Assessment of Cyanobacteria and Associated Toxins in West Michigan Lakes.

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MDEQ
SARAH LESAGE, MDEQ
PROJECT MANAGER

LAKE SUPERIOR STATE UNIVERSITY
JUDY WESTRICK, CHEMIST

AWRI
RICHARD R. REDISKE, PH.D., PROJECT MANAGER
JANEL HAGAR, GRADUATE STUDENT
YING HONG, AQUATIC BIOLOGIST
JIM O'KEEFE, CHEMIST
ALAN STEINMAN, PH.D., LIMNOLOGIST

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Table of Contents

List of Tables

List of Figures

Executive Summary

Cyanobacteria populations and their associated toxins were investigated in seven drowned river mouth lakes in west Michigan during the summer of 2006. A gradient of low mesotrophic to hypereutrophic systems were examined to determine if concentrations of cyanotoxins exceeded the World Health Organization (WHO) guidelines and to evaluate the performance of three analytical methods. Bear Lake, Spring Lake, Mona Lake, and Lake Macatawa are hypereutrophic systems with extensive histories of cyanobacteria blooms. Muskegon Lake, White Lake, and Duck Lake are mesotrophic/eutrophic systems with increasing reports of algal bloom corresponding to the invasion of zebra mussels. These seven lakes are connected either directly or indirectly to Lake Michigan, and used extensively for boating, skiing, fishing, and swimming.

Six of the seven lakes were found to have summer cyanobactera blooms and contained low levels of cyanotoxins throughout July and August (2006). Duck Lake, a mesotrophic system, had no samples with microcystin LR above the detection limit (0.001 µg/l). None of the lakes had microcystin LR concentrations above the WHO recreational water guideline of 20 µg/l and only two of the seven lakes had concentrations > 1 µg/l. A summary of the data is shown below:

Microcystin LR by HPLC/MS	Bear Lake	Duck Lake	Lake Macatawa	Mona Lake	Muskegon Lake	Spring Lake	White Lake
# Analyzed	29	29	30	28	31	28	28
> 0.01 µg/l	29	0	30	24	31	28	20
> 0.1 µg/l	29	0	20	0	21	0	7
> 1 µg/l	7	0	0	0	3	0	0
>20 µg/l	0	0	0	0	0	0	0

The WHO secondary guidelines for chlorophyll-*a* and cyanobacteria cell counts were found to be unreliable indicators of cyanotoxin concentrations as 60% of the samples exceeded the cyanobacteria cell counts guideline of > 100,000/ ml and 27% exceeded the chlorophyll-*a* guideline of 50 µg/l. Diverse populations of cyanobacteria were found in each lake and seasonal changes in species and abundance were observed. A significant difference in cyanotoxin levels was not observed between beach and open water samples.

Three methods were used to measure cyanotoxins in the investigation. Enzyme-linked immunosorbent assay (ELISA), protein phosphatase inhibition assay (PPIA), and high performance liquid chromatography with mass spectrometry (HPLC/MS) were used to evaluate microcystins. In addition, HPLC/MS was used to measure anatoxin-a and cylindrospermopsin. The latter two toxins were not detected in the 2006 samples. PPIA is an inexpensive screening method ($20-$30/test) that measures total microcystin activity and all congeners respond in a 1:1 ratio. ELISA also is an inexpensive screening tool ($10-$20/test) that is more focused on microcystin LR. Other congeners can cross react with the method and consequently, the data cannot be used as an absolute indicator of LR concentration. HPLC/MS is a more accurate analytical method that identifies individual microcystin congeners based on retention time and molecular weight. While HPLC/MS has clear advantages with respect to accuracy and sensitivity, it requires expensive

analytical equipment ($150,000) and only a few microcystin standards are commercially available. PPIA and ELISA were found to significantly overestimate the concentration of microcystin LR in most of the samples. Muskegon Lake was an exception as it contained mostly microcystin LR and the three methods yielded relatively similar results. ELISA and PPIA results were similar for three lakes, however Bear Lake and Lake Macatawa had PPIA concentrations significantly greater than ELISA. These data suggest that the congener composition for each lake is unique and related to community composition and/or limnological characteristics of each lake. Only one sample from a cyanobacteria bloom in Bear Lake had PPIA results of >20 µg/l.

The diversity of cyanobacteria communities and analytical results suggests that no single analytical method can be used to assess cyanotoxin levels. In Muskegon Lake, conditions in 2006 showed that the three methods produced comparable results. Based on the variability of cyanobacteria communities observed in the other lakes, this relationship needs to be evaluated with several years of data to determine if it is consistent over time. Data from the other lakes suggest that a combination of HPLC/MS and a broad screening method such as PPIA may be necessary to accurately measure LR concentrations and evaluate the total amount of cyanotoxins present. Since PPIA and ELISA consistently overpredicted the level of microcystin LR, these methods appear to be good conservative screening tools for the cost effective evaluation of large numbers of samples. If levels of cyanotoxins above 20 µg/l are measured by PPIA or ELISA, analysis by HPLC/MS is recommended to provide an accurate determination of the microcystin LR concentration.

All of the lakes studied in 2006 had cyanotoxin levels below the WHO guidelines for microcystin LR. Although toxin producing organisms were present in most of lakes at cell counts above the WHO guideline, the recreational value of the water was not impacted by elevated cyanotoxin concentrations. Since cyanobacteria blooms and toxin production are influenced by a variety of local and regional factors, the use of single year of data may not be representative of future conditions.

1.0 Introduction

Cyanobacterial blooms are occurring more frequently as a result of an increasing trend in cultural eutrophication (Briand et al. 2003; Chorus et al. 2000; Jacoby 2003) and the introduction of exotic species (Juhel et al. 2006; Vanderploeg et al. 2001). Increased cultural eutrophication can be attributed to agricultural and urban run-off, sewage discharges, stormwater, groundwater contamination from septic systems, and atmospheric loading of nutrients (Paerl 1998; Pitois et al. 2001). Excess nutrients can contribute to a proliferation of cyanobacteria species and the formation of blooms (Bennett 2002; Briand et al. 2003). Cyanobacterial blooms impact freshwater environments by reducing oxygen concentrations, increasing turbidity, and disrupting the food web (Bennett 2002; WHO 2003). They also are capable of causing aesthetic problems and producing potent cyanotoxins, making freshwater unfit for consumption by wildlife and livestock, and undesirable for most human uses including drinking and recreation (Bennett 2002; Johnston and Jacoby 2003; WHO 1999). In addition, the invasive species, *Dreissena polymorpha* (zebra mussel) can influence the abundance of cyanobacteria due to its selective feeding. Studies have shown that zebra mussels will selectively reject *Microcystis*, which is then embedded in mucus and discharged in a more nutrient enriched form in the zebra mussel's pseudofeces. This selective feeding process can result in a disproportionate amount of toxin producing cyanobacteria in the water (GRERL 2002; Juhel et al. 2006; Vanderploeg et al. 2001).

Three common toxins that are produced in freshwater lakes are: anatoxin-a, cylindrospermopsin, and microcystin (Chorus et al. 2000; Leflaive 2007; Zurawell et al. 2005). Anatoxin-a is a neurotoxin and has the ability to bind to neural nicotinic acetylcholine receptors and cause depolarization of nerve cells, blocking further depolarization. This can result in paralysis, asphyxiation, and death (Briand et al. 2003; Carmichael 1992; Zurawell et al. 2005). Cylindrospermopsin acts as both a hepatotoxin and a cytotoxin. This toxin is discharged from the cell and extracellular concentrations are often higher than intracellular concentrations, increasing toxin exposure (Griffiths amd Saker 2003). Cylindrospermopsin has the ability to inhibit protein synthesis largely affecting the liver and kidney but could possibly affect all tissues that rapidly synthesize protein (Briand et al. 2003, Falconer 1999). Microcystin is the most common algal toxin in temperate freshwater systems and acts as a highly toxic hepatotoxin (Chorus et al. 2000). Exposure to hepatotoxins can cause nausea, vomiting, acute liver failure, and the growth of liver and other tumors. Microcystin is the cyanotoxin most often cited as the cause of human and livestock poisoning related to cyanobacteria blooms. It has the ability to be transported across the ileum where it can enter the blood stream via the bile acid transporter. It can then enter hepatocytes in the liver again via the bile acid transporter and cause disruption in liver cell structure by protein phosphatase 1 and 2a inhibition (Bogialli 2005; Briand et al. 2003, Carmichael 1992; Dittman et al. 2001; Zurawell et al. 2005). Humans and animals can be exposed to these toxins during recreational activities via inhalation or accidental ingestion or by dermal contact with toxins while wading, swimming, skiing and canoeing (USEPA 2001; WDHHS 2004; WHO 1999, WHO 2003). Because of health risks, the World Health Organization (WHO) has established a recommended guideline for recreational water exposure to microcystin LR at 20 μg/l or less (Table 1.1; WHO 1999).

TABLE 1.1. WORLD HEALTH ORGANIZATION GUIDELINES FOR RECREATIONAL WATER EXPOSURE TO CYANOBACTERIA AND MICROCYSTIN LR.

Advisory Level	Microsystin LR	Chlorophyll-*a*	Cyanobacteria Density
Moderate	20 µg/l	50 µg/l	100,000/ml
High	1,000 µg/l	100 µg/l	10,000,000/ml

Presently, there are no known physiological or biochemical functions for cyanotoxins and factors contributing to the formation of toxins are under investigation (Chorus et al. 2000: Kaebernick 2000). Environmental conditions such as nutrient enrichment, light, temperature, essential metal availability, and the activity of selective grazers are all thought to initiate bloom growth and toxin production (Long et al. 2001, Paerl 1988). Understanding how environmental factors influence the production of microcystins has confronted scientists for nearly 40 years and the results among the many reports have been highly variable (Orr and Jones 1998). Until recently, it had been assumed that cyanotoxins were produced as secondary metabolites (Carmichael 1992; Dittmann et al. 2001) and not linked to growth, development, or reproduction. However, their production has been correlated with growth and larger quantities are often produced during cyanobacterial blooms (Long et al. 2001; Orr and Jones 1998; Rogers et al. 2005; Rolland et al. 2005). This may provide a possible explanation as to why many environmental and physiological factors may play a role in toxin production. In field and laboratory studies, environmental parameters such as warm water temperatures (21^{o} – 27^{o}C), elevated phosphorus, elevated nitrogen, low stoichiometric ratio of available nitrogen to phosphorus, and high pH (6-9), have been to shown to play a role in microcystin production (Crayton 1993; Johnston and Jacoby 2003; Oberholster et al. 2004; Paerl 1998; Rolland et al. 2005, Zurawell et al., 2005). Because cyanotoxins carry potential health hazards, it is important to know not only whether or not they are being produced, but also what might influence their production.

West Michigan contains a number of lakes that have extensive recreational use and histories of cyanobacteria blooms and the presence of zebra mussels. Very little data are available on the composition of cyanobacteria and toxin concentrations in these systems. Spring Lake, Mona Lake, and Lake Macatawa are hypereutrophic systems with long histories of cyanobacteria blooms. While *Microcystis* is usually the dominant genus, *Planktothrix sp. and Cylindrospermopsis sp.* have recently been found in Mona Lake (Hong et al. 2006). These genera are known to produce anatoxins and cylindrospermopsin, which are potent hepatotoxins and neurotoxins (Briand et al., 2003; Osswald et al., 2007). In addition, blooms were reported in 2004 on a number of lakes that had limited or no history of cyanobacteria problems (Duck Lake, White Lake, Muskegon Lake, and Blue Lake). During a bloom in September 2004, levels of microcystin LR ranging from 20 µg/l – 238 µg/l were reported in Muskegon Lake (G. Fahnenstiel, unpublished data).

Currently, a standard method for the measurement of cyanotoxins in environmental samples has not been proposed. Three methods that have been historically used to measure microcystin include the enzyme-linked immunosorbent assay (ELISA), the protein phosphatase inhibition assay (PPIA), and high performance liquid chromatography with mass spectrometry (HPLC/MS; Fastner et al. 2002). The ELISA method is typically used as a

screening tool. It is a biochemical assay that measures competitive binding to antibodies raised in animals against microcystin (commonly LR). This method provides rapid results at a low cost (McElhiney et al. 2005). However, a recent study has identified a number of potential interferences such as pH, salinity, adherence to plastic and false positives due to high methanol concentrations (Metcalf et al. 2000). Also, due to the format of the test, different microcystin analogues can bind with different affinities and cross-reactivity has been documented (Envirologix, 2005, McElhiney et al. 2005). As well, this method does not differentiate between analogues, and therefore does not offer evidence of toxicity (Metcalf et al. 2000). The PPIA method also is typically used as a screening tool and offers a somewhat sensitive and inexpensive way to determine total microcystin concentration as well as toxicity (bioactivity). It operates by measuring the inhibitory affect of a sample on the release of a phosphate from a non-specific substrate: para-nitrophenol phosphate (PNPP). All microcystin congeners react in a 1:1 ratio with the substrate so it provides an indication of total activity (Carmichael 1999). However, it is not specific for microcystin and can show cross reactivity with other protein phosphatase inhibitors that may be present in environmental samples (Carmichael 1999; McElhiney et al. 2005; Metcalf et al. 2000). HPLC/MS couples the ability of HPLC (high pressure liquid chromatography) to separate microcystins with the ability of MS (mass spectrometry) to identify a molecular weight "fingerprint" based on a known standard. This method provides the most sensitive detection and accurate identification of toxin analogues. However, it is technically challenging, expensive, and requires the availability of standards of which only a few are available commercially (McElhiney et al. 2005; Mathys et al. 2004). This method can also be used to measure anatoxin-a and cylindrospermopsin.

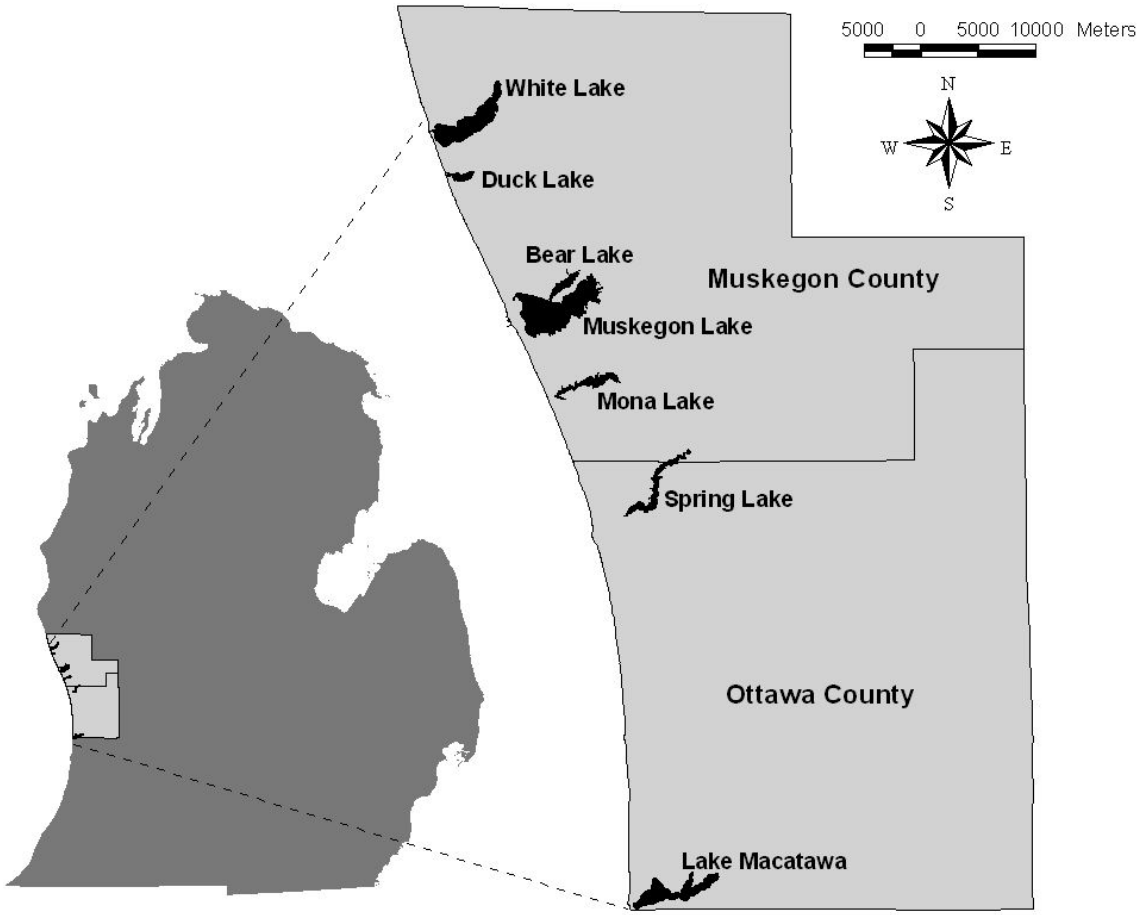
This study was used to determine if microcystin, anatoxin-a, and/or cylindrospermopsin were present in recreational water samples from a group of seven west Michigan lakes with heavy recreational use, using three different methods of analysis: ELISA, PPIA, and HPLC/MS. Cyanobacteria species and density, chlorophyll-*a*, and a suite of limnological parameters (pH, dissolved oxygen, temperature, turbidity, total and soluble reactive phosphorus, nitrate, ammonia, and total nitrogen) also were determined. The data collected as part of this project were used to determine the occurrence and potential significance of cyanobacteria and their associated toxins in these seven lakes. These data can be useful to state and local public health agencies that are involved in establishing standards for recreational exposure. In addition to providing data for public health assessment, this project utilized a methodology that can serve as a model for evaluating the nature and extent of cyanobacteria and their toxins in aquatic systems. This assessment is critical in the assessment of public health impacts and the development of water quality management programs to ameliorate their hazards.

1.1 Project Objectives and Task Elements

The main project goal was to develop information on the occurrence of cyanobacteria and their related toxins in seven west Michigan lakes with heavy recreational use and to determine how numbers and concentrations compared to the World Health Organization

(WHO) guidelines (WHO 1999). The locations of lakes investigated are shown in Figure 1.1. Bear Lake, Spring Lake, Mona Lake, and Lake Macatawa are upper eutrophic -

FIGURE 1.1. LAKES SELECTED FOR CYANOBACTERIA MONITORING (2006).



hypereutrophic systems with extensive histories of cyanobacteria blooms. Muskegon Lake, White Lake, and Duck Lake are mesotrophic/eutrophic systems with increasing algal bloom reports corresponding to the invasion of zebra mussels. These seven lakes are drowned river mouths, connecting either directly or indirectly to Lake Michigan, and used extensively for boating, skiing, fishing, and swimming.

This project assessed the significance of cyanobacteria blooms by three types of assessments:

- Cyanobacteria species composition, abundance, and biovolume
- Chlorophyll-*a*
- Cyanobacteria toxin measurement

Since cyanobacteria produce a variety of toxins, it was necessary to determine the composition, abundance, and biovolume of taxa present in each lake. Based on these values and chlorophyll-*a* concentrations, the data were analyzed to determine if correlations existed between these parameters and toxin concentrations. Three analytical methods were used for the measurement of algal toxins:

- ELISA was used as a screening tool to identify samples that contained microcystin. ELISA is an inexpensive and rapid test method that provides qualitative information related to the presence of the microcystin LR equivalents and concentration ranges.
- PPIA was used as a second microcystin screening method. PPIA is an inexpensive and rapid test that provides qualitative information on the presence of the microcystin LR equivalents related to the bioactivity of the microcystin toxin.
- HPLC/MS was used to confirm microcystin values determined by both ELISA and PPIA.
- HPLC/MS also was used to determine the concentration of anatoxin-a and cylindrospermopsin.

This approach provided a high level of quality assurance and provided data that can be used for decision making and public health assessment. Additional information related to the analytical methods is provided in Section 2. Project tasks are described below.

Task 1: Sampling of selected locations for cyanobacteria.

- AWRI sampled three open water sites and one public bathing beach on each lake. The beach was sampled at the three locations used by the MDEQ for *E. coli* monitoring. Integrated 1-meter water samples were collected at each location (Sutherland et al., 1992). In addition, one of the locations was collected in duplicate. Each lake was sampled at two-week intervals during July and August 2006 (four sampling events; 196 samples total). AWRI also established a system of contacts with designated lake association and riparian owners and were updated on a weekly basis about the occurrence of cyanobacteria blooms. If an algal bloom was reported, surface scum and/or the integrated water samples were collected. Samples for cyanobacteria analysis were stored on ice in the field in 1 L plastic opaque bottles. All samples were returned to the AWRI laboratory on a daily basis for further processing and storage. In the laboratory, samples for phytoplankton identification were preserved with Lugol's solution and unpreserved samples were filtered in triplicate for toxin analysis and stored at -20^{o}C. Samples for chlorophyll-*a* were filtered in the laboratory and stored in foil covered plastic centrifuge tubes at -20°C.

