

FINAL REPORT

**Monitoring and Predicting Concentrations of Cyanobacterial Toxins in
Michigan Lakes**

Orlando Sarnelle

Howard Wandell

**Department of Fisheries and Wildlife
Michigan State University**

January 2008

ACKNOWLEDGEMENTS

This project was made possible with funding from the Michigan Department of Environmental Quality, Water Bureau. We thank Jamie Morrison, RajReni Kaul and Geoffrey Horst for assistance with field and laboratory work, and Jamie Morrison for help with project management. We especially thank citizens of the Michigan Cooperative Lakes Monitoring Program (CLMP) for collecting the bulk of the samples for this project, and the Department of Fisheries and Wildlife for logistical and technical support.

TABLE OF CONTENTS

| | |
|------------------------|----|
| Acknowledgements..... | 2 |
| List of Figures..... | 4 |
| Executive Summary..... | 5 |
| Introduction..... | 7 |
| Methods..... | 8 |
| Results..... | 11 |
| Discussion..... | 20 |
| Literature Cited..... | 23 |
| Appendix A..... | 25 |
| Appendix B..... | 32 |
| Appendix C..... | 35 |
| Appendix D..... | 37 |
| Appendix E..... | 40 |

LIST OF FIGURES

| | | |
|-----------|--|----|
| Figure 1 | Comparison between chlorophyll <i>a</i> concentrations ($\mu\text{g L}^{-1}$) for replicate samples analyzed at MSU versus analyzed at the MDEQ..... | 12 |
| Figure 2 | Comparison between toxin (microcystin) concentrations for samples collected by CLMP volunteers versus by MSU personnel..... | 12 |
| Figure 3 | Comparison between toxin (microcystin) concentrations measured at MSU versus at Greenwater Laboratories (GWL)..... | 13 |
| Figure 4 | Effect of sample storage in insulated shipping container on microcystin concentrations..... | 13 |
| Figure 5 | Effect of frozen storage on microcystin concentrations..... | 14 |
| Figure 6 | Effect of boiling on microcystin concentrations..... | 14 |
| Figure 7 | Frequency distributions of microcystin concentrations at the shoreline and in the euphotic zone for CLMP lakes with and without zebra mussels..... | 15 |
| Figure 8 | Relationship between microcystin concentrations at the shoreline and in the euphotic zone away from shore for CLMP lakes..... | 16 |
| Figure 9 | Relationships between microcystin and chlorophyll <i>a</i> concentrations at the shoreline for CLMP lakes with and without zebra mussels..... | 17 |
| Figure 10 | Relationships between microcystin in the euphotic zone and TP for CLMP lakes with and without zebra mussels..... | 17 |
| Figure 11 | Relationships between microcystin at the shoreline and TP for CLMP lakes with and without zebra mussels..... | 18 |
| Figure 12 | Mean microcystin concentrations for lakes with and without zebra mussels..... | 18 |
| Figure 13 | Relationship between chlorophyll <i>a</i> in the euphotic zone and total phosphorus for CLMP lakes..... | 19 |
| Figure 14 | Relationships between chlorophyll <i>a</i> at the shoreline and total phosphorus for CLMP lakes with and without zebra mussels..... | 19 |

EXECUTIVE SUMMARY

We conducted a survey of 77 lakes from 38 Michigan counties in August-September, 2006 to assess concentrations of the cyanobacterial toxin, microcystin, in nearshore surface waters. The lakes ranged across the state from Marquette and Dickinson counties in the Upper Peninsula to Cass and Lenawee counties in the south. Samples for microcystin, chlorophyll *a* and total phosphorus (TP) were collected by citizen volunteers via the Cooperative Lake Monitoring Program administered by the Michigan Department of Environmental Quality (MDEQ). We examined predictive relationships among microcystin, chlorophyll *a*, total phosphorus, latitude and maximum depth. In addition, we explicitly examined the influence of zebra mussel invasion on these relationships and on toxin concentrations in general.

In contrast to many limnological monitoring programs, we were particularly interested in measuring toxin levels at the most likely point of human recreational contact, namely at the water's surface along the shoreline. Toxicogenic cyanobacteria can regulate their buoyancy, a property that can lead to the formation of thin layers of high concentration at the surface (scums). As a result, the potential exists for large concentrations of cyanobacteria (and accompanying toxins) to be blown toward the shoreline. Survey results were compared to standards promulgated by the World Health Organization (WHO); the 1 $\mu\text{g L}^{-1}$ limit for drinking water and the 20 $\mu\text{g L}^{-1}$ limit for recreational exposure.

The major findings of the project are as follows:

- 1) citizen volunteers are an effective means of collecting samples for accurate estimates of microcystin.
- 2) microcystin samples should be shipped to the laboratory via Express Mail or other 2-day delivery method, should be stored in the freezer, and should either be boiled before ELISA analysis or corrected for not boiling by a factor of 1.23.
- 3) Based on WHO guidelines, almost all lakes sampled by CLMP volunteers during 2006 posed little or no public health risk to recreational users at the time of sampling.
- 4) microcystin concentrations above the recreational standard of 20 $\mu\text{g L}^{-1}$ appear to be rare in Michigan subject to the following caveats:
 - a) very few lakes with TP > 25 $\mu\text{g L}^{-1}$ were included in the survey.
 - b) lakes were only sampled once, mostly in September.
 - c) it is not known whether 2006 was representative of typical climatic conditions that affect the development of toxicogenic cyanobacterial blooms in Michigan.
- 5) concentrations of microcystin tend to be higher at the shoreline than in depth-integrated samples of the entire euphotic zone.
- 6) microcystin concentrations are substantially higher and more variable in lakes that have been invaded by zebra mussels.
- 7) rare episodic events of very high toxin levels at the shoreline are possible in lakes with low TP if zebra mussels are present.
- 8) it is unknown whether rare episodes of high toxin levels in lakes with zebra mussels are restricted to particular lakes or may occur in any invaded lake in any given year.
- 9) standard monitoring procedures based on depth-integrated sampling away from shore may give no indication of toxin concentrations that exceed recreational standards at the shoreline.
- 10) total phosphorus is a positive predictor of microcystin concentrations at the shoreline, but only in lakes that lack zebra mussels.

- 11) latitude and maximum depth were not significant predictors of toxin concentrations.
- 12) shoreline microcystin concentrations above $1 \mu\text{g L}^{-1}$ appear to be unlikely in lakes with $\text{TP} < 15 \mu\text{g L}^{-1}$ that lack zebra mussels.
- 13) shoreline microcystin concentrations above $1 \mu\text{g L}^{-1}$ appear to be unlikely in lakes with shoreline chlorophyll *a* levels below $10 \mu\text{g L}^{-1}$.
- 14) more research is needed with respect to findings 4, 8, 10, 12, and 13.

INTRODUCTION

Filamentous and colonial cyanobacteria are the most important taxa causing harmful phytoplankton blooms in lakes (Reynolds 1984, Paerl 1988). Cyanobacterial blooms reduce water transparency and recreational value, cause odor and taste problems, and can be toxic to both terrestrial and aquatic organisms (Carmichael and Falconer 1993, Chorus and Bartram 1999). In addition, cyanobacteria are often avoided or poorly assimilated by herbivores (DeMott 1989) and so may reduce the efficiency of planktonic food chains, and as a consequence, negatively impact pelagic-based fisheries.

A long-standing tenet of freshwater ecology is that summer blooms of potentially-toxic cyanobacteria (typically species belonging to the genera *Anabaena*, *Aphanizomenon*, *Microcystis* and *Oscillatoria*) are a characteristic response to nutrient (particularly phosphorus) enrichment and a symptom of eutrophication (Smith 1983, Trimbee and Prepas 1987, Watson et al. 1997, Downing et al. 2001). As a result, much effort and expenditure has been directed toward reducing nutrient loading to alleviate water-quality problems associated with toxic cyanobacterial blooms. However, recent studies indicate that invasion by the zebra mussel (*Dreissena polymorpha*) results in a substantial increase in toxin-producing cyanobacteria in lakes with low-moderate nutrient levels. For example, Gull Lake in southwest Michigan has oligo-mesotrophic levels of phosphorus and was virtually free of toxin-producing cyanobacteria in the early 1990's. Since being invaded by *Dreissena* in 1994, *Microcystis aeruginosa* has become one of the dominant phytoplankton species in mid-late summer (Sarnelle et al. 2005).

Experimental studies have confirmed that *Dreissena* invasion is the primary driver of increases in *M. aeruginosa* in lakes with low-moderate phosphorus concentrations (Sarnelle et al. 2005). More recently, we surveyed inland lakes in Michigan with and without *Dreissena* (2002-2003) and found that the biomass of *Microcystis aeruginosa* was, on average, three times higher in invaded lakes having low-moderate phosphorus concentrations (total phosphorus, TP <25 µg/L). Further, we observed that concentrations of microcystins, a class of toxins commonly produced by *M. aeruginosa*, were also about three times higher in invaded lakes (Knoll et al. *in press*) with low-moderate TP. Thus, there is reason to be concerned that the ongoing *Dreissena* invasion of inland lakes is having a major impact on water quality in otherwise high-quality lakes in Michigan, through the promotion of a toxin-producing species of phytoplankton.

Microcystins are generally classified as hepatotoxins and have been responsible for lethal and sub-lethal poisonings of humans, livestock and other animals throughout the world (Chorus and Bartram 1999). We did not encounter microcystin concentrations near 1 µg/L, the drinking-water limit of the World Health Organization, in our recent lake survey (Knoll et al. *in press*), but our sampling regime (depth-integrated sampling from the deepest point of each lake) was inadequate for estimating toxin concentrations at the primary point of contact between lake water and recreational users or terrestrial animals, namely at the water surface along the shore. Toxin-producing cyanobacteria are well-known to accumulate at the surface on calm summer days via their buoyancy-regulating abilities (Reynolds 1984). As a consequence, it is possible that toxin concentrations at the surface are much higher than we have estimated from depth-integrated samples. Further, scums of cyanobacteria are commonly observed to accumulate along the shoreline and in bays and canals. Indeed, we have observed visible scums of *Microcystis* on the eastern shore of oligo-mesotrophic Gull Lake in August. Thus, although we have established that toxin concentrations are higher in *Dreissena*-invaded lakes, we do not have reliable estimates of toxin concentrations at likely points of human contact, and thus have no basis from

which to begin to calculate health risks. This project will generate a large set of toxin data from Michigan lakes with which to assess these risks.

Goals and Objectives

This project has two major themes. The first is to assess toxin concentrations at likely points of human contact for a large number of Michigan lakes. The monitored lakes will encompass a large range of potential risk with respect to toxic cyanobacteria, including, but not limited to, lakes with high TP (> 50 µg/L) and lakes with low-moderate TP that have been invaded by zebra mussels. We are particularly interested in the interaction between phosphorus and *Dreissena* invasion, as our previous research has suggested that these two factors interact strongly and in unexpected ways (Raikow et al. 2004, Sarnelle et al. 2005) in affecting the abundance of toxic cyanobacteria.

The second major theme is to build a general framework for understanding and predicting cyanobacterial toxin concentrations at likely points of human contact in Michigan lakes. In the past, such a framework would emphasize phosphorus concentrations or loadings as the primary driver of cyanobacterial biomass and consequently, of toxin concentrations. In a post-*Dreissena* world, we must now include exotic invasion as an additional strong driver that may interact with phosphorus to determine lake-wide levels of toxins. In addition, the prediction of toxin levels at points of human contact will require, at a minimum, data on the spatial distributions of toxin within lakes for a large set of inland lakes that vary in latitude, area, depth, fetch, orientation with respect to prevailing winds, etc. Finally, we seek reliable, empirical predictors of toxin concentrations across lakes by collecting data on easily measured trophic variables (water clarity, TP, chlorophyll *a*) in concert with our proposed toxin measurements.

Objectives:

- Measure concentrations of microcystins at likely points of human contact in a large number Michigan lakes in late summer via a Toxin Assessment Partnership (TAP) with the Cooperative Lakes Monitoring Program (CLMP) of Michigan.
- Collect data on trophic-state variables (Secchi depth, TP and chlorophyll *a*) simultaneous with toxin measurements for this same set of lakes.
- Model relationships between trophic-state variables, lake-morphometry variables and levels of microcystins.
- Provide input to lake communities, local government and state agencies on the above relationships and the potential impact upon recreational use of Michigan inland lakes.
- Publish results from this project in peer-reviewed journals.

METHODS

Sample collection and processing

Samples were collected by citizen volunteers in conjunction with the existing Cooperative Lakes Monitoring Program (CLMP) administered by the Michigan Department of Environmental Quality (MDEQ). Volunteers were trained at the annual conference of the Michigan Lake and Stream Association and provided with sampling containers, shipping supplies, an instruction manual (Appendix A) and a data sheet (Appendix B). Each of 77 lakes was sampled once, during either September (1-29 Sept) or late August (25-31 Aug, northern lakes, Appendix C), as specified by standard CLMP procedures (unpublished CLMP operating manual, 2006).

Volunteers were asked to note the presence/absence of zebra mussels in their lake on the data sheet (Appendix B). We checked this information, where possible, against a database of zebra mussel occurrence maintained by Michigan Sea Grant. A total of 33 lakes in the survey were listed in Michigan Sea Grant's database. The zebra mussel characterization by CLMP volunteers matched Sea Grant's database in 30 of 33 cases (91% accuracy), indicating that volunteer characterizations were generally reliable. Of the 3 mismatches, one lake was listed as uninvaded by volunteers but as recently invaded (in 2005) by Sea Grant (Pickerel Lake, Kalkaska County). This lake was scored as invaded in our analyses. Two lakes (Gilletts Lake, Jackson County and Hubbard Lake, Alcona County) were listed as invaded by CLMP volunteers but uninvaded by Sea Grant. According to the Sea Grant database however, these lakes have not been monitored for zebra mussel status since 1997. We assumed that they were invaded at some point between 1997 and 2005.

Detailed descriptions of sampling procedures are described in the CLMP Harmful Algae Monitoring Procedures manual (Appendix A). In each lake, samples were taken from a deep station away from shore with a depth-integrating sampler ("euphotic zone" samples, depth = 2 x Secchi Depth), and from four shoreline stations ("shoreline" samples), on the same day. Euphotic-zone samples were collected as specified by standard CLMP procedures. Shoreline samples were collected from the water surface by submerging a 250ml opaque polyethylene bottle just below the water surface. Volunteers were instructed to take one shoreline sample from the north, south, east and west shores of the lake and to sample close to shore where water depth was about 0.6 m.

Water samples from the euphotic zone were used to assess chlorophyll and total phosphorus (TP), following standard CLMP processing procedures, as well as to assess the cyanobacterial toxin, microcystin. Chlorophyll and TP analyses on euphotic-zone samples were performed by MDEQ. Water samples from the shoreline were used to assess surface concentrations of chlorophyll and microcystin. Shoreline chlorophyll and all microcystin analyses were conducted at the research laboratories of the Department of Fisheries and Wildlife at Michigan State University (MSU).

