrations (Figure 57). Since unionids were absent in fall samples from LTL and SLL, and entirely from NLL, it is unknown if unionids and zebra mussels contained similar concentrations of MC-eq.

MC-eq concentrations were measured in six species of fish, from three basins and across two seasons. Unfortunately, not all species were collected in each basin and season. Moreover, samples of white muscle and liver were analyzed for MC-eq in piscivores, whereas whole
individual minnows, \textit{(Notropis texanus)} Girard, were analyzed for MC-eq. Mean MC-eq including liver and white muscle, was greatest in walleye (SLL), followed by whitefish (NLL) and smallmouth bass (SLL) (Figure 58). In walleye and smallmouth bass, mean fall MC-eq was greater than spring MC-eq (Figure 58). Fall MC-eq concentrations were greater in \textit{N. texanus} collected in LTL as compared to SLL (Figure 58).

Fishes from NLL and SLL are commonly consumed by humans, especially walleye and whitefish. Samples of white muscle indicate some fishes contained MC-eq in excess of the WHO recommended total daily intake (TDI) (Table 15) based on a consumption of 226.8 g (8 oz). Mean white muscle MC-eq from many fishes indicated men, women and children would consume MC-eq in excess of WHO TDI even in spring, prior to \textit{M. aeruginosa} blooms (Table 15). As with whole water MC-eq however, WHO TDI is based on MC-LR. Therefore, MC-eq quantified in fish samples may overestimate the concentration of MC-LR.

In zebra mussel effect basins, mean MC-eq measured in sediment, macroinvertebrate tissue, bivalves and piscivores was greater in fall than in spring (Table 16). Such seasonal differences also occurred in LGL (Table 16). A bloom of \textit{M. aeruginosa} did not occur in BGL during the summer of 2004, and differences in MC-eq from spring to fall were not expected. Of all ecosystem components, macroinvertebrates contained the greatest concentrations of MC-eq in both spring and fall (Table 16). \textit{Hexagenia} spp. contained MC-eq in concentrations approximately an order of magnitude greater than other macroinvertebrate taxa, and were responsible for the high mean MC-eq for macroinvertebrates. Mean MC-eq was quantified in sediment, macroinvertebrates and bivalves across depths and large standard error was expected. Although fishes were not collected across depths, there were species specific differences that contribute to large standard error for MC-eq of piscivores. Mean MC-eq calculated across
seasons and depths indicate that MC-eq approximately increased with trophic level (Figure 59). Macroinvertebrates again contained higher mean concentrations of MC-eq as a result of extremely high concentrations of MC-eq measured in *Hexagenia* spp. (Figure 59).

Comparisons of mean MC-eq and MC-LR in *Hexagenia* spp. and sediment indicate that a small percentage of MC-eq concentrations likely consisted of MC-LR (Table 17). The relationship between MC-eq and MC-LR was significant when comparing samples of *Hexagenia* spp., sediment or *Hexagenia* spp. and sediment (Table 18), with *Hexagenia* spp. containing greater concentrations of measured MC-eq and MC-LR (Figures 60 & 61). It is unknown if this relationship would exist for other ecosystem components such as whole water, or fish white muscle tissue. In both LTL and NLL, mean MC-LR was greater following *M. aeruginosa* blooms, with high variability in NLL (Figure 62). In a similar pattern, the size of *Hexagenia* spp. collected from LTL was greater in fall than spring, whereas a large overlap of standard error occurred in NLL.
CHAPTER III: DISCUSSION

Temperatures during the summer of 2004 were very mild, especially in late summer (unpublished data). Ideal temperatures for *M. aeruginosa* growth are above 25 °C (Robarts and Zohary 1987). Temperatures in LGL, LTL and SLL were so low that these basins turned over in August only to restratify in September. Since *M. aeruginosa* blooms in Leelanau lakes in late summer, it is not surprising that densities of *M. aeruginosa* were lower in 2004 than in previous years (Chapter I). Although NLL did not completely turnover in late August, densities of *M. aeruginosa* followed a very similar pattern to that of water temperature. For example, in NLL (2002), maximum *M. aeruginosa* density was over 4,400 cells ml⁻¹ in August, whereas in 2004, a maximum of 400 cells ml⁻¹ of *M. aeruginosa* was recorded (Figure 13). It is unknown if *M. aeruginosa* densities would have continued to climb had water temperatures remained constant, or increased, nor if this would have resulted in greater concentrations of MC.

It was not surprising that *M. aeruginosa* densities and MC concentrations varied over time and did not follow similar patterns from basin to basin. Toxin production by cyanobacteria fluctuates. In addition, whole water samples collected for MC analyses were from the deepest locations of lake basins and therefore unlikely to contain the highest concentrations of MC (Ozawa *et al.* 2005). Inshore areas are likely to contain the highest concentrations of MC, based on wind and nutrients (Ishikawa *et al.* 2002). Since basin morphology, location and orientation will influence wind exposure, all study basins will experience these effects differently.

Although not reported in most literature as a MC producing genus, *Aphanocapsa* spp. may contribute to MC concentrations. Domingos *et al.* (1998) presents evidence for MC production by *Aphanocapsa cumulus* Komarek and Cronberg. Kankaanpaa *et al.* (2005) studied culture ponds containing a number of different species of cyanobacteria including both
Microcystis and Aphanocapsa. These authors indicated that Aphanocapsa was not a toxin producing genus, yet both MC and Aphanocapsa were recorded from a pond that did not contain genera considered potentially toxic (Kankaanpaa et al. 2005). In this study, all basins contained Aphanocapsa spp. to varying degrees (unpublished data). Since mean MC was related to mean M. aeruginosa, however, it appears that other cyanobacteria rarely contributed to MC production in Leelanau lake basins during 2004.

The relationship among MC concentrations measured in whole water (total MC), filtered water (dissolved/extracellular MC) and on filters (cell bound MC) may be the result of sampling within lake basins. On four dates in LTL and SLL and only three in NLL, water samples were collected for comparison of extracellular and bound MC. Based on when those dates coincided with M. aeruginosa blooms, the relative concentrations of dissolved MC and MC within cells of M. aeruginosa would likely change. Sivonen and Jones (1999) reported that during periods of healthy growth, more than 80% of MC was cell bound and therefore, high densities of M. aeruginosa in study basins were expected to coincide with high cell-bound MC measured on filters. As blooms of M. aeruginosa declined, the amount of MC in the filtered water was expected to increase, since MC would be released into the water upon lysis (Sivonen and Jones 1999). The increase in dissolved MC with decreasing M. aeruginosa was recorded in LTL in late September, but not for NLL or SLL. Although it is unlikely that free MC was broken down in NLL and SLL in between data collection (September 8th - 15th), since these samples were collected from the water surface, MC may have been exposed to full sunlight and undergone rapid breakdown (Sivonen and Jones 1999). Regardless, such comparisons require larger datasets to fully understand MC concentrations relative to M. aeruginosa blooms.
Since MC was related to *M. aeruginosa* densities, and *M. aeruginosa* densities were greatest in late summer and early fall in study basins, seasonal increases in MC measured in lake sediments were expected. Since breakdown of free MC can occur quickly and less than 20% is typically adsorbed to natural suspended soils (Sivonen and Jones 1999, Ihle *et al.* 2005), MC measured in spring sediments was higher than predicted. In study basins, the majority of sediment MC concentrations determined for late spring and early summer were likely within over-wintering cells of *M. aeruginosa*. Surviving *M. aeruginosa* may have been resuspended in spring and early summer from the sediment into the water column (Ihle *et al.* 2005); depending on environmental factors such as temperature, light (Hansson 1993) and potentially wind energy for mixing. Therefore, in shallow basins such as LGL and perhaps LTL, where water temperatures increased at greater rates (unpublished data) and light penetrated a greater proportion of the lake volume than in deeper basins, resuspension of *M. aeruginosa* may have occurred earlier in the year. It is possible that spring transects in LGL and LTL, followed resuspension and thus reflect a smaller concentration of sediment MC in spring as compared to BGL, NLL and SLL which may have been sampled following resuspension. The spring sampling dates of all basins could therefore have resulted in increases in sediment MC that appear either greater or smaller than others.

Sediment MC concentrations tend to increase with depth (Ihle *et al.* 2005). This trend occurred in BGL and LGL in the spring, and LTL, NLL and SLL in spring and fall. Fewer samples were collected in LGL and BGL, relative to the zebra mussel effect basins. In addition, these analyses required pooling data from different basins, and hence the use of percent depth. Although LTL differs morphologically from NLL and SLL to an extent, the difference between BGL and LGL is more extensive. BGL is the deepest of all study basins at 40 meters, whereas
LGL is the most shallow at four meters. Moreover, the slope of BGL is much greater than LGL. Pooling data from these two basins may be more problematic than the more similar zebra mussel effect basins and may contribute, along with small sample size, to the lack of significance between spring sediment MC and percent depth.