Task 2: Analysis of cyanobacteria species, water quality parameters, and chlorophyll-*a*.

- All samples were analyzed for cyanobacteria species and numbers, chlorophyll-*a*, and water quality parameters. Cyanobacteria species were identified and enumerated using an inverted microscope and counting chamber (USEPA, 1997a). Biovolume estimates were made according to WHO (1999) guidelines.

Chlorophyll-*a* was measured by spectrophotometric methods (USEPA, 1997b). Analytical methods are described in Section 2.

Task 3: Analysis of Cyanobacteria Toxins

- All samples were analyzed for cyanobacteria toxins by ELISA, PPI, and HPLC/MS. Methods are summarized below and described in more detail in Section 2.
  - ELISA analyses were conducted according to methods outlined by Fastner et al. (1998). Samples were lyophilized and then sonicated in 75% aqueous methanol. Microcystin LR equivalents were measured ELISA kits (Envirologix; Portland, Maine).
  - PPIA analyses were conducted according to methods outlined by Carmichael (1999). PPIA utilized the same extracts prepared for ELISA analysis. The rate of phosphate hydrolysis was calculated from the change in absorbance at 405 nm over 1 hour and compared to the control.
  - The concentrations of microcystin LR, RR, YR, LA, LW, and LF, cylindrospermopsin and anatoxin-a were determined by liquid chromatography-tandem mass spectrometry (Li et al. 2006). Nodularin was added to extracts and used as an internal standard.

2.0 Methods

2.1 Field Methods

Three open water sites and one public bathing beach were sampled on each lake (Figures 2.1-2.6). The beach was sampled at the three locations used by the MDEQ for *E. coli* monitoring. GPS coordinates were taken at each station during the initial sampling survey and used as reference points for subsequent events (Table 2.1). Each lake was sampled at two-week intervals during July and August 2006 (four sampling events; 196 samples total). Integrated epilimnetic (1 m) water samples (Sutherland et al., 1992) were collected for the analysis of chlorophyll-*a*, microcystins, anatoxin-a, cylindrospermopsin, and phytoplankton identification. A 1.5 m polycarbonate tube (10 cm O.D.) was lowered to a 1 m depth was used to collect the integrated samples. Before the tube was pulled from the water, the bottom was sealed with a rubber stopper. One to several integrated water samples were collected and pooled to provide a 2 liter composite sample for the analyses described in Section 2. Aliquots of the composite were transferred into two one-liter amber plastic bottles, and stored on ice. The samples were returned to the laboratory after each sampling event for processing. A Hydrolab DataSonde 4a was used in the field to determine pH, dissolved oxygen, and at each station.

TABLE 2.1. GPS COORDINATES FOR LAKE SAMPLING LOCATIONS.

	Open Water Site #1	Open Water Site #2	Open Water Site #3	Beach Sample Site #1	Beach Sample Site #2	Beach Sample Site #3
Muskegon Lake	N 43.25108	N 43.23865	N 43.23094	N 43.23077	N 43.23040	N 43.23004
Site Coordinates	W 086.26005	W 086.27346	W 086.31462	W 086.32610	W 086.32593	W 086.32560
Bear Lake	N 43.25993	N 43.24965	N 43.24374	N 43.26109	N 43.26091	N 43.26081
Site Coordinates	W 086.27588	W 086.29173	W 086.29616	W 086.27697	W 086.27701	W 086.27704
Duck Lake	N 43.34169	N 43.33989	N 43.34159	N 43.34275	N 43.34272	N 43.34268
Site Coordinates	W 086.38412	W 086.39693	W 086.40508	W 086.39700	W 086.39738	W 086.39767
White Lake	N 43.39896	N 43.38449	N 43.37621	N 43.40112	N 43.40108	N 43.40114
Site Coordinates	W 086.35702	W 086.37868	W 086.399540	W 086.35868	W 086.35920	W 086.35981
Mona Lake	N 43.18395	N 43.18028	N 43.17373	N 43.17580	N 43.17599	N 43.17638
Site Coordinates	W 086.23070	W 086.24585	W 086.28030	W 086.24736	W 086.24623	W 086.24554
Spring Lake	N 43.09098	N 43.08750	N 43.08404	N 43.08129	N 43.08131	N 43.08140
Site Coordinates	W 086.17135	W 086.18502	W 086.20164	W 086.18613	W 086.18604	W 086.18582
Lake Macatawa	N 42.79431	N 42.78302	N 42.77980	N 42.77870	N 42.77862	N 42.77842
Site Coordinates	W 086.12024	W 086.15098	W 086.17945	W 086.19807	W 086.19813	W 086.19820

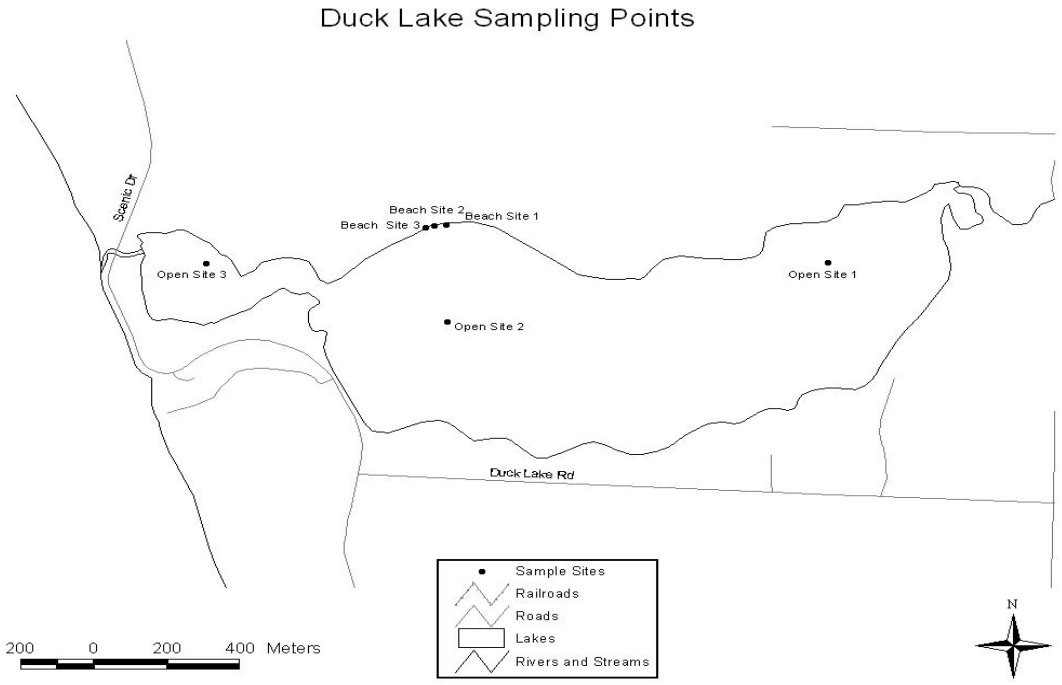


FIGURE 2.1. DUCK LAKE SAMPLING LOCATIONS (2006).

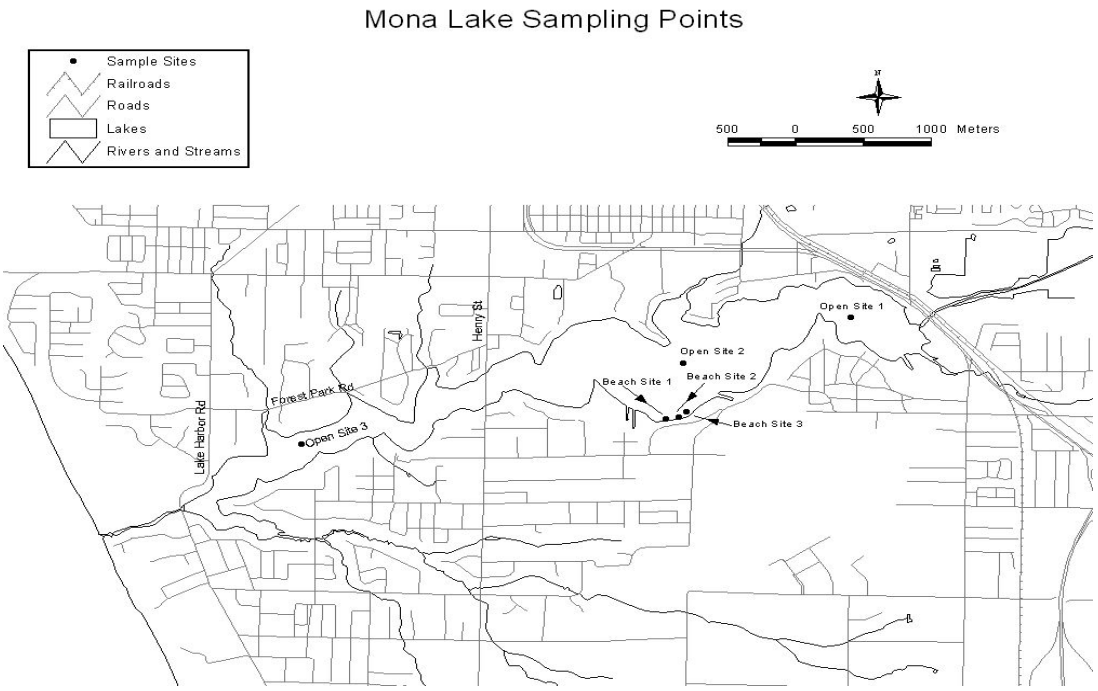


FIGURE 2.2. MONA LAKE SAMPLING LOCATIONS (2006).

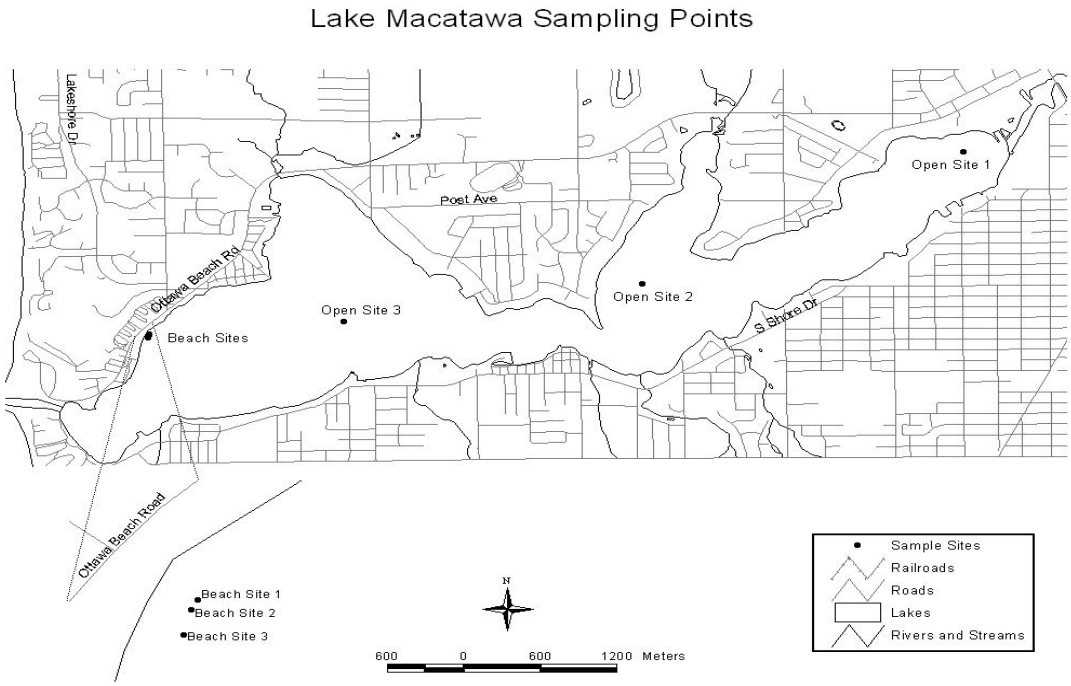


FIGURE 2.3. LAKE MACATAWA SAMPLING LOCATIONS (2006).

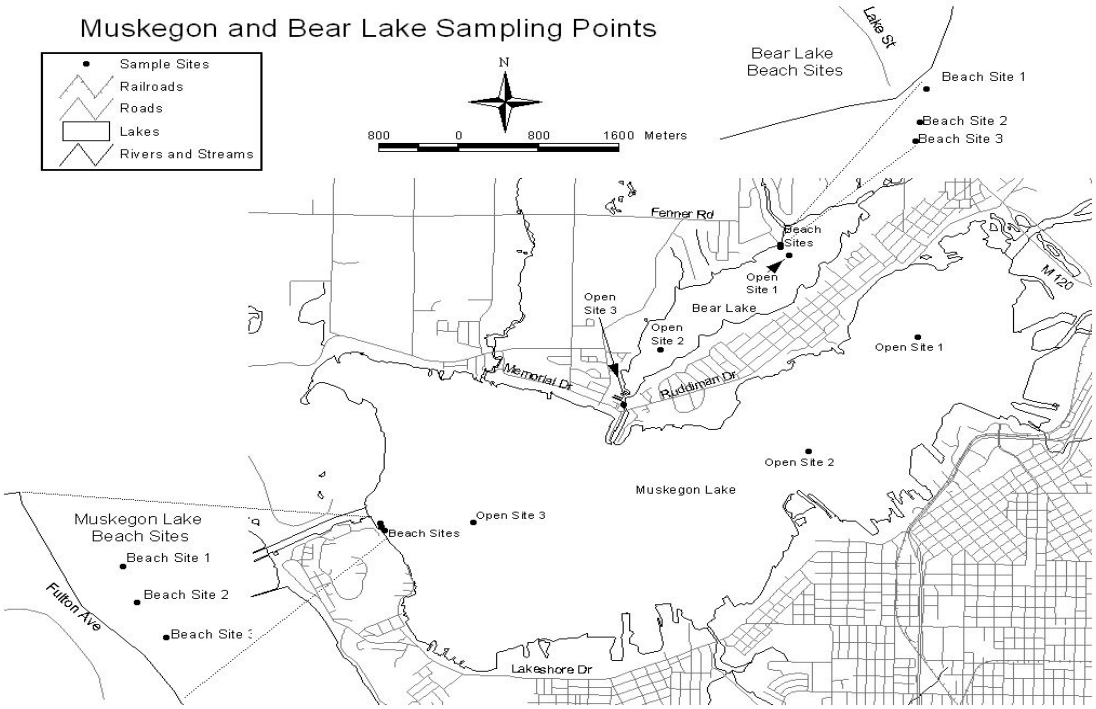


FIGURE 2.4. MUSKEGON LAKE AND BEAR LAKE SAMPLING LOCATIONS (2006).

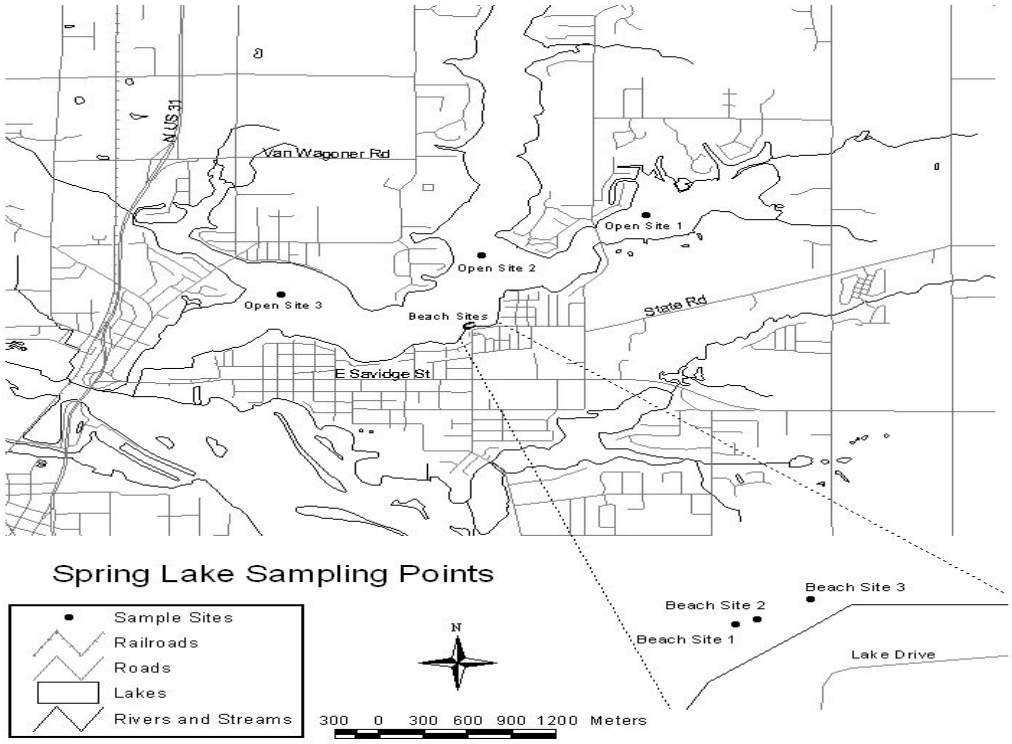


FIGURE 2.5. SPRING LAKE SAMPLING LOCATIONS (2006).

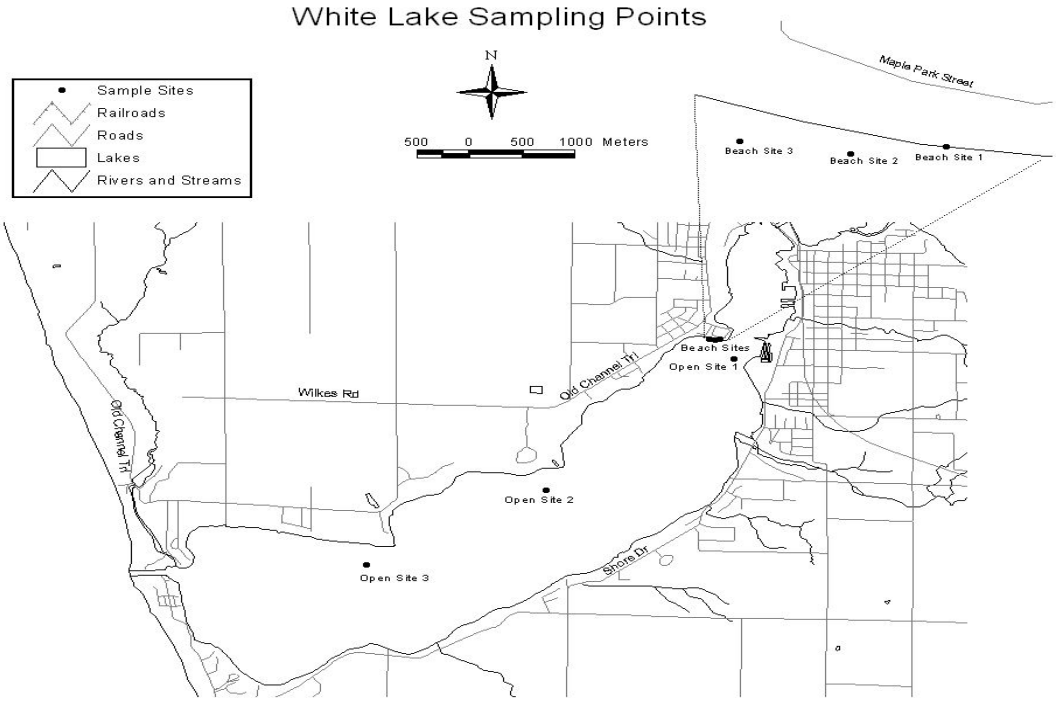


FIGURE 2.6. WHITE LAKE SAMPLING LOCATIONS (2006).

2.2 Analytical Methods

A summary of analytical methods and detection limits is provided in Table 2.2. Instrument conditions and a summary of quality assurance procedures are provided in the following sections.

TABLE 2.2. ANALYTICAL METHODS AND DETECTION LIMITS.