Toxin samples were minimally processed. Whole water was poured from the collection container into a new Nalgene 60 ml polyethylene bottle by volunteers and immediately frozen in the volunteers' home. Chlorophyll samples were filtered by volunteers according to standard CLMP procedures and immediately frozen. Frozen samples to be analyzed at MSU (water for microcystin and filters for chlorophyll) were shipped to MSU within 8 days of collection in insulated shipping containers with 2 frozen "blue ice" packs via Express Mail (U. S. Postal Service). Samples destined for MDEQ (euphotic-zone: water for TP, filters for chlorophyll) were transported to MDEQ according to standard CLMP procedures. Upon arrival at MSU, samples were transferred immediately to a lab freezer and held at -20°C until analyzed. Notes were also recorded about shipping and arrival date and condition of samples. In most cases, water samples were still frozen upon arrival at MSU.

Sample analysis

Details about MDEQ analytical procedures are given in MDEQ SOP's (2006 a, b). Chlorophyll was measured at MSU via overnight extraction of filters in 95% ethanol followed by fluorometric measurement of extracted chlorophyll *a* (Welschmeyer 1994). Microcystin was measured on whole water via Enzyme-Linked ImmunoSorbent Assay (ELISA), in most cases

within 30-60 days after collection. See the project QAPP for further details about analytical procedures.

Data on TP was taken from Annual Summary Reports of the CLMP (2004, 2005, 2006). We used late summer TP data because it was a better predictor of microcystin concentrations than spring TP. For three lakes, no summer TP data for 2006 was available, so we estimated these values from mean summer TP in 2004 and 2005 and an empirical relationship between averaged 2004-2005 TP and 2006 TP for all CLMP lakes ($y = 2.0 + 0.82x$, $R^2 = 0.63$). Chlorophyll *a* data was a combination of data from MDEQ (most of the euphotic-zone samples) and samples collected by CLMP and analyzed by MSU (a few euphotic-zone samples and all shoreline samples). As described below, there was good agreement between MDEQ and MSU analyses of chlorophyll *a*.

Quality Assurance/Quality Control Assessments

Data quality was evaluated and assured using four general approaches, side-by-side comparisons of data from samples analyzed by MDEQ versus MSU (chlorophyll *a*) and collected by CLMP volunteers versus MSU personnel (microcystin), comparisons of split samples analyzed for microcystin by MSU and an independent commercial laboratory (Greenwater Labs, Palatka, Florida), examination of the effects of sample handling and processing procedures on microcystin, and routine use of analytical blanks and standards. See the project QAPP for further details about these procedures.

1. Side-by-side comparisons, MDEQ versus MSU analysis of chlorophyll *a* concentration.

Samples were collected by CLMP volunteers and stored frozen at the MDEQ lab. One replicate filter was analyzed by MDEQ for all CLMP lakes. For 52 lakes selected to vary widely in chlorophyll *a* concentration (based on MDEQ results), one replicate filter was brought to MSU and analyzed for chlorophyll *a* with the same basic method used by MDEQ (i. e., ethanol extraction, fluorometric analysis).

2. Side-by-side comparisons, CLMP versus MSU sample collection, microcystin analysis.

MSU personnel visited 10 lakes on the days of CLMP sample collection, accompanying the volunteers and sampling in the same locations, at the same time and with the same methods as the volunteers. Samples collected by volunteers were stored and delivered to MSU according to the CLMP Harmful Algae Monitoring Procedures document (Appendix A). Samples collected by MSU were returned to the lab on the day of collection and frozen until analyzed.

3. Split samples, MSU versus independent commercial laboratory, microcystin.

Samples were collected from an oligotrophic lake with zebra mussels (Gull Lake, Barry County) and a highly eutrophic lake on the MSU campus (Lake #2, Inland Lakes Research Area) on 19 July 2007. The samples were split, with half of each sample shipped immediately to Greenwater Laboratories in Palatka, Florida for microcystin analysis. The other half was frozen and subsequently analyzed at MSU. Both laboratories used an ELISA method to quantify microcystin. Greenwater labs also used High Performance Liquid Chromatography (HPLC) to characterize the dominant microcystin variant.

4. Effects of sample handling and processing procedures on microcystin.

We examined the influence of several laboratory procedures on microcystin (toxin) measurement using samples collected from Lake #2 of the Inland Lakes Research Area. The goal of these tests was to determine the extent to which our methods resulted in underestimates of toxin concentrations in the field at the time of collection. Lake #2 was chosen for logistical reasons (it is minutes from the MSU lab) and because it has high toxin concentrations. Underestimating toxin concentrations is a potential problem only when concentrations are high, since low concentrations are not a public health concern. The procedures investigated included: storage time in insulated shipping containers (shipping test), time in frozen storage (freezer test), boiling of samples to release toxin (boiling test), and extraction of samples in methanol (extraction test). Given that microcystin is a relatively stable, intracellular toxin (Chorus and Bartram 1999), we expected that incomplete release of toxin from cells would be the most likely source of underestimation.

To examine the effects of storage in insulated shipping containers, we collected a large volume of water from Lake #2 and dispensed replicate aliquots into 35 sample bottles. The shipping containers and sample bottles used were identical to those used by CLMP volunteers to collect and ship toxin samples to MSU. Each of seven shipping containers was packed with five sample bottles at the bottom, two frozen "blue ice" packs on top of the samples, and styrofoam peanuts on top of the ice packs, such that the container was completely full. A small temperature recorder was also included in each container. Containers were sealed with packing tape and placed in a room without air conditioning to simulate summer transport conditions. Containers were opened after 1, 2, 3, 4, 7 and 8 days and bottles were removed and immediately frozen. Results were compared to bottles filled and frozen on the day of collection (day 0 samples).

To examine the effect of storage time in the freezer, we collected a large volume of water from Lake #2 and dispensed replicate aliquots into 76 sample bottles. Half the bottles were made of glass, the other half were polyethylene. Samples were frozen at -20°C for varying amounts of time (7 - 77 days) before being analyzed for microcystin. No significant differences were found between the two bottle types, so the data were pooled to examine loss over time in the freezer.

To examine the effect of boiling on microcystin concentrations, we selected 42 CLMP samples of widely-varying toxin concentrations and simultaneously analyzed replicate aliquots for microcystin with our standard protocol (direct analysis of untreated water) versus immersing the samples in a boiling water bath for 30 minutes before analysis. To examine the effect of methanol extraction on microcystin concentrations, we selected 12 CLMP samples and simultaneously analyzed replicate aliquots for microcystin with our standard protocol (direct analysis of untreated water) versus evaporating 25 ml of the sample to dryness and then extracting the residue in 75% methanol.

RESULTS

Quality Assurance/Quality Control

1. Side-by-side comparisons, MDEQ versus MSU analysis for chlorophyll *a* concentration.

The results of this comparison show good overall correspondence between laboratories (Figure 1, intercept of regression line not significantly different from 0), but evidence of a slight bias, with MSU values slightly higher than MDEQ values (slope of regression significantly greater than 1). This bias, however, was driven by one sample with high chlorophyll (MSU =

38, DEQ = 26). Exclusive of this sample, the slope of the regression (1.09) was not significantly different from 1.

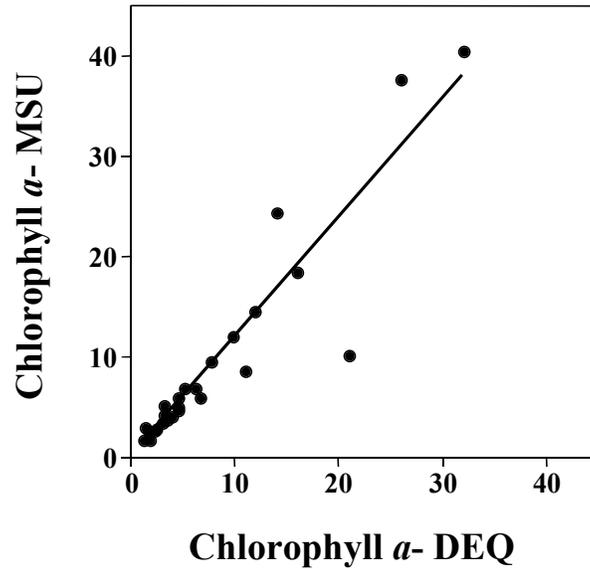


Figure 1. Comparison between chlorophyll *a* concentrations ($\mu\text{g L}^{-1}$) for replicate samples analyzed at MSU versus analyzed at the MDEQ. Equation for the fitted line: $y = -0.06 + 1.19x$, $R^2 = 0.86$.

2. Side-by-side comparisons, CLMP versus MSU sample collection, microcystin analysis.

The results of this comparison show good overall correspondence in microcystin concentrations between samples collected by CLMP volunteers versus MSU personnel (Figure 2). There were no significant differences in mean concentrations between the two groups for all samples ($N = 50$) or for samples collected from the euphotic zone (paired t-tests, $P > 0.30$).

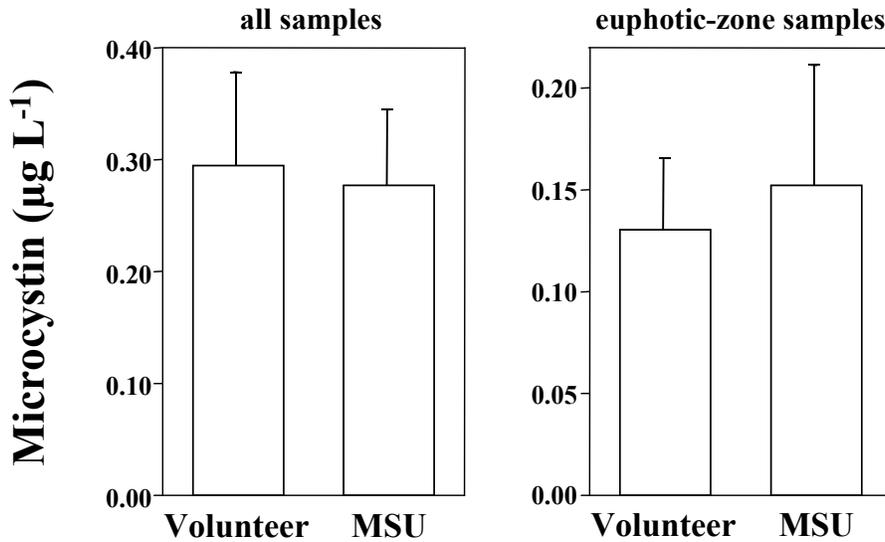


Figure 2. Comparison between toxin (microcystin) concentrations for samples collected by CLMP volunteers versus by MSU personnel.

3. Split samples, MSU versus independent commercial laboratory, microcystin.

There was good agreement between analyses performed by the different laboratories (Figure 3), especially considering that ELISA analysis is inherently variable and the exact methods used by each lab were not identical. Analytical error was not higher for MSU than the commercial lab (Figure 3). The dominant variant in both samples, as assessed by HPLC was microcystin-LR, a common variant with relatively high toxicity (Chorus and Bartram 1999).

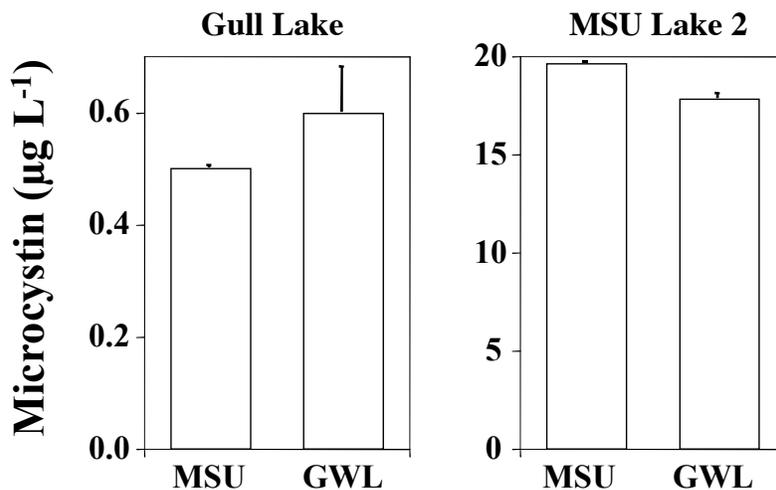


Figure 3. Comparison between toxin (microcystin) concentrations measured at MSU versus at Greenwater Laboratories (GWL). Standard error bars represent analytical error.

4. Effects of sample handling and processing procedures on microcystin.

Results from the shipping test showed no significant breakdown of toxin for the first 2 days in the shipping containers, after which toxin concentrations began to decrease (Figure 4). Temperatures in the containers remained below 5°C for the first 24 hours and below 20°C for the first 40 hours. Examination of shipping records indicated that none of the samples shipped by volunteers spent more than 2 days in transit and most of the bottles were frozen upon delivery, so our standard method of shipping samples likely did not result in any toxin degradation.

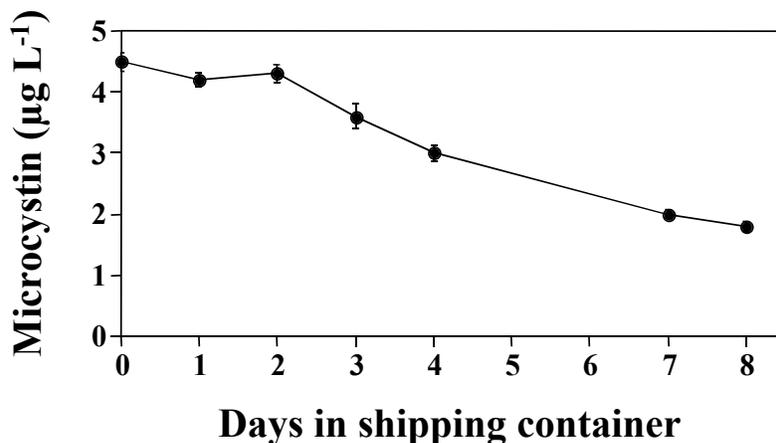


Figure 4. Effect of sample storage in insulated shipping container on microcystin concentrations. Means and standard errors for five replicate bottles are depicted.

Results of the freezer test showed a statistically significant decrease in toxin concentrations over time, but the magnitude of the loss was very small and highly variable, and largely driven by data from day 7 (Figure 5). Fitting an exponential decay function to the data, we estimated a loss rate of 0.003 day^{-1} . In general, CLMP samples were analyzed for toxin within 30-60 days of collection. Within this window, we can expect measured concentrations to underestimate true values by ~10-15% in the majority of cases. We consider this to be a negligible source of variation relative to spatial and temporal variation in nature (see *Toxin concentrations in surface waters and euphotic zone*, below). Given the small effect of freezer storage and the uncertainty of the test results, we did not correct samples for this potential loss factor.

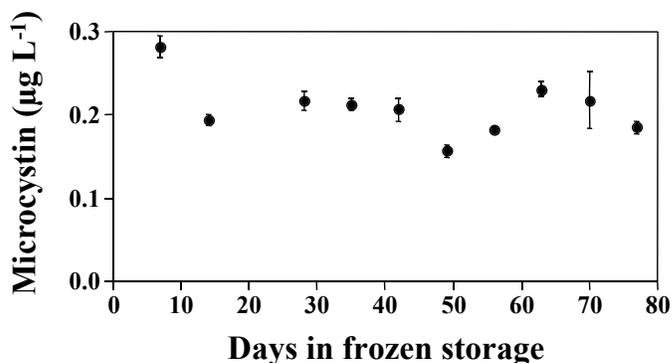


Figure 5. Effect of frozen storage on microcystin concentrations. Means and standard errors for 5-8 replicate bottles are depicted.