Samples for macroinvertebrates did not contain the same taxa in both spring and fall nor across basins or depths, so comparisons were thus limited to the most common taxa. Although seasonal increases in MC concentrations were measured they were difficult to assess because of the small sample size.

Macroinvertebrates are likely exposed to MC in lake sediments. Many taxa consume sediments directly, such as amphipods and oligochaetes. Both fall MC measured in amphipods and chironomids increased with percent depth, similar to sediment MC. Interestingly, basins with the greatest sediment MC concentrations do not always contain macroinvertebrates with high MC concentrations. The highest fall sediment MC was collected from LTL, for example, yet the macroinvertebrate from LTL with the highest mean MC was crayfish. This may indicate differences in accumulation/depuration of MC among macroinvertebrates, or could simply reflect spatial variability of sampling.

Some macroinvertebrate taxa increase with zebra mussel populations in part because of the ample pseudofeces (Greenwood et al. 2001). Fecal (and pseudofecal) material may contain excreted *M. aeruginosa* and if consumed, contribute to MC concentrations. Oligochaetes consume primarily sediment, but also plant and animal detritus (Pennak 1989). Although it is expected that if sediment MC concentrations increased over seasons that MC in oligochaetes would also increase, some variability may be explained by oligochaete diversity. There are a few carnivorous forms of oligochaetes such as the genus *Chaetogaster* that consume insect
larvae, protozoans and even other oligochaetes (Pennak 1989). With 10 families of oligochaetes (Pennak 1989) it is possible that samples of oligochaetes contained various species or genera made up of individuals with different diets and thus different exposures to MC.

The macroinvertebrate taxa that contained the greatest spring MC concentrations and accumulated the most MC during the summer of 2004 were *Hexagenia* spp. Bioturbation by *Hexagenia* spp. was reported by Bartsch et al. (1999) to result in the resuspension of sediment into the water column. Since *Hexagenia* spp. burrows are located near the sediment water interface, bioturbation is likely to include sediment with the greatest MC concentrations (Ihle et al. 2005). *Hexagenia* spp. may therefore be exposed to MC throughout the year, with resuspension of overwintering cells of *M. aeruginosa* available for filtration. MC concentration in *Hexagenia* spp. is of particular concern as they can contribute to the trophic transfer of contaminants associated with sediment (Finley 1985). As adults, *Hexagenia* spp. are prey for many species of birds (Smits et al. 2005), and therefore MC contained in *Hexagenia* spp. may result in MC concentrations in some terrestrial species.

If bioturbation of macroinvertebrates such as *Hexagenia* spp., influences MC concentrations near the sediment water interface, MC may be available to bivalves throughout the year, or in the absence of *M. aeruginosa* in the water column. Ihle et al. (2005) observed a decrease in sediment MC immediately after sedimentation of *M. aeruginosa*. The dead or dying cells likely release MC upon lysis and the extracellular MC may be available for direct consumption by benthic species such as bivalves.

Vanderploeg et al. (2001) reported that zebra mussels selectively reject *M. aeruginosa* thereby contributing to *M. aeruginosa* blooms in basins containing established populations of zebra mussels. Zebra mussels produce less pseudofeces when fed green algae versus toxic
cyanobacteria (Babcock-Johnson et al. 2002), further supporting rejection of *M. aeruginosa*. Pires *et al.* (2004) however provide evidence that zebra mussels regularly consume toxic strains of *M. aeruginosa* and assimilate free, unbound MC with a subsequent depuration period of approximately 4-6 weeks. When Pires *et al.* (2004) fed zebra mussels toxic *Microcystis* spp. as a single food item however, complete depuration of MC did not occur during the study period. In this study, zebra mussels were likely to ingest some *M. aeruginosa* while rejecting other cells with pseudofeces. If MC was present in pseudofeces, it may have been available to macroinvertebrates or other bivalves.

Different species of unionids have been shown to accumulate toxins. *Anodonta cygnea* Linnaeus for example, accumulates large amounts of toxins produced by *Oscillatoria* (Eriksson *et al.* 1989). MC in *Anodonta grandis simpsoniana* Lea was measured between 24,000-257,000 ppb (dw) by Prepas *et al.* (1997) and 130,000 - 250,000 ppb (dw) were determined in *Unio douglasiae* Gray by Yokokama and Park (2003). Unionids were rarely collected from transect locations, making increases at depths and within basins difficult to decipher. It is possible that with the establishment of the zebra mussel in study basins, unionid populations have declined (Mackie 1991) and are unlikely to be found outside of Glen Lake (BGL or LGL).

A large sampling effort for fish was made during spring in NLL and SLL. In fall, fish were only collected in SLL and the total number collected declined. This was a function of timing, as the MDNR was unable to provide similar staff time and equipment in both seasons. Accumulation of MC by different species was therefore difficult to assess. Many different types of fish accumulate MC and suffer liver damage as a result. Impaired liver function, decreased growth, inhibition of gill ion transport, change in blood chemistry (Bury *et al.* 1995, Kotak *et al.* 1996) and cardiovascular effects (Best *et al.* 2001) have been reported. More specifically,
diffuse necrosis and megalocytosis in the whole liver of Atlantic salmon was observed following exposure to high doses of MC-LR (Anderson *et al.* 1993). Brown trout fry decrease growth rates when chronically exposed to toxic and non-toxic *M. aeruginosa*, with a greater effect caused by toxic strains (Bury *et al.* 1995).

Growth rates for Michigan sport fishes reported by the MDNR indicate that walleye grow at a rate greater than that of smallmouth or largemouth bass (Schneider 2000). MC enters hepatocytes from the gut (Falconer *et al.* 1992), therefore consumption is required for uptake. Although their diets are similar, higher growth rates of walleye may reflect greater consumption of prey fishes containing MC and subsequent accumulation.

The presence of MC can limit fish consumption of cyanobacteria (Soares *et al.* 2004). However, Soares *et al.* (2004) examined juvenile tilapia fed only toxic *M. aeruginosa*. Planktivorous fishes, where phytoplankton both capable and incapable of producing toxin are present may readily accumulate MC. Phytoplanktivorous fish are more likely to resist MC exposure than piscivores based on tissue dynamics (Xie *et al.* 2004). MC concentrations of walleye and smallmouth bass may therefore be a function of diets including high amounts of planktivorous fish containing low MC and piscivorous fish with high MC concentrations. Detoxification also differs among fish species (Xie *et al.* 2004). Therefore higher MC in walleye may be a reflection of slower toxin depuration when compared to smallmouth bass.

There is a considerable potential for MC to be magnified in the aquatic food web (Sivonen and Jones 1999). Mean values from all measured components indicate that MC concentrations in NLL, SLL, LTL, BGL and LGL biomagnify through the food web (Figure 59), with highest MC concentrations in *Hexagenia* spp. and piscivorous fishes. The mean macroinvertebrate concentration excluding *Hexagenia* spp. values (760 ppb) was approximately
half that of the piscivorous fishes. Ibelings et al. (2005) observed no evidence for biomagnification when measuring phytoplankton, *Daphnia* sp., zebra mussels and fish; however, no covalently bound MC was included in the study. It was estimated that the maximum for covalently bound MC in zebra mussels was approximately 38% (Ibelings et al. 2005), a potentially high amount of error. It is possible that if MC is present throughout the year in ecosystem components that critical concentrations will be reached. If so, multiple levels of the food web may be threatened to varying degrees. Christoffersen et al. (2002) reported that when the presence of MC continues year to year, the bacterial community can adapt to include greater numbers of heterotrophic bacteria capable of effectively degrading free MC. This adaptation of microbes to cyanobacterial metabolites is necessary for MC degradation (Rapala 1994), but will not influence bound MC within living cells.

Samples of *Hexagenia* spp. and sediment from LTL and NLL were chosen for analyses of MC using both ELISA and HPLC-MS, because sufficient samples existed, and because of the remarkably high concentrations of MC-eq quantified in *Hexagenia* spp. It was surprising that the amount of MC-LR was related to the concentration of MC-eq, and that this relationship was determined for two very different types of samples in *Hexagenia* spp. and sediment. Although it is unknown if this relationship exists in other ecosystem components measured using ELISA, it seems very likely that the concentration of MC-LR represented a small fraction of the MC-eq measured using ELISA. It appears that the size of *Hexagenia* spp. collected influenced the amount of MC-LR accumulated in LTL and NLL in 2004. Consumption rates were likely greater by larger individuals and may have resulted in greater MC-LR exposure.