Parameter	Preparation	Description	Methods Reference
Phytoplankton Identification	Settling Chamber Lugol's Solution	Light Microscopy	USEPA (1997b)
Soluble Reactive Phosphorus	0.45 µm filter in field	Automated ascorbic acid	4500-P F*
Total Phosphorus	Persulfate digestion	Automated ascorbic acid	4500-P B.5 and F*
Ammonia	-	Automated phenate	4500-NH_3 H*
Total Kjeldahl Nitrogen	Digestion	Automated phenate	4500-N_{ORG} B*
Nitrate, Chloride, Sulfate	0.45 µm filter	Ion Chromatography	4100 C*
Turbidity	-	Nephelometric	2130-B
Chlorophyll-*a*	GF filter in field	Spectrophotometric	USEPA (1997a)
Microcystin by ELISA	Filtration, 75% methanol extraction	Colorimetric	Envirologix**
Microcystin LR by PPIA	Filtration, 75% methanol extraction	Colorimetric	Carmichael (1999)
Microcystin LR, RR, YR, LA, LW, and LF, cylindrospermopsin and anatoxin-a	Filtration, 75% methanol extraction	High Pressure Liquid Chromatography/Mass Spectrometry	Li et al. (2006)

* Standard Methods (APHA 1999).
** Envirologix Portland Maine. 96-Well-QuantiPlate Test Kit

2.2.1. Chlorophyll-*a*

Chlorophyll-*a* was analyzed by modifications to spectrophotometric methods (USEPA, 1997a). A sample aliquot (10 – 100 ml) was vacuum-filtered through a 0.45 µm membrane

filter (Millipore) and placed in foil wrapped centrifuge tubes, and frozen. For analysis, 7 ml of aqueous acetone solution was added to each centrifuge tube. Aqueous acetone solution was comprised of 90% acetone (reagent grade) and 10% magnesium carbonate solution (1.0 g finely powered $MgCO_3$ dissolved in 100 ml DI H_2O). Samples were then sonicated for 20 seconds on ice and 3 mL of aqueous acetone was added for rinsing. Samples were steeped overnight in the dark at 4°C. The sample was then centrifuged (500 rpm) for 10 minutes and the supernatant transferred to a near-UV cuvette (5 cm path length) and read at 750, 663, 645, and 630 nm using a Shimadzu UV-1601 spectrophotometer with a 2 nm bandwidth. The sample was then acidified with one drop of 0.1 N HCl and read again to determine the absorbance at 665 nm and 750 nm for the Pheophytin *a* correction. Chlorophyll-*a* concentrations were determined using the trichromatic chlorophyll equation with Pheophytin *a* correction.

2.2.2 Sample Preparation for Microcystin and Algal Toxin Analysis

Water samples for the analyses of microcystin were prepared according to modifications to a method previously described (Fastner et al. 1998). A 50-200 mL aliquot from each water sample was filtered on a 25 mm Whatman GF/F glass microfiber filter (Fisher Scientific cat # 09-874-64) in triplicate. Each filter was folded and placed in separate 2.0 mL plastic microfuge tubes and stored at 4°C. Prior to extraction, samples were lyophilized (Labconco, FREEZONE6) overnight. Filters were then placed in separate 15 mL glass vials. 3.0 mL of 75% aqueous methanol was added to each filter followed by water-bath sonication (Branson, Bransonic 5200) for 45 minutes. Samples were then centrifuged (Fisher Scientific, Marathon 3000) for 15 minutes at 3000 rpm. Supernatant (extract) was removed and transferred to a graduated glass centrifuge tube and stored at 4°C. To the remaining filter, 3.0 mL 75% aqueous methanol was added and frozen overnight. The following day, the filter was water-bath sonicated again for 45 minutes and centrifuged for 10 minutes at 3000 rpm. The supernatant was removed and pooled with stored extract. Extracts were then taken to dryness using a hot water bath and nitrogen gas. The extract was resuspended with 1 mL of methanol followed by 1 mL of water. Extracts were then divided into two parts for toxin analysis. One ml was used for HPLC/MS examination and one ml was used for ELISA and PPIA testing. Extracts were transferred to 2 mL HPLC vials and stored at 4°C.

2.2.3 Microcystin LR Equivalents by ELISA

ELISA analyses for microcystin were performed on all samples using commercially available 96-Well-QuantiPlate test kits (Envirologix) for microcystins as described in kit instructions (EnviroLogix Inc. Portland, ME). The test is a competitive Enzyme-Linked ImmunoSorbent Assay (ELISA) with a quantitation range of 0.16 to 2.5 parts per billion (ppb). All samples were diluted with pyrogen-free de-ionized water to 10x and 100x dilutions to decrease methanol concentrations and to ensure concentrations were within the range of detection for the ELISA test. All environmental samples, a negative control, and three calibrators (2.5 ppb, 0.6 ppb, and 0.16 ppb) were run in duplicate. A standard curve was created using the three calibrators against which all unknown samples were measured. Before beginning the assay, the 96-well-plates and all reagents were allowed to reach room temperature. 125 µl of

diluent was then added to each well using a multichannel pipette. Immediately after, 20 µl of each sample or standard was added to separate wells. The 96-well-plate was covered with parafilm and incubated (0.5 h, room temp.) on an orbital shaker (~200 rpm) to allow binding of microcystin in the samples and standards to bind to the microcystin-antibody coated in the wells. Following incubation, microcystin-enzyme conjugate (100 µl) was added to each well and incubated again (0.5 h, room temp.) on an orbital shaker (~200 rpm) to allow microcystin-enzyme conjugate binding to any free microcystin-antibody sites not occupied by the samples or microcystin standards. Well contents were emptied and the plates were rinsed (3X) with a saline wash solution to remove unbound material. 100 µl of the chromogen-substrate was added to each well before a final incubation (0.5 h, room temp.) to allow chromogen-substrate, a colored indicator, to bind to the microcystin enzyme-conjugate previously bound to the microcystin-antibody. The binding of chromagen substrate to the microcystin-enzyme conjugate produces a blue color in the wells. Finally, stop solution (100 µl of 1.0 N HCl) was added to each well to produce a yellow color. The OD of the 96-well-plate was read on an Awareness Technologies Plate Reader at a wavelength of 450 nm. Microcystin concentrations were calculated based on a semi-log standard curve. The 96-well-plate was also read at a dual wavelength of 630 nm as a reference to remove any interference from bubbles in the sample or scratches on the plastic.

2.2.4 Microcystin LR Equivalents by PPIA

PP1 activity was determined by measuring the amount of color production from the liberation of *p*-nitrophenol from *p*-nitrophenol phosphate. This assay was carried out in a 96-well microtitre plate (falcon cat # 3070) using a plate reader set to read a single wavelength, Λ = 404 nm) according to a modified PPIA protocol (Carmichael 1999) provided by Satchwell and Boyer (personal communication). The test was performed by adding 10 µL of 50% MeOH to blank and control wells, 10 µL of each microcystin standard, or 10 µL of each sample (in 50% aqueous MeOH) to each well followed by 90 µL of Solution D to blank wells or 90 µL of PP1 (New England Biolabs, cat # P0754L) solution (1:800) to standard, control, and unknown wells. Following a 5 minute pre-incubation at 37^{o}C, 100 µL of PNPP (Fisher Scientific, cat # ICN980701) substrate solution was added. The plates were then read for the initial reading (t=0). Samples were incubated for 60 minutes at 37^{o}C and color production was measured. Samples were then read again for the final reading (t=60). All environmental samples, a negative control, a positive control, and four calibrators (40 ppb, 20 ppb, 12 ppb, and 6 ppb) were run in duplicate. A standard curve was created using the % control activity vs. the concentrations of the four calibrators. The % control activity = V(standard) – V(blank) / V(control) – V(blank). V (reaction rate) = final abs - initial abs / assay time. Some samples were diluted with 50% MeOH to 10x and 100 x dilutions to ensure concentrations were within the range of detection for the PPIA test.

2.2.5 Microcystin LR, YR, RR, and LA by HPLC/MS

Analysis for microcystins was performed using a Thermo Surveyor MSQ Single Quadrupole Mass Selective Detector and Thermo Spectrasystem HPLC system according to a modified method described by Li et al. (2006). Arginine containing microcystin compounds LR, RR

and YR were analyzed on a Phenomenex Gemini 150mm column. Non-arginine containing Microcystin LA was analyzed on a Thermo ODS-2 Hypersil 150 mm column. A tertiary gradient program (Table 2.3) was employed for both arginine and non-arginine containing microcystins that consisted of (A) HPLC water, (B) Acetonitrile and (C) 0.10% Formic acid in Acetonitrile. The MS was operated in the Single Ion Monitoring Mode (SIM) under one set of conditions for arginine containing compounds (Table 2.4) and another set of conditions for the non-arginine containing compound (Table 2.5). Nodularin was used as an internal standard.

TABLE 2.3 GRADIENT PROGRAM FOR MICROCYSTINS

Time (min)	Flow (ml/min)	A(%)	B(%)	C(%)
0	0.40	90	0	10
1	0.40	90	0	10
8	0.40	5	85	10
13	0.40	5	85	10
13	0.40	90	0	10
21	0.40	90	0	10

TABLE 2.4. HPLC/MS CONDITIONS FOR ARGININE CONTAINING MICROCYSTINS.

Mass	Compound	Dwell Time(sec)	Cone(volts)	Span(amu)
825.5	ISTD (Nodularin)	0.20	120	0.20
519.8	Microcystin-RR	0.40	120	0.40
995.8	Microcystin-LR	0.20	120	0.20
1045.6	Microcystin-YR	0.20	120	0.20

TABLE 2.5. HPLC/MS CONDITIONS FOR NON-ARGININE CONTAINING MICROCYSTINS.

Mass	Compound	Dwell Time(sec)	Cone(volts)	Span(amu)
825.5	ISTD (Nodularin)	0.20	120	1.00
910.46	Microcystin-LA	0.20	120	1.00

2.2.6 Anatoxin a and Cylindrospermopsin by HPLC/MS

The concentrations of cylindrospermopsin and anatoxin-a were determined by liquid chromatography-tandem mass spectrometry using a Waters Quattro Micro LC/MS/MS. Nodularin was added to extracts and used as an internal standard. Compounds were separated on a Betabasic C18 column at 50^{o}C. The mobile phase was a binary gradient of water and methanol, both containing 0.1% formic acid. The initial gradient started at 95% water and

5% methanol, followed by a step change to 50% water and 50% methanol at 3 minutes, with a linear gradient from 5 to 20 minutes to 5% water and 95% methanol. Instrument detection limits for these toxins was determined to be near 20 picograms on-column. For calibration, a series of 6 solutions were prepared with the internal standard at 1000 pg/µl and the analytes in the range of 1 to 500 ng/ml in final volumes of 1 ml of 90:10 water:methanol.

2.2.7 Identification and Enumeration of Cyanobacteria

Cyanobacteria species were identified and enumerated using an inverted microscope and counting chamber (USEPA, 1997b). The analysis and enumeration of preserved samples was carried out utilizing a Nikon Eclipse TE200 inverted microscope. An aliquot (5-10 mL) of preserved sample was sedimented in a settling chamber. Identification was made using magnifications of 450 and 1000x with phase contrast illumination. In all the samples 200-300 units (colonies or filaments) were counted. The cell volume of each species was computed by using average dimensions and simulating with geometrical shapes most closely resembling the species. Photomicrography was performed using a Spot Insight digital camera, and cell measurements were made from digital image analyses using Image –Pro plus software. Cyanobacteria were identified to the lowest possible taxonomic level.

2.2.8 Statistical Analyses

Statistical analyses were conducted with SPSS (SPSS, Inc.). The Wilcoxon Signed-Rank Test was used to evaluate paired data. Independent data was evaluated with the Mann-Whitney Test. When data sets included values that were less than the detection limit (DL), a numerical value of 0.5DL was used in all statistical tests and the computation of means.

3.0 Results for Individual Lakes

3.1 Duck Lake

The results of water chemistry and cyanobacteria analyses for Duck Lake are summarized in Table 3.1. Dissolved nitrogen compounds (NO_3-N and NH_3-N) were low and mean summer nitrate and ammonia concentrations were < 0.01 mg/l and 0.02 mg/l, respectively. Dissolved phosphorus (SRP) was below the detection limit (< 0.005 mg/l) in all samples. Mean total phosphorus (TP) and total Kjeldahl nitrogen (TKN) concentrations were 0.01 mg/l and 0.53 mg/l, respectively. TP concentrations ranged from <0.010-0.040 mg/l while TKN results ranged from 0.24-0.98 mg/l. The mean summer chlorophyll-*a* was 3.2 µg/l and ranged from 1.6-6.6 µg/l. Limnological assessment methods utilize chlorophyll-*a* and total phosphorus concentrations to determine lake trophic status. Thus, based on standard values of these parameters used to assess lake trophic status (Cooke et al. 2003), chlorophyll-*a* and total phosphorus values would indicate a lower mesotrophic status for Duck Lake. Carlson (1977) developed a Trophic Status Index (TSI) that uses chlorophyll-*a* and total phosphorus as indicators. The summer mean TSI values for TP and chlorophyll-*a* were 42 and 41, respectively. These TSI values again indicate that Duck Lake is a mesotrophic system.

TABLE 3.1. WATER CHEMISTRY RESULTS FOR DUCK LAKE (2006). (TSI=CARLSON TROPHIC STATE INDEX).

Date	Event	Site	Water Temp °C	DO (mg/L)	pH	Turb (NTU)	Cl (mg/L)	SO4 (mg/L)	NO3-N (mg/L)	NH3-N (mg/L)	TKN-N (mg/L)	SRP-P (mg/L)	TP-P (mg/L)	TP-P (ug/L)	TSI TP	Ratio TN:TP	Chl *a* (ug/L)	TSI Chl *a*
07/03/06	1	Open 1	24.1	7.57	8.70	3.8	23.0	13.5	< 0.01	0.03	0.73	< 0.005	0.011	11	39	152	2.4	39
07/03/06	1	Open 2	23.9	7.36	8.72	2.5	21.0	13.4	< 0.01	0.02	0.24	< 0.005	0.012	12	40	47	3.2	42
07/03/06	1	Open 2 D	23.9	7.36	8.72	3.2	31.7	13.8	< 0.01	0.03	0.62	< 0.005	0.010	10	37	143	2.7	40
07/03/06	1	Open 3	24.0	7.49	8.72	2.7	25.4	13.8	< 0.01	0.03	0.80	< 0.005	0.012	12	40	152	1.7	36
07/18/06	2	Open 1	26.5	7.53	8.78	3.9	14.2	13.7	< 0.01	0.02	0.60	< 0.005	0.019	19	47	72	2.5	39
07/18/06	2	Open 2	26.6	8.12	8.69	3.5	14.1	14.1	0.01	0.01	0.38	< 0.005	0.016	16	44	56	2.2	38
07/18/06	2	Open 2 D	26.6	8.12	8.69	3.2	18.5	14.3	< 0.01	0.01	0.58	< 0.005	0.016	16	44	82	2.0	37
07/18/06	2	Open 3	26.9	7.32	8.70	2.5	16.5	13.8	< 0.01	0.02	0.60	< 0.005	0.014	14	42	98	1.6	35
08/02/06	3	Open 1	28.4	7.62	8.70	4.9	14.1	13.4	< 0.01	0.02	0.35	< 0.005	0.013	13	41	63	2.1	38
08/02/06	3	Open 2	28.3	7.81	8.68	6.6	16.4	12.9	< 0.01	0.01	0.38	< 0.005	0.012	12	40	73	4.1	45
08/02/06	3	Open 3	29.0	7.84	8.66	5.5	14.8	13.0	< 0.01	0.02	0.35	< 0.005	0.011	11	39	73	2.4	39
08/02/06	3	Open 3 D	29.0	7.84	8.66	5.4	16.5	13.3	< 0.01	0.02	< 0.1	< 0.005	0.011	11	39	4	3.4	43
08/23/06	4	Open 1	24.2	7.97	8.50	2.5	15.8	15.5	< 0.01	< 0.01	0.44	< 0.005	0.035	35	55	28	6.6	49
08/23/06	4	Open 1 D	24.2	7.97	8.50	2.5	15.6	13.3	< 0.01	< 0.01	0.43	< 0.005	0.027	27	52	35	5.5	47
08/23/06	4	Open 2	24.3	7.87	8.46	2.2	18.1	15.4	< 0.01	< 0.01	0.48	< 0.005	0.012	12	40	89	4.4	45
08/23/06	4	Open 3	23.7	7.34	8.38	1.9	13.9	10.9	< 0.01	< 0.01	0.38	< 0.005	< 0.01	NA	N/A	N/A	3.2	42
07/03/06	1	Beach 1	26.3	8.47	8.86	4.6	21.2	13.8	< 0.01	0.02	0.98	< 0.005	0.014	14	42	158	2.9	41
07/03/06	1	Beach 2	25.9	8.07	8.87	3.5	17.7	11.1	< 0.01	0.02	0.74	< 0.005	0.010	10	37	169	2.2	38
07/03/06	1	Beach 3	26.4	8.13	8.87	5.7	30.6	13.9	< 0.01	0.02	0.50	< 0.005	0.010	10	37	116	2.6	40
07/19/06	2	Beach 1	26.8	7.64	8.77	4.2	14.6	14.2	< 0.01	0.02	0.74	< 0.005	0.014	14	42	120	2.7	40
07/19/06	2	Beach 2	27.0	7.51	8.77	4.7	13.8	13.9	< 0.01	0.01	0.61	< 0.005	0.011	11	39	125	3.0	41
07/19/06	2	Beach 3	27.3	7.47	8.72	7.7	13.9	14.1	< 0.01	0.02	0.52	< 0.005	0.013	13	41	91	1.9	37
08/07/06	3	Beach 1	27.9	8.35	8.80	3.7	22.6	14.6	< 0.01	0.02	0.46	< 0.005	0.017	17	45	63	4.3	45
08/07/06	3	Beach 2	27.8	8.42	8.81	3.0	16.1	14.7	< 0.01	0.02	0.40	< 0.005	0.012	12	40	77	2.9	41
08/07/06	3	Beach 3	28.6	8.26	8.81	2.6	14.5	13.2	< 0.01	0.05	0.33	< 0.005	0.014	14	42	60	2.6	40
08/21/06	4	Beach 1	24.7	7.66	8.51	2.0	15.6	15.6	< 0.01	< 0.01	0.56	< 0.005	0.010	10	37	124	4.2	45
08/21/06	4	Beach 2	24.7	7.45	8.54	1.9	15.9	15.0	< 0.01	0.02	0.46	< 0.005	< 0.01	N/A	N/A	N/A	4.2	45
08/21/06	4	Beach 3	25.1	7.51	8.48	5.9	17.0	15.5	< 0.01	0.02	0.55	< 0.005	< 0.01	N/A	N/A	N/A	5.7	48
Mean			26.15	7.79	8.68	3.8	18.0	13.8	0.01	0.02	0.53	0.002	0.01	14	42	91	3.2	41
Standard Error			0.33	0.07	0.03	0.3	0.9	0.2	0.000	0.002	0.03	0.000	0.00	1	1	9	0.2	1
Min			23.74	7.32	8.38	1.9	13.8	10.9	0.005	0.01	0.24	0.005	0.005	10	37	4	1.6	35
Max			29.03	8.47	8.87	7.7	31.7	15.6	0.01	0.05	0.98	0.002	0.04	35	55	169	6.6	49

Total nitrogen to total phosphorus ratios (TN:TP) are often used as a relative indicator of nitrogen or phosphorus limitation in aquatic ecosystems (Smith 1982, Downing and McCauley 1992). A number of studies have attempted to determine the ratio at which phytoplankton are most likely to be nitrogen or phosphorus limited (Sakamoto 1966, Smith 1982, 1983). In general, these studies suggest that for phytoplankton growing during the summer, N-limitation was most likely when the epilimnion TN:TP ratio (molar) was less than 22:1, whereas P-limitation was most likely when the epilimnion TN:TP ratio was greater than 37:1. The mean molar TN:TP ratio for Duck Lake was 91, suggesting that the system appears to be phosphorus limited.

The distribution of phytoplankton organisms is shown in Figure 3.1. Duck Lake is dominated by cyanobacteria during the summer months with biovolumes for open water and beach samples of 5 x 10^5 μm^3/ml and 1 x 10^6 μm^3/ml, respectively. Diatoms, dinoflagellates, and green algae also were significant components of the phytoplankton community. The composition of the cyanobacteria population for the beach and open water locations are given in Figures 3.2 and 3.3, respectively. *Aphanocapsa conferta* dominated the phytoplankton of both beach and open water locations, however biovolumes were greater at the beach locations. *Cylindrospermopsis* was not found in Duck Lake. *Woronichinia naegelianum, Anabaena flos-aquae, Aphanizomenon flos-aquae, Aphanocapsa delicatissima,* and *Microcystis wesenbergii* also are present in the beach locations. Cyanobacteria numbers increased by a factor of ten from July to August. The *Anabaena* and *Microcystis* species are capable of producing microcystins (Chorus et al. 2000).