In the boiling test, there were significantly higher toxin concentrations in boiled samples (mean \pm SE for standard protocol, $1.15 \pm 0.45 \mu\text{g L}^{-1}$, for boiling method, $1.52 \mu\text{g L}^{-1} \pm 0.57$, paired t-test, $P < 0.05$). Linear regression fitted to the relationship between boiled and not-boiled concentrations yielded an intercept that was not significantly different from zero. Consequently, we fitted a regression with zero intercept to the data to estimate a factor to account for the underestimation of toxin levels in samples that were not boiled (Figure 6). All CLMP concentrations in this report were multiplied by 1.23 for this underestimation. In the extraction test, we found no significant difference in mean concentration for the two methods (mean \pm SE for standard protocol, $0.44 \pm 0.24 \mu\text{g L}^{-1}$, for methanol extraction, $0.46 \mu\text{g L}^{-1} \pm 0.28$, paired t-test, $P > 0.70$), so no correction was warranted.

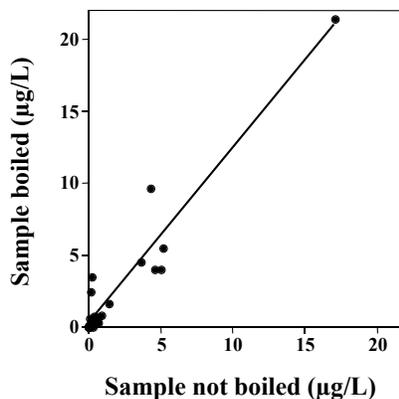


Figure 6. Effect of boiling on microcystin concentrations. The equation for the regression line fitted to the data is: $y = 1.23x$.

Toxin concentrations in surface waters and the euphotic zone

From 77 lakes, we obtained a total of 75 samples from the euphotic zone (one sample per lake, 2 lakes with missing samples) and 303 samples from the water surface at the shoreline (4 per lake, 5 missing samples). More than half of the samples ($N = 44$) were collected from lakes that have been invaded by zebra mussels (*Dreissena sp.*), hereafter denoted as lakes with "mussels". Given that a previous survey of Michigan lakes reported higher levels of the microcystin-producing species, *Microcystis aeruginosa*, and microcystin in lakes with mussels (Knoll et al. *in press*), we classified lakes by mussel presence/absence in analyzing the data from the CLMP survey. Data distributions were highly skewed, with many low values and a very small number of very high values (Figure 7). Consequently, microcystin concentrations were \log_{10} transformed for all statistical tests. We present untransformed data in some cases to facilitate visual interpretation.

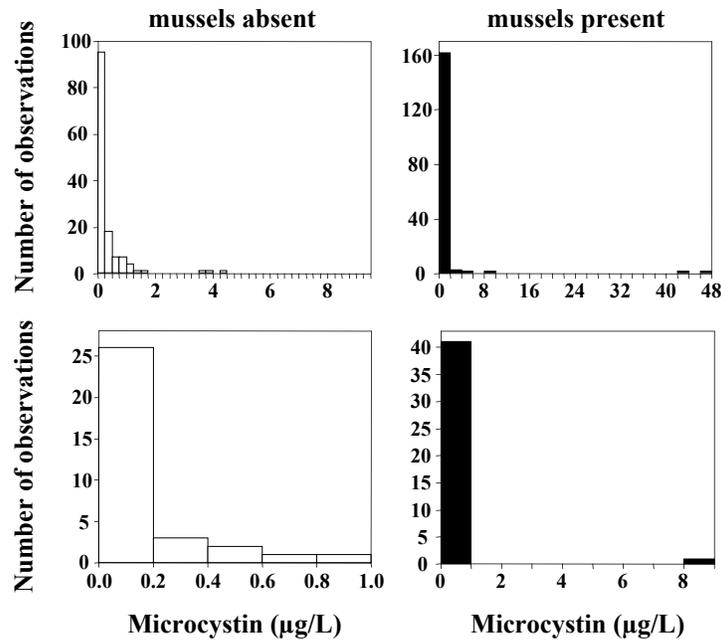


Figure 7. Frequency distributions of microcystin concentrations at the shoreline (top panels) and in the euphotic zone (bottom panels) for CLMP lakes with and without zebra mussels. Note the different scales in each panel. The shoreline data are based on individual samples from each site (north, south, east, west).

All but one of the euphotic-zone samples had a microcystin concentration $< 1.0 \mu\text{g L}^{-1}$ (Figure 7, Appendix D). One euphotic-zone sample from a mussel-infested lake (Bills Lake, Newago County) had a euphotic-zone concentration of $8 \mu\text{g L}^{-1}$. Microcystin concentrations were usually higher at the shoreline and in lakes with mussels (Figure 8). Across all lakes, mean concentrations at the shoreline ($0.6 \mu\text{g L}^{-1}$) were two times higher than concentrations in the euphotic zone ($0.3 \mu\text{g L}^{-1}$, means significantly different by paired t-test, $P < 0.0001$).

As seen for the euphotic-zone samples, the vast majority of shoreline samples had concentrations $< 1.0 \mu\text{g L}^{-1}$ (Figure 7, Appendix E). However, a few shoreline samples had concentrations $> 2 \mu\text{g L}^{-1}$, with three samples in two mussel-infested lakes (Lakeville Lake, Oakland County and Bills Lake, Newago County) having concentrations > 8 and $> 40 \mu\text{g L}^{-1}$ (Figure 7). Across all lakes, there were no significant differences in shoreline concentrations among the four sampling locations (means for north, south, east and west not different at $P >$

0.80). Although shoreline concentrations were about 2.5 times higher in lakes with mussels than in lakes without mussels (mean \pm SE, for lakes with mussels = $0.83 \pm 0.54 \mu\text{g L}^{-1}$, for lakes without mussels = $0.34 \pm 0.08 \mu\text{g L}^{-1}$), this difference was not statistically different across all lakes ($P > 0.70$). The influence of mussels, however, was highly variable (Figure 7) and complicated by other factors, as discussed below.

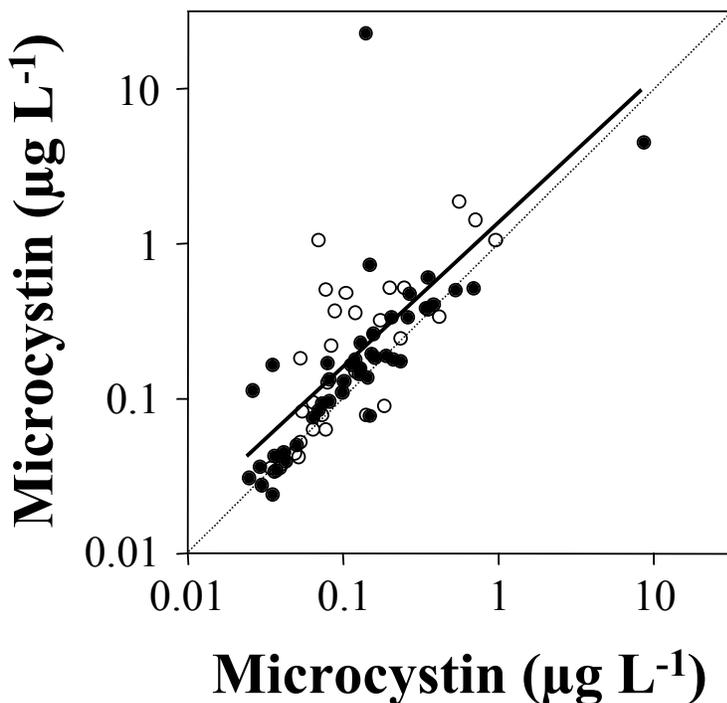


Figure 8. Relationship between microcystin concentrations at the shoreline (y axis) and in the euphotic zone away from shore (x-axis) for CLMP lakes. The dotted line is the one-to-one line. Equation for the log-log regression (solid line): $y = 0.12 + 0.93x$, $R^2 = 0.56$, $P < 0.0001$. Solid circles are lakes with zebra mussels, open circles are lakes without zebra mussels.

Predicting toxin concentrations

Based on existing research (Watson et al. 1997, Downing et al. 2001, Raikow et al. 2004, Knoll et al. *in press*), we expected three factors (among the limited variables available for analysis) to have the greatest influence on microcystin concentrations- total phosphorus, chlorophyll *a*, and presence/absence of zebra mussels. We also examined the relationship between shoreline and euphotic-zone toxin concentrations, the influences of lake depth and latitude, and the relationship between chlorophyll *a* and total phosphorus (TP). For these analyses, shoreline toxin data was averaged across the four sites in each lake.

We found a strong positive relationship between microcystin concentrations at the shoreline and in the euphotic zone, and there was no influence of mussel presence/absence on the predictive relationship (Figure 8). Microcystin concentrations at the shoreline were also positively correlated with chlorophyll *a* concentration at the shoreline (Figure 9). In this case, lakes with mussels had significantly higher microcystin concentrations per unit of chlorophyll *a* (Figure 9). In contrast, there was no significant relationship between microcystin and chlorophyll *a* concentrations in the euphotic zone (ANOVA, $P > 0.10$).

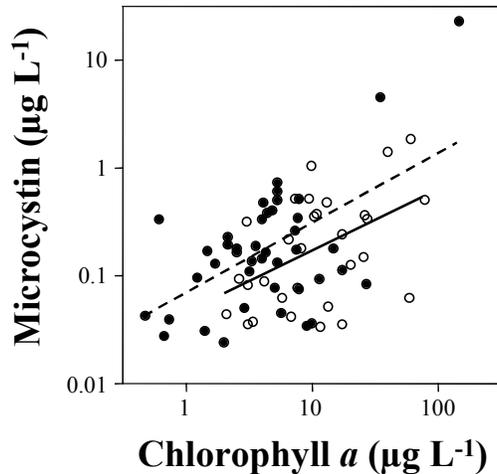


Figure 9. Relationships between microcystin and chlorophyll *a* concentrations at the shoreline for CLMP lakes with (solid circles, dotted line, regression equation: $y = -1.18 + 0.65x$, $R^2 = 0.30$, $P < 0.001$) and without zebra mussels (open circles, solid line, regression equation: $y = -1.35 + 0.57x$, $R^2 = 0.21$, $P < 0.01$). Regression slopes were not significantly different ($P > 0.50$). Influence of mussel presence/absence was statistically significant by ANCOVA ($P < 0.05$).

Microcystin concentrations in the euphotic zone and at the shoreline were positively related to TP, but an analysis of heterogeneity of slopes suggested that this relationship may differ for lakes with and without mussels (ANOVA test of differences in slopes, $P < 0.09$ for euphotic-zone data, $P < 0.11$ for shoreline data). Consequently, we examined the relationships between microcystin and TP separately for the two classes of lakes. We found significant positive relationships between microcystin and TP for lakes without mussels but no relationships for lakes with mussels (Figures 10, 11). However, it should be noted that the range of TP was narrower for lakes with mussels (Appendix E). Restricting the data set to lakes with TP less than $15 \mu\text{g L}^{-1}$, there were significantly higher toxin levels in lakes with mussels (Fig. 12). In contrast, we found no significant influences of lake depth or latitude on toxin levels ($P > 0.15$).

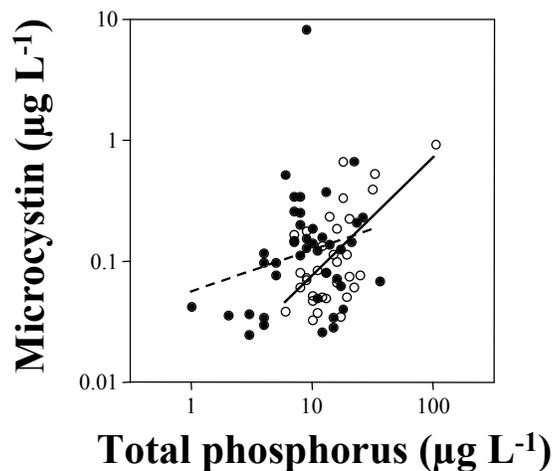


Figure 10. Relationships between microcystin in the euphotic zone and TP for CLMP lakes with (solid circles, dotted line, regression equation: $y = -1.26 + 0.35x$, $R^2 = 0.05$, $P > 0.10$) and without zebra mussels (open circles, solid line, regression equation: $y = -2.11 + 0.98x$, $R^2 = 0.37$, $P < 0.001$).

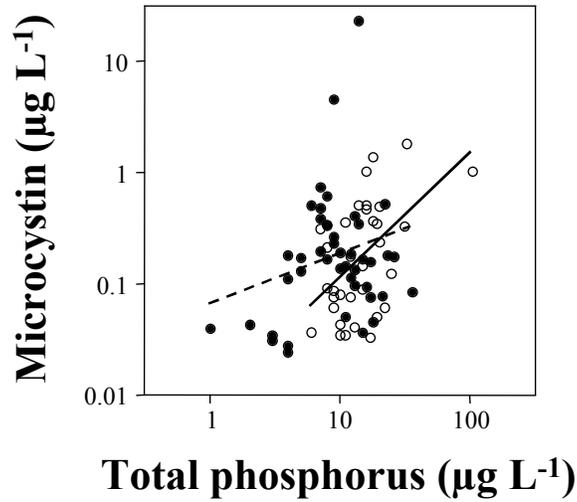


Figure 11. Relationships between microcystin at the shoreline and TP for CLMP lakes with (solid circles, dotted line, regression equation: $y = -1.13 + 0.39x$, $R^2 = 0.04$, $P > 0.15$) and without zebra mussels (open circles, solid line, regression equation: $y = -2.03 + 1.10x$, $R^2 = 0.30$, $P < 0.001$).

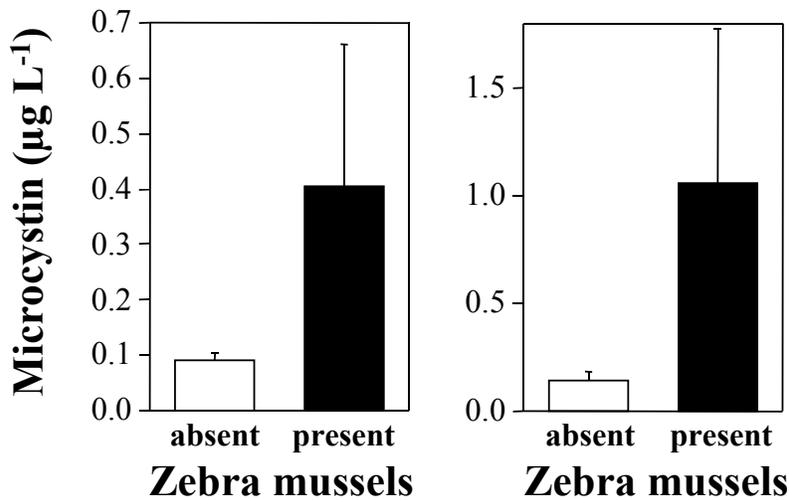


Figure 12. Mean microcystin concentrations for lakes with ($N = 33$) and without ($N = 16$) zebra mussels. Data were restricted to lakes with $TP < 15 \mu\text{g L}^{-1}$. Left panel: euphotic-zone concentrations (means significantly different at $P < 0.05$). Right panel: shoreline concentrations (means significantly different at $P < 0.04$).