In aquatic systems, toxins remain in cyanobacterial cells until lysis, making consumption the pathway through which other organisms, including humans are likely to accumulate toxin
(Sivonen and Jones 1999). The human exposure risk in Leelanau lake basins to MC is difficult to assess based on concentrations of MC-eq as opposed to MC-LR. Lakes in Leelanau County are not used for drinking water by humans; therefore, human exposure is likely to occur through recreation (ingestion/inhalation of whole water) and fish consumption. If MC-eq measured in whole water in 2004, contained relatively high concentrations of MC-LR, human exposure may have exceeded the low WHO recreational exposure limit in LTL. In addition, the recorded maximum value of 213 ppb MC in LTL (assuming 100-200 ml of water consumed with recreation [Falconer et al. 1999]) equates to over 10-20 times the WHO guideline tolerable daily intake for an adult (60 kg) and over 30-60 times that of a child (15 kg). Since tourism in Leelanau County occurs mainly in summer months, when cyanobacterial blooms are likely to occur, greater numbers of people, including children, could be exposed to MC. Recreation by children also tends to occur in shallow areas, where MC concentrations, depending on weather patterns, may be greatest. MC is lower in fish muscle than in liver as the liver is the target organ (Soares et al. 2004). Although humans typically consume only muscle, Eaglesham et al. (2002) indicated that in prawns, muscle had no detectable MC when raw, but when cooked toxin was redistributed between the viscera and flesh. If fish tissue responds to cooking in a similar manner, human consumption would not be advised unless toxin is not present in muscle tissue or the liver. Larger and hence more desirable fish are likely to contain higher MC concentrations.
CHAPTER III: CONCLUSIONS

MC was present in all measured components in spring, months after *M. aeruginosa* blooms had occurred the previous summer. These data suggest that regardless of the breakdown of free MC by heterotrophic bacteria, regardless of the depuration period of these taxa, and regardless of the solubility of MC, it exists in these ecosystems in the absence of *M. aeruginosa* blooms. Lauren-Matta *et al.* (1995) demonstrated that low persistence of MC, caused repeated stress to organisms with required production of detoxication enzymes. Decreased survivorship resulting from MC exposure has been reported in many different taxa (Lauren-Maatta *et al.* 1995) even at low concentrations (Rohrlack *et al.* 2001). More specifically in fish and mammals, hepatocyte damage reaches apoptosis at low concentrations of MC (200 ppb) and proceeds towards necrosis at high (500 ppb) concentrations (Li Li and Chen 2005). In addition, chironomids, decapods and oligochaetes are negatively impacted by cyanobacterial blooms (Krzyanek *et al.* 1993). High concentrations of MC measured in *Hexagenia* spp. indicate the potential for MC exposure to terrestrial species.

Although MC-LR was likely a small fraction of measured MC-eq, and the potential for acute exposure to MC-LR by humans is unlikely in study basins, the threat of human sub lethal chronic exposure should be monitored. The long term risks associated with *M. aeruginosa* blooms and MC production may be of greater concern (Kuiper-Goodman *et al.* 1999) since experimentation using animal (non-human) subjects indicated that even chronic low-level exposure to MC-LR caused chronic liver injury, possible carcinogenesis and tumor growth (Kuiper-Goodman *et al.* 1999).
PROJECT CONCLUSIONS AND FUTURE RESEARCH

The results from this project demonstrate that these lake basins with established populations of zebra mussels, Little Traverse Lake, North Lake Leelanau and South Lake Leelanau, are changing, and that zebra mussel establishment is one driving factor for these changes. The summer phytoplankton are shifting from communities dominated by diatoms and chrysophytes to cyanobacteria, mainly *Microcystis aeruginosa*. Microcystin produced by these cyanobacteria appears to bioaccumulate in these basins, and is present throughout the year (before and after cyanobacterial blooms). The *Hexagenia* spp. and walleye contain the greatest concentrations of microcystin. This is important since *Hexagenia* spp. provide a link between aquatic and terrestrial ecosystems, and walleye are a sport fish regularly consumed by humans.

Microcystin concentrations in measured components were greater than many reported in the literature. Most of these studies did not include aquatic ecosystems with established populations of zebra mussels. Therefore, the potential influence of zebra mussels on microcystin production and fate should be further explored. Specifically of interest is the potential influence of zebra mussel nutrient enrichment of lake sediments via fecal and pseudofecal deposition, on both the viability of overwintering *M. aeruginosa*, and the amount of microcystin produced. In addition, microcystin should be quantified in winged adult *Hexagenia* spp. and potentially in birds or other predators of *Hexagenia* spp. Finally, the ability of *Dinobryon* to photosynthesize and phagocytize, may provide it with a unique opportunity in basins with blooms of *M. aeruginosa*. Research documenting the potential of *Dinobryon* to consume toxic and non-toxic *M. aeruginosa* might provide insight into how phytoplankton in these lake basins may continue to change and perhaps adapt to zebra mussel populations.
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Leelanau Historical Society, PO Box 246, Leland, MI 49654. Personal Communication.
Lowe, Rex, Phycologist, Department of Biology, Bowling Green State University, Bowling Green, Ohio 43403. Personal Communication.


APPENDIX A. TABLES AND FIGURES
<table>
<thead>
<tr>
<th>Year</th>
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Table 1. Year of zebra mussel introduction (I), transitional period (T), and estimated population establishment (E) for study lake basins (Big Glen Lake [BGL], Little Glen Lake [LGL], Lime Lake [LL], Little Traverse Lake [LTL], and North [NLL] and South [SLL] Lake Leelanau).
Figure 1. Study location in Leelanau County, Michigan. Big Glen Lake (BGL), Little Glen Lake (LGL), Lime Lake (LL), Little Traverse Lake (LTL), and North (NLL) and South (SLL) Lake Leelanau. Source: political boundaries and hydrology provided by Michigan Department of National Resources, MDNR, (MIRIS 1:24,000 scale).
<table>
<thead>
<tr>
<th></th>
<th>Total Phosphorus (ug L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Total Nitrogen (ug L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Chlorophyll-a (ug L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>Secchi Transparency (m)</th>
<th>Dissolved Oxygen (mg L&lt;sup&gt;-1&lt;/sup&gt;)</th>
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<tr>
<td>BGL</td>
<td>mean ± SE 5.3 ± 0.2 55 ± 5 1.8 ± 0.1 5.8 ± 0.1 5.7 ± 0.50</td>
<td>range 1.0 - 50.0 0 - 740 0.5 - 2.0 1.7 - 9.5 0 - 13.36</td>
<td>n 364 363 63 83</td>
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<td>LGL</td>
<td>mean ± SE 6.8 ± .2 54 ± 5 2.2 ± 0.1 2.3 ± 0.1 9.6 ± 0.2</td>
<td>range 1.0 - 38.7 0 - 450 0.7 - 4.8 0.8 - 3.8 6.9 - 13.97</td>
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<td>LL</td>
<td>mean ± SE 4.4 ± .2 236 ± 8 2.0 ± 0.1 3.3 ± 0.1 5.2 ± 0.4</td>
<td>range 0.0 - 27.3 2 - 890 0.5 - 4.3 1.7 - 9.5 0 - 12.9</td>
<td>n 309 271 83</td>
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<td>LTL</td>
<td>mean ± SE 5.2 ± 0.1 142 ± 7 2.4 ± 0.1 3.5 ± 0.1 4.6 ± 0.4</td>
<td>range 0.2 - 24.7 1 - 1000 0.0 - 6.8 1.8 - 9.8 0 - 12.9</td>
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<td>NLL</td>
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<td>SLL</td>
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<td>n 317 269 79</td>
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Table 2. Summary table including mean values, associated standard error (SE), range and total number of measurements (n) of total phosphorus (ug L<sup>-1</sup>), total nitrogen (ug L<sup>-1</sup>), chlorophyll-a (ug L<sup>-1</sup>), secchi disk transparency (m), and dissolved oxygen (mg L<sup>-1</sup>) in Big Glen Lake [BGL], Little Glen Lake [LGL], Lime Lake [LL], Little Traverse Lake [LTL], North Lake Leelanau [NLL] and South Lake Leelanau [SLL] from 1989 through 2004.
<table>
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<th>Basin</th>
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<th>p-value</th>
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Table 3. Regression models describing total phosphorus (TP) in study lake basins from 1990 to 2004 (Big Glen Lake [BGL], Lime Lake [LL], Little Traverse Lake [LTL], North Lake Leelanau [NLL] and South Lake Leelanau [SLL]) in the epilimnion (depth = 0) and hypolimnion.
A. Big Glen Lake