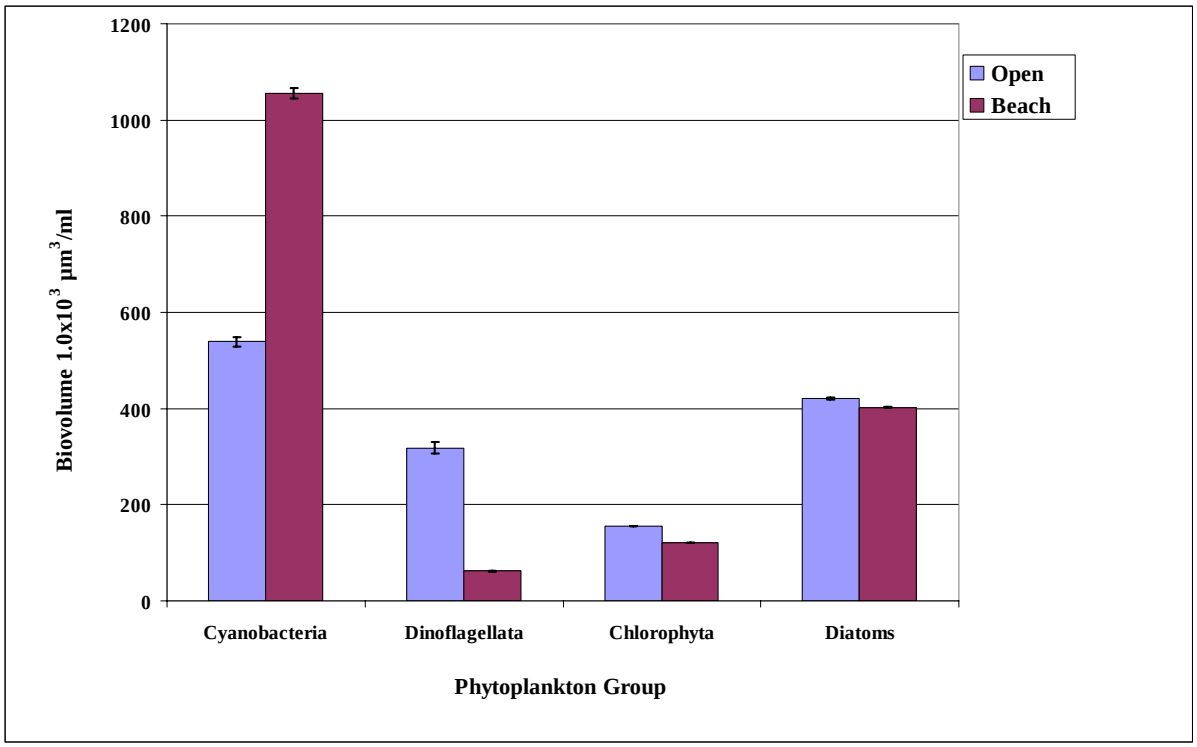


FIGURE 3.1. DISTRIBUTION OF PHYTOPLANKTON ORGANISMS IN DUCK LAKE (2006).

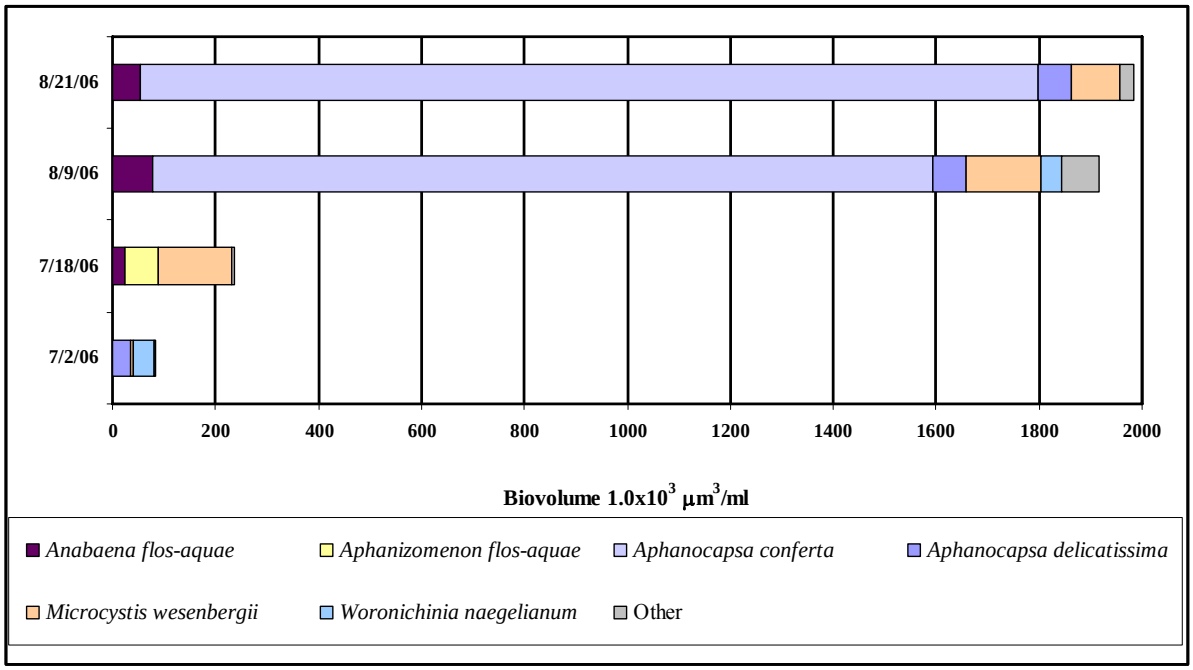


FIGURE 3.2. MEAN CYANOBACTERIA POPULATION COMPOSITION IN DUCK LAKE BEACH LOCATIONS (2006).

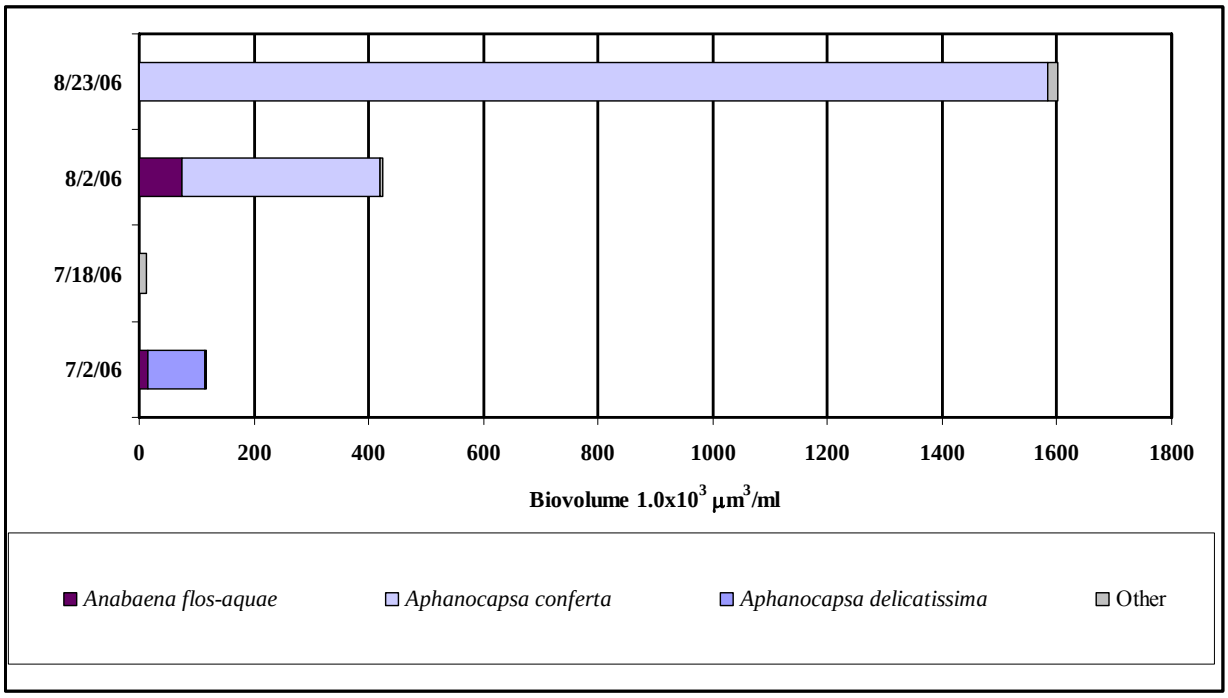


FIGURE 3.3. MEAN CYANOBACTERIA POPULATION COMPOSITION IN DUCK LAKE OPEN WATER LOCATIONS (2006).

The cyanotoxin and cyanobacteria results for Duck Lake are summarized in Table 3.2. Mean microcystin activity by PPIA was 0.075 µg/l and results ranged from <0.01-0.107 µg/l. The mean microcystin LR concentration was 0.002 µg/l with a range of <0.001 – 0.004 µg/l. Mean microcystin LR equivalents by ELISA and mean total microcystins by HPLC/MS were both 0.002 µg/l, indicating good agreement between methods. Microcystin LR and LA were the only congeners detected by HPLC/MS, however the fact that PPIA results were 30 times higher indicated that other congeners were present. Maximum microcystin LR (0.004 µg/l), cyanobacteria cell counts (9.78 x 10^3/ml), and chlorophyll-*a* concentration (6.6 µg/l) were well below the moderate WHO advisory levels of 20 µg/l, 1.0 x 10^5, and 50 µg/l, respectively. Anatoxin-a and cylindrospermopsin were not detected.

TABLE 3.2 CYANOTOXIN AND CYANOBACTERIA RESULTS FOR DUCK LAKE (2006).

Date	Event	Site	Anatoxin-a (ug/L)	Cylindrospermopsin (ug/L)	PPIA (ug/L)	ELISA Conc. (ug/L)	HPLC/MS Total Conc. (ug/L)	HPLC/MS RR (ug/L)	HPLC/MS YR (ug/L)	HPLC/MS LA (ug/L)	HPLC/MS LR (ug/L)	Cyanobac Total # Cells per mL	Cyanobac Biovolume µm^3/ml	Chl *a* (ug/L)
07/03/06	1	Open 1	<0.01	<0.01	0.073	<0.01	< 0.005	<0.001	<0.001	<0.001	0.002	2.23E+03	2.45E+05	2.4
07/03/06	1	Open 2	<0.01	<0.01	<0.01	<0.01	< 0.005	<0.001	<0.001	<0.001	<0.001	4.71E+02	9.94E+04	3.2
07/03/06	1	Open 2 D	<0.01	<0.01	<0.01	<0.01	< 0.005	<0.001	<0.001	<0.001	<0.001	-	-	2.7
07/03/06	1	Open 3	<0.01	<0.01	<0.01	<0.01	<0.005	<0.001	<0.001	<0.001	<0.001	2.89E+03	2.86E+03	1.7
07/18/06	2	Open 1	<0.01	<0.01	0.062	<0.01	< 0.005	<0.001	<0.001	<0.001	<0.001	4.28E+00	4.07E+02	2.5
07/18/06	2	Open 2	<0.01	<0.01	<0.01	<0.01	< 0.005	<0.001	<0.001	<0.001	<0.001	3.47E+02	3.30E+04	2.2
07/18/06	2	Open 2 D	<0.01	<0.01	<0.01	<0.01	< 0.005	<0.001	<0.001	<0.001	<0.001	-	-	2.0
07/18/06	2	Open 3	<0.01	<0.01	0.077	<0.01	<0.005	<0.001	<0.001	<0.001	0.002	4.70E+01	6.74E+03	1.6
08/02/06	3	Open 1	<0.01	<0.01	0.062	<0.01	< 0.005	<0.001	<0.001	<0.001	<0.001	8.78E+02	5.95E+04	2.1
08/02/06	3	Open 2	<0.01	<0.01	<0.01	<0.01	< 0.005	<0.001	<0.001	<0.001	<0.001	1.55E+03	8.98E+05	4.1
08/02/06	3	Open 3	<0.01	<0.01	<0.01	<0.01	< 0.005	<0.001	<0.001	<0.001	<0.001	1.32E+03	3.13E+05	2.4
08/02/06	3	Open 3 D	<0.01	<0.01	<0.01	<0.01	<0.005	<0.001	<0.001	<0.001	<0.001	-	-	3.4
08/23/06	4	Open 1	<0.01	<0.01	<0.01	<0.01	< 0.005	<0.001	<0.001	<0.001	0.001	4.64E+03	4.75E+06	6.6
08/23/06	4	Open 1 D	<0.01	<0.01	<0.01	<0.01	< 0.005	<0.001	<0.001	<0.001	0.001	9.97E+02	2.58E+04	5.5
08/23/06	4	Open 2	<0.01	<0.01	0.081	<0.01	< 0.005	<0.001	<0.001	<0.001	0.001	2.42E+03	2.82E+04	4.4
08/23/06	4	Open 3	<0.01	<0.01	0.107	<0.01	<0.005	<0.001	<0.001	<0.001	<0.001	-	-	3.2
07/03/06	1	Beach 1	<0.01	<0.01	0.067	<0.01	< 0.005	<0.001	<0.001	<0.001	<0.001	5.31E+02	6.83E+04	2.9
07/03/06	1	Beach 2	<0.01	<0.01	<0.01	<0.01	< 0.005	<0.001	<0.001	<0.001	0.003	2.93E+03	6.94E+04	2.2
07/03/06	1	Beach 3	<0.01	<0.01	<0.01	<0.01	<0.005	<0.001	<0.001	<0.001	<0.001	6.25E+02	1.16E+05	2.6
07/19/06	2	Beach 1	<0.01	<0.01	0.073	<0.01	< 0.005	<0.001	<0.001	<0.001	0.002	6.30E+02	4.23E+04	2.7
07/19/06	2	Beach 2	<0.01	<0.01	<0.01	<0.01	< 0.005	<0.001	<0.001	<0.001	<0.001	6.05E+03	6.32E+05	3.0
07/19/06	2	Beach 3	<0.01	<0.01	<0.01	<0.01	<0.005	<0.001	<0.001	<0.001	<0.001	5.01E+02	3.56E+04	1.9
08/07/06	3	Beach 1	<0.01	<0.01	<0.01	<0.01	< 0.005	<0.001	<0.001	<0.001	0.001	9.02E+03	7.65E+05	4.3
08/07/06	3	Beach 2	<0.01	<0.01	<0.01	<0.01	< 0.005	<0.001	<0.001	<0.001	0.002	1.04E+03	4.72E+05	2.9
08/07/06	3	Beach 3	<0.01	<0.01	<0.01	<0.01	<0.005	<0.001	<0.001	<0.001	<0.001	9.78E+03	4.51E+06	2.6
08/21/06	4	Beach 1	<0.01	<0.01	<0.01	<0.01	< 0.005	<0.001	<0.001	<0.001	0.004	1.61E+03	1.65E+06	4.2
08/21/06	4	Beach 2	<0.01	<0.01	<0.01	<0.01	< 0.005	<0.001	<0.001	<0.001	<0.001	5.95E+03	1.48E+06	4.2
08/21/06	4	Beach 3	<0.01	<0.01	<0.01	<0.01	<0.005	<0.001	<0.001	<0.001	0.003	4.92E+03	2.82E+06	5.7
Mean			0.005	0.005	0.025	0.005	0.005	0.001	0.001	0.001	0.001	2.56E+03	7.97E+05	3.2
Standard Error			0.000	0.000	0.006	0.000	0.000	0.000	0.000	0.000	0.000	5.66E+02	2.78E+05	0.2
Min			0.005	0.005	0.005	0.005	0.005	0.001	0.001	0.001	0.001	4.28E+00	4.07E+02	1.6
Max			0.005	0.005	0.107	0.005	0.005	0.001	0.001	0.001	0.004	9.78E+03	4.75E+06	6.6
World Health Organization Moderate Advisory Level											20	1.0E+05	-	50

3.2 Lake Macatawa

The results of water chemistry and cyanobacteria analyses for Lake Macatawa are summarized in Table 3.3. Nitrate and ammonia were low and mean summer nitrate and ammonia concentrations were 0.05 mg/l and 0.10 mg/l, respectively. Mean SRP was 0.0008 mg/l and ranged from <0.005-0.035 mg/l. Mean TP and TKN concentrations were 0.14 mg/l and 1.78 mg/l, respectively. TP concentrations ranged from 0.054-0.305 mg/l while TKN results ranged from 0.89-3.40 mg/l. The mean summer chlorophyll-*a* was 65 µg/l and ranged from 38-85 µg/l. Based on standard values for chlorophyll-*a* and TP used to assess lake

TABLE 3.3. WATER CHEMISTRY RESULTS FOR LAKE MACATAWA (2006). (TSI=CARLSON TROPHIC STATE INDEX).

Date	Event	Site	Water Temp °C	DO (mg/L)	pH	Turb (NTU)	Cl (mg/L)	SO4 (mg/L)	NO3-N (mg/L)	NH3-N (mg/L)	TKN-N (mg/L)	SRP-P (mg/L)	TP-P (mg/L)	TP-P (ug/L)	TSI TP	Ratio TN:TP	Chl *a* (ug/L)	TSI Chl *a*
07/14/06	1	Open 1	26.8	8.43	8.12	47	58	33	0.32	0.17	2.10	< 0.005	0.187	187	80	31	75	73
07/14/06	1	Open 2	26.4	11.34	8.61	20	60	35	0.13	0.04	3.00	0.008	0.186	186	80	38	59	71
07/14/06	1	Open 3	25.4	9.74	8.61	25	51	34	0.02	0.03	2.44	< 0.005	0.114	114	72	49	69	72
07/22/06	2	Open 1	26.8	5.03	7.71	52	89	35	0.40	0.53	2.52	0.009	0.217	217	82	35	56	70
07/22/06	2	Open 2	25.9	5.65	8.14	59	76	32	0.03	0.18	2.06	0.012	0.268	268	85	19	61	71
07/22/06	2	Open 2 D	25.9	5.65	8.14	54	63	36	< 0.01	0.19	2.06	0.011	0.247	247	84	20	52	69
07/22/06	2	Open 3	25.1	6.23	8.48	28	46	31	< 0.01	0.04	1.52	0.007	0.108	108	72	32	57	70
08/11/06	3	Open 1	24.0	5.70	7.88	56	49	28	0.11	0.25	1.82	0.012	0.179	179	79	27	73	73
08/11/06	3	Open 2	25.6	8.29	8.39	69	70	30	0.09	0.09	2.02	< 0.005	0.112	112	72	43	68	72
08/11/06	3	Open 3	23.8	8.08	8.60	39	43	25	< 0.01	0.03	1.59	< 0.005	0.105	105	71	34	65	72
08/11/06	3	Open 3 D	23.8	8.08	8.60	36	44	26	0.01	0.04	1.47	< 0.005	0.092	92	69	37	62	71
08/25/06	4	Open 1	24.5	6.84	7.73	41	61	29	0.19	0.53	2.90	< 0.005	0.305	305	87	26	85	74
08/25/06	4	Open 1 D	24.5	6.84	7.73	43	44	24	0.14	0.54	3.40	< 0.005	0.256	256	84	35	82	74
08/25/06	4	Open 2	24.0	9.29	8.48	30	46	24	0.02	0.07	1.48	< 0.005	0.085	85	68	41	70	72
08/25/06	4	Open 3	22.7	8.13	8.55	28	45	27	< 0.01	0.01	1.18	< 0.005	0.068	68	65	39	64	71
07/13/06	1	Beach 1	27.4	13.72	9.16	21	48	32	< 0.01	0.02	0.89	< 0.005	0.064	64	64	32	59	71
07/13/06	1	Beach 2	26.5	13.67	9.23	30	47	32	< 0.01	0.02	1.68	< 0.005	0.069	69	65	54	80	74
07/13/06	1	Beach 3	26.6	14.24	9.27	23	47	32	< 0.01	0.02	1.78	< 0.005	0.072	72	66	55	52	69
07/13/06	1	Beach 3 D	26.6	14.24	9.27	34	50	33	< 0.01	0.02	1.70	0.005	0.054	54	62	70	72	73
07/13/06	1	Beach 4	27.1	13.03	9.19	27	54	33	< 0.01	0.02	1.52	< 0.005	0.064	64	64	53	ND	ND
07/21/06	2	Beach 1	25.5	7.93	8.75	21	47	31	< 0.01	0.02	1.24	0.007	0.147	147	76	19	38	66
07/21/06	2	Beach 2	25.5	7.87	8.79	21	41	30	< 0.01	0.03	1.54	0.021	0.147	147	76	24	68	72
07/21/06	2	Beach 3	25.5	8.15	8.82	22	42	28	< 0.01	0.02	1.30	0.012	0.144	144	76	20	60	71
08/08/06	3	Beach 1	26.2	9.32	8.92	15	48	32	< 0.01	0.03	1.64	0.035	0.194	194	80	19	61	71
08/08/06	3	Beach 2	26.3	9.10	8.93	10	32	24	< 0.01	0.02	1.82	0.029	0.210	210	81	19	66	72
08/08/06	3	Beach 3	26.3	9.25	8.93	7	43	32	0.03	0.03	1.80	0.021	0.208	208	81	20	75	73
08/08/06	3	Beach 4	25.4	8.86	8.90	45	37	28	0.01	0.02	1.37	0.017	0.193	193	80	16	ND	ND
08/22/06	4	Beach 1	22.9	10.49	8.84	35	38	26	< 0.01	0.01	1.19	< 0.005	0.072	72	66	37	62	71
08/22/06	4	Beach 2	22.9	10.07	8.86	31	55	30	< 0.01	0.01	1.04	< 0.005	0.060	60	63	39	57	70
08/22/06	4	Beach 3	22.6	10.74	8.93	47	39	27	< 0.01	0.01	1.39	< 0.005	0.073	73	66	43	62	71
Mean			25.27	9.13	8.62	34	50	30	0.05	0.10	1.78	0.008	0.143	143	74	34	65	71
Standard Error			0.26	0.48	0.08	3	2	1	0.02	0.03	0.11	0.002	0.013	13	1	2	2	0.3
Min			22.64	5.03	7.71	7	32	24	0.01	0.01	0.89	0.002	0.054	54	62	16	38	66
Max			27.40	14.24	9.27	69	89	36	0.40	0.54	3.40	0.035	0.305	305	87	70	85	74

trophic status (Cooke et al. 2003), concentrations in Lake Macatawa would indicate hypereutrophic status. The summer mean Carlson TSI values for TP and chlorophyll-*a* were 74 and 71, respectively. These TSI values again indicate that Lake Macatawa is a hypereutrophic system. The mean molar TN:TP ratio for Lake Macatawa was 34, indicating phosphorus limitation.