Lastly, there were significant positive relationships between TP and chlorophyll a in the euphotic zone and at the shoreline (Figures 13, 14). There was no mussel influence on euphotic-zone chlorophyll a , whereas shoreline chlorophyll a was significantly lower in lakes with mussels (Figure 13), even though toxin concentrations were higher (Figure 9).

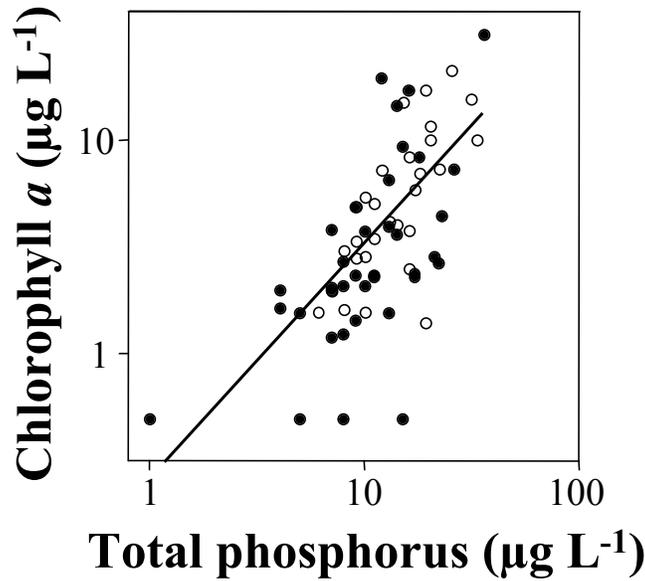


Figure 13. Relationship between chlorophyll *a* in the euphotic zone and total phosphorus for CLMP lakes. Equation for the log-log regression: $y = -0.58 + 1.07x$, $R^2 = 0.43$, $P < 0.0001$. Solid circles are lakes with zebra mussels, open circles are lakes without zebra mussels.

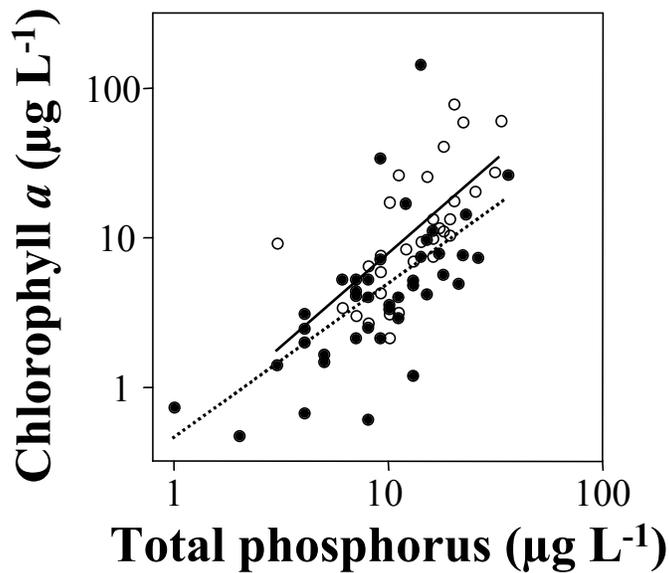


Figure 14. Relationships between chlorophyll *a* at the shoreline and total phosphorus for CLMP lakes with (solid circles, dotted line, regression equation: $y = -0.36 + 1.03x$, $R^2 = 0.44$, $P < 0.0001$) and without zebra mussels (open circles, solid line, regression equation: $y = -0.38 + 1.25x$, $R^2 = 0.44$, $P < 0.0001$). Regression slopes were not significantly different ($P > 0.50$). Influence of mussel presence/absence was statistically significant by ANCOVA ($P < 0.02$).

DISCUSSION

Quality control testing indicated that sample collection by volunteers provided data on microcystin concentrations that was comparable to sampling by MSU personnel. Our testing also revealed that shipping water samples on ice in insulated shipping containers via Express Mail (U. S. Postal Service, maximum 2 day delivery) was satisfactory for preventing sample degradation. Finally, we found that water samples should be boiled before analysis to avoid underestimation of microcystin, and that a correction factor of 1.23 should be applied to samples that are not boiled. The latter finding was probably a function of the release of toxin from phytoplankton cells during boiling. Assuming that intracellular toxin is released in the digestive system of humans upon ingestion of contaminated water, concentrations based on boiled samples seem to be the most appropriate for assessing public health risks.

The vast majority of samples we analyzed were very low in microcystin relative to current drinking water ($1 \mu\text{g L}^{-1}$) or recreational ($20 \mu\text{g L}^{-1}$) standards promulgated by the WHO. For water samples taken with a depth-integrating sampler from the euphotic zone, only 1.3% (1 out of 75 samples) had a microcystin concentration above $1 \mu\text{g L}^{-1}$ and none were above $20 \mu\text{g L}^{-1}$. For surface water samples taken from the shoreline, which we expected to have higher concentrations than depth-integrated samples, 6% (18 of 303) were above $1 \mu\text{g L}^{-1}$ and 0.6% (2 of 303) were above $20 \mu\text{g L}^{-1}$. Thus, it is safe to conclude that most lakes sampled by CLMP volunteers during 2006 posed little or no public health risk to recreational users at the time of sampling. It should be noted however, that we have no specific data on seasonal dynamics of phytoplankton in these lakes, so we do not know the likelihood that our one-time sampling missed toxicogenic blooms. We targeted our sampling program at late summer because toxicogenic cyanobacteria tend to dominate late in the growth season (Sarnelle 1993), but we do not know whether peak abundance of cyanobacteria in 2006 occurred in September, the month in which most samples were collected. We also do not know whether 2006 was a year in which toxicogenic blooms tended to be more or less common during the summer in Michigan. Data on seasonal dynamics and interannual variation of toxin concentrations would enhance our ability to characterize public health risks and predict blooms.

Two lakes with mussels (Lakeville Lake, Oakland County and Bills Lake, Newago County) had high microcystin concentrations at the time of sampling, relative to drinking water or recreational standards. In both lakes, concentrations were highly variable among the four shoreline sampling stations, with a 40-fold range ($1 - 46 \mu\text{g L}^{-1}$) in Lakeville Lake and 30-fold range ($0.3 - 9 \mu\text{g L}^{-1}$) in Bills lake (Appendix D). In comparison, the most eutrophic lake in the survey (Crystal Lake, Dickinson County, TP = $103 \mu\text{g L}^{-1}$) had microcystin concentrations of $\sim 1 \mu\text{g L}^{-1}$ at the shoreline (Appendix D, E). In Bills Lake, microcystin concentration in the euphotic zone ($8 \mu\text{g L}^{-1}$) was indicative of levels at the shoreline. This was not the case in Lakeville Lake, where standard CLMP sampling from the euphotic zone ($0.1 \mu\text{g L}^{-1}$) was not indicative of the high toxin concentrations along the shore.

We suggest that high toxin concentrations and variability at the shore reflect the episodic and spatially variable nature of floating scums of bloom-forming cyanobacteria. It would appear that there was little or no scum formation in Bills Lake at the time of sampling, despite generally high cyanobacterial concentrations in the lake as a whole, given that shoreline and euphotic-zone concentrations were similar. In contrast, the data from Lakeville Lake suggest relatively low concentrations of cyanobacteria in the lake as a whole (based on the low euphotic-zone concentration), but an intense scum at the surface accumulated along the east and west shorelines

(Appendix D, E). It is also possible that the shoreline samples in Lakeville Lake were taken from bays or marinas with limnological conditions that are atypical of the lake as a whole. Lakeville Lake is an impoundment that consists of several lake basins, and shoreline samples were taken from well-protected bays. This might help to explain why shoreline and euphotic-zone toxin levels differed so much.

Of considerable interest from the perspective of existing limnological paradigms is the fact that the two lakes with high cyanobacterial toxin concentrations were not eutrophic based on existing classification indices. Total phosphorus at the time of toxin sampling in Bills Lake was $9 \mu\text{g L}^{-1}$ and summer TP did not exceed $9 \mu\text{g L}^{-1}$ in any of the last 3 years (CLMP Annual Reports, 2004, 2005, 2006). Likewise, spring TP has averaged $8 \mu\text{g L}^{-1}$ over the last 3 years (CLMP Annual Reports, 2004, 2005, 2006c). Similarly, TP in Lakeville Lake was $14 \mu\text{g L}^{-1}$ during toxin sampling in 2006 and has averaged $6 \mu\text{g L}^{-1}$ (spring) and $13 \mu\text{g L}^{-1}$ (summer) over the last 3 years (CLMP Annual Reports, 2004, 2005, 2006c). Based on these TP levels, both lakes would be characterized as oligo-mesotrophic (Carlson 1977), and neither would be expected to support large blooms of toxicogenic cyanobacteria (Kalff 2002). Chlorophyll *a* concentrations in the euphotic zones of these two lakes were also indicative of oligo-mesotrophic conditions. Average chlorophyll *a* in 2004 - 2006 was 2.1 - 3.0 in Bills Lake and 1.5 - 2.4 in Lakeville Lake (2004, 2005, 2006).

The finding of high cyanobacterial toxin concentrations in lakes with relatively low phosphorus subsequent to zebra mussel invasion reiterates previous research (Raikow et al. 2004, Sarnelle et al. 2005, Knoll et al. in press). More importantly with respect to public health risk, this finding suggests that monitoring that is solely based on existing indices of eutrophication (for example, open water TP samples of the euphotic zone) may not detect potentially hazardous conditions in regions where zebra mussels are invading. The survey data suggest that such conditions are rare in Michigan, but this conclusion must be tempered by the data limitations noted above, and by the fact that few high TP lakes were included in our survey (Appendix E). In lakes without mussels, TP is a positive predictor of microcystin concentrations (Figure 10, 11), as discussed below.

As found in a previous survey of Michigan lakes (Knoll et al. *in press*), the presence of zebra mussels is a major factor influencing microcystin concentrations in lakes with low phosphorus (Figure 12). Experimental research has demonstrated that zebra mussel invasion is the cause of elevated microcystin in such lakes (Sarnelle et al. 2005). However, levels of microcystin in invaded lakes tended to be highly variable (Figure 12, Knoll et al. *in press*) and we currently have no information about what drives this variability. Survey data indicate that TP is of no value in explaining this variability (Figure 10, 11), which confirms indications from an earlier survey (Raikow et al. 2004). One factor that may play a role is temperature, with warmer temperatures tending to favor toxicogenic cyanobacteria (Paerl 1988, Weyhenmeyer 2001, Park et al. 2004). We were unable to detect any influence of latitude on toxin levels in the survey, but given the unique meteorological conditions characteristic of Michigan, it may prove more fruitful to examine the influence of mean annual temperature rather than latitude. We hope to obtain the requisite temperature data to enable such an analysis in the future. In any case, we do not know whether the high concentrations of toxin seen in Lakeville Lake and Bills Lake are likely to recur in those particular lakes in subsequent years, or if such events can occur in any lake with zebra mussels in any given year. More research and monitoring will be needed to answer that question.

In lakes lacking zebra mussels, euphotic-zone TP is a significant predictor of microcystin concentrations at the shoreline (Figure 11), although the strength of the predictive relationship is somewhat lower than for chlorophyll *a* (Figure 14). A weaker relationship is not surprising since microcystin is produced by only a small number of phytoplankton species which are likely to respond to phosphorus enrichment less predictably than total phytoplankton biomass. Average shoreline concentrations of microcystin above $1 \mu\text{g L}^{-1}$ were never found in uninvaded lakes with $\text{TP} < 15 \mu\text{g L}^{-1}$, suggesting that monitoring for microcystin may be unnecessary in uninvaded lakes below this TP level. In lakes with higher TP or zebra mussels, the survey data suggest that shoreline chlorophyll *a* may prove useful as a relatively inexpensive "early-warning" monitoring tool, given the positive relationship between shoreline chlorophyll *a* and microcystin (Figure 9). The latter relationship was relatively weak, but average shoreline concentrations of microcystin above $1 \mu\text{g L}^{-1}$ were never found in uninvaded lakes with average shoreline concentrations of chlorophyll *a* below $10 \mu\text{g L}^{-1}$. Although more data are needed to confirm this threshold, it may be efficient to target toxin monitoring efforts at lakes with shoreline concentrations of chlorophyll *a* that exceed $10 \mu\text{g L}^{-1}$.

LITERATURE CITED

- Carlson, R. E. 1977. Trophic State Index for Lakes. *Limnology and Oceanography* **22**:361-369.
- Carmichael, W. W., and I. R. Falconer. 1993. Diseases related to freshwater blue-green algal toxins and control measures. Pages 187-209 in I. R. Falconer, editor. *Algal toxins in seafood and drinking water*. Academic Press, London, UK.
- Chorus, I., and J. Bartram, editors. 1999. *Toxic cyanobacteria in water: a guide to their public health consequences, monitoring and management*. E & FN Spon, London, UK.
- DeMott, W. R. 1989. The role of competition in zooplankton succession. Pages 195-252 in U. Sommer, editor. *Plankton ecology: succession in plankton communities*. Springer-Verlag, Berlin, Germany.
- Downing, J. A., S. B. Watson, and E. McCauley. 2001. Predicting cyanobacteria dominance in lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **58**:1905-1908.
- Kalff, J. 2002. *Limnology: inland water ecosystems*. Prentice-Hall.
- Knoll, L. B., O. Sarnelle, S. K. Hamilton, C. E. H. Scheele, A. E. Wilson, J. B. Rose, and M. R. Morgan. in press. Invasive zebra mussels (*Dreissena polymorpha*) increase cyanobacterial toxin concentrations in low-nutrient lakes. *Canadian Journal of Fisheries and Aquatic Sciences*.
- MDEQ. 2004. Cooperative Lakes Monitoring Program. Annual Summary Report.
- MDEQ. 2005. Cooperative Lakes Monitoring Program. Annual Summary Report.
- MDEQ. 2006a. Laboratory Services Standard Operating Procedure Document. Total Phosphorus in Water.
- MDEQ. 2006b. Laboratory Services Standard Operating Procedure Document. Chlorophyll a.
- MDEQ. 2006c. Cooperative Lakes Monitoring Program. Annual Summary Report.
- Paerl, H. W. 1988. Nuisance phytoplankton blooms in coastal, estuarine and inland waters. *Limnology and Oceanography* **33**:823-847.
- Park, S., M. T. Brett, A. Muller-Solger, and C. R. Goldman. 2004. Climatic forcing and primary productivity in a subalpine lake: interannual variability as a natural experiment. *Limnology and Oceanography* **49**:614-619.
- Raikow, D. E., O. Sarnelle, A. E. Wilson, and S. K. Hamilton. 2004. Dominance of the noxious cyanobacterium *Microcystis aeruginosa* in low nutrient lakes is associated with exotic zebra mussels. *Limnology and Oceanography* **49**:482-487.
- Reynolds, C. S. 1984. *The ecology of freshwater phytoplankton*. Cambridge University, Cambridge.
- Sarnelle, O. 1993. Herbivore effects on phytoplankton succession in a eutrophic lake. *Ecological Monographs* **63**:129-149.
- Sarnelle, O., A. E. Wilson, S. K. Hamilton, L. B. Knoll, and D. E. Raikow. 2005. Complex interactions between the zebra mussel, *Dreissena polymorpha*, and the harmful phytoplankter, *Microcystis aeruginosa*. *Limnology and Oceanography* **50**:896-904.
- Smith, V. H. 1983. Low nitrogen to phosphorus favors dominance by bluegreen algae in lake phytoplankton. *Science* **221**:669-671.
- Trimbee, A. M., and E. E. Prepas. 1987. Evaluation of total phosphorus as a predictor of relative biomass of blue-green algae with an emphasis on Alberta lakes. *Canadian Journal of Fisheries and Aquatic Sciences* **44**:1337-1342.