B. Little Glen Lake

C. Lime Lake

Figure 2. Total phosphorus (ug L⁻¹) over time from 1990 through 2004 in Big Glen Lake (A), Little Glen Lake (B) and Lime Lake (C) in the epilimnion (black circles) and hypolimnion (grey squares). Trend lines indicate significant relationships.
A. Little Traverse Lake

B. North Lake Leelanau

C. South Lake Leelanau

Figure 3. Total phosphorus (ug L$^{-1}$) over time from 1990 through 2004 in Little Traverse Lake (A), North Lake Leelanau (B) and South Lake Leelanau (C) in the epilimnion (black circles) and hypolimnion (grey squares). Trend lines indicate significant relationships; vertical red lines represent approximate time of zebra mussel establishment.
Figure 4. Nitrate/nitrite (ug L⁻¹) over time from 1990 through 2004 in Big Glen Lake (A), Little Glen Lake (B) and Lime Lake (C) in the epilimnion (black circles) and hypolimnion (grey squares).
A. Little Traverse Lake

B. North Lake Leelanau

C. South Lake Leelanau

Figure 5. Nitrate/nitrite (ug L$^{-1}$) over time from 1990 through 2004 in Little Traverse Lake (A), North Lake Leelanau (B) and South Lake Leelanau (C) in the epilimnion (black circles) and hypolimnion (grey squares). Trend line indicates significant relationship; vertical red lines represent approximate time of zebra mussel establishment.
A. Little Traverse Lake

B. North Lake Leelanau

C. South Lake Leelanau

Figure 6. Mean surface water temperature (°C) pre and post zebra mussel establishment (June, July and August) in Little Traverse Lake (A), North Lake Leelanau (B) and South Lake Leelanau (C). Bars indicate standard error.
Figure 7. Summer secchi transparency (m) of Big Glen Lake (A), Little Glen Lake (B) and Lime Lake (C) over time. Points represent raw data unless two or more samples were collected within a month, and then points represent the mean value with bars plus or minus standard error. Shaded area represents the three year time period of transition, with time of zebra mussel introduction estimated by the left side of the rectangle and the right side approximating the time after which basin-wide zebra mussel effects were expected.
Figure 8. Summer secchi transparency (m) of Little Traverse Lake (A), North Lake Leelanau (B) and South Lake Leelanau (C) and over time. Points represent raw data unless two or more samples were collected within a month, and then points represent the mean value with bars plus or minus standard error. Shaded area represents the three year time period of transition, with time of zebra mussel introduction estimated by the left side of the rectangle and the right side approximating the time after which basin-wide zebra mussel effects were expected.
### Secchi Disk Transparency

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<th>Comparison</th>
<th>June</th>
<th>July</th>
<th>August</th>
</tr>
</thead>
<tbody>
<tr>
<td>BGL</td>
<td>By Month Only</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LGL</td>
<td>Pre ZM and Transition</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>LL</td>
<td>Pre ZM and Transition</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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</tbody>
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<table>
<thead>
<tr>
<th>Zebra Mussel Effect</th>
<th>Comparison</th>
<th>June</th>
<th>July</th>
<th>August</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTL</td>
<td>Pre ZM, Post ZM</td>
<td>****</td>
<td>***</td>
<td>NS</td>
</tr>
<tr>
<td>NLL</td>
<td>Pre ZM, Post ZM</td>
<td>***</td>
<td>***</td>
<td>NS</td>
</tr>
<tr>
<td>SLL</td>
<td>Pre ZM, Post ZM</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Table 4. Summer (June, July and August) secchi disk transparency compared using repeated measures ANOVA in control basins (Big Glen Lake [BGL], Little Glen Lake [LGL] and Lime Lake [LL]) and zebra mussel effect basins (Little Traverse Lake [LTL], North Lake Leelanau [NLL] and South Lake Leelanau [SLL]). Comparisons varied based on time of zebra mussel [ZM] introduction in each basin. In BGL, transparencies were compared by month; in LGL and LL, transparencies were compared pre ZM introduction and during transition periods; in LTL, NLL and SLL, transparencies were compared pre ZM introduction and post ZM establishment; (p-value < 0.001***, < 0.00001****).

<table>
<thead>
<tr>
<th>Basin</th>
<th>Regression Model</th>
<th>$R^2$</th>
<th>p-value</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>BGL</td>
<td>Log Chlorophyll = 1.791 - 0.000037*time</td>
<td>12.8%</td>
<td>0.004</td>
<td>58</td>
</tr>
<tr>
<td>LGL</td>
<td>Log Chlorophyll = 1.370 - 0.000024*time</td>
<td>8.7%</td>
<td>0.021</td>
<td>57</td>
</tr>
<tr>
<td>LL</td>
<td>Chlorophyll = 10.56 - 0.000235*time</td>
<td>19.2%</td>
<td>&lt; 0.001</td>
<td>68</td>
</tr>
<tr>
<td>LTL</td>
<td>Log Chlorophyll = 1.563 - 0.000029*time</td>
<td>6.7%</td>
<td>0.003</td>
<td>115</td>
</tr>
<tr>
<td>NLL</td>
<td>Chlorophyll = 6.46 - 0.000137*time</td>
<td>10.0%</td>
<td>0.004</td>
<td>77</td>
</tr>
<tr>
<td>SLL</td>
<td>Log Chlorophyll = 1.359 - 0.000027*time</td>
<td>6.9%</td>
<td>0.019</td>
<td>75</td>
</tr>
</tbody>
</table>

Table 5. Regression models describing chlorophyll-a in study lake basins from 1993 to 2004 (Big Glen Lake [BGL], Little Glen Lake [LGL], Lime Lake [LL], Little Traverse Lake [LTL], North Lake Leelanau [NLL] and South Lake Leelanau [SLL]).
A. Big Glen Lake

Figure 9. Chlorophyll-a (ug L⁻¹) over time from 1993 through 2004 in Big Glen Lake (A) and Lime Lake (B). Labeled peaks are those that exceed the third quartile (Q3). Also included are the median (Med) and first quartile (Q1). Insert represents the frequency of chlorophyll-a determinations by month (March – November).
Figure 10. Chlorophyll-a (µg L⁻¹) over time from 1993 through 2004 in Little Traverse Lake (A), North Lake Leelanau (B) and South Lake Leelanau (C). Labeled peaks are those that exceed the third quartile (Q₃). Also included are the median (Med) and first quartile (Q₁); vertical red lines approximate time of zebra mussel establishment.
A. Big Glen Lake and Lime Lake

B. Little Traverse Lake, North Lake Leelanau and South Lake Leelanau

Figure 11. Frequency of chlorophyll-a peaks (concentrations exceeding the third quartile, per lake basin) and total samples from 1989 to 2004 (March through November) in Big Glen Lake and Lime Lake (A: zebra mussels absent) and Little Traverse Lake, and North Lake Leelanau and South Lake Leelanau (B: pre and post zebra mussel establishment).
Figure 12. Images of a foaming event that occurred in South Lake Leelanau in August (2002). The extent of the event (left) and close up of the foam showing pollen-like substance (right).
A. Big Glen Lake [BGL], Little Glen Lake [LGL] and Lime Lake [LL]

Figure 13. *M. aeruginosa* (cells ml\(^{-1}\)) over time (2001 – 2004) at the surface in Big Glen Lake [BGL], Little Glen Lake [LGL] and Lime Lake [LL] (A) and Little Traverse Lake [LTL], North Lake Leelanau [NLL] and South Lake Leelanau [SLL] (B).