The distribution of phytoplankton organisms is shown in Figure 3.4. Lake Macatawa is dominated by cyanobacteria during the summer months with biovolumes for open water and beach samples of 1.7 x 10^7 μm^3/ml and 2.1 x 10^7 μm^3/ml, respectively. Dinoflagellates, diatoms, and green algae also were significant components of the phytoplankton community. The composition of the cyanobacteria population for the beach and open water locations are given in Figures 3.5 and 3.6, respectively. Phytoplankton species varied during each sampling event. *Microcystis aeruginosa* and *Planktothrix agardhii* were the dominant organisms on July 13, 2006 in the open water locations. Beach samples on this date had greater cyanobacteria biovolumes with the addition of *Anabaena flos-aquae* and *Planktothrix rubescens* to the dominant group of organisms. Cyanobacteria biovolumes at the open water locations dropped to 1 x 10^6

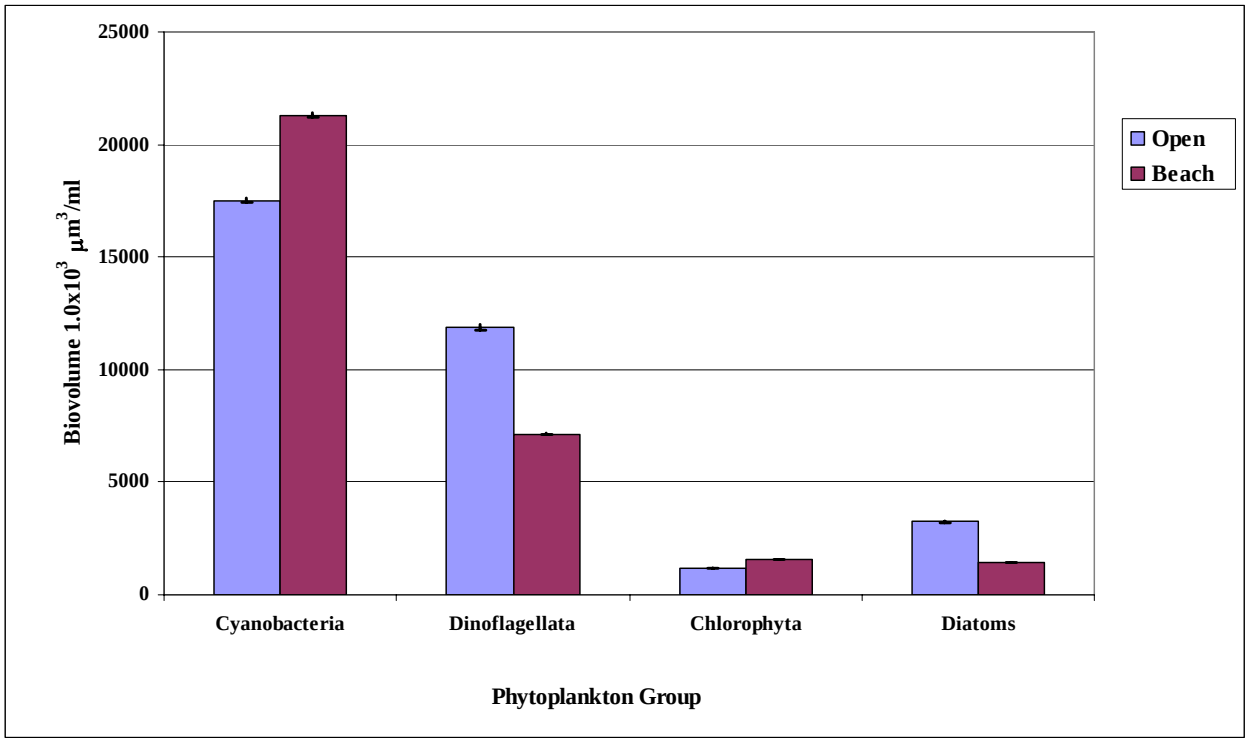


FIGURE 3.4. DISTRIBUTION OF PHYTOPLANKTON ORGANISMS IN LAKE MACATAWA (2006).

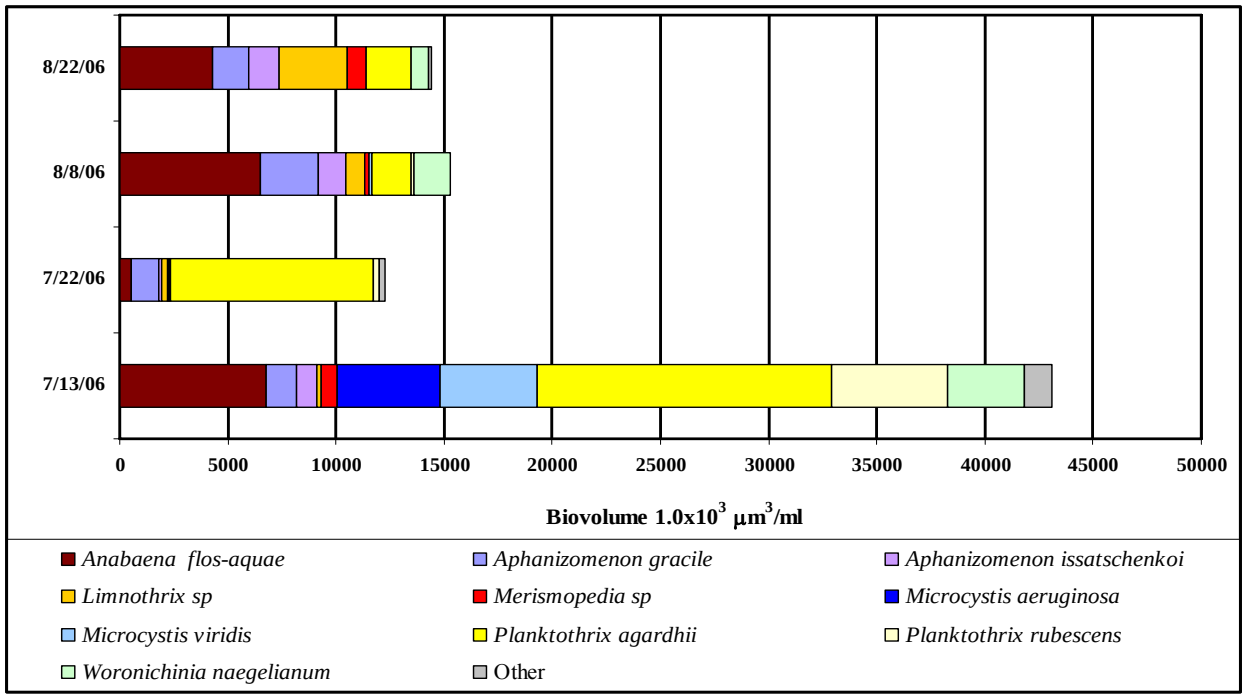


FIGURE 3.5. MEAN CYANOBACTERIA POPULATION COMPOSITION IN LAKE MACATAWA BEACH LOCATIONS (2006).

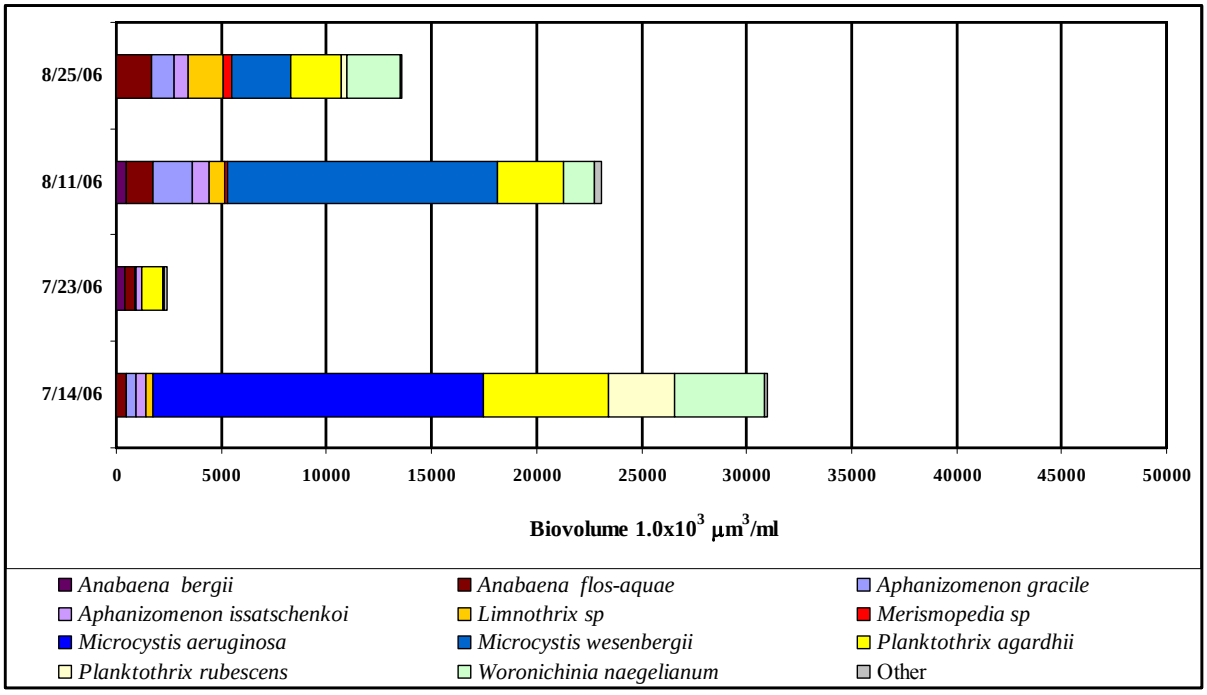


FIGURE 3.6. MEAN CYANOBACTERIA POPULATION COMPOSITION IN LAKE MACATAWA OPEN WATER LOCATIONS (2006).

μm^3/ml on July 22, 2007 and were replaced by a bloom of dinoflagelates (*Gymnodinium aeruginosum* and *Gymnodinium excavatum*). August phytoplankton populations also varied between open water and beach locations. Dominant open water cyanobacteria were *Microcystis wesenbergii, Planktothrix agardhii,* and *Microcystis aeruginosa* while populations at beach locations contained greater amounts of *Anabaena flos-aquae* and *Limnothrix sp*. *Cylindrospermopsis* was not found in Lake Macatawa.

The cyanotoxin and cyanobacteria results for Lake Macatawa are summarized in Table 3.4. Mean microcystin activity by PPIA was 0.97 µg/l and results ranged from 0.16-1.87 µg/l. The mean microcystin LR concentration was 0.19 µg/l with a range of 0.048-0.40 µg/l. There was no significant difference in microcystin LR concentrations between beach and open water samples (Mann-Whitney p=0.64). Mean microcystin LR equivalents by ELISA and mean total microcystins by HPLC/MS were 0.54 µg/l and 0.20 µg/l, indicating that the ELISA method overestimated the total concentration. ELISA also overestimated the LR concentration by factor of three. Microcystin LR was the most abundant congener detected with small amounts of LA, YR, and RR. PPIA results were two times higher than ELISA and eight times higher than HPLC/MS, indicating that other congeners were present. The maximum microcystin LR (0.43 µg/l) was less than the WHO moderate advisory level of 20 µg/l. Cyanobacteria cell counts (22 of 24 samples), and chlorophyll-*a* concentration (23 of 24 samples) were above the moderate WHO advisory levels of 1.0 x 10^5/ml, and 50 µg/l, respectively. The maximum cyanobacteria cell count was 1.0 x 10^5/ml. Although cyanobacteria numbers and chlorophyll-*a* exceed the WHO guideline, many of the dominant

organisms such as *Microcystis wesenbergii*, *Planktothrix agardhii*, and *Limnothrix sp*. are not known for producing high levels of microcystins (Chorus et al. 2000). Anatoxin-a and cylindrospermopsin were not detected.

TABLE 3.4 CYANOTOXIN AND CYANOBACTERIA RESULTS FOR LAKE MACATAWA (2006).

Date	Event	Site	Anatoxin-a (ug/L)	Cylindrospermopsin (ug/L)	PPIA (ug/L)	ELISA Conc. (ug/L)	HPLC/MS Total Conc. (ug/L)	HPLC/MS RR (ug/L)	HPLC/MS YR (ug/L)	HPLC/MS LA (ug/L)	HPLC/MS LR (ug/L)	Cyanobac Total # Cells per mL	Cyanobac Biovolume $\mu m^3/ml$	Chl *a* (ug/L)
07/14/06	1	Open 1	<0.01	<0.01	0.433	0.3162	0.052	< 0.001	< 0.001	< 0.001	0.052	8.45E+05	5.76E+07	75
07/14/06	1	Open 2	<0.01	<0.01	0.541	0.4299	0.086	< 0.001	< 0.001	< 0.001	0.086	1.35E+05	5.28E+06	59
07/14/06	1	Open 3	<0.01	<0.01	1.722	0.4667	0.094	< 0.001	< 0.001	< 0.001	0.094	8.50E+05	3.00E+07	69
07/22/06	2	Open 1	<0.01	<0.01	0.156	0.1611	0.049	0.001	< 0.001	< 0.001	0.048	8.79E+04	9.32E+05	56
07/22/06	2	Open 2	<0.01	<0.01	0.271	0.2418	0.093	0.001	< 0.001	< 0.001	0.092	1.12E+05	5.39E+06	61
07/22/06	2	Open 2 D	<0.01	<0.01	0.954	0.2275	0.087	0.001	< 0.001	< 0.001	0.086	-	-	52
07/22/06	2	Open 3	<0.01	<0.01	0.430	0.1902	0.093	0.002	< 0.001	< 0.001	0.091	2.73E+04	8.42E+05	57
08/11/06	3	Open 1	<0.01	<0.01	0.453	0.4657	0.214	< 0.001	< 0.001	< 0.001	0.214	5.53E+05	2.25E+07	73
08/11/06	3	Open 2	<0.01	<0.01	1.370	0.7502	0.406	< 0.001	0.012	< 0.001	0.394	3.60E+05	2.17E+07	68
08/11/06	3	Open 3	<0.01	<0.01	1.687	0.6986	0.366	< 0.001	< 0.001	< 0.001	0.366	4.77E+05	2.52E+07	65
08/11/06	3	Open 3 D	<0.01	<0.01	0.627	0.7355	0.393	< 0.001	< 0.001	< 0.001	0.393	-	-	62
08/25/06	4	Open 1	<0.01	<0.01	0.632	1.0091	0.254	0.002	0.005	< 0.001	0.247	-	-	85
08/25/06	4	Open 1 D	<0.01	<0.01	0.713	0.6196	0.227	0.002	< 0.001	< 0.001	0.225	4.30E+05	9.18E+06	82
08/25/06	4	Open 2	<0.01	<0.01	0.760	1.2868	0.434	0.003	0.006	0.022	0.403	6.12E+05	2.35E+07	70
08/25/06	4	Open 3	<0.01	<0.01	0.712	0.8837	0.312	0.005	0.004	0.011	0.292	4.68E+05	8.07E+06	64
07/13/06	1	Beach 1	<0.01	<0.01	0.738	0.6022	0.104	0.003	< 0.001	< 0.001	0.101	8.48E+05	3.06E+07	59
07/13/06	1	Beach 2	<0.01	<0.01	1.652	0.5741	0.168	< 0.001	0.007	< 0.001	0.161	1.68E+06	6.80E+07	80
07/13/06	1	Beach 3	<0.01	<0.01	1.874	0.5350	0.117	< 0.001	< 0.001	< 0.001	0.117	7.53E+05	3.07E+07	52
07/13/06	1	Beach 3 D	<0.01	<0.01	1.704	0.6200	0.117	< 0.001	< 0.001	< 0.001	0.117	-	-	72
07/13/06	1	Beach 4	<0.01	<0.01	1.677	0.5968	0.117	< 0.001	< 0.001	< 0.001	0.117	-	-	-
07/21/06	2	Beach 1	<0.01	<0.01	1.388	0.5515	0.106	0.003	0.004	< 0.001	0.099	3.73E+06	1.68E+06	38
07/21/06	2	Beach 2	<0.01	<0.01	1.177	0.7568	0.095	0.002	< 0.001	< 0.001	0.093	6.61E+05	2.23E+07	68
07/21/06	2	Beach 3	<0.01	<0.01	0.990	0.6993	0.096	0.002	0.004	< 0.001	0.090	4.37E+05	1.31E+07	60
08/08/06	3	Beach 1	<0.01	<0.01	0.377	0.2700	0.207	< 0.001	0.013	< 0.001	0.194	2.49E+05	1.44E+07	61
08/08/06	3	Beach 2	<0.01	<0.01	0.546	0.3416	0.293	< 0.001	< 0.001	< 0.001	0.293	3.91E+05	1.49E+07	66
08/08/06	3	Beach 3	<0.01	<0.01	1.659	0.3416	0.234	< 0.001	< 0.001	< 0.001	0.234	3.95E+05	1.68E+07	75
08/08/06	3	Beach 4	<0.01	<0.01	0.484	0.3740	0.194	< 0.001	< 0.001	< 0.001	0.194	-	-	-
08/22/06	4	Beach 1	<0.01	<0.01	1.337	0.4818	0.276	0.003	0.005	< 0.001	0.268	1.92E+06	1.89E+07	62
08/22/06	4	Beach 2	<0.01	<0.01	1.376	0.4686	0.302	0.004	0.004	< 0.001	0.294	9.06E+05	1.31E+07	57
08/22/06	4	Beach 3	<0.01	<0.01	0.749	0.4915	0.279	0.003	0.004	< 0.001	0.272	7.91E+05	1.12E+07	62
Mean			<0.01	<0.01	0.973	0.540	0.196	0.002	0.003	0.002	0.191	7.38E+05	1.94E+07	65
Standard Error			<0.01	<0.01	0.096	0.046	0.021	0.000	0.001	0.001	0.020	1.59E+05	3.32E+06	2
Min			<0.01	<0.01	0.156	0.161	0.049	0.001	0.001	0.001	0.048	2.73E+04	8.42E+05	38
Max			<0.01	<0.01	1.874	1.287	0.434	0.005	0.013	0.022	0.403	3.73E+06	6.80E+07	85
World Health Organization Moderate Advisory Level											20	1.0E+05	-	50

3.3 Spring Lake

The results of water chemistry and cyanobacteria analyses for Spring Lake are summarized in Table 3.5. Nitrate and ammonia were low and mean summer concentrations were 0.12 mg/l and 0.06 mg/l, respectively. Mean SRP was 0.003 mg/l and ranged from <0.005-0.014 mg/l. Mean TP and TKN concentrations were 0.030 mg/l and 1.14 mg/l, respectively. TP concentrations ranged from 0.017-0.051 mg/l while TKN results ranged from 0.60-1.4 mg/l. Mean molar TN:TP ratio for Spring Lake was 99, suggesting phosphorus limitation. The mean summer chlorophyll-*a* was 52 µg/l and ranged from 35-75 µg/l. The summer mean Carlson TSI values for TP and chlorophyll-*a* were 53 and 69, respectively. The TP TSI value would indicate eutrophic status while the chlorophyll-*a* TSI would indicate hypereutrophic conditions. Spring Lake was treated with alum in the fall of 2005 to reduce phosphorus availability in the sediments and lower internal loading. While TP levels are lower by 50% compared to historic data (Steinman et al. 2004), lower nutrient concentrations

are not reflected in the chlorophyll-*a* results. A similar trend of low TP and high chlorophyll-*a* was noted by Steinman and Ogdahl (in press). Some species of cyanobacteria are capable of adjusting their buoyancy in the water column (Paerl and Ustach 1982; Pearl et al. 2001) and accumulate phosphorus at the sediment/water interface through luxury consumption (Pearl 1996). The shallow bathymetry of Spring Lake and the high levels of phosphorus in the sediment (Steinman et al. 2004) are ideal conditions for certain cyanobacteria species to move vertically from the sediment to the surface and form blooms.