- Watson, S. B., E. McCauley, and J. A. Downing. 1997. Patterns in phytoplankton taxonomic composition across temperate lakes of differing nutrient status. *Limnology and Oceanography* **42**:487-495.
- Welschmeyer, N. A. 1994. Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and phaeopigments. *Limnology and Oceanography* **39**:1985-1992.
- Weyhenmeyer, G. A. 2001. Warmer winters: Are planktonic algal populations in Sweden's largest lakes affected? *Ambio* **30**:565-571.



Cooperative
Lakes
Monitoring
Program

Harmful Algae Shoreline Samples CHLOROPHYLL and MICROCYSTIN

PROJECT PARTICIPATION REQUIREMENTS

To participate in the Harmful Algae Monitoring Project individuals must have completed the necessary training and be participating in the Cooperative Lakes Monitoring Program's (CLMP) Secchi Disk, Total Phosphorus and Chlorophyll Monitoring Projects. In addition to these written procedures for harmful algae, participants must carefully follow all CLMP written monitoring procedures, especially the procedures for chlorophyll.

In addition to the routine CLMP sampling for Secchi disk, total phosphorus and chlorophyll the volunteer monitors will be sampling for chlorophyll at four locations along the shoreline during the last chlorophyll sampling event (late August or September). They will also collect water samples, for microcystin, the toxin produced by cyanobacteria, at these same four locations and at the deep station sampling site. The shoreline chlorophyll samples will be turned in with the regular CLMP chlorophyll samples to the DEQ collection sites. The microcystin water samples will be mailed directly, by the volunteer sampler, to Michigan State University.

EQUIPMENT CHECKLIST

Review the Equipment Checklist on Page 1 of the CLMP Chlorophyll Project written procedures and be sure you have all the equipment listed. In addition to the equipment listed in the Chlorophyll procedures you should have the following for the Harmful Algae Project:

- 1 copy of the Harmful Algae monitoring procedures
- 3 harmful algae data forms
- 5 additional white membrane filter disks
- 5 additional sample storage vials and caps
- 4 additional dark brown rectangular sample storage bottles, labeled N (north), S (south), E (east) and W (west)
- 5 clear/white microcystin sample bottles with labels on
- 5 chlorophyll sample labels for the chlorophyll shoreline sample vials
- 2 blue-ice freezer packs- keep frozen until ready to use
- 3 zip-lock bags
- 1 bag of packing peanuts, to use for mailing the sample to the MSU laboratory
- 1 shipping container (insulated cooler)
- 1 shipping label with postage

(Note: Some samplers will receive one additional filter disk (6 total), vial and cap (6 total), label (6 total) and brown rectangular storage bottle (5 total), in order to collect a replicate sample at one of the four shoreline sampling sites.)

SAFETY

As with all CLMP sampling, the harmful algae samples should be collected when the weather conditions are safe. Be sure to sample with all of your safety equipment onboard (life jackets, back-up oars etc). Sample with a partner and remain low in the boat when collecting samples.

TRAINING

Onsite training is provided and recommended for the Harmful Algae Project, but not required. Onsite training is required to participate in the CLMP Chlorophyll Project. Some of the benefits of this training include discussing sampling concerns with resource people and other volunteers as well as increased data quality control. This training is offered at Michigan Lake and Stream Associations' annual conference the last weekend in April. Training may be offered at other locations. Contact the individual identified in the Technical Support section below for training requirements and the location of alternative training sites.

QUALITY ASSURANCE/QUALITY CONTROL

As part of the quality control for this project, MSU staff may conduct side-by-side sampling for selected lakes enrolled in Harmful Algae monitoring. If your lake is selected for the QA/QC process, you will be contacted prior to sampling to coordinate the side-by-side sampling. For some lakes (chosen randomly), replicate samples will be analyzed as part of the QA/QC process.

SAMPLING

When collecting samples for the Harmful Algae Project, please carefully follow the procedures describe here, and the CLMP written procedures for chlorophyll sampling.

Sample Collection Dates.

The last chlorophyll sampling event date, September for most lakes, late August for northern lakes, will be the time to collect samples for the Harmful Algae Project. Harmful Algae samples will be collected **only** during this last sampling event. Sampling two or three days before or after the August/September target date is acceptable. Samples should be collected in the morning, starting about 10:00 am, so the sun will be high enough for a good Secchi disk reading, and you will have time to collect samples from all stations and filter the chlorophyll samples.

Sample Collection Locations.

The CLMP chlorophyll samples are collected as a composite sample from the deep basin of the lake. At this site monitors will use the CLMP depth-compositing sampler to fill the two regular brown chlorophyll sample bottles and one microcystin toxin clear/white sample bottle. To measure toxin levels at the most likely points of human contact, the monitor will collect samples of surface water for microcystin and chlorophyll from four sites around the lake shoreline. These four sample sites should roughly approximate the four compass points: north, south, east and west. If the lake has a canal or public beach, include the canal or beach as one of the four sites. These four sampling sites should be close enough to shore to be in about 2 feet of water.

Since samples are collected in 2 feet of water, take caution not to stir up bottom sediments. If boating to the collection site, shut the motor off and row carefully when nearing the site. If driving or walking to the collection sites, use a dock if available, and the

owner approves, to collect the sample or wade very carefully to avoid suspending sediments and fill the collection bottle by holding it well away from the body. If the bottom is disturbed and the water becomes cloudy with sediments, move a short distance to a new location.

A. Sample Collection

1. Organize sampling equipment. Before proceeding to the sampling stations use the chlorophyll and harmful algae equipment list to organize all of the equipment that you will need to obtain both the deep station and shoreline samples. Make sure all the sampling equipment as well as your boating safety equipment and anchor are on-board before leaving the dock. With the number of deep station and shoreline sample bottles that you will have; you will probably need a second or larger cooler to store samples while on the lake. If you don't have a second or larger cooler, you can use the mailer cooler for the microcystin samples. Just be sure not to get it wet, because you will need to mail the microcystin samples in it later. (**Note:** The chlorophyll sample filtering apparatus and materials should be organized at a convenient indoor location (i.e. kitchen, laundry room, or garage with sink and ready to use upon returning).

2. Proceed to your sampling stations. Once all of the sampling equipment and your boating safety equipment are on-board, proceed **first** to the deepest basin of the lake to collect the deep basin chlorophyll samples and one microcystin sample. When you are directly over the deep basin, orient the boat so it is facing the breeze and move upwind until slightly past the deepest point in the lake. Lower the anchor and allow the boat to drift back over the deepest point of the lake before securing the anchor line. This allows you to sample the water column outside the area where sediments may have re-suspended when the anchor hit bottom. When in position, take out and fill in the CLMP chlorophyll and harmful algae sample data forms (**Note:** Use a soft lead pencil, or fine tip permanent marker pen when recording information on the data forms. Avoid using inks that will fade or run when wet.)

Label the five white/clear microcystin sample bottles. Use a fine tip permanent black marker to complete the label for the microcystin sample bottles. The information on the label should include the Sampling Date, Volunteer's Initials, the Location which is the lake's name, the Field ID which is the sampling station in the lake such as the "deep basin", "N" (north) site, "E" (east) site, "S" (south) site, "W" (west) site and Parameter Code should already be on the label. Leave the Chemicals Added section of the label blank.

3. Measure Secchi transparency. Follow the CLMP Secchi Disk Project procedures to measure the Secchi disk transparency.

4. Prepare composite sampler for sample collection. Remove the sampler bottle from the weighted container and rinse the bottle with lake water. Shake out any residual lake water from the sampler bottle and secure the sampler bottle back into the weighted container. Make sure the weight is in the container, the retaining chain is around the neck

of the sampler bottle, the stopper assembly is secure, the bottle cap is tightened, and the measured line is securely fastened to the composite sampler.

5. Use the composite sampler to collect water for chlorophyll and microcystin samples. Fill the one liter composite sampler bottle to nearly fill, but not completely. Follow the directions provided in the CLMP chlorophyll monitoring procedures to collect the water sample.

6. Place chlorophyll samples in the brown rectangular bottles and the microcystin sample in the clear/white bottle. Remove the sampler bottle from the weighted container. Swirl bottle gently to mix the sample. Use a small portion of the composite sample to rinse both of the rectangular (brown) sample storage bottles and the deep station microcystin bottle. Swirl or gently shake the sampler bottle again to mix sample and then fill both chlorophyll bottles and the microcystin bottle. Fill the bottles only to the bottom of the neck leaving some head space in the bottles. Place the microcystin sample bottle in the insulated cooler bag. Continue to process the chlorophyll samples as described in the Chlorophyll Project procedures. (**Note: No preservative should be added to the microcystin sample bottle, just keep it cool.**)

7. Prepare for shoreline sample collection. Before leaving the deep station sampling site remove the one liter composite sampler bottle from the composite sampler. Remove the two-hole stopper and cap from the bottle and secure them in your cooler bag, so they are not lost. Organize your chlorophyll and microcystin sample bottles so you will be using the correct bottles, at the proper sampling site (the south labeled bottles at the south sampling site).

8. Proceed to the first of the four shoreline sampling sites. Boat to the first shoreline sampling site. (**Note:** For a large lake you may choose to drive a car to the four sampling sites and use a dock or waders or a swim suit to wade out into 2 feet of water to collect the samples. Remember to avoid stirring up the bottom sediments.) Boat in close enough to shore to be in 2 feet of water. You do not need to anchor. Staying low in the boat, carefully lean over the side of the boat and fill the one liter bottle with water and empty out the water. This first bottle fill is a rinse for this sampling site. Now refill the bottle again, for the sample. (**Note:** Do not use the “dead spider” grip to push the bottle below the water surface, like when collecting the total phosphorus sample. The objective of shoreline sampling is to collect water right at the surface, where the cyanobacteria concentrations are greatest.) Ease the bottle into the water and let water from the surface run into the bottle. Gradually lower the back of the bottle as it fills. There should be no bubbling as the bottle fills. If there is bubbling the bottle is too deep in the water.

9. Place chlorophyll sample in the brown rectangular bottle and the microcystin sample in the clear/white bottle. Swirl the one liter sampler bottle gently to mix the sample. (**Note:** At the shoreline sampling stations you have only one brown chlorophyll bottle instead of two like at the deep station.) Use a small portion of the sample to rinse both the brown sample storage bottle and the (clear/white) microcystin bottle. Swirl or gently shake sampler bottle again to mix sample and then fill the chlorophyll bottle and the

microcystin bottle. Fill the bottles only to the bottom of the neck leaving some head space in the bottles. Place the microcystin in the insulated cooler bag. Continue to process the chlorophyll sample as described in the Chlorophyll Project procedures. **Before leaving the site complete the information required on the Harmful Algae sampling data form and mark your map on the back of the form identifying the location of the sample site.**

10. Complete all four shoreline sampling sites and replicate sample, if you have a replicate kit. Repeat steps 8 and 9 at the remaining three shoreline sampling sites. If you have a replicate kit you will have an extra brown chlorophyll bottle (five total) and an extra clear/white microcystin bottle (six total). At one of your four shoreline sampling sites collect an extra or replicate chlorophyll and microcystin sample. Process these replicate samples just as you would the other samples. Fill in the back of the field form with the replicate sample information.

11. Prepare to return to shore. After stabilizing and storing the samples, empty the sampler bottle, rinse with clean water, and secure the sampler bottle back into the weighted container. Retrieve the two-hole stopper and cap from the cooler bag and place on the sampler bottle. Return to shore to filter your chlorophyll samples and freeze your microcystin samples.

B. Sample Filtering and Handling

1. Organize samples. After returning to shore, separate the chlorophyll samples from the microcystin samples. Place the microcystin samples in the freezer. (**Note:** be sure the bottle is not completely full with water, so it will not crack when frozen.) Take the chlorophyll samples to your filtering location. Use the CLMP chlorophyll procedures to process the samples.

2. Filter the deep chlorophyll sample. **First process the deep station chlorophyll samples.** Wrap the two vials from the deep station with a piece of aluminum foil to protect the filters from light. Mark the lake name and month on the outside of the foil. Put the “foiled” sample vial into a zip-lock bag and label the bag with your name, the lake name, county, and township. Store the vials with filter disks in your freezer. (**Note:** You will take both your deep station and shoreline chlorophyll frozen filters to the designated DEQ District office **no later than noon on the scheduled turn-in date.**)

3. Filter the shoreline chlorophyll samples. **After the deep station chlorophyll samples have been processed,** process the four (five if you were asked to do a replicate) chlorophyll samples from the shoreline sampling sites just as you did the chlorophyll samples from the deep station using the chlorophyll procedures. Be sure to rinse your syringe and plastic tubing with tap water between each shoreline chlorophyll sample. Use a fine tip permanent marker to complete the label for the shoreline chlorophyll vials. The information on the label should include the Sampling Date, Volunteer’s Initials, the Location which is the lake’s name, the Field ID which is the sampling station in the lake such as the “N (north) site, E (east) site, S (south) site, or W (west) site”, the Parameter

Code is “CA” and the Chemicals Added is “MgCO₃”. Wrap the four (five if you were asked to do a replicate) labeled shoreline chlorophyll filter vials together in aluminum foil. Mark the **lake name** and the word “**shoreline**” on the outside of the foil. Put the “foiled” sample vials into a zip-lock bag and label the bag with the **lake name** and **county**. (**Note:** Do not include the shoreline chlorophyll filter vials with the regular CLMP deep station vials, because they are going to different laboratories.) Include one of the “Harmful Algae” data sheets in the zip-lock bag with the foil wrapped vials.

IMPORTANT NOTE: For many shoreline samples it may not be possible to push 50 cc through the filter. Many algal cells or soil particles in the shoreline water, may clog the filter before 50 cc can be pushed through. If the plunger becomes extremely hard to push, don’t force it. STOP the filtering process and note the amount of cc’s that have been pushed through the filter (50 cc minus the cc’s of sample remaining). In the Data Form section for that shoreline sample, record the amount (cc’s) of water that was successfully passed through the filter. Write large and clearly so the sample processor will see and read your note. The sample is still good. The laboratory will adjust their calculations to account for the smaller sample volume.