B. Little Traverse Lake [LTL], North Lake Leelanau [NLL] and South Lake Leelanau [SLL]
Figure 14. Relative abundance of phytoplankton divisions (Cyanophyta and sum of Bacillariophyta, Chlorophyta and Chrysophyta) and *M. aeruginosa* density (cells ml$^{-1}$) in composite samples collected from Big Glen Lake (A) and Lime Lake (B) in 1993, 2002 and 2003.
Figure 15. Relative abundance of phytoplankton divisions (Cyanophyta and sum of Bacillariophyta, Chlorophyta and Chrysophyta) and *M. aeruginosa* density (cells ml$^{-1}$) in composite samples collected from Little Traverse Lake (A), North Lake Leelanau (B) and South Lake Leelanau (C) in 1993, 2002 and 2003.
Figure 16. Biovolume (um$^3$) of *Aphanocapsa* spp., *Chroococcus* spp. and *M. aeruginosa* in composite samples collected from Big Glen Lake (A) and Lime Lake (B) in 1993, 2002 and 2003.
A. Little Traverse Lake

B. North Lake Leelanau

C. South Lake Leelanau

Figure 17. Biovolume ($\text{um}^3$) of *Aphanocapsa* spp., *Chroococcus* spp. and *M. aeruginosa* in composite samples collected from Little Traverse Lake (A), North Lake Leelanau (B) and South Lake Leelanau (C) in 1993, 2002 and 2003.
Figure 18. Non-metric multidimensional scaling plot categorized by lake basin (Big Glen Lake [BGL], Lime Lake [LL], Little Traverse Lake [LTL], North Lake Leelanau [NLL] and South Lake Leelanau [SLL]) of composite samples of phytoplankton species collected in 1993, 2002 and 2003.
A. Big Glen Lake and Lime Lake

B. Little Traverse Lake, North Lake Leelanau and South Lake Leelanau

Figure 19. Non-metric multidimensional scaling plot categorized by sampling year (1993 and 2002/2003) and season (spring/early summer: March 1st – July 15th; late summer/early fall: July 16th – October 31st) of composite samples of phytoplankton species from Big Glen Lake and Lime Lake (A) and Little Traverse Lake, North Lake Leelanau and South Lake Leelanau (B).
<table>
<thead>
<tr>
<th>Basins</th>
<th>Global R</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control Basins (BGL, LL)</strong></td>
<td>0.579</td>
<td>0.001</td>
</tr>
<tr>
<td>Early Season</td>
<td>0.292</td>
<td>0.214</td>
</tr>
<tr>
<td>Late Season</td>
<td>0.66</td>
<td>0.001</td>
</tr>
<tr>
<td><strong>Zebra Mussel Effect Basins (LTL, NLL, SLL)</strong></td>
<td>0.604</td>
<td>0.001</td>
</tr>
<tr>
<td>Early Season</td>
<td>0.621</td>
<td>0.001</td>
</tr>
<tr>
<td>Late Season</td>
<td>0.655</td>
<td>0.001</td>
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</table>

Table 6. ANOSIM results comparing seasonal phytoplankton assemblages (early: March 1st – July 15th; late: July 16th – October 31st) collected in 1993 with those collected in 2002/2003, in control basins (Big Glen Lake, Lime Lake) and zebra mussel effect basins (Little Traverse Lake, North Lake Leelanau, South Lake Leelanau).
### A. Early Season

<table>
<thead>
<tr>
<th>Division</th>
<th>Species List</th>
<th>Division</th>
<th>Species List</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacillariophyta</td>
<td>Cyclotella spp.</td>
<td>Bacillariophyta</td>
<td>Cyclotella spp.</td>
</tr>
<tr>
<td>Chrysophyta</td>
<td>Dinobryon divergens</td>
<td>Bacillariophyta</td>
<td>Synedra spp.</td>
</tr>
<tr>
<td>Bacillariophyta</td>
<td>Misc. penate diatoms</td>
<td>Chrysophyta</td>
<td>Dinobryon sertularia</td>
</tr>
<tr>
<td>Bacillariophyta</td>
<td>Synedra spp.</td>
<td>Cyanophyta</td>
<td>Aphanocapsa spp.</td>
</tr>
<tr>
<td>Chrysophyta</td>
<td>Dinobryon sociale</td>
<td>Cyanophyta</td>
<td>Chroococcus spp.</td>
</tr>
<tr>
<td>Chrysophyta</td>
<td>Dinobryon sertularia</td>
<td>Bacillariophyta</td>
<td>Asterionella formosa</td>
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<tr>
<td></td>
<td></td>
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<td></td>
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</tbody>
</table>

### B. Late Season

<table>
<thead>
<tr>
<th>Division</th>
<th>Species List</th>
<th>Division</th>
<th>Species List</th>
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</thead>
<tbody>
<tr>
<td>Bacillariophyta</td>
<td>Cyclotella spp.</td>
<td>Cyanophyta</td>
<td>Aphanocapsa spp.</td>
</tr>
<tr>
<td>Cyanophyta</td>
<td>Chroococcus spp.</td>
<td>Cyanophyta</td>
<td>Chroococcus spp.</td>
</tr>
<tr>
<td>Cryptophyta</td>
<td>Rhodomonas minuta</td>
<td>Bacillariophyta</td>
<td>Cyclotella spp.</td>
</tr>
<tr>
<td>Chrysophyta</td>
<td>Dinobryon sociale</td>
<td>Cyanophyta</td>
<td>Microcystis aeruginosa</td>
</tr>
<tr>
<td>Cyanophyta</td>
<td>Aphanocapsa spp.</td>
<td>Cyanophyta</td>
<td>Merismopedia spp.</td>
</tr>
<tr>
<td>Bacillariophyta</td>
<td>Synedra spp.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 7. Phytoplankton taxa with greatest influence on overall community change from 1993 to 2002/2003, early in the season (A: March 1st – July 15th) and late in the season (B: July 16th – October 31st), in spatial control basins (Big Glen Lake [BGL] and Lime Lake [LL]) and zebra mussel effect basins (Little Traverse Lake [LTL], North Lake Leelanau [NLL], South Lake Leelanau [SLL]).
Figure 20. Discrete (A) and continuous (B) bathymetric data from Big Glen Lake. Source: discrete bathymetry provided by Michigan Department of National Resources, MDNR (1:24,000 scale).
A. Discrete Bathymetry

![Discrete Bathymetry Diagram]

B. Continuous Bathymetry

![Continuous Bathymetry Diagram]

Figure 21. Discrete (A) and continuous (B) bathymetric data from Little Glen Lake. Source: discrete bathymetry provided by Michigan Department of Natural Resources, MDNR (1:24,000 scale).
Figure 22. Discrete (A) and continuous (B) bathymetric data from Lime Lake. Source: discrete bathymetry provided by Michigan Department of Natural Resources, MDNR (1:24,000 scale).
A. Discrete Bathymetry

B. Continuous Bathymetry

Figure 23. Discrete (A) and continuous (B) bathymetric data from Little Traverse Lake. Source: discrete bathymetry provided by Michigan Department of National Resources, MDNR (1:24,000 scale).
A. Slope

B. Aspect

Figure 24. Slope (A) and aspect (B) of the Little Traverse Lake basin. Source: based on discrete bathymetry provided by Michigan Department of Natural Resources, MDNR (1:24,000 scale).
A. Distance from In/Outflows

B. Distance from Boat Launch

Figure 25. Estimated Euclidean distance from in/outflows (A) and maintained boat launches (B) in Lime Lake. Source: hydrology provided by Michigan Department of Natural Resources, MDNR (1:24,000 scale).
Figure 26. Discrete bathymetric data from North Lake Leelanau. Source: provided by Michigan Department of Natural Resources, MDNR (1:24,000 scale).
Figure 27. Continuous bathymetric data from North Lake Leelanau. Source: based on discrete bathymetry provided by Michigan Department of Natural Resources, MDNR (1:24,000 scale).
Figure 28. Slope of the North Lake Leelanau basin. Source: based on discrete bathymetry provided by Michigan Department of Natural Resources, MDNR (1:24,000 scale).
Figure 29. Aspect of the North Lake Leelanau basin. Source: based on discrete bathymetry provided by Michigan Department of Natural Resources, MDNR (1:24,000 scale).
Figure 30. Estimated Euclidean distance from in/outflows in North Lake Leelanau. Source: hydrology provided by Michigan Department of Natural Resources, MDNR (1:24,000 scale).
Figure 31. Estimated Euclidean distance from maintained boat launches in North Lake Leelanau. Source: hydrology provided by Michigan Department of Natural Resources, MDNR (1:24,000 scale).
Figure 32. Discrete bathymetric data from South Lake Leelanau. Source: provided by Michigan Department of Natural Resources, MDNR (1:24,000 scale).
Figure 33. Continuous bathymetric data from South Lake Leelanau. Source: based on discrete bathymetry provided by Michigan Department of Natural Resources, MDNR (1:24,000 scale).
Figure 34. Slope of the South Lake Leelanau basin. Source: based on discrete bathymetry provided by Michigan Department of Natural Resources, MDNR (1:24,000 scale).
Figure 35. Aspect of the South Lake Leelanau basin. Source: based on discrete bathymetry provided by Michigan Department of Natural Resources, MDNR (1:24,000 scale).
Figure 36. Estimated Euclidean distance from in/outflows in South Lake Leelanau. Source: hydrology provided by Michigan Department of Natural Resources, MDNR (1:24,000 scale).
Figure 37. Estimated Euclidean distance from maintained boat launches in South Lake Leelanau. Source: hydrology provided by Michigan Department of Natural Resources, MDNR (1:24,000 scale).
<table>
<thead>
<tr>
<th>Location</th>
<th>Mean ± SE (Ind m⁻²)</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>BGL</td>
<td>0 ± 0</td>
<td>2004</td>
</tr>
<tr>
<td>LGL</td>
<td>14 ± 3</td>
<td>2004</td>
</tr>
<tr>
<td>LL</td>
<td>196 ± 26</td>
<td>2004</td>
</tr>
<tr>
<td>LTL</td>
<td>1342 ± 86</td>
<td>2003</td>
</tr>
<tr>
<td>NLL</td>
<td>2766 ± 222</td>
<td>2002</td>
</tr>
<tr>
<td>SLL</td>
<td>1307 ± 89</td>
<td>2002</td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 - 0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 - 617</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 - 4849</td>
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<tr>
<td></td>
<td>0 - 10939</td>
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<tr>
<td></td>
<td>0 - 32461</td>
<td></td>
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<tr>
<td></td>
<td>0 - 22743</td>
<td></td>
</tr>
</tbody>
</table>