TABLE 3.5. WATER CHEMISTRY RESULTS FOR SPRING LAKE (2006). (TSI=CARLSON TROPHIC STATE INDEX).

Date	Event	Site	Water Temp °C	DO (mg/L)	pH	Turb (NTU)	Cl (mg/L)	SO4 (mg/L)	NO3-N (mg/L)	NH3-N (mg/L)	TKN-N (mg/L)	SRP-P (mg/L)	TP-P (mg/L)	TP-P (ug/L)	TSI TP	Ratio TN:TP	Chl *a* (ug/L)	TSI Chl *a*
07/14/06	1	Open 1	27.2	8.97	8.60	16	44	38	0.11	0.03	1.32	< 0.005	0.040	40	57	75	46	68
07/14/06	1	Open 2	26.5	8.74	8.47	7	41	38	0.19	0.03	1.14	< 0.005	0.025	25	51	104	49	69
07/14/06	1	Open 3	26.1	8.60	8.46	9	45	38	0.20	0.05	1.34	< 0.005	0.027	27	52	114	47	68
07/14/06	1	Open 3 D	26.1	8.60	8.46	8	45	40	0.20	0.04	1.36	< 0.005	0.025	25	51	124	53	70
07/22/06	2	Open 1	25.2	6.63	8.38	27	48	38	0.11	0.08	1.38	< 0.005	0.043	43	58	75	38	66
07/22/06	2	Open 2	25.7	6.12	8.18	10	47	39	0.25	0.16	1.26	< 0.005	0.028	28	52	112	36	66
07/22/06	2	Open 2 D	25.7	6.12	8.18	9	49	39	0.28	0.14	1.26	< 0.005	0.032	32	54	97	44	68
07/22/06	2	Open 3	25.7	6.63	8.29	13	53	41	0.34	0.09	1.14	< 0.005	0.040	40	57	68	51	69
08/11/06	3	Open 1	25.5	5.80	8.06	26	37	27	0.03	0.13	1.27	< 0.005	0.017	17	45	182	51	69
08/11/06	3	Open 2	26.5	8.53	8.49	15	45	25	0.04	0.03	1.04	< 0.005	0.025	25	51	94	59	71
08/11/06	3	Open 3	26.5	8.73	8.51	17	45	37	0.05	0.03	1.14	< 0.005	0.036	36	56	72	57	70
08/11/06	3	Open 3 D	26.5	8.73	8.51	20	43	36	0.05	0.03	1.15	< 0.005	0.030	30	53	87	62	71
08/25/06	4	Open 1	24.5	7.79	8.32	28	71	38	0.02	0.21	0.75	0.006	0.041	41	58	52	75	73
08/25/06	4	Open 1 D	24.5	7.79	8.32	27	82	51	< 0.01	0.01	1.29	< 0.005	0.039	39	57	74	67	72
08/25/06	4	Open 2	24.7	8.08	8.42	16	51	29	< 0.01	< 0.01	0.600	0.014	0.024	24	50	55	64	71
08/25/06	4	Open 3	24.6	8.03	8.38	14	16	14	< 0.01	0.01	0.94	< 0.005	0.024	24	50	88	58	70
07/13/06	1	Beach 1	26.3	8.30	8.48	6	47	40	0.22	0.07	1.14	< 0.005	0.030	30	53	105	35	65
07/13/06	1	Beach 2	26.3	7.86	8.45	11	45	34	0.19	0.09	0.82	< 0.005	0.023	23	49	105	41	67
07/13/06	1	Beach 3	26.4	8.13	8.47	5	46	37	0.19	0.05	1.12	< 0.005	0.027	27	52	112	61	71
07/21/06	2	Beach 1	26.2	7.80	8.54	18	48	40	0.27	0.05	1.32	< 0.005	0.038	38	57	95	41	67
07/21/06	2	Beach 2	26.3	7.73	8.55	14	48	40	0.27	0.12	1.14	< 0.005	0.037	37	56	92	57	70
07/21/06	2	Beach 3	26.4	7.60	8.55	10	49	40	0.25	0.05	1.24	< 0.005	0.027	27	52	126	43	68
08/13/06	3	Beach 1	25.7	8.57	8.54	13	47	37	0.06	0.02	1.08	< 0.005	0.021	21	48	122	48	69
08/13/06	3	Beach 2	25.9	8.68	8.64	12	41	34	0.05	0.01	1.13	< 0.005	0.018	18	46	146	53	70
08/13/06	3	Beach 3	25.8	8.82	8.62	13	47	38	0.05	0.02	1.09	< 0.005	0.021	21	48	122	66	72
08/27/06	4	Beach 1	25.1	8.63	8.39	19	27	25	< 0.01	0.03	1.19	< 0.005	0.024	24	50	112	54	70
08/27/06	4	Beach 2	24.9	7.78	8.32	12	30	28	0.01	0.03	0.95	< 0.005	0.021	21	48	104	57	70
08/27/06	4	Beach 3	24.0	7.98	8.34	17	51	44	< 0.01	0.03	1.02	< 0.005	0.029	29	53	80	51	69
07/22/06	2	Boat Launch	24.9	9.19	8.53	18	50	36	0.16	0.04	1.40	< 0.005	0.051	51	61	70	ND	ND
Mean			25.7	7.96	8.43	15	46	36	0.124	0.06	1.14	0.003	0.030	30	53	99	52	69
Standard Error			0.1	0.17	0.03	1	2	1	0.021	0.01	0.04	0.0005	0.001	1	1	5	2	0.4
Min			24.00	5.80	8.06	5	16	14	0.01	0.01	0.60	0.002	0.017	17	45	52	35	65
Max			27.23	9.19	8.64	28	82	51	0.34	0.21	1.40	0.014	0.051	51	61	182	75	73

The distribution of phytoplankton organisms is shown in Figure 3.7. Spring Lake is dominated by cyanobacteria during the summer months with biovolumes for open water and beach samples of 8.9 x 10^6 μm^3/ml and 7.6 x 10^6 μm^3/ml, respectively. Dinoflagellates and green algae also were significant components of the phytoplankton community. The diatom assemblage was relatively low (5 x 10^5 μm^3/ml). Open water samples contained greater cyanobacteria biovolumes than the beach samples. The composition of the cyanobacteria population for the beach and open water locations are given in Figures 3.8 and 3.9, respectively. *Limnothrix sp.* was the dominant organism in all of the samples. *Cylindrospermopsis sp.* and *Aphanizomenon gracile* were more abundant in August than July. *Cylindrospermopsis sp.* is a subtropical, toxin-producing, cyanobacteria that was recently found in Mona Lake (Hong et al. 2006). The maximum density of *Cylindrospermopsis* was 4,721 trichomes/ml.

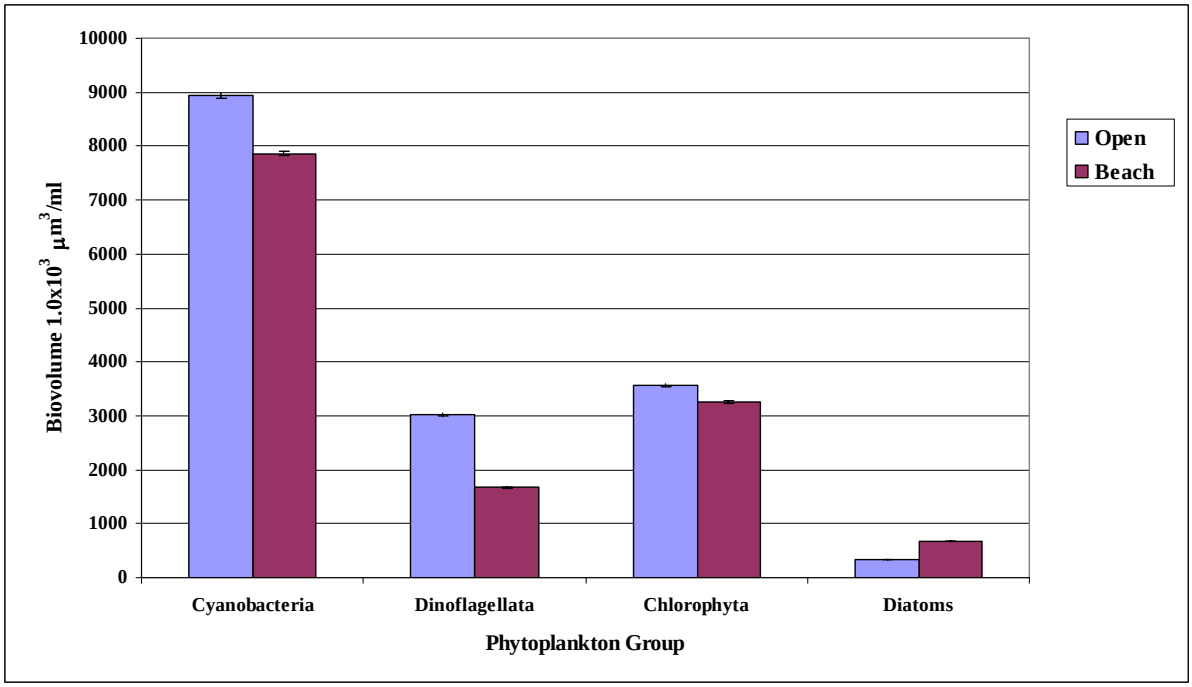


FIGURE 3.7. DISTRIBUTION OF PHYTOPLANKTON ORGANISMS IN SPRING LAKE (2006).

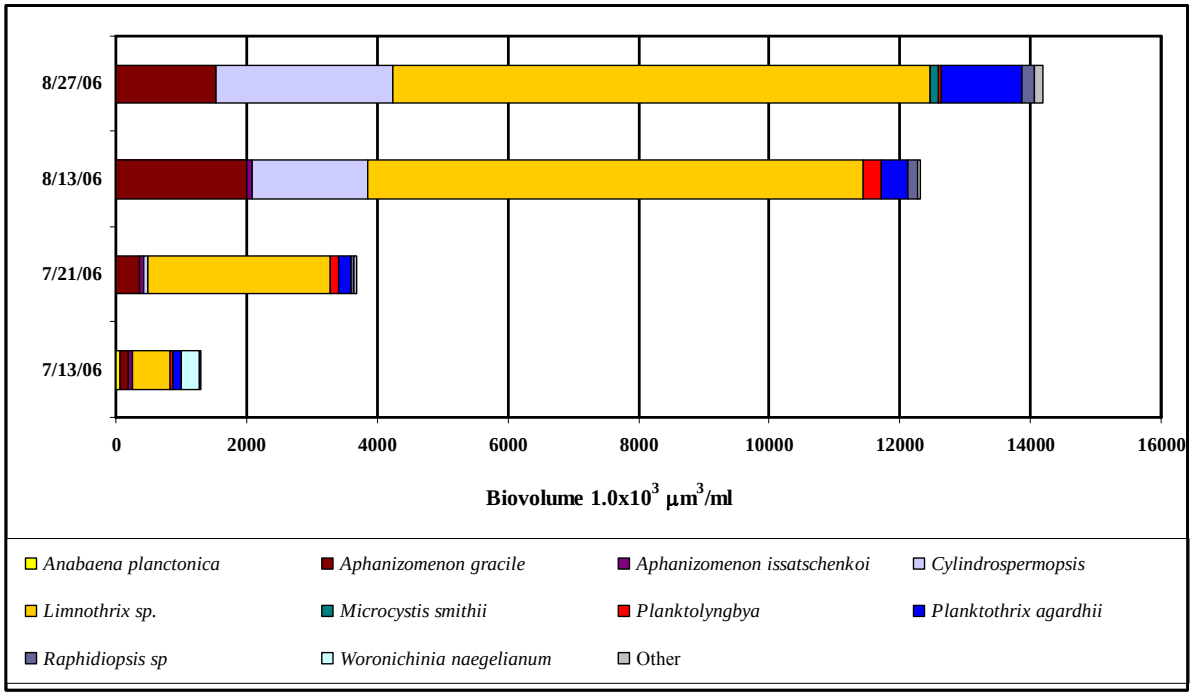


FIGURE 3.8. MEAN CYANOBACTERIA POPULATION COMPOSITION IN SPRING LAKE BEACH LOCATIONS (2006).

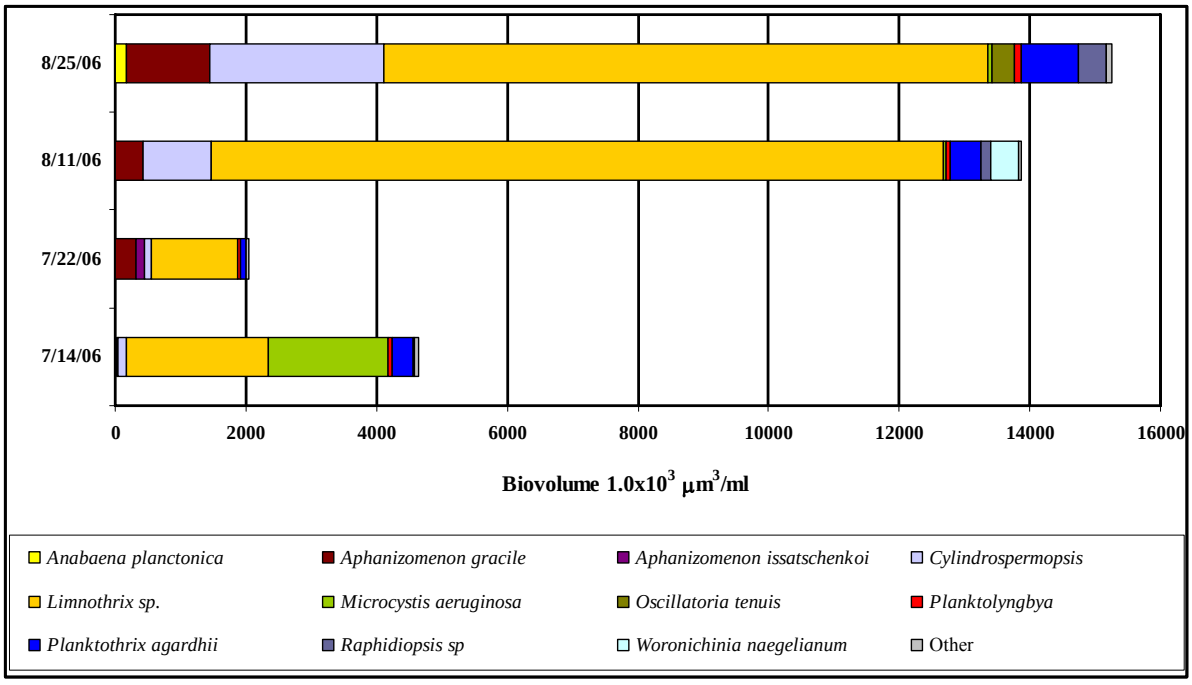


FIGURE 3.9. MEAN CYANOBACTERIA POPULATION COMPOSITION IN SPRING LAKE OPEN WATER LOCATIONS (2006).

Cylindrospermopsis densities in Spring Lake were similar to populations reported in Florida lakes (Chapman and Schelske 1997) and ten times greater historical data from Mona Lake (Hong et al. 2006).

The cyanotoxin and cyanobacteria results for Spring Lake are summarized in Table 3.6. Mean microcystin activity by PPIA was 0.090 µg/l and results ranged from <0.01-0.187 µg/l. The mean microcystin LR concentration was 0.035 µg/l with a range of 0.005-0.070 µg/l. There was no significant difference in microcystin LR concentrations between beach and open water samples (Mann-Whitney p=0.98). Mean microcystin LR equivalents by ELISA and mean total microcystins by HPLC/MS were 0.068 µg/l and 0.057 µg/l, indicating that the ELISA method slightly overestimated the total concentration. ELISA also overestimated the LR concentration by factor of two. Microcystin LR was the most abundant congener detected with small amounts of LA, YR, and RR. Mean LA and RR results were 50% of the LR concentration. PPIA results were two times higher than ELISA and HPLC/MS concentrations, indicating that other congeners were present. The maximum microcystin LR (0.070 µg/l) was less than the WHO moderate advisory level of 20 µg/l. Mean cyanobacteria cell counts (8.4 x 10^6/ml) and chlorophyll-*a* concentration (52 µg/l) were above the moderate WHO advisory levels of 1.0 x 10^5/ml, and 50 µg/l, respectively. Although cyanobacteria numbers and chlorophyll-*a* exceed the WHO guideline, many of the dominant organisms such as *Limnothrix sp*. *Cylindrospermopsis sp.*, and *Aphanizomenon gracile* are not known for producing high levels of microcystins (Chorus et al. 2000). Anatoxin-a and cylindrospermopsin were not detected.

TABLE 3.6 CYANOTOXIN AND CYANOBACTERIA RESULTS FOR SPRING LAKE (2006).