4. Clean and dry chlorophyll sampling and filtering equipment. After you have filtered the samples and stored them in the freezer, clean the chlorophyll filtering equipment by rinsing each component with tap water (**DO NOT USE DETERGENTS**) and letting them air dry. When your equipment is clean and dry, loosely reassemble the filtering components and composite sampler and store the equipment in a convenient place for your next sampling season. (**Note:** Your insulated cooler bag can be used to store your chlorophyll filtering equipment and sample storage bottles.)

C. Sample Delivery

1. Packing the microcystin samples for mailing. On a Monday, Tuesday, Wednesday, or Thursday after the microcystin samples are completely frozen, pack the five samples in the provided mailing cooler with the frozen cooler packs, which had been frozen beforehand. Include one of the Harmful Algae data forms in a zip-lock bag in the cooler. Completely fill the void space in the cooler with the bag of packing peanuts provided and crumpled newspaper. Tape the mailing cooler closed and attach the provided mailing label to the shipping cooler.

2. Mail microcystin samples. Take the packed shipping cooler to a local post office and mail it “Next Day” mail, to MSU, at the address provided. Postage is provided so there is no cost to the volunteer. Next day mail will insure that the samples arrive at MSU in near frozen state. Mailing on a Monday through Thursday will insure that the samples arrive when the MSU office is open. **Do not take your shoreline water samples to the DEQ collection center with your chlorophyll samples. They will NOT accept these samples. They must be mailed directly to MSU.**

3. Deliver chlorophyll filters. Deliver the deep station and shoreline chlorophyll samples to the designated DEQ District office before the designated turn-in time. (**Note:** Refer to

the schedule of turn-in dates and drop-off locations for your samples.) **Your chlorophyll samples must be frozen when you drop them off at the DEQ District.** Place the completed data forms (one for the deep station samples and one for the shoreline samples) in the zip-lock bag with your frozen chlorophyll filters. (Note: Your insulated cooler bag with freezer ice pack can be used to keep your chlorophyll filters frozen while transporting them to the designated DEQ District office.) **Your frozen samples must be received no later than noon on the scheduled turn-in date at your designated DEQ District office.** For your convenience you may turn in samples any time the DEQ District office is open until noon on the scheduled turn-in date. Samples turned in late or not frozen will not be accepted.

TECHNICAL SUPPORT

Should you have any questions or comments about the Harmful Algae monitoring procedures or problems during sampling, sample handling, or sample delivery, contact:

Mr. Howard Wandell, Department of Fisheries and Wildlife, 13 Natural Resources Building,
Michigan State University, East Lansing, MI 48824-1222

Phone: 517-432-1491

FAX: 517-432-1699

Email: wandellh@msu.edu

APPENDIX B. Data sheet used by CLMP volunteers.



Cooperative
Lakes
Monitoring
Program

**Harmful Algae
Shoreline Samples
CHLOROPHYLL and MICROCYSTIN**

Lake Name: _____ County: _____

Township: _____

Volunteer Monitor Name(s): _____ **Date**

Sampled: _____

Weather Conditions (sunny, cloudy, windy, etc.): _____

Unusual Conditions (heavy rain, boating, etc.): _____

Are zebra mussels in the lake? Yes. No.

North Sampling Site

Time: _____

Location (GPS latitude/longitude coordinates or physically describe site location)

Water Condition at sampling site (check all that apply)

Clear Turbid Green Brown Noticeable algae sheen and/or scum

Filtering Sample (if 50 cc could not be filtered for this sample, indicate amount filtered) _____

East Sampling Site

Time: _____

Location (GPS latitude/longitude coordinates or physically describe site location)

Water Condition at sampling site (check all that apply)

Clear Turbid Green Brown Noticeable algae sheen and/or scum

Filtering Sample (if 50 cc could not be filtered for this sample, indicate amount filtered) _____

South Sampling Site

Time: _____

Location (GPS latitude/longitude coordinates or physically describe site location)

Water Condition at sampling site (check all that apply)

Clear Turbid Green Brown Noticeable algae sheen and/or scum

Filtering Sample (if 50 cc could not be filtered for this sample, indicate amount filtered) _____

West Sampling Site

Time: _____

Location (GPS latitude/longitude coordinates or physically describe site location) _____

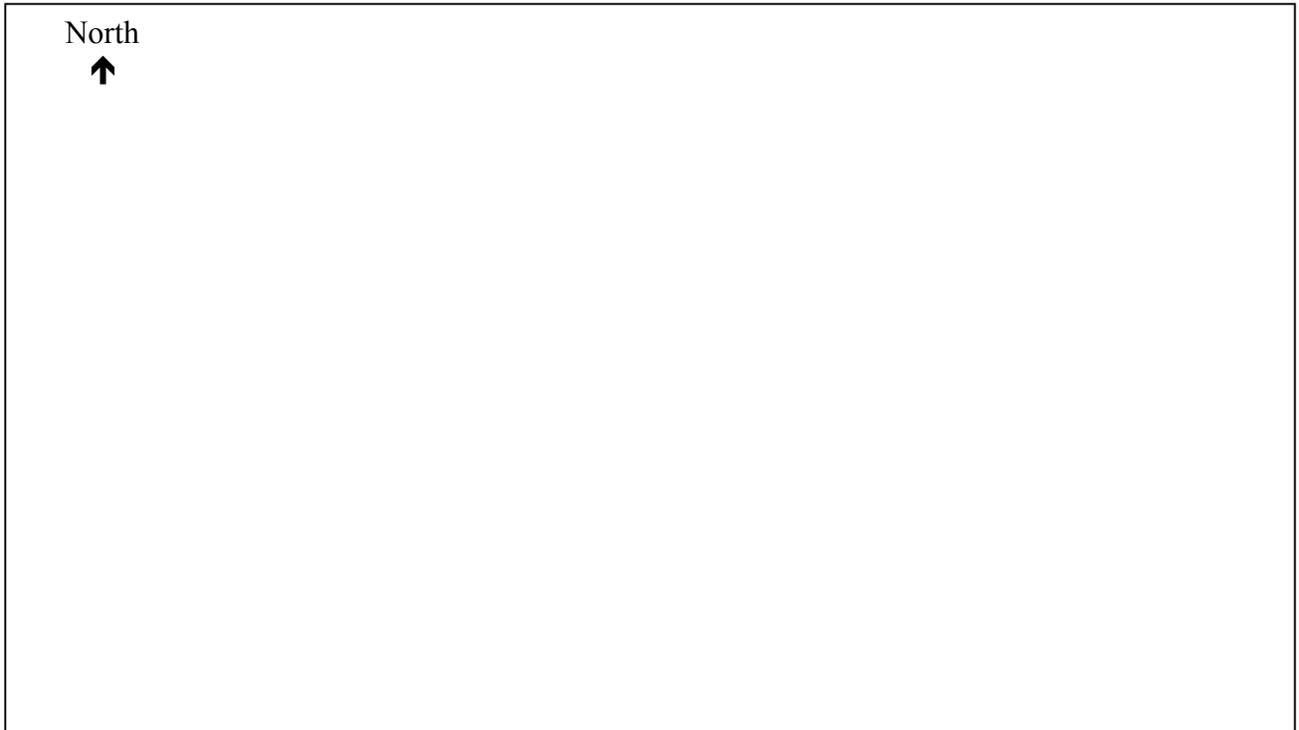
Water Condition at sampling site (check all that apply)

Clear Turbid Green Brown Noticeable algae sheen and/or scum

Filtering Sample (if 50 cc could not be filtered for this sample, indicate amount filtered) _____

- ❖ In the box below draw an outline of your lake (i.e. lake map)
- ❖ On the lake map mark your four harmful algae sampling locations

North
↑



FOR REPLICATE SAMPLE IF ASKED TO COLLECT

(If your sampling kit has a note asking you to collect replicate samples, you will also have an extra brown bottle (5) and an extra white/clear bottle (6) in your kit.)

Sampling Site: N (north) _____, E (east) _____, S (south) _____, W (west) _____.

(Mark at which sampling site location you collected the replicate sample.)

Filtering Sample (if 50 cc could not be filtered for this sample, indicate amount filtered) _____

- ❖ **Mail one copy of the completed data form with your microcystin water samples to MSU. Send a second copy with your shoreline chlorophyll samples to the DEQ collection center. Keep one copy of the completed data form for your records.**

APPENDIX C. Harmful Algae Monitoring, 2006, Data Summary
 Background information for lakes lacking zebra mussels (*Dreissena*).

| Lake | County | ID# | Latitude | Longitude | Surface area km ² | Max. depth m | Date sampled |
|------------|------------|--------|----------|-----------|---------------------------------|-----------------|-----------------|
| Baldwin | Montcalm | 590171 | 43.16445 | -85.26723 | | 10.7 | 15-Sep-06 |
| Bear | Kalkaska | 400026 | 44.74519 | -84.90227 | 1.2788 | 18.0 | 9-Sep-06 |
| Big Star | Lake | 430022 | 43.83278 | -85.95001 | 3.6908 | 7.6 | 8-Sep-06 |
| Cowan | Kent | 410550 | 43.11556 | -85.42139 | | 16.2 | 12-Sep-06 |
| Cowboy | Dickenson | 220128 | 45.81188 | -88.12098 | | 7.6 | 25-Aug-06 |
| Crockery | Ottawa | 700422 | 43.16584 | -85.85639 | 0.4371 | 16.5 | 26-Sep-06 |
| Crooked | Kalamazoo | 390599 | 42.20355 | -84.70847 | | | 24-Sep-06 |
| Crystal | Oceana | 640062 | 43.65334 | -86.38056 | 0.3076 | 11.3 | 15-Sep-06 |
| Crystal | Dickenson | | 45.81165 | -88.07762 | | 2.4 | 25-Aug-06 |
| Cub | Kalkaska | 400031 | 44.71889 | -84.95278 | 0.2145 | 7.0 | 9-Sep-06 |
| Deer | Alger | 20126 | 46.47688 | -86.96156 | 1.0765 | 18.3 | 27-Aug-06 |
| Earl | Livingston | 470554 | 42.60191 | -83.89588 | | 6.7 | 17-Sep-06 |
| Freska | Kent | 410702 | 43.11080 | -85.64000 | 0.2509 | 7.6 | 17-Sep-06 |
| George | Clare | 180056 | 43.95584 | -84.93834 | 0.5423 | 7.6 | 11-Sep-06 |
| Goshorn | Allegan | 30650 | 42.68250 | -86.18380 | 0.0900 | 18.0 | 22-Sep-06 |
| Hess | Newaygo | 620032 | 43.38862 | -85.76806 | 3.0554 | 8.5 | 15-Sep-06 |
| Hicks | Osceola | | 44.02306 | -85.28417 | 0.6273 | 10.1 | 10-Sep-06 |
| Indian | Osceola | 670227 | 43.96610 | -85.40500 | 0.3440 | 15.2 | 9-Sep-06 |
| Jewell | Alcona | 10041 | 44.67917 | -83.61056 | 0.7815 | 10.4 | 15-Sep-06 |
| Kimball | Newaygo | | 43.45639 | -85.82862 | 0.6192 | 15.8 | 16-Sep-06 |
| Little | Marquette | 520210 | 46.27362 | -87.35778 | | 15.2 | 27-Aug-06 |
| Mehl | Marquette | 520451 | 46.27686 | -87.37477 | 0.3683 | 6.1 | 27-Aug-06 |
| Moon | Gogebic | 270120 | 46.17195 | -89.21223 | 0.3764 | 12.2 | 24-Aug-06 |
| Murray | Kent | 410268 | 43.03056 | -85.37278 | 1.2950 | 21.9 | 15-Sep-06 |
| Osterhout | Allegan | 30263 | 42.43945 | -86.03889 | 0.6799 | 9.1 | 23-Sep-06 |
| Pickerel | Kalkaska | 400035 | 44.80056 | -84.97667 | 0.4047 | 21.9 | 16-Sep-06 |
| Round | Clinton | 190146 | 42.87660 | -84.44520 | 0.3440 | 7.3 | 15-Sep-06 |
| Shafer | Van Buren | | | | 0.3278 | 21.0 | 24-Sep-06 |
| Shingle | Clare | 180108 | 43.96306 | -84.95028 | 0.1416 | 12.2 | 11-Sep-06 |
| Sweezey | Jackson | 380470 | 42.16210 | -84.16811 | 0.4249 | 7.0 | 23-Sep-06 |
| Upper Long | Oakland | 631118 | 42.59648 | -83.32253 | | | 26-Sep-06 |
| Viking | Otsego | 690136 | 44.89389 | -84.62000 | 0.1611 | 7.6 | 25-Aug-06 |
| Webinguaw | Newaygo | 620283 | 43.67350 | -85.77712 | 0.2469 | 4.9 | 16-Sep-06 |
| Wells | Osceola | 670121 | 43.99728 | -85.41303 | 0.1943 | 25.0 | 8-Sep-06 |

APPENDIX C. Harmful Algae Monitoring, 2006, Data Summary
 Background information for lakes with zebra mussels (*Dreissena*).