Table 8. Summary table including mean values, associated standard error (SE), range, total number of counted video images (n) and year of video collection of zebra mussel densities (individuals m⁻²), in Big Glen Lake (BGL), Little Glen Lake (LGL), Lime Lake (LL), Little Traverse Lake (LTL), North Lake Leelanau (NLL) and South Lake Leelanau (SLL).
Table 9. Regression models describing zebra mussel density (individuals m$^{-2}$ [ZMD]) based on slope (%), aspect (degrees), continuous bathymetry (meters [cBath]) and distance from in/outflows (meters [DIO]) in Little Traverse Lake (LTL), North Lake Leelanau (NLL) and South Lake Leelanau (SLL).

<table>
<thead>
<tr>
<th>Basin</th>
<th>Regression Model</th>
<th>$R^2$</th>
<th>p-value</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTL</td>
<td>LogZMD = 2.631 + 0.4201 * LogSlope</td>
<td>12.0</td>
<td>0.008</td>
<td>55</td>
</tr>
<tr>
<td>NLL</td>
<td>LogZMD = 3.604 - 0.002143 * Aspect</td>
<td>17.8</td>
<td>0.004</td>
<td>42</td>
</tr>
<tr>
<td>SLL</td>
<td>SqrtZMD = 34.6 - 14.0 * sqrtcBath + 0.714 * sqrtDIO</td>
<td>40.3</td>
<td>&lt; 0.001</td>
<td>66</td>
</tr>
</tbody>
</table>
Figure 38. Estimated zebra mussel density in Little Traverse Lake using regression analysis (Log individuals m$^{-2}$). Source: hydrology provided by Michigan Department of Natural Resources, MDNR (1:24,000 scale).
Figure 39. Estimated zebra mussel density (Log individuals m$^{-2}$) in North Lake Leelanau using regression analysis. Source: hydrology provided by Michigan Department of Natural Resources, MDNR (1:24,000 scale).
Figure 40. Estimated zebra mussel density (square root individuals m$^{-2}$) in South Lake Leelanau using multiple regression. Source: hydrology provided by Michigan Department of Natural Resources, MDNR (1:24,000 scale).
Figure 41. Estimated zebra mussel density in Little Traverse Lake using the kernel technique (individuals m\(^{-2}\)km\(^{-2}\)). Source: hydrology provided by Michigan Department of Natural Resources, MDNR (1:24,000 scale).
Figure 42. Estimated zebra mussel density (individuals m$^{-2}$ km$^{-2}$) in North Lake Leelanau using the kernel technique. Source: hydrology provided by Michigan Department of Natural Resources, MDNR (1:24,000 scale).
Figure 43. Estimated zebra mussel density (individuals m⁻²km⁻²) in South Lake Leelanau using the kernel technique. Source: hydrology provided by Michigan Department of Natural Resources, MDNR (1:24,000 scale).
Table 10. Regression models comparing zebra mussel densities estimated using the kernel function (Kernel) and actual zebra mussel densities counted using underwater video (ZMD) in Little Traverse Lake (LTL), North Lake Leelanau (NLL) and South Lake Leelanau (SLL).

<table>
<thead>
<tr>
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<th>Regression Model</th>
<th>R²</th>
<th>p-value</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>LTL</td>
<td>LogKernel = 3.607 + 0.2497 * LogZMD</td>
<td>17.1%</td>
<td>0.003</td>
<td>58</td>
</tr>
<tr>
<td>NLL</td>
<td>LogKernel = 2.215 + 0.4733 * LogZMD</td>
<td>40.7%</td>
<td>&lt; 0.001</td>
<td>43</td>
</tr>
<tr>
<td>SLL</td>
<td>LogKernel = 3.289 + 0.01630 * SqrtZMD</td>
<td>30.1%</td>
<td>&lt; 0.001</td>
<td>59</td>
</tr>
</tbody>
</table>
Figure 44. Error plots for kernel density estimates of zebra mussels (individuals m\(^{-2}\) km\(^{-2}\)) compared to zebra mussel densities (individuals m\(^{-2}\)) collected by underwater video in Little Traverse Lake (A), North Lake Leelanau (B) and South Lake Leelanau (C).
<table>
<thead>
<tr>
<th>Basin</th>
<th>Year</th>
<th>Mean Thermocline (m)</th>
<th>Volume of Epilimnion (L)</th>
<th>Area of Zebra Mussel Habitat (m²)</th>
<th>Zebra Mussel density (indi m²)</th>
<th>Total Zebra Mussels</th>
<th>Filtering Capacity (Turnover day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BGL</td>
<td>2004</td>
<td>3.88</td>
<td>7322944.80</td>
<td>3967000</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
<td>LGL</td>
<td>2004</td>
<td>NA</td>
<td>763306.67</td>
<td>5725000</td>
<td>14</td>
<td>80150000</td>
<td>105.00</td>
</tr>
<tr>
<td>LL</td>
<td>2004</td>
<td>2.91</td>
<td>1052652.00</td>
<td>1574000</td>
<td>196</td>
<td>308504000</td>
<td>293.07</td>
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<td>LTL</td>
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<td>1342</td>
<td>1975424000</td>
<td>2373.39</td>
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<tr>
<td>NLL</td>
<td>2002</td>
<td>3.00</td>
<td>4469206.67</td>
<td>2693000</td>
<td>2766</td>
<td>7448838000</td>
<td>1666.70</td>
</tr>
<tr>
<td>SLL</td>
<td>2002</td>
<td>3.24</td>
<td>3947736.00</td>
<td>4277000</td>
<td>1307</td>
<td>5590039000</td>
<td>1416.01</td>
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</table>

Table 11. Estimated filtering capacities (frequency of epilimnetic turnover via zebra mussel filtration) of zebra mussel populations measured from 2002 to 2004 in Big Glen Lake (BGL), Little Glen Lake (LGL), Lime Lake (LL), Little Traverse Lake (LTL), North Lake Leelanau (NLL) and South Lake Leelanau (SLL), based on the volume of the epilimnion, amount of suitable habitat and mean zebra mussel density.
Figure 45. Comparison of *M. aeruginosa* maxima and zebra mussel population filtering capacity (frequency of epilimnetic turnover) in Big Glen Lake (BGL), Little Glen Lake (LGL), Lime Lake (LL), Little Traverse Lake (LTL), North Lake Leelanau (NLL) and South Lake Leelanau (SLL). Density = 199.5 + 1.498 * FC, p = 0.058
A. Big Glen Lake

![Graph A]

B. Little Glen Lake

![Graph B]

C. Lime Lake

![Graph C]

Figure 46. *Microcystis aeruginosa* densities (cells ml$^{-1}$) [solid lines] and microcystin equivalents (ppb) [dashed lines] measured from the surface in Big Glen Lake (A), Little Glen Lake (B) and Lime Lake (C) over time (2004).
Figure 47. *Microcystis aeruginosa* densities (cells ml\(^{-1}\)) [solid lines] and microcystin equivalents (ppb) [dashed lines] measured from the surface in Little Traverse Lake (A), North Lake Leelanau (B) and South Lake Leelanau (C) over time (2004). Red dashed line indicates World Health Organization limit for drinking water of one ppb.
Figure 48. Mean microcystin equivalents (ppb) as a function of the mean *Microcystis aeruginosa* (cells ml\(^{-1}\)) (log microcystin-eq = -2.865 + 1.172 *log *M. aeruginosa*, \(R^2 = 82.9\%\), \(p = 0.012\)) as measured in Big Glen Lake (BGL), Little Glen Lake (LGL), Lime Lake (LL), Little Traverse Lake (LTL), North Lake Leelanau (NLL) and South Lake Leelanau (SLL) in 2004.