Date	Event	Site	Anatoxin-a (ug/L)	Cylindrospermopsin (ug/L)	PPIA (ug/L)	ELISA Conc. (ug/L)	HPLC/MS Total Conc. (ug/L)	HPLC/MS RR (ug/L)	HPLC/MS YR (ug/L)	HPLC/MS LA (ug/L)	HPLC/MS LR (ug/L)	Cyanobac Total # Cells per mL	Cyanobac Biovolume $\mu m^3/ml$	Chl *a* (ug/L)
07/14/06	1	Open 1	<0.01	<0.01	0.099	0.0397	0.037	0.010	< 0.001	< 0.001	0.027	6.03E+05	5.36E+06	46
07/14/06	1	Open 2	<0.01	<0.01	0.094	0.0733	0.055	0.014	0.008	< 0.001	0.033	2.14E+05	1.89E+06	49
07/14/06	1	Open 3	<0.01	<0.01	0.100	0.0662	0.055	0.011	0.007	< 0.001	0.037	2.27E+05	6.66E+06	47
07/14/06	1	Open 3 D	<0.01	<0.01	0.109	0.0513	0.061	0.012	0.006	< 0.001	0.043	-	-	53
07/22/06	2	Open 1	<0.01	<0.01	0.137	0.1006	0.092	0.025	0.016	< 0.001	0.051	1.66E+05	1.52E+06	38
07/22/06	2	Open 2	<0.01	<0.01	<0.01	0.0360	0.042	0.013	0.004	< 0.001	0.025	2.01E+05	1.84E+06	36
07/22/06	2	Open 2 D	<0.01	<0.01	0.068	0.0391	0.044	0.014	0.004	< 0.001	0.026	-	-	44
07/22/06	2	Open 3	<0.01	<0.01	0.075	0.0493	0.046	0.010	0.005	< 0.001	0.031	2.11E+05	2.75E+06	51
08/11/06	3	Open 1	<0.01	<0.01	0.109	0.0872	0.051	0.018	0.000	< 0.001	0.033	1.36E+06	1.34E+07	51
08/11/06	3	Open 2	<0.01	<0.01	0.187	0.0834	0.067	0.018	0.000	< 0.001	0.049	1.42E+06	1.36E+07	59
08/11/06	3	Open 3	<0.01	<0.01	0.113	0.1014	0.090	0.022	0.009	< 0.001	0.059	1.44E+06	1.46E+07	57
08/11/06	3	Open 3 D	<0.01	<0.01	0.120	0.0963	0.069	0.019	0.000	< 0.001	0.050	-	-	62
08/25/06	4	Open 1	<0.01	<0.01	0.112	0.0767	0.042	0.011	0.005	< 0.001	0.026	1.09E+06	1.32E+07	75
08/25/06	4	Open 1 D	<0.01	<0.01	0.101	0.0594	0.055	0.022	0.005	< 0.001	0.028	-	-	67
08/25/06	4	Open 2	<0.01	<0.01	0.101	0.0459	0.041	0.012	0.004	< 0.001	0.025	8.23E+05	1.22E+07	64
08/25/06	4	Open 3	<0.01	<0.01	0.114	0.0726	0.054	0.016	0.005	< 0.001	0.033	1.76E+06	2.03E+07	58
07/13/06	1	Beach 1	<0.01	<0.01	<0.01	0.0395	0.051	0.011	0.005	< 0.001	0.035	2.87E+05	1.24E+06	35
07/13/06	1	Beach 2	<0.01	<0.01	<0.01	0.0392	0.044	0.016	0.006	< 0.001	0.022	2.14E+05	1.91E+06	41
07/13/06	1	Beach 3	<0.01	<0.01	<0.01	0.0252	0.024	0.006	0.003	< 0.001	0.015	3.31E+04	7.20E+05	61
07/21/06	2	Beach 1	<0.01	<0.01	0.092	0.0792	0.057	0.015	0.004	< 0.001	0.038	9.87E+04	9.94E+05	41
07/21/06	2	Beach 2	<0.01	<0.01	0.096	0.0715	0.065	0.014	0.005	< 0.001	0.046	6.41E+05	6.03E+06	57
07/21/06	2	Beach 3	<0.01	<0.01	0.125	0.0959	0.065	0.022	0.004	< 0.001	0.039	4.52E+05	4.00E+06	43
08/13/06	3	Beach 1	<0.01	<0.01	0.159	0.1145	0.098	0.018	0.010	< 0.001	0.070	2.48E+06	3.04E+06	48
08/13/06	3	Beach 2	<0.01	<0.01	0.142	0.1124	0.096	0.030	0.013	< 0.001	0.053	1.31E+06	1.55E+07	53
08/13/06	3	Beach 3	<0.01	<0.01	0.104	0.1135	0.068	0.016	0.005	< 0.001	0.047	1.78E+06	1.84E+07	66
08/27/06	4	Beach 1	<0.01	<0.01	0.094	0.0656	0.042	0.015	0.003	< 0.001	0.024	1.31E+06	1.78E+07	54
08/27/06	4	Beach 2	<0.01	<0.01	<0.01	0.0583	0.059	0.009	0.003	0.025	0.022	1.17E+06	1.59E+07	57
08/27/06	4	Beach 3	<0.01	<0.01	0.106	0.0648	0.083	0.025	0.003	0.024	0.031	7.68E+05	8.89E+06	51
07/22/06	2	Boat Launch	<0.01	<0.01	<0.01	0.0190	0.006	0.001	0.000	0.000	0.005	-	-	ND
Mean			<0.01	<0.01	0.090	0.068	0.057	0.015	0.005	0.003	0.035	8.36E+05	8.41E+06	52
Standard Error			<0.01	<0.01	0.009	0.005	0.004	0.001	0.001	0.003	0.003	1.42E+05	1.41E+06	2
Min			<0.01	<0.01	0.010	0.019	0.006	0.001	0.001	0.001	0.005	3.31E+04	7.20E+05	35
Max			<0.01	<0.01	0.187	0.114	0.098	0.030	0.016	0.025	0.070	2.48E+06	2.03E+07	75
World Health Organization Moderate Advisory Level											20	1.0E+05	-	50

3.4 Mona Lake

The results of water chemistry and cyanobacteria analyses for Mona Lake are summarized in Table 3.7. Mean summer nitrate and ammonia concentrations were 0.01 mg/l and 0.03 mg/l, respectively. Mean SRP was <0.005 mg/l and ranged from <0.005-0.006 mg/l. Mean TP and TKN concentrations were 0.060 mg/l and 1.29 mg/l, respectively. TP concentrations ranged from 0.03-0.0.23 mg/l while TKN results ranged from 0.96-1.6 mg/l. Mean molar TN:TP ratio for Mona Lake was 53, suggesting phosphorus limitation. The mean summer chlorophyll-*a* was 56 µg/l and ranged from 33-83 µg/l. Based on standard values for chlorophyll-*a* and TP used to assess lake trophic status (Cooke et al. 2003), concentrations in Mona Lake would indicate upper eutrophic to hypereutrophic status. The summer mean Carlson TSI values for TP and chlorophyll-*a* were 62 and 70, respectively. These TSI values again indicate that Mona Lake is a upper eutrophic to hypereutrophic system.

TABLE 3.7. WATER CHEMISTRY RESULTS FOR MONA LAKE (2006). (TSI=CARLSON TROPHIC STATE INDEX).

Date	Event	Site	Water Temp °C	DO (mg/L)	pH	Turb (NTU)	Cl (mg/L)	SO4 (mg/L)	NO3-N (mg/L)	NH3-N (mg/L)	TKN-N (mg/L)	SRP-P (mg/L)	TP-P (mg/L)	TP-P (ug/L)	TSI TP	Ratio TN:TP	Chl *a* (ug/L)	TSI Chl *a*
07/06/06	1	Open 1	24.3	10.42	8.99	8	48	38	< 0.01	0.02	1.52	< 0.005	0.060	60	63	57	46	68
07/06/06	1	Open 2	23.9	8.68	8.86	7	46	37	< 0.01	0.03	1.20	< 0.005	0.041	41	58	66	56	70
07/06/06	1	Open 3	23.8	8.20	8.75	6	49	38	< 0.01	0.03	1.08	< 0.005	0.033	33	55	74	33	65
07/06/06	1	Open 3 D	23.8	8.20	8.75	7	48	38	< 0.01	0.02	1.16	< 0.005	0.041	41	58	48	40	67
07/29/06	2	Open 1	26.9	12.52	8.95	31	47	33	< 0.01	< 0.01	1.28	< 0.005	0.059	59	63	48	60	71
07/29/06	2	Open 2	26.0	8.66	8.81	26	57	40	< 0.01	< 0.01	1.06	< 0.005	0.061	61	63	38	39	67
07/29/06	2	Open 3	25.5	7.53	8.52	15	55	38	< 0.01	0.01	1.07	< 0.005	0.048	48	60	50	56	70
07/29/06	2	Open 1 D	26.9	12.52	9.05	30	54	40	< 0.01	< 0.01	1.40	< 0.005	0.058	58	63	53	56	70
08/10/06	3	Open 1	26.6	10.65	8.97	30	55	36	0.06	0.02	1.48	< 0.005	0.073	73	66	47	61	71
08/10/06	3	Open 2	26.4	8.71	8.89	29	63	34	< 0.01	0.02	1.39	< 0.005	0.055	55	62	57	67	72
08/10/06	3	Open 2 D	26.4	8.71	8.89	27	49	34	< 0.01	0.02	1.52	< 0.005	0.057	57	62	60	62	71
08/10/06	3	Open 3	26.1	7.16	8.59	22	49	32	< 0.01	0.02	1.40	< 0.005	0.043	43	58	73	55	70
08/25/06	4	Open 1	23.8	6.62	8.46	23	38	26	0.02	0.06	1.31	< 0.005	0.107	107	72	29	78	73
08/25/06	4	Open 1 D	23.8	6.62	8.46	22	50	32	< 0.01	0.07	1.38	< 0.005	0.077	77	67	42	83	74
08/25/06	4	Open 2	23.8	6.51	8.45	21	29	20	< 0.01	0.09	1.14	< 0.005	0.066	66	65	41	62	71
08/25/06	4	Open 3	23.8	8.51	8.67	21	19	14	0.01	< 0.01	1.16	< 0.005	0.061	61	63	42	50	69
07/07/06	1	Beach 1	24.6	9.66	8.94	11	99	38	< 0.01	0.04	1.12	0.006	0.049	49	60	52	52	69
07/07/06	1	Beach 2	24.7	9.42	8.95	13	35	29	< 0.01	0.02	1.28	0.005	0.045	45	59	64	54	70
07/07/06	1	Beach 3	25.0	9.50	8.96	12	50	38	< 0.01	0.01	0.96	< 0.005	0.028	28	52	77	57	70
07/29/06	2	Beach 1	26.5	10.32	9.06	25	54	40	< 0.01	< 0.01	1.48	< 0.005	0.048	48	60	68	59	71
07/29/06	2	Beach 2	26.5	10.20	9.06	24	45	34	< 0.01	< 0.01	1.43	< 0.005	0.053	53	61	60	46	68
07/29/06	2	Beach 3	26.5	10.75	9.06	26	53	38	< 0.01	< 0.01	1.27	< 0.005	0.052	52	61	54	58	70
08/10/06	3	Beach 1	26.4	9.33	8.94	26	51	35	< 0.01	0.02	1.30	< 0.005	0.047	47	60	62	54	70
08/10/06	3	Beach 2	26.4	8.90	8.95	28	50	33	< 0.01	0.03	1.51	0.006	0.054	54	62	63	45	68
08/10/06	3	Beach 3	26.6	9.08	8.96	28	48	33	< 0.01	0.02	1.60	< 0.005	0.057	57	62	63	61	71
08/27/06	4	Beach 1	25.2	10.43	8.87	24	49	36	< 0.01	0.03	1.04	< 0.005	0.057	57	62	42	55	70
08/27/06	4	Beach 2	25.2	10.28	8.89	23	51	38	< 0.01	0.02	1.32	< 0.005	0.059	59	63	50	71	72
08/27/06	4	Beach 3	25.5	10.38	8.92	24	50	37	< 0.01	0.03	1.29	< 0.005	0.230	230	83	13	52	69
Mean			25.38	9.23	8.84	21.0	49.8	34.2	0.01	0.02	1.29	0.001	0.06	61	62	53	56	70
Standard Error			0.22	0.30	0.04	1.5	2.4	1.1	0.00	0.004	0.03	0.000	0.01	7	1	3	2	0.4
Min			23.75	6.51	8.45	5.8	19.3	13.6	0.01	0.01	0.96	0.001	0.03	28	52	13	33	65
Max			26.90	12.52	9.06	31.2	99.0	39.8	0.06	0.09	1.60	0.006	0.23	230	83	77	83	74

The distribution of phytoplankton organisms is shown in Figure 3.10. Mona Lake is dominated by cyanobacteria during the summer months with biovolumes for open water and beach samples of 2.9 x 10^6 μm^3/ml and 2.6 x 10^6 μm^3/ml, respectively. Dinoflagellates also were a significant component of the phytoplankton community. The diatom and green algae assemblages were low (≈2 x 10^5 μm^3/ml). Open water samples contained greater cyanobacteria biovolumes than the beach samples. The composition of the cyanobacteria population for the beach and open water locations are given in Figures 3.11 and 3.12, respectively. *Anabaena flos-aquae*, *Limnothrix sp. Aphanizomenon gracile, Microcystis aeruginosa,* and *Planktothrix agardhii* were the dominant organism in all of the samples. *Anabaena flos-aquae* was more abundant in August than July and became the dominant organism for both August sampling events. *Cylindrospermopsis sp.* also was present in August. The maximum density of *Cylindrospermopsis* was 2,424 trichomes/ ml. *Cylindrospermopsis* densities in Mona Lake were similar to populations reported in Florida lakes (Chapman and Schelske 1997) and ten times greater historical data from Mona Lake (Hong et al. 2006).

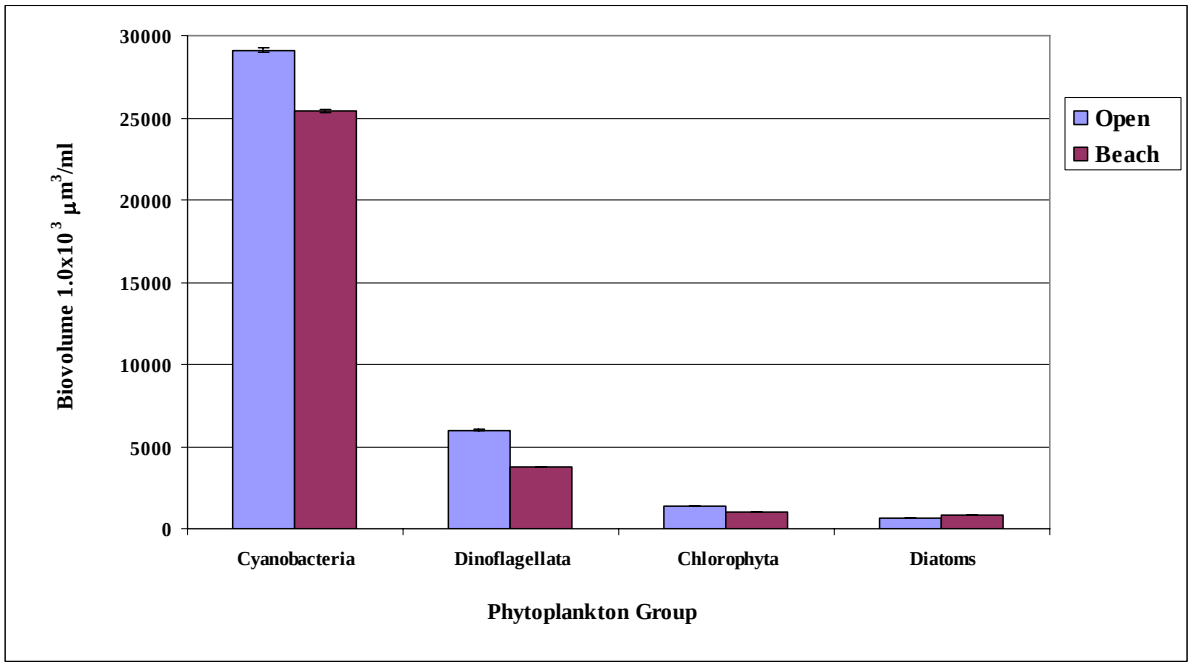


FIGURE 3.10. DISTRIBUTION OF PHYTOPLANKTON ORGANISMS IN MONA LAKE (2006).

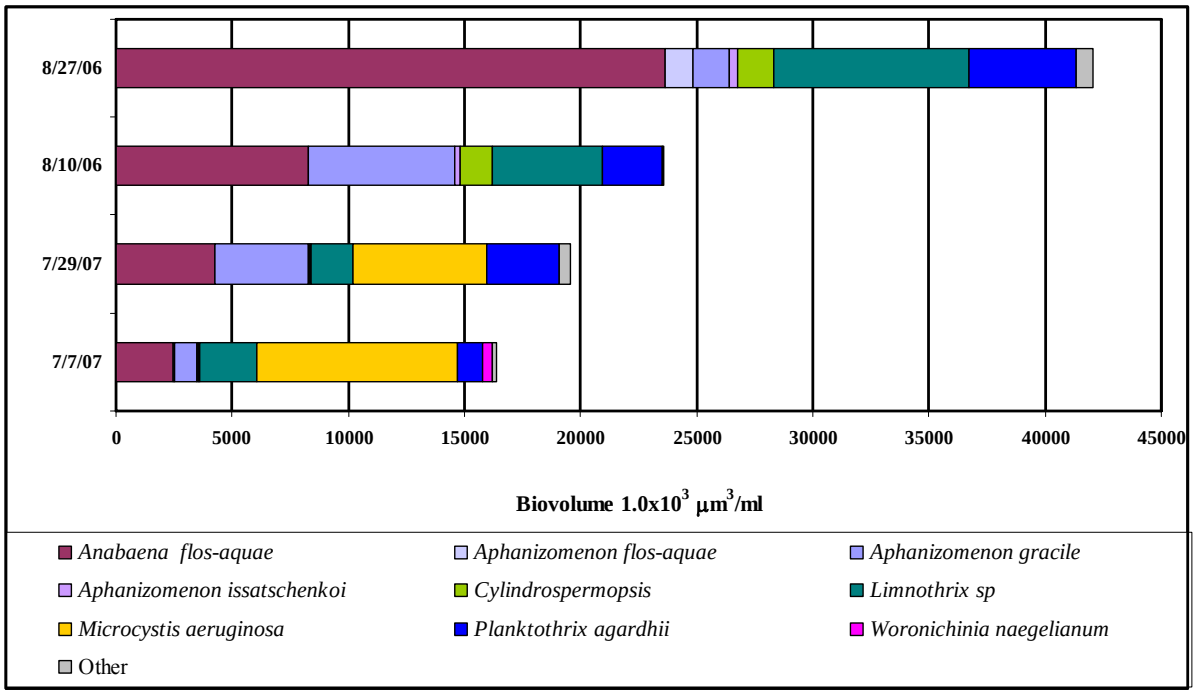


FIGURE 3.11. MEAN CYANOBACTERIA POPULATION COMPOSITION IN MONA LAKE BEACH LOCATIONS (2006).

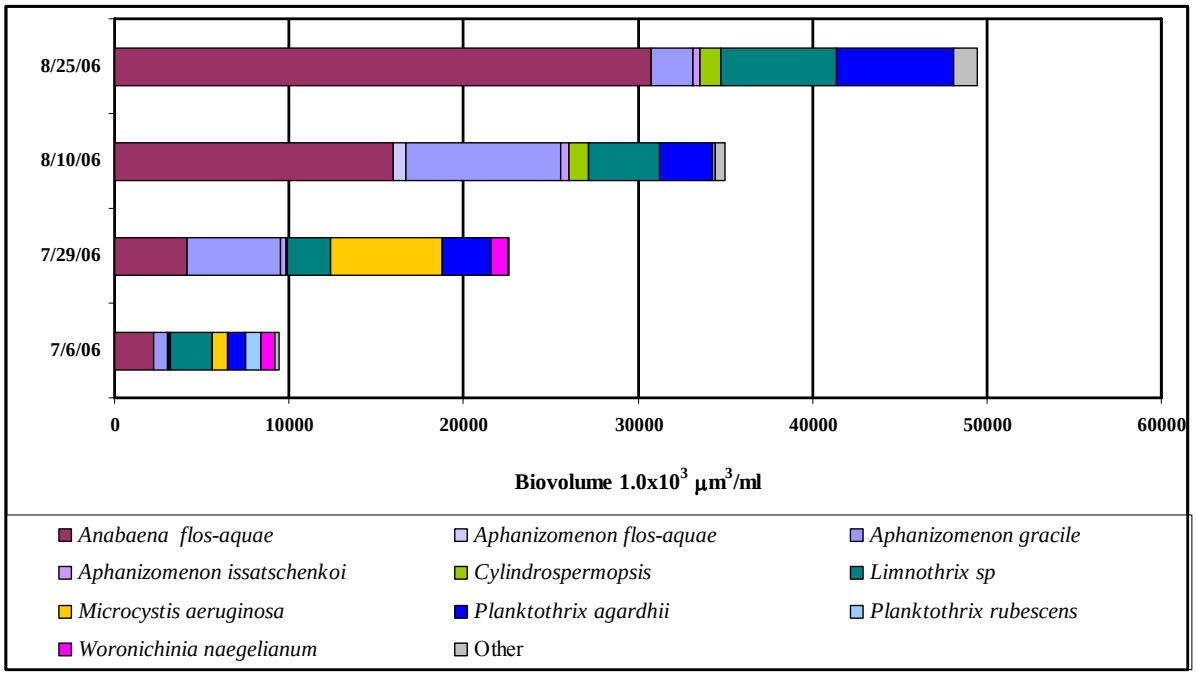


FIGURE 3.12. MEAN CYANOBACTERIA POPULATION COMPOSITION IN MONA LAKE OPEN WATER LOCATIONS (2006).