| Lake | County | ID# | Latitude | Longitude | Surface area km ² | Max. depth m | Date sampled |
|---------------|----------------|--------|----------|-----------|---------------------------------|-----------------|-----------------|
| Ann | Benzie | 100082 | 44.71195 | -85.84334 | 2.1327 | 21.3 | 10-Sep-06 |
| Antoine | Dickenson | 220028 | 45.83806 | -88.03195 | 3.0271 | 7.6 | 25-Aug-06 |
| Barlow | Barry | 80176 | 42.67056 | -85.52042 | | 19.2 | 24-Sep-06 |
| Beaver | Alpena | 40097 | 44.93612 | -83.80000 | 2.6912 | 23.5 | 31-Aug-06 |
| Bellaire | Antrium | 50052 | 44.95334 | -85.21889 | 7.1833 | 29.0 | 1-Sep-06 |
| Big Fisher | Leelanau | 450224 | 44.89428 | -85.94887 | | 4.9 | 7-Sep-06 |
| Big Glen | Leelanau | 450049 | 44.87889 | -85.96278 | 19.6884 | 39.6 | 6-Sep-06 |
| Big | Osceola | 670056 | 43.86695 | -85.19917 | 0.8256 | 25.9 | 10-Sep-06 |
| Bills | Newaygo | 620062 | | | 0.8256 | 27.0 | 15-Sep-06 |
| Birch | Cass | 140061 | 41.88139 | -85.85834 | 1.1938 | 29.0 | 21-Sep-06 |
| Brooks | Leelanau | 450222 | 44.87462 | -85.93284 | | | 5-Sep-06 |
| Cedar | Alcona/Iosco | 10017 | 44.52751 | -83.33195 | 4.3505 | 3.0 | 9-Sep-06 |
| Clam | Antrium | 50101 | 44.93612 | -85.27334 | 1.6997 | 8.8 | 31-Aug-06 |
| Clark | Jackson | 380173 | 42.11945 | -84.31306 | 2.2663 | 16.8 | 21-Sep-06 |
| Deer | Oakland | 630708 | 42.73056 | -83.43084 | 0.5544 | 19.2 | 21-Sep-06 |
| Derby | Montcalm | 590144 | 43.27389 | -85.12945 | 0.4775 | 26.5 | 14-Sep-06 |
| Diamond | Cass | 140039 | 41.90380 | -85.96660 | 4.1279 | 19.5 | 23-Sep-06 |
| Evans | Lenawee | 460309 | 42.05778 | -84.11306 | 0.8134 | 12.8 | 21-Sep-06 |
| Fisher | St. Joseph | 750139 | 41.99500 | -85.57250 | 1.3234 | 12.8 | 23-Sep-06 |
| Gilletts | Jackson | | 42.25639 | -84.31028 | | 9.1 | 17-Sep-06 |
| Gourdneck | Kalamazoo | 390541 | 42.15973 | -85.57389 | 0.8984 | 15.8 | 20-Sep-06 |
| Hamlin- Lower | Mason | 530073 | 44.05528 | -86.46834 | 20.1943 | 24.4 | 10-Sep-06 |
| Hamlin- Upper | Mason | 530074 | 44.07306 | -86.44417 | 20.1943 | 11.3 | 10-Sep-06 |
| Houghton | Roscommon | 720163 | 44.32667 | -84.68083 | 81.1170 | 6.1 | 10-Sep-06 |
| Hubbard | Alcona | 10020 | 44.83334 | -83.60000 | 35.8155 | 22.9 | 5-Sep-06 |
| Klinger | St. Joseph | 750136 | 41.80278 | -85.54389 | 3.3590 | 21.9 | 23-Sep-06 |
| Lakeville | Oakland | 630670 | 42.82917 | -85.15195 | 1.8616 | 20.1 | 24-Sep-06 |
| Little Fisher | Leelanau | 340223 | 44.89770 | -85.95052 | 5.6657 | 4.0 | 7-Sep-06 |
| Little Glen | Leelanau | 450050 | 44.86500 | -86.00876 | 1.8130 | 4.0 | 5-Sep-06 |
| Magician | Cass | 140065 | 42.06500 | -86.18389 | 2.3100 | 17.0 | 29-Sep-06 |
| Margrethe | Crawford | 200036 | 44.62778 | -84.78750 | 7.7701 | 19.8 | 3-Sep-06 |
| Mullett | Cheboygan | 160050 | 45.48445 | -84.56028 | 70.2550 | 36.6 | 2-Sep-06 |
| Nepessing | Lapeer | 440094 | 43.01223 | -83.37445 | 1.6754 | 7.6 | 14-Sep-06 |
| Orion | Oakland | 630555 | 42.78140 | -83.25376 | 1.9021 | | 24-Sep-06 |
| Parke | Oakland | 631119 | 42.73847 | -83.41385 | 0.0931 | 15.2 | 21-Sep-06 |
| Robinson | Newaygo | 620061 | 43.53195 | -85.85612 | 0.5544 | 9.1 | 15-Sep-06 |
| Round | Mecosta | 540073 | 43.62417 | -85.30834 | 0.6273 | 13.7 | 14-Sep-06 |
| Silver | Grand Traverse | 280116 | 44.70528 | -85.68667 | 2.4282 | 29.9 | 9-Sep-06 |
| Stony Lake | Oceana | 640049 | 43.56060 | -86.48610 | 1.1251 | 12.5 | 14-Sep-06 |
| Torch North | Antrium | 50055 | 45.02778 | -85.31556 | 35.4917 | 76.2 | 3-Sep-06 |
| Torch South | Antrium | 50240 | 44.91590 | -85.30280 | 35.4917 | 86.9 | 3-Sep-06 |
| Van Etten | Iosco | 350074 | 44.46417 | -83.35389 | 5.3420 | 10.1 | 8-Sep-06 |
| Vineyard | Jackson | 380263 | 42.07500 | -84.20417 | 2.0437 | 12.8 | 24-Sep-06 |

APPENDIX D. Harmful Algae Monitoring, 2006, Data Summary
 Shoreline (surface water) samples *

| Lake | County | ID# | <i>Dreissena</i> status | north Chl µg/L | north Toxin µg/L | east Chl µg/L | east Toxin µg/L | south Chl µg/L | south Toxin µg/L | west Chl µg/L | west Toxin µg/L | mean Chl µg/L | mean Toxin µg/L |
|-----------------|------------|--------|----------------------------|----------------------|------------------------|---------------------|-----------------------|----------------------|------------------------|---------------------|-----------------------|---------------------|-----------------------|
| Baldwin | Montcalm | 590171 | no | 8.3 | 0.08 | 7.7 | 0.09 | 7.8 | 0.14 | 15.1 | 3.94 | 9.7 | 1.06 |
| Bear Lake | Kalkaska | 400026 | no | 3.0 | 0.04 | 3.8 | 0.03 | 3.7 | 0.04 | 2.8 | 0.04 | 3.3 | 0.04 |
| Big Star | Lake | 430022 | no | 4.6 | 0.07 | 6.9 | 0.08 | 6.8 | 0.05 | 4.7 | 0.05 | 5.7 | 0.06 |
| Cowan | Kent | 410550 | no | 21.2 | 0.10 | 30.6 | 0.39 | 62.7 | 0.70 | 195.1 | 0.86 | 77.4 | 0.51 |
| Cowboy | Dickenson | 220128 | no | | 0.09 | | 0.07 | | 0.08 | | 0.08 | | 0.08 |
| Crockery | Ottawa | 700422 | no | 12.6 | 0.05 | 14.3 | 0.05 | 11.6 | 0.06 | 14.2 | 0.05 | 13.2 | 0.05 |
| Crooked | Kalamazoo | 390599 | no | 5.5 | 0.08 | 5.3 | 0.08 | 8.3 | 0.08 | 11.0 | 0.08 | 7.5 | 0.08 |
| Crystal | Oceana | 640062 | no | 0.2 | 0.13 | 11.8 | 0.06 | 8.0 | 0.09 | 12.6 | 0.45 | 8.2 | 0.18 |
| Crystal | Dickenson | | no | | 0.98 | | 0.89 | | 1.25 | | 1.15 | | 1.07 |
| Cub Lake | Kalkaska | 400031 | no | 2.3 | 0.28 | 4.9 | 0.25 | 2.1 | 0.20 | 2.7 | 0.58 | 3.0 | 0.33 |
| Deer | Alger | 20126 | no | 50.1 | 0.03 | 9.7 | 0.04 | 0.3 | 0.04 | 8.0 | 0.03 | 17.0 | 0.04 |
| Earl | Livingston | 470554 | no | 15.5 | 0.30 | 9.4 | 0.32 | 10.1 | 0.44 | 8.4 | 0.48 | 10.8 | 0.38 |
| Freska | Kent | 410702 | no | 72.5 | 0.89 | 8.2 | 0.07 | 14.7 | 0.41 | 8.0 | 0.10 | 25.9 | 0.37 |
| George | Clare | 180056 | no | 3.3 | 0.04 | 4.9 | 0.05 | 12.1 | 0.05 | 7.0 | 0.04 | 6.8 | 0.04 |
| Goshorn | Allegan | 30650 | no | 22.4 | 0.40 | 23.0 | 0.29 | 38.1 | 0.28 | 24.7 | 0.41 | 27.0 | 0.34 |
| Hess | Newaygo | 620032 | no | 31.4 | 1.08 | 21.7 | 0.44 | 158.2 | 4.29 | 26.8 | 1.70 | 59.5 | 1.88 |
| Hicks | Osceola | | no | 18.1 | 0.26 | 17.7 | 0.25 | 18.5 | 0.22 | 14.3 | 0.23 | 17.1 | 0.24 |
| Indian Lake | Osceola | 670227 | no | 4.3 | 0.06 | 4.1 | 0.06 | 4.1 | 0.06 | 4.0 | 0.18 | 4.1 | 0.09 |
| Jewell Lake | Alcona | 10041 | no | 5.0 | 0.43 | 8.5 | 1.50 | 6.7 | 0.07 | 8.8 | 0.13 | 7.2 | 0.53 |
| Kimball | Newaygo | | no | 17.6 | 0.04 | 18.0 | 0.05 | 21.9 | 0.06 | 176.1 | 0.10 | 58.4 | 0.06 |
| Little | Marquette | 520210 | no | 4.3 | 0.12 | 0.2 | 0.07 | 3.8 | 0.07 | 3.8 | 0.06 | 3.1 | 0.08 |
| Mehl | Marquette | 520451 | no | 12.7 | 0.61 | 4.9 | 0.09 | 4.3 | 0.11 | 3.9 | 0.08 | 6.4 | 0.22 |
| Moon | Gorebic | 270120 | no | 3.4 | 0.04 | 2.4 | 0.03 | 2.8 | 0.03 | 3.8 | 0.04 | 3.1 | 0.04 |
| Murray | Kent | 410268 | no | 13.0 | 0.86 | 6.2 | 0.33 | 7.2 | 0.19 | 10.9 | 0.72 | 9.3 | 0.53 |
| Osterhout | Allegan | 30263 | no | 6.7 | 1.03 | 29.9 | 0.12 | 6.7 | 0.61 | 8.6 | 0.18 | 13.0 | 0.48 |
| Pickernel | Kalkaska | 400035 | no | 5.5 | 0.04 | 13.2 | 0.04 | 10.3 | 0.03 | 6.7 | 0.03 | 8.9 | 0.04 |
| Round | Clinton | 190146 | no | 22.6 | 0.12 | 12.3 | 0.14 | 39.8 | 0.22 | 25.4 | 0.12 | 25.0 | 0.15 |
| Shafer | Van Buren | | no | | 0.07 | | 0.09 | | 0.11 | | 0.09 | | 0.09 |
| Shingle | Clare | 180108 | no | | 0.04 | 9.6 | 0.04 | 10.0 | 0.03 | 14.6 | 0.03 | 11.4 | 0.03 |
| Sweezy | Jackson | 380470 | no | 3.0 | 0.04 | 2.0 | 0.06 | 1.7 | 0.04 | 1.6 | 0.04 | 2.1 | 0.04 |
| Upper Long Lake | Oakland | 631118 | no | 28.5 | 0.79 | 23.8 | 3.52 | 79.6 | 0.64 | 26.4 | 0.72 | 39.6 | 1.42 |
| Viking | Otsego | 690136 | no | 20.7 | 0.10 | 19.1 | 0.08 | 20.8 | 0.11 | 19.8 | 0.22 | 20.1 | 0.13 |
| Webinguaw | Newaygo | 620283 | no | 8.9 | 0.42 | 4.9 | 0.14 | 6.4 | 0.09 | 20.6 | 0.79 | 10.2 | 0.36 |
| Wells | Osceola | 670121 | no | 2.6 | 0.09 | 1.1 | 0.10 | 4.8 | 0.10 | 2.0 | 0.10 | 2.6 | 0.10 |

* Analytical toxin results are increased by a factor of 1.23 to adjust for sample preparation.

APPENDIX D. Harmful Algae Monitoring, 2006, Data Summary
 Shoreline (surface water) samples *

| Lake | County | ID# | <i>Dreissena</i> status | north Chl µg/L | north Toxin µg/L | east Chl µg/L | east Toxin µg/L | south Chl µg/L | south Toxin µg/L | west Chl µg/L | west Toxin µg/L | mean Chl µg/L | mean Toxin µg/L |
|---------------|--------------|--------|----------------------------|----------------------|------------------------|---------------------|-----------------------|----------------------|------------------------|---------------------|-----------------------|---------------------|-----------------------|
| Ann | Benzie | 100082 | yes | 2.0 | 0.32 | 1.8 | 0.09 | 1.1 | 0.05 | 1.8 | 0.06 | 1.7 | 0.13 |
| Antoine | Dickenson | 220028 | yes | | 0.21 | | 0.22 | | 0.15 | | 0.18 | | 0.19 |
| Barlow | Barry | 80176 | yes | 4.3 | 0.16 | 3.8 | 0.17 | 1.6 | 0.25 | 4.4 | 0.19 | 3.5 | 0.19 |
| Beaver | Alpena | 40097 | yes | 2.3 | 0.15 | 2.0 | 0.24 | 1.9 | 0.20 | 2.3 | 0.21 | 2.1 | 0.20 |
| Bellaire | Antrium | 50052 | yes | 2.3 | 0.25 | 2.7 | 0.18 | 2.2 | 0.11 | 2.7 | 0.19 | 2.5 | 0.18 |
| Big Fisher | Leelanau | 450224 | yes | 1.8 | 0.03 | 3.1 | 0.02 | 1.1 | 0.03 | | 0.02 | 2.0 | 0.02 |
| Big Glen | Leelanau | 450049 | yes | 1.3 | 0.03 | 1.6 | 0.04 | 1.3 | 0.03 | 1.5 | 0.02 | 1.4 | 0.03 |
| Big Lake | Osceola | 670056 | yes | 3.0 | 0.25 | 2.6 | 0.22 | 9.9 | 0.63 | 3.7 | 0.56 | 4.8 | 0.41 |
| Bills Lake | Newaygo | 620062 | yes | 30.3 | 5.15 | 27.7 | 3.40 | 75.4 | 9.39 | 3.8 | 0.30 | 34.3 | 4.56 |
| Birch | Cass | 140061 | yes | 3.8 | 0.12 | 3.5 | 0.14 | 2.9 | 0.13 | 3.1 | 0.16 | 3.3 | 0.14 |
| Brooks | Leelanau | 450222 | yes | 24.5 | 0.41 | 16.4 | 0.02 | 7.9 | 0.02 | 18.8 | 0.02 | 16.9 | 0.12 |
| Cedar | Alcona/Iosco | 10017 | yes | 2.3 | 0.03 | 5.2 | 0.15 | 4.1 | 0.17 | 4.3 | 0.24 | 4.0 | 0.15 |
| Clam | Antrium | 50101 | yes | 2.7 | 0.09 | 1.4 | 0.08 | 6.2 | 0.47 | 6.0 | 1.31 | 4.1 | 0.49 |
| Clark | Jackson | 380173 | yes | 1.9 | 0.13 | 3.6 | 0.66 | 1.3 | 0.07 | 1.7 | 0.08 | 2.1 | 0.23 |
| Deer | Oakland | 630708 | yes | 2.4 | 0.16 | 2.1 | 0.18 | 3.1 | 0.23 | 2.5 | 0.09 | 2.5 | 0.17 |
| Derby | Montcalm | 590144 | yes | 5.9 | 1.57 | 2.1 | 0.26 | 8.5 | 0.79 | 4.5 | 0.36 | 5.2 | 0.75 |
| Diamond | Cass | 140039 | yes | 6.5 | 0.72 | 4.0 | 0.37 | 1.7 | 0.10 | 3.8 | 0.17 | 4.0 | 0.34 |
| Evans | Lenawee | 460309 | yes | 1.1 | 0.07 | 1.1 | 0.15 | 1.4 | 0.10 | 1.2 | 0.08 | 1.2 | 0.10 |
| Fisher | St. Joseph | 750139 | yes | 5.5 | 0.75 | 4.3 | 0.36 | 6.5 | 0.88 | 4.7 | 0.45 | 5.2 | 0.61 |
| Gilletts | Jackson | | yes | 4.1 | 0.15 | 0.0 | 0.13 | 6.5 | 0.16 | | 0.20 | | 0.16 |
| Gourdneck | Kalamazoo | 390541 | yes | 5.1 | 0.19 | 4.8 | 0.14 | 5.4 | 0.06 | 5.4 | 0.16 | 5.2 | 0.14 |
| Hamlin- Lower | Mason | 530073 | yes | 3.3 | 0.06 | 1.3 | 0.08 | 11.8 | 0.06 | 3.6 | 0.13 | 5.0 | 0.08 |
| Hamlin- Upper | Mason | 530074 | yes | 3.2 | 0.18 | 16.0 | 0.16 | 2.5 | 0.18 | 7.8 | 0.20 | 7.4 | 0.18 |
| Houghton | Roscommon | 720163 | yes | 14.1 | 0.16 | 14.2 | 0.19 | 12.5 | 0.18 | 16.9 | 0.20 | 14.5 | 0.18 |
| Hubbard | Alcona | 10020 | yes | 0.7 | 0.19 | 0.7 | 0.32 | 0.9 | 0.26 | 0.1 | 0.61 | 0.6 | 0.34 |
| Klinger | St. Joseph | 750136 | yes | 5.7 | 0.16 | 15.3 | | 1.9 | 0.51 | 5.9 | 0.14 | 7.2 | 0.27 |
| Lakeville | Oakland | 630670 | yes | 20.6 | 3.68 | 308.7 | 46.35 | 6.0 | 1.00 | 234.6 | 43.26 | 142.5 | 23.57 |
| Little Fisher | Leelanau | 340223 | yes | 0.6 | 0.03 | 0.7 | 0.03 | 0.8 | 0.03 | 0.7 | 0.03 | 0.7 | 0.03 |
| Little Glen | Leelanau | 450050 | yes | 4.0 | 0.84 | 1.6 | 0.26 | 4.3 | 0.82 | 11.1 | 0.10 | 5.2 | 0.51 |
| Magician | Cass | 140065 | yes | 9.2 | 0.30 | 15.3 | 0.51 | 5.5 | 0.38 | 0.2 | 0.22 | 7.5 | 0.35 |
| Margrethe | Crawford | 200036 | yes | 2.3 | 0.26 | 4.5 | 0.40 | 6.3 | 0.41 | 4.2 | 0.47 | 4.3 | 0.39 |
| Mullett | Cheboygan | 160050 | yes | 1.1 | 0.27 | 0.0 | 0.22 | 2.6 | 0.13 | 2.1 | 0.07 | 1.5 | 0.17 |
| Nepessing | Lapeer | 440094 | yes | 2.6 | 0.12 | 1.5 | 0.35 | 17.3 | 1.17 | 9.3 | 0.47 | 7.7 | 0.53 |
| Orion | Oakland | 630555 | yes | 3.0 | 0.26 | 1.3 | 0.04 | 1.3 | 0.33 | 11.3 | 0.04 | 4.2 | 0.17 |