<table>
<thead>
<tr>
<th>Basin</th>
<th>Mean (Microcystis)</th>
<th>Standard Error</th>
<th>n</th>
<th>Mean (Microcystin-eq)</th>
<th>Standard Error</th>
<th>n</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>BGL</td>
<td>0.0</td>
<td>0.0</td>
<td>3</td>
<td>&lt; 0.00</td>
<td>0.0</td>
<td>3</td>
<td>&lt; 0.00</td>
</tr>
<tr>
<td>LGL</td>
<td>87.5</td>
<td>51.5</td>
<td>4</td>
<td>0.06</td>
<td>0.04</td>
<td>4</td>
<td>0.16</td>
</tr>
<tr>
<td>LL</td>
<td>30.0</td>
<td>30.0</td>
<td>5</td>
<td>0.06</td>
<td>0.02</td>
<td>5</td>
<td>0.11</td>
</tr>
<tr>
<td>LTL</td>
<td>704.6</td>
<td>147.8</td>
<td>14</td>
<td>15.65</td>
<td>15.25</td>
<td>14</td>
<td>213.93</td>
</tr>
<tr>
<td>NLL</td>
<td>119.0</td>
<td>47.1</td>
<td>12</td>
<td>0.14</td>
<td>0.02</td>
<td>12</td>
<td>0.21</td>
</tr>
<tr>
<td>SLL</td>
<td>42.0</td>
<td>28.5</td>
<td>12</td>
<td>0.15</td>
<td>0.01</td>
<td>12</td>
<td>0.19</td>
</tr>
</tbody>
</table>

Table 12. Summary table including mean values, associated standard error (SE) and total number of measurements (n) of *Microcystis aeruginosa* (cells ml\(^{-1}\)) and microcystin equivalents (ppb) in Big Glen Lake (BGL), Little Glen Lake (LGL), Lime Lake (LL), Little Traverse Lake (LTL), North Lake Leelanau (NLL) and South Lake Leelanau (SLL) in 2004.
A. Little Traverse Lake

B. North Lake Leelanau

C. South Lake Leelanau

Figure 49. *M. aeruginosa* (cells ml\(^{-1}\)) and microcystin equivalents (ppb) measured in 20 ml of whole water, filtered water and collected on filters from Little Traverse Lake (A), North Lake Leelanau (B) and South Lake Leelanau (C).
Figure 50. Mean microcystin equivalents measured in sediment collected from Big Glen Lake (BGL), Little Glen Lake (LGL), Little Traverse Lake (LTL), North Lake Leelanau (NLL) and South Lake Leelanau (SLL) in spring and fall (2004). Bars indicate standard error, data labels represent the number of samples.

<table>
<thead>
<tr>
<th>Group</th>
<th>Regression Model</th>
<th>( R^2 )</th>
<th>p-value</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>SC</td>
<td>Log Fall Sediment MC-eq = 0.1948 + 0.01422 * %Depth</td>
<td>75.2</td>
<td>0.005</td>
<td>8</td>
</tr>
<tr>
<td>ZM</td>
<td>Log Spring Sediment MC-eq = -0.5565 + 1.129 *Log %Depth</td>
<td>29.5</td>
<td>0.005</td>
<td>25</td>
</tr>
<tr>
<td>ZM</td>
<td>Log Fall Sediment MC-eq = -0.1211 + 1.268 *Log %Depth</td>
<td>42.6</td>
<td>&lt; 0.001</td>
<td>25</td>
</tr>
</tbody>
</table>

Table 13. Regression models describing microcystin equivalents (MC-eq) in spring and fall sediments collected from spatial control basins (SC: Big Glen Lake and Little Glen Lake), and zebra mussel effect basins (ZM: Little Traverse Lake, North Lake Leelanau and South Lake Leelanau) in 2004.
Figure 51. Fall sediment microcystin equivalents (MC) as a function of percent depth in spatial control basins (Big Glen Lake [BGL] and Little Glen Lake [LGL]). Trend line indicates significant relationship.
Figure 52. Spring (A) and fall (B) sediment microcystin equivalents (MC) as a function of percent depth in zebra mussel effect basins (Little Traverse Lake [LTL], North Lake Leelanau [NLL] and South Lake Leelanau [SLL]). Trend lines indicate significant relationships.
A. Amphipoda

B. Chironomidae

Figure 53. Mean microcystin equivalents (ppb) measured in amphipods (A) and chironomids (B) collected from Big Glen Lake (BGL), Little Glen Lake (LGL), Little Traverse Lake (LTL), North Lake Leelanau (NLL) and South Lake Leelanau (SLL) in spring and fall (2004). Bars indicate standard error, data labels represent the number of samples.
A. Crayfish

B. Hexagenia spp.

Figure 54. Mean microcystin equivalents (ppb) measured in Crayfish (A) and Hexagenia spp. (B) collected from Big Glen Lake (BGL), Little Glen Lake (LGL), Little Traverse Lake (LTL), North Lake Leelanau (NLL) and South Lake Leelanau (SLL) in spring and fall (2004). Bars indicate standard error, data labels represent the number of samples.
Figure 55. Mean microcystin equivalents (ppb) measured in Oligochaeta collected from Big Glen Lake (BGL), Little Glen Lake (LGL), Little Traverse Lake (LTL), North Lake Leelanau (NLL) and South Lake Leelanau (SLL) in spring and fall (2004). Bars indicate standard error, data labels represent the number of samples.

### Table 14

<table>
<thead>
<tr>
<th>Component</th>
<th>Regression Model</th>
<th>$R^2$</th>
<th>p-value</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphipoda</td>
<td>Log MC-eq = -0.3087 + 2.101 *Log %Depth</td>
<td>67.7%</td>
<td>p &lt; 0.044</td>
<td>6</td>
</tr>
<tr>
<td>Chironomidae</td>
<td>Log MC-eq = 0.9326 + 1.229 *Log %Depth</td>
<td>23.1%</td>
<td>p &lt; 0.013</td>
<td>23</td>
</tr>
</tbody>
</table>

Table 14. Regression models describing microcystin equivalents (MC-eq, ppb) in Amphipods and Chironomids collected from zebra mussel effect basins (Little Traverse Lake, North Lake Leelanau and South Lake Leelanau) in 2004.
Figure 56. Microcystin equivalents (ppb) as a function of percent depth measured in amphipods (A) and chironomids (B) in zebra mussel effect basins (Little Traverse Lake [LTL], North Lake Leelanau [NLL] and South Lake Leelanau [SLL]) in spring and fall (2004). Trend lines indicate significant relationships.
A. Unionidae

B. Zebra Mussels

Figure 57. Mean microcystin equivalents (ppb) measured in unionids (A) and zebra mussels (B) collected from Big Glen Lake (BGL), Little Glen Lake (LGL), Little Traverse Lake (LTL), North Lake Leelanau (NLL) and South Lake Leelanau (SLL) in spring and fall (2004). Bars indicate standard error, data labels represent the number of samples.
Figure 58. Mean microcystin equivalents (ppb) in liver and muscle tissue (walleye, smallmouth bass, whitefish, lake trout and largemouth bass) and whole fish (*Notropis texanus*) collected in spring and fall from South Lake Leelanau (SLL), North Lake Leelanau (NLL) and Little Traverse Lake (LTL) (2004). Bars indicate standard error.

Table 15. The amount of fish (g) that can be safely consumed by men (80 kg), women (66 kg) and children (30 kg) per day based on the mean microcystin equivalents (MC-eq) measured in fish muscle tissue from North and South Lake Leelanau (2004). Determined using the total daily intake recommended by the World Health Organization of 0.04 ug kg$^{-1}$. 

<table>
<thead>
<tr>
<th>Season</th>
<th>Fish</th>
<th>Mean MC-eq (ppm)</th>
<th>fish (g) can be consumed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>muscle</td>
<td>men</td>
</tr>
<tr>
<td>Spring</td>
<td>Whitefish</td>
<td>0.0241</td>
<td>132.54</td>
</tr>
<tr>
<td>Spring</td>
<td>Lk Trout</td>
<td>0.0195</td>
<td>163.95</td>
</tr>
<tr>
<td>Spring</td>
<td>Walleye</td>
<td>0.0203</td>
<td>157.71</td>
</tr>
<tr>
<td>Spring</td>
<td>Smallmouth Bass</td>
<td>0.0134</td>
<td>238.17</td>
</tr>
<tr>
<td>Fall</td>
<td>Walleye</td>
<td>0.0244</td>
<td>131.14</td>
</tr>
<tr>
<td>Fall</td>
<td>Smallmouth Bass</td>
<td>0.0272</td>
<td>117.67</td>
</tr>
<tr>
<td>Fall</td>
<td>Largemouth Bass</td>
<td>0.0087</td>
<td>369.04</td>
</tr>
</tbody>
</table>
### Table 16. Summary table including mean values, associated standard error (SE), range and total number of measurements (n) of microcystin equivalents (MC-eq) (ppb) measured in lake sediment, macroinvertebrates, bivalves, and piscivorous fish, in Big Glen Lake (BGL), Little Glen Lake (LGL), Little Traverse Lake (LTL), North Lake Leelanau (NLL) and South Lake Leelanau (SLL) in 2004.

<table>
<thead>
<tr>
<th></th>
<th>Sediment MC-eq (ppb)</th>
<th>Macroinvertebrate MC-eq (ppb)</th>
<th>Bivalve MC-eq (ppb)</th>
<th>Piscivorous Fish MC-eq (ppb)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Spring</td>
<td>Fall</td>
<td>Spring</td>
<td>Fall</td>
</tr>
<tr>
<td>BGL</td>
<td>mean ± SE</td>
<td>5.7 ± 1.9</td>
<td>7.25 ± 5.2</td>
<td>24.2 ± 4.9</td>
</tr>
<tr>
<td>range</td>
<td>1.5 - 9.4</td>
<td>1.2 - 22.7</td>
<td>14 - 38</td>
<td>5.7 - 42</td>
</tr>
<tr>
<td>n</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>mean ± SE</td>
<td>2.1 ± 0.7</td>
<td>28.2 ± 11.9</td>
<td>43.9 ± 10</td>
<td>1317.3 ± 674.4</td>
</tr>
<tr>
<td>range</td>
<td>1.1 - 4.1</td>
<td>3.9 - 60.8</td>
<td>15.6 - 63</td>
<td>317.7 - 3307</td>
</tr>
<tr>
<td>n</td>
<td>9</td>
<td>9</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>mean ± SE</td>
<td>63.0 ± 21.3</td>
<td>335.1 ± 130.7</td>
<td>2171.1 ± 663</td>
<td>15388.5 ± 4864.7</td>
</tr>
<tr>
<td>range</td>
<td>4.1 - 206.9</td>
<td>46.5 - 988.1</td>
<td>7.7 - 5547</td>
<td>10.2 - 46522</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>9</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>mean ± SE</td>
<td>12.6 ± 5.5</td>
<td>70.4 ± 50.3</td>
<td>7618.1 ± 2893.9</td>
<td>19794.1 ± 12472.2</td>
</tr>
<tr>
<td>range</td>
<td>0.7 - 50</td>
<td>1.5 - 471.4</td>
<td>574.7 - 33798</td>
<td>283.5 - 105723</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>9</td>
<td>11</td>
<td>8</td>
</tr>
<tr>
<td>mean ± SE</td>
<td>50.2 ± 28.8</td>
<td>210.9 ± 119.3</td>
<td>2336.6 ± 807.4</td>
<td>9823.4 ± 3151.3</td>
</tr>
<tr>
<td>range</td>
<td>0.5 - 328.7</td>
<td>5.2 - 712.9</td>
<td>129.9 - 6772</td>
<td>306.8 - 25181</td>
</tr>
<tr>
<td>n</td>
<td>11</td>
<td>7</td>
<td>9</td>
<td>7</td>
</tr>
</tbody>
</table>

The table above shows the concentration of microcystin equivalents (MC-eq) in different samples collected from Big Glen Lake [BGL], Little Glen Lake [LGL], Little Traverse Lake [LTL], North Lake Leelanau [NLL] and South Lake Leelanau [SLL] during the years 2004. The data includes the mean value, standard error, range and total number of measurements (n) for each sample type (sediment, macroinvertebrates, bivalves, and piscivorous fish) for each lake in the spring and fall seasons.
Figure 59. Food web schematic including mean microcystin equivalents (MC-eq) per component (ppb). Whole water MC-eq refers to dissolved and cell bound MC-eq; planktivorous fishes are minnows (*N. texanus*) and piscivores include lake trout, smallmouth and largemouth bass, walleye and whitefish. Mean values are calculated using data collected in spring and fall (before and after blooms of *M. aeruginosa*) and across depths.
### Table 17. Summary table including mean values, associated standard error (SE), range and total number of measurements (n) of microcystin equivalents (MC-eq) measured using ELISA (ppb), microcystin-LR (MC-LR) measured using HPLC-MS (ppb) and percent microcystin-LR (relative to MC-eq) measured in *Hexagenia spp.* and lake sediment, collected from Little Traverse Lake and North Lake Leelanau in 2004.

<table>
<thead>
<tr>
<th>Component</th>
<th>MC-eq (ppb)</th>
<th>MC-LR (ppb)</th>
<th>Percent MC-LR (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hexagenia spp.</strong></td>
<td>mean ± SE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>range</td>
<td>30981 ± 8835</td>
<td>384 ± 157</td>
<td>4 ± 2</td>
</tr>
<tr>
<td>n</td>
<td>25</td>
<td>27</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>1097 - 211344</td>
<td>1 - 4177</td>
<td></td>
</tr>
<tr>
<td><strong>Sediment</strong></td>
<td>mean ± SE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>range</td>
<td>164 ± 56</td>
<td>11 ± 4</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>n</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>1 - 988</td>
<td>0 - 93</td>
<td>1 - 56</td>
</tr>
</tbody>
</table>

Table 18. Regression models describing the relationship between microcystin-LR (MC-LR, ppb) measured using HPLC-MS and microcystin equivalents (MC-eq, ppb) measured using ELISA in Little Traverse Lake and North Lake Leelanau (2004).

<table>
<thead>
<tr>
<th>Component</th>
<th>Regression Model</th>
<th>R²</th>
<th>p-value</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexagenia spp. &amp; sediment</td>
<td>Log MC-LR = -0.7717 + 0.6855 * Log MC-eq</td>
<td>87.10% P &lt; 0.001</td>
<td>38</td>
<td></td>
</tr>
<tr>
<td>Hexagenia spp.</td>
<td>Log Hexagenia MC-LR = -0.2198 + 0.5535 * Log Hexagenia MC-eq</td>
<td>78.80% P &lt; 0.001</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Sediment</td>
<td>Log Sediment MC-LR = -0.9263 + 0.7919 * Log Sediment MC-eq</td>
<td>66.30% P &lt; 0.001</td>
<td>23</td>
<td></td>
</tr>
</tbody>
</table>
Figure 60. The relationship between microcystin-LR (ppb) measured using HPLC and microcystin equivalents (ppb) measured using ELISA in *Hexagenia spp.* and lake sediment collected from Little Traverse Lake and North Lake Leelanau in 2004. Trend line indicates significant relationship.
A. *Hexagenia* spp. Only

![Graph showing the relationship between microcystin-LR (ppb) measured using HPLC-MS and microcystin equivalents (ppb) measured using ELISA in *Hexagenia* spp. (A) and lake sediments (B) collected from Little Traverse Lake and North Lake Leelanau in 2004. Trend lines indicate significant relationships.]

B. Sediment Only

![Graph showing the relationship between microcystin-LR (ppb) measured using HPLC-MS and microcystin equivalents (ppb) measured using ELISA in *Hexagenia* spp. (A) and lake sediments (B) collected from Little Traverse Lake and North Lake Leelanau in 2004. Trend lines indicate significant relationships.]

Figure 61. The relationship between microcystin-LR (ppb) measured using HPLC-MS and microcystin equivalents (ppb) measured using ELISA in *Hexagenia* spp. (A) and lake sediments (B) collected from Little Traverse Lake and North Lake Leelanau in 2004. Trend lines indicate significant relationships.
Figure 62. Mean microcystin-LR (ppb) in *Hexagenia spp.* collected from Little Traverse Lake (LTL) and North Lake Leelanau (NLL) in spring and fall of 2004. Bars indicate standard error.

Figure 63. Mean length of *Hexagenia spp.* (mm) collected from Little Traverse Lake (LTL) and North Lake Leelanau (NLL) in spring and fall of 2004, for microcystin analyses.