APPENDIX D. Harmful Algae Monitoring, 2006, Data Summary
 Shoreline (surface water) samples

| Lake | County | ID# | <i>Dreissena</i> status | north Chl µg/L | north Toxin µg/L | east Chl µg/L | east Toxin µg/L | south Chl µg/L | south Toxin µg/L | west Chl µg/L | west Toxin µg/L | mean Chl µg/L | mean Toxin µg/L |
|-------------|----------------|--------|----------------------------|----------------------|------------------------|---------------------|-----------------------|----------------------|------------------------|---------------------|-----------------------|---------------------|-----------------------|
| Parke | Oakland | 631119 | yes | 9.4 | 0.08 | 6.9 | 0.09 | 7.1 | 0.08 | 7.8 | 0.07 | 7.8 | 0.08 |
| Robinson | Newaygo | 620061 | yes | 9.0 | 0.06 | 8.6 | 0.07 | 17.7 | 0.18 | 9.6 | 0.07 | 11.2 | 0.09 |
| Round | Mecosta | 540073 | yes | 5.1 | 0.05 | 4.1 | 0.05 | 8.0 | 0.05 | 5.2 | 0.04 | 5.6 | 0.05 |
| Silver | Grand Traverse | 280116 | yes | 3.5 | 0.08 | 3.6 | 0.09 | 1.9 | 0.21 | 3.4 | 0.07 | 3.1 | 0.11 |
| Stony Lake | Oceana | 640049 | yes | 5.4 | 0.04 | 2.3 | 0.03 | 17.5 | 0.04 | 13.9 | 0.03 | 9.8 | 0.04 |
| Torch North | Antrium | 50055 | yes | 0.5 | 0.05 | | | | | 0.5 | 0.04 | 0.5 | 0.04 |
| Torch South | Antrium | 50240 | yes | | | 0.7 | 0.04 | 0.8 | 0.04 | | | 0.7 | 0.04 |
| Van Etten | Iosco | 350074 | yes | 22.8 | 0.08 | 63.3 | 0.06 | 9.2 | 0.06 | 11.2 | 0.15 | 26.6 | 0.08 |
| Vineyard | Jackson | 380263 | yes | 1.9 | 0.04 | 3.4 | 0.07 | 3.0 | 0.05 | 3.1 | 0.04 | 2.9 | 0.05 |

* Analytical toxin results are increased by a factor of 1.23 to adjust for sample preparation.

APPENDIX E. Harmful Algae Monitoring, 2006, Data Summary
Euphotic zone samples

| Lake | County | ID# | Surface area km ² | Max. depth m | <i>Dreissena</i> status | Chl µg L ⁻¹ | Toxin µg L ⁻¹ | TP µg L ⁻¹ |
|------------|------------|--------|---------------------------------|-----------------|----------------------------|---------------------------|-----------------------------|--------------------------|
| Baldwin | Montcalm | 590171 | | 10.7 | no | 8.3 | 0.07 | 16 |
| Bear | Kalkaska | 400026 | 1.2788 | 18.0 | no | 1.6 | 0.04 | 6 |
| Big Star | Lake | 430022 | 3.6908 | 7.6 | no | 2.8 | 0.08 | 9 |
| Cowan | Kent | 410550 | | 16.2 | no | 10.0 | 0.08 | 20 |
| Cowboy | Dickenson | 220128 | | 7.6 | no | | 0.14 | 12 |
| Crockery | Ottawa | 700422 | 0.4371 | 16.5 | no | 17.0 | 0.05 | 19 |
| Crooked | Kalamazoo | 390599 | | | no | 3.4 | 0.07 | 9 |
| Crystal | Oceana | 640062 | 0.3076 | 11.3 | no | 7.2 | 0.05 | 12 |
| Crystal | Dickenson | | | 2.4 | no | | 0.95 | 103 |
| Cub | Kalkaska | 400031 | 0.2145 | 7.0 | no | 2.0 | 0.17 | 7 |
| Deer | Alger | 20126 | 1.0765 | 18.3 | no | 5.4 | 0.03 | 10 |
| Earl | Livingston | 470554 | | 6.7 | no | 7.0 | 0.34 | 18 |
| Freska | Kent | 410702 | 0.2509 | 7.6 | no | 5.1 | 0.09 | 11 |
| George | Clare | 180056 | 0.5423 | 7.6 | no | 4.2 | 0.05 | 13 |
| Goshorn | Allegan | 30650 | 0.0900 | 18.0 | no | 15.5 | 0.40 | 31 |
| Hess | Newaygo | 620032 | 3.0554 | 8.5 | no | 10.0 | 0.54 | 33 |
| Hicks | Osceola | | 0.6273 | 10.1 | no | 11.5 | 0.23 | 20 |
| Indian | Osceola | 670227 | 0.3440 | 15.2 | no | 4.9 | 0.18 | 9 |
| Jewell | Alcona | 10041 | 0.7815 | 10.4 | no | 3.8 | 0.19 | 16 |
| Kimball | Newaygo | | 0.6192 | 15.8 | no | 7.4 | 0.06 | 22 |
| Little | Marquette | 520210 | | 15.2 | no | 2.9 | 0.05 | 10 |
| Mehl | Marquette | 520451 | 0.3683 | 6.1 | no | 3.1 | 0.08 | 8 |
| Moon | Gorebic | 270120 | 0.3764 | 12.2 | no | 3.5 | 0.04 | 11 |
| Murray | Kent | 410268 | 1.2950 | 21.9 | no | 4.0 | 0.24 | 14 |
| Osterhout | Allegan | 30263 | 0.6799 | 9.1 | no | 2.5 | 0.10 | 16 |
| Pickerel | Kalkaska | 400035 | 0.4047 | 21.9 | no | | 0.04 | 3 |
| Round | Clinton | 190146 | 0.3440 | 7.3 | no | 15.0 | 0.12 | 15 |
| Shafer | Van Buren | | 0.3278 | 21.0 | no | | | 15 |
| Shingle | Clare | 180108 | 0.1416 | 12.2 | no | 5.8 | 0.04 | 17 |
| Swezey | Jackson | 380470 | 0.4249 | 7.0 | no | 1.6 | 0.05 | 10 |
| Upper Long | Oakland | 631118 | | | no | | 0.69 | 18 |
| Viking | Otsego | 690136 | 0.1611 | 7.6 | no | 21.1 | 0.08 | 25 |
| Webinguaw | Newaygo | 620283 | 0.2469 | 4.9 | no | 1.4 | 0.12 | 19 |
| Wells | Osceola | 670121 | 0.1943 | 25.0 | no | 1.6 | 0.06 | 8 |

APPENDIX E. Harmful Algae Monitoring, 2006, Data Summary
Euphotic zone samples

| Lake | County | ID# | Surface area km ² | Max. depth m | <i>Dreissena</i> status | Chl µg L ⁻¹ | Toxin µg L ⁻¹ | TP µg L ⁻¹ |
|---------------|----------------|--------|---------------------------------|-----------------|----------------------------|---------------------------|-----------------------------|--------------------------|
| Ann | Benzie | 100082 | 2.1327 | 21.3 | yes | 1.6 | 0.10 | 5 |
| Antoine | Dickenson | 220028 | 3.0271 | 7.6 | yes | | 0.16 | 12 |
| Barlow | Barry | 80176 | | 19.2 | yes | 3.8 | 0.19 | 10 |
| Beaver | Alpena | 40097 | 2.6912 | 23.5 | yes | 2.0 | 0.15 | 7 |
| Bellaire | Antrium | 50052 | 7.1833 | 29.0 | yes | 2.0 | 0.12 | 4 |
| Big Fisher | Leelanau | 450224 | | 4.9 | yes | | 0.03 | 4 |
| Big Glen | Leelanau | 450049 | 19.6884 | 39.6 | yes | | 0.02 | 3 |
| Big | Osceola | 670056 | 0.8256 | 25.9 | yes | 1.6 | 0.38 | 13 |
| Bills | Newaygo | 620062 | 0.8256 | 27.0 | yes | 2.4 | 8.37 | 9 |
| Birch | Cass | 140061 | 1.1938 | 29.0 | yes | 2.1 | 0.14 | 10 |
| Brooks | Leelanau | 450222 | | | yes | 19.5 | 0.03 | 12 |
| Cedar | Alcona/Iosco | 10017 | 4.3505 | 3.0 | yes | 2.4 | 0.12 | 11 |
| Clam | Antrium | 50101 | 1.6997 | 8.8 | yes | 2.1 | 0.26 | 7 |
| Clark | Jackson | 380173 | 2.2663 | 16.8 | yes | 1.5 | 0.13 | 9 |
| Deer | Oakland | 630708 | 0.5544 | 19.2 | yes | 1.3 | 0.11 | 8 |
| Derby | Montcalm | 590144 | 0.4775 | 26.5 | yes | 1.2 | 0.14 | 7 |
| Diamond | Cass | 140039 | 4.1279 | 19.5 | yes | 2.7 | 0.25 | 8 |
| Evans | Lenawee | 460309 | 0.8134 | 12.8 | yes | 6.6 | 0.08 | 13 |
| Fisher | St. Joseph | 750139 | 1.3234 | 12.8 | yes | 2.1 | 0.34 | 8 |
| Gilletts | Jackson | | | 9.1 | yes | 2.4 | 0.13 | 17 |
| Gourdneck | Kalamazoo | 390541 | 0.8984 | 15.8 | yes | 4.0 | 0.08 | 13 |
| Hamlin- Lower | Mason | 530073 | 20.1943 | 24.4 | yes | 2.9 | 0.15 | 21 |
| Hamlin- Upper | Mason | 530074 | 20.1943 | 11.3 | yes | 7.3 | 0.23 | 26 |
| Houghton | Roscommon | 720163 | 81.1170 | 6.1 | yes | 4.4 | 0.21 | 23 |
| Hubbard | Alcona | 10020 | 35.8155 | 22.9 | yes | 0.5 | 0.20 | 8 |
| Klinger | St. Joseph | 750136 | 3.3590 | 21.9 | yes | 4.9 | 0.16 | 9 |
| Lakeville | Oakland | 630670 | 1.8616 | 20.1 | yes | 3.7 | 0.14 | 14 |
| Little Fisher | Leelanau | 340223 | 5.6657 | 4.0 | yes | | 0.03 | 4 |
| Little Glen | Leelanau | 450050 | 1.8130 | 4.0 | yes | | 0.53 | 6 |
| Magician | Cass | 140065 | 2.3100 | 17.0 | yes | 14.6 | | 14 |
| Margrethe | Crawford | 200036 | 7.7701 | 19.8 | yes | 3.8 | 0.34 | 7 |
| Mullett | Cheboygan | 160050 | 70.2550 | 36.6 | yes | 0.5 | 0.08 | 5 |
| Nepessing | Lapeer | 440094 | 1.6754 | 7.6 | yes | 2.7 | 0.68 | 22 |
| Orion | Oakland | 630555 | 1.9021 | | yes | 0.5 | 0.03 | 15 |
| Parke | Oakland | 631119 | 0.0931 | 15.2 | yes | 2.3 | 0.06 | 17 |
| Robinson | Newaygo | 620061 | 0.5544 | 9.1 | yes | 17.0 | 0.07 | 16 |
| Round | Mecosta | 540073 | 0.6273 | 13.7 | yes | 8.4 | 0.04 | 18 |
| Silver | Grand Traverse | 280116 | 2.4282 | 29.9 | yes | 1.7 | 0.10 | 4 |
| Stony Lake | Oceana | 640049 | 1.1251 | 12.5 | yes | 9.3 | 0.03 | 15 |
| Torch North | Antrium | 50055 | 35.4917 | 76.2 | yes | | 0.04 | 2 |
| Torch South | Antrium | 50240 | 35.4917 | 86.9 | yes | 0.5 | 0.04 | 1 |
| Van Etten | Iosco | 350074 | 5.3420 | 10.1 | yes | 31.0 | 0.07 | 36 |
| Vineyard | Jackson | 380263 | 2.0437 | 12.8 | yes | 2.3 | 0.05 | 11 |