The cyanotoxin and cyanobacteria results for Mona Lake are summarized in Table 3.8. Mean microcystin activity by PPIA was 0.16 µg/l and results ranged from 0.064-0.41 µg/l. The mean microcystin LR concentration was 0.054 µg/l with a range of 0.005-0.096 µg/l. There was no significant difference in microcystin LR concentrations between beach and open water samples (Mann-Whitney p=0.94). Mean microcystin LR equivalents by ELISA and mean total microcystins by HPLC/MS were 0.083 µg/l and 0.057 µg/l, indicating that the ELISA method overestimated the total concentration. ELISA also overestimated the LR concentration by factor of 1.5. Microcystin LR was the most abundant congener detected with small amounts of YR and RR. PPIA results were two times higher than ELISA and HPLC/MS concentrations, indicating that other congeners were present. The maximum microcystin LR (0.096 µg/l) was less than the WHO moderate advisory level of 20 µg/l. Mean cyanobacteria cell counts (1.3 x 10^6/ml) and chlorophyll-*a* concentration (56 µg/l) were above the moderate WHO advisory levels of 1.0 x 10^5/ml, and 50 µg/l, respectively. Although cyanobacteria numbers and chlorophyll-*a* exceed the WHO guideline, many of the dominant organisms such as *Limnothrix sp*. *Planktothrix agardhii*, and *Aphanizomenon gracile* are not known for producing high levels of microcystins (Chorus et al. 2000). Anatoxin-a and cylindrospermopsin were not detected.

TABLE 3.8 CYANOTOXIN AND CYANOBACTERIA RESULTS FOR MONA LAKE (2006).

Date	Event	Site	Anatoxin-a (ug/L)	Cylindrospermopsin (ug/L)	PPIA (ug/L)	ELISA Conc. (ug/L)	HPLC/MS Total Conc. (ug/L)	HPLC/MS RR (ug/L)	HPLC/MS YR (ug/L)	HPLC/MS LA (ug/L)	HPLC/MS LR (ug/L)	Cyanobac Total # Cells per mL	Cyanobac Biovolume $\mu m^3/ml$	Chl *a* (ug/L)
07/06/06	1	Open 1	<0.01	<0.01	<0.01	0.024	0.010	< 0.001	< 0.001	< 0.001	0.010	3.51E+05	7.23E+06	46
07/06/06	1	Open 2	<0.01	<0.01	<0.01	0.022	0.006	< 0.001	< 0.001	< 0.001	0.006	5.85E+05	1.59E+07	56
07/06/06	1	Open 3	<0.01	<0.01	0.064	0.022	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	2.80E+05	5.07E+06	33
07/06/06	1	Open 3 D	<0.01	<0.01	<0.01	0.024	0.005	< 0.001	< 0.001	< 0.001	0.005	-	-	40
07/29/06	2	Open 1	<0.01	<0.01	0.252	0.090	0.059	0.005	< 0.001	< 0.001	0.054	8.39E+05	3.86E+07	60
07/29/06	2	Open 2	<0.01	<0.01	0.208	0.111	0.067	0.005	< 0.001	< 0.001	0.062	6.54E+05	1.92E+07	39
07/29/06	2	Open 3	<0.01	<0.01	0.140	0.075	0.041	0.004	< 0.001	< 0.001	0.037	3.60E+05	9.98E+06	56
07/29/06	2	Open 1 D	<0.01	<0.01	0.167	0.083	0.044	0.003	< 0.001	< 0.001	0.041	-	-	56
08/10/06	3	Open 1	<0.01	<0.01	0.124	0.074	0.071	< 0.001	< 0.001	< 0.001	0.071	1.06E+06	1.31E+07	61
08/10/06	3	Open 2	<0.01	<0.01	0.118	0.078	0.073	< 0.001	< 0.001	< 0.001	0.073	2.94E+06	6.47E+07	67
08/10/06	3	Open 2 D	<0.01	<0.01	0.091	0.068	0.057	< 0.001	< 0.001	< 0.001	0.057	-	-	62
08/10/06	3	Open 3	<0.01	<0.01	0.098	0.051	0.048	0.006	< 0.001	< 0.001	0.042	4.36E+06	2.71E+07	55
08/25/06	4	Open 1	<0.01	<0.01	0.167	0.106	0.082	0.006	< 0.001	< 0.001	0.076	2.90E+06	8.61E+07	78
08/25/06	4	Open 1 D	<0.01	<0.01	0.200	0.122	0.091	0.006	< 0.001	< 0.001	0.085	-	-	83
08/25/06	4	Open 2	<0.01	<0.01	0.170	0.093	0.089	0.004	0.002	< 0.001	0.083	2.18E+06	3.97E+07	62
08/25/06	4	Open 3	<0.01	<0.01	0.164	0.176	0.092	0.005	< 0.001	< 0.001	0.087	2.32E+06	2.26E+07	50
07/07/06	1	Beach 1	<0.01	<0.01	<0.01	0.026	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	4.68E+05	1.55E+07	52
07/07/06	1	Beach 2	<0.01	<0.01	0.104	0.027	0.011	< 0.001	< 0.001	< 0.001	0.011	6.29E+05	2.27E+07	54
07/07/06	1	Beach 3	<0.01	<0.01	<0.01	0.023	0.007	< 0.001	< 0.001	< 0.001	0.007	3.99E+05	1.08E+07	57
07/29/06	2	Beach 1	<0.01	<0.01	0.134	0.105	0.057	0.004	< 0.001	< 0.001	0.053	4.10E+05	7.13E+06	59
07/29/06	2	Beach 2	<0.01	<0.01	0.144	0.111	0.067	0.004	< 0.001	< 0.001	0.063	3.90E+05	1.19E+07	46
07/29/06	2	Beach 3	<0.01	<0.01	0.156	0.121	0.066	0.004	< 0.001	< 0.001	0.062	8.89E+05	3.96E+07	58
08/10/06	3	Beach 1	<0.01	<0.01	0.097	0.061	0.057	< 0.001	< 0.001	< 0.001	0.057	9.26E+05	2.60E+07	54
08/10/06	3	Beach 2	<0.01	<0.01	0.098	0.084	0.061	< 0.001	< 0.001	< 0.001	0.061	6.50E+05	2.01E+07	45
08/10/06	3	Beach 3	<0.01	<0.01	0.231	0.062	0.041	< 0.001	< 0.001	< 0.001	0.041	9.46E+05	2.47E+07	61
08/27/06	4	Beach 1	<0.01	<0.01	0.150	0.202	0.108	0.007	0.005	< 0.001	0.096	1.74E+06	4.44E+07	55
08/27/06	4	Beach 2	<0.01	<0.01	0.406	0.114	0.104	0.006	0.005	< 0.001	0.093	2.34E+06	5.33E+07	71
08/27/06	4	Beach 3	<0.01	<0.01	0.137	0.164	0.076	0.006	0.000	< 0.001	0.070	1.59E+06	2.85E+07	52
Mean			<0.01	<0.01	0.157	0.083	0.053	0.003	0.001	0.001	0.050	1.26E+06	2.73E+07	56
Standard Error			<0.01	<0.01	0.015	0.009	0.006	0.001	0.001	0.001	0.006	2.18E+05	4.04E+06	2
Min			<0.01	<0.01	0.064	0.022	0.001	0.001	0.001	0.001	0.001	2.80E+05	5.07E+06	33
Max			<0.01	<0.01	0.406	0.202	0.108	0.007	0.005	0.001	0.096	4.36E+06	8.61E+07	83
World Health Organization Moderate Advisory Level											20	1.0E+05	-	50

3.5 White Lake

The results of water chemistry and cyanobacteria analyses for White Lake are summarized in Table 3.9. Mean summer nitrate and ammonia concentrations were 0.03 mg/l and 0.04 mg/l, respectively. Mean SRP was <0.005 mg/l and ranged from <0.005-0.010 mg/l. Mean TP and TKN concentrations were 0.030 mg/l and 0.72 mg/l, respectively. TP concentrations ranged from 0.02-0.0.05 mg/l while TKN results ranged from 0.23-1.66 mg/l. Mean molar TN:TP ratio for White Lake was 64, suggesting phosphorus limitation. The mean summer chlorophyll-*a* was 19 µg/l and ranged from 8-35 µg/l. Based on standard values for chlorophyll-*a* and TP used to assess lake trophic status (Cooke et al. 2003), concentrations in White Lake would indicate eutrophic status. The summer mean Carlson TSI values for TP and chlorophyll-*a* were 52 and 59, respectively. These TSI values again indicate that White Lake is a low to mid eutrophic system.

TABLE 3.9. WATER CHEMISTRY RESULTS FOR WHITE LAKE (2006). (TSI=CARLSON TROPHIC STATE INDEX).

Date	Event	Site	Water Temp °C	DO (mg/L)	pH	Turb (NTU)	Cl (mg/L)	SO4 (mg/L)	NO3-N (mg/L)	NH3-N (mg/L)	TKN-N (mg/L)	SRP-P (mg/L)	TP-P (mg/L)	TP-P (ug/L)	TSI TP	Ratio TN:TP	Chl *a* (ug/L)	TSI Chl *a*
07/03/06	1	Open 1	23.5	6.68	8.50	4	30	18	0.02	0.03	0.96	0.007	0.022	22	49	102	18	59
07/03/06	1	Open 2	23.0	8.20	8.69	6	35	18	0.01	0.03	0.88	0.007	0.020	20	47	102	11	54
07/03/06	1	Open 3	22.2	8.26	8.63	5	34	19	< 0.01	0.02	0.84	< 0.005	0.016	16	44	119	13	56
07/18/06	2	Open 1	25.4	6.61	8.12	3	27	19	0.14	0.10	0.60	0.007	0.032	32	54	58	11	54
07/18/06	2	Open 1 D	25.4	6.61	8.12	5	24	19	0.13	0.10	0.66	0.007	0.034	34	55	58	12	55
07/18/06	2	Open 2	25.6	6.62	8.53	3	22	18	0.02	0.08	0.68	0.006	0.045	45	59	38	13	56
07/18/06	2	Open 3	25.1	6.58	8.55	4	27	19	0.01	0.05	0.64	< 0.005	0.025	25	51	62	13	56
08/02/06	3	Open 1	27.4	6.49	8.34	12	25	16	0.04	0.04	0.52	< 0.005	0.031	31	54	43	19	59
08/02/06	3	Open 2	26.5	7.97	8.50	8	24	17	< 0.01	0.02	0.54	< 0.005	0.024	24	50	52	16	58
08/02/06	3	Open 3	26.2	8.33	8.44	9	23	16	< 0.01	0.02	0.49	< 0.005	0.027	27	52	42	15	57
08/02/06	3	Open 2 D	26.5	7.97	8.50	9	23	18	< 0.01	0.02	0.49	< 0.005	0.026	26	51	44	15	57
08/23/06	4	Open 1	23.8	9.46	8.62	6	24	19	< 0.01	< 0.01	0.48	< 0.005	0.019	19	47	56	33	65
08/23/06	4	Open 1 D	23.8	9.46	8.62	9	28	19	< 0.01	< 0.01	0.50	< 0.005	0.020	20	47	55	32	65
08/23/06	4	Open 2	24.2	9.17	8.63	6	24	21	< 0.01	< 0.01	0.23	< 0.005	0.022	22	49	23	30	64
08/23/06	4	Open 3	23.7	8.92	8.58	8	24	21	< 0.01	< 0.01	0.54	< 0.005	0.017	17	45	70	27	63
07/03/06	1	Beach 1	23.8	8.53	8.68	25	40	18	0.02	0.02	1.66	< 0.005	0.050	50	61	75	15	57
07/03/06	1	Beach 2	23.7	8.38	8.69	28	47	18	0.02	0.02	1.16	< 0.005	0.032	32	54	83	13	56
07/03/06	1	Beach 3	23.9	8.42	8.72	20	31	19	0.02	0.05	1.04	< 0.005	0.031	31	54	80	13	56
07/03/06	1	Beach 3 D	23.9	8.42	8.72	22	37	18	0.02	0.02	1.20	< 0.005	0.039	39	57	70	12	55
07/19/06	2	Beach 1	24.7	6.94	8.30	8	24	19	0.06	0.04	0.54	< 0.005	0.027	27	52	53	11	54
07/19/06	2	Beach 2	24.5	6.25	8.21	7	24	19	0.07	0.05	0.72	< 0.005	0.023	23	49	81	10	53
07/19/06	2	Beach 3	24.6	6.18	8.19	5	23	19	0.06	0.05	0.74	< 0.005	0.021	21	48	89	8	51
08/07/06	3	Beach 1	27.7	10.94	8.80	6	33	20	< 0.01	0.02	0.78	0.006	0.043	43	58	41	20	60
08/07/06	3	Beach 2	28.0	11.01	8.89	22	30	20	< 0.01	0.03	0.61	< 0.005	0.041	41	58	34	29	64
08/07/06	3	Beach 3	27.9	10.06	8.88	19	27	20	< 0.01	0.03	0.61	< 0.005	0.035	35	55	40	22	61
08/18/06	4	Beach 1	23.9	8.20	8.48	9	40	22	< 0.01	0.06	0.62	< 0.005	0.023	23	49	66	35	65
08/18/06	4	Beach 2	23.9	7.95	8.48	6	46	21	< 0.01	0.06	0.68	< 0.005	0.019	19	47	86	30	64
08/18/06	4	Beach 3	23.9	7.49	8.47	7	30	21	< 0.01	0.07	0.69	< 0.005	0.030	30	53	56	31	64
Mean			24.86	8.08	8.53	9.9	29.5	18.9	0.03	0.04	0.72	0.003	0.03	28	52	64	19	59
Standard Error			0.29	0.25	0.04	1.4	1.3	0.3	0.01	0.01	0.05	0.000	0.00	2	1	4	2	1
Min			22.15	6.18	8.12	2.6	22.4	15.7	0.01	0.01	0.23	0.002	0.02	16	44	23	8	51
Max			27.96	11.01	8.89	27.7	46.8	21.9	0.14	0.10	1.66	0.01	0.05	50	61	119	35	65

The distribution of phytoplankton organisms is shown in Figure 3.13. White Lake is dominated by cyanobacteria during the summer months with biovolumes for open water and beach samples of 7.2 x 10^6 μm^3/ml and 5.5 x 10^6 μm^3/ml, respectively. Dinoflagellates and diatoms also were components of the phytoplankton community. The green algae assemblages were low (≈6 x 10^4 μm^3/ml). Open water samples contained greater cyanobacteria biovolumes than the beach samples. The composition of the cyanobacteria population for the beach and open water locations are given in Figures 3.14 and 3.15, respectively. *Microcystis wesenbergii* was the dominant organism in all but one of the sampling events *Anabaena flos-aquae*, *Anabaena mendotae*, and *Microcystis aeruginosa*, also were abundant. Both species of *Microcystis* appeared to crash on July 18-19, 2006 and were replaced by *Aphanizomenon issatschenkoi* in the open water and beach locations. *Cylindrospermopsis* was not found in White Lake.

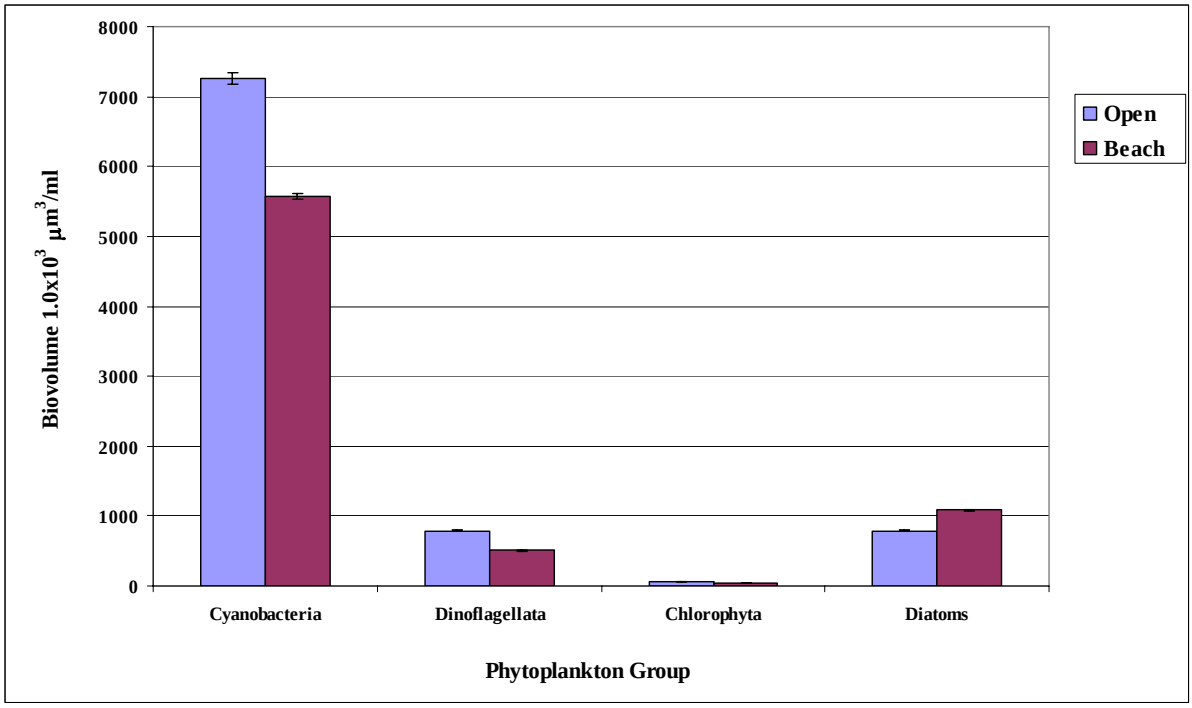


FIGURE 3.13. DISTRIBUTION OF PHYTOPLANKTON ORGANISMS IN WHITE LAKE (2006).

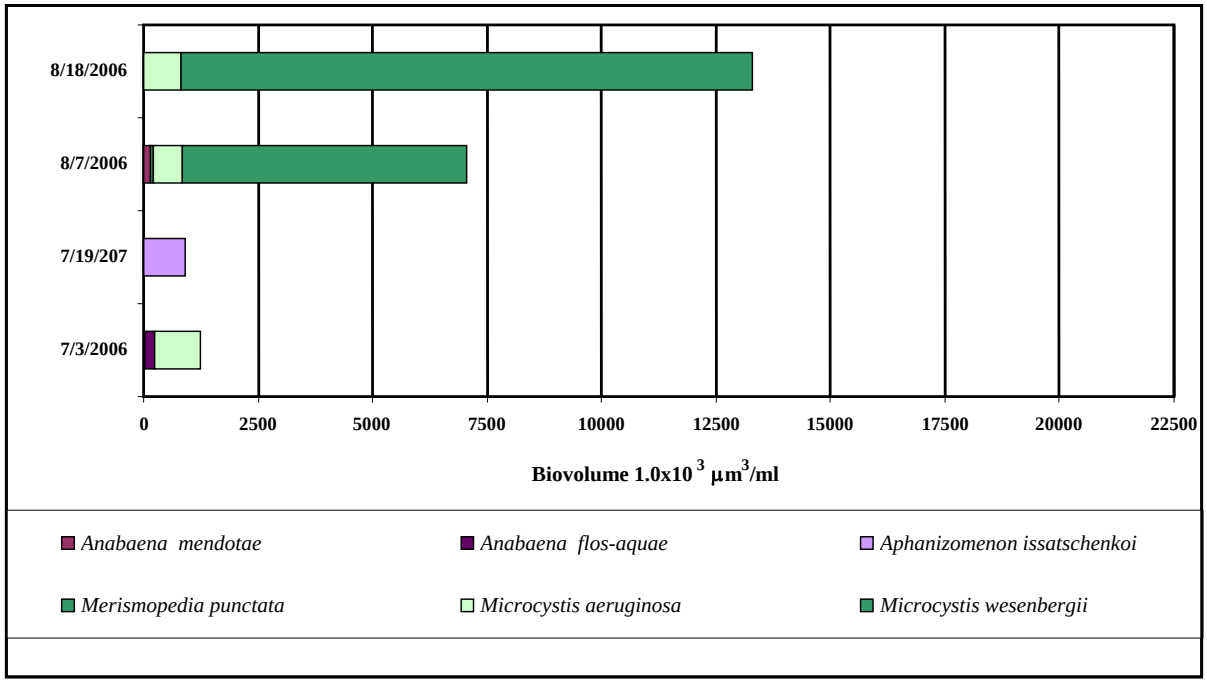


FIGURE 3.14. MEAN CYANOBACTERIA POPULATION COMPOSITION IN WHITE LAKE BEACH LOCATIONS (2006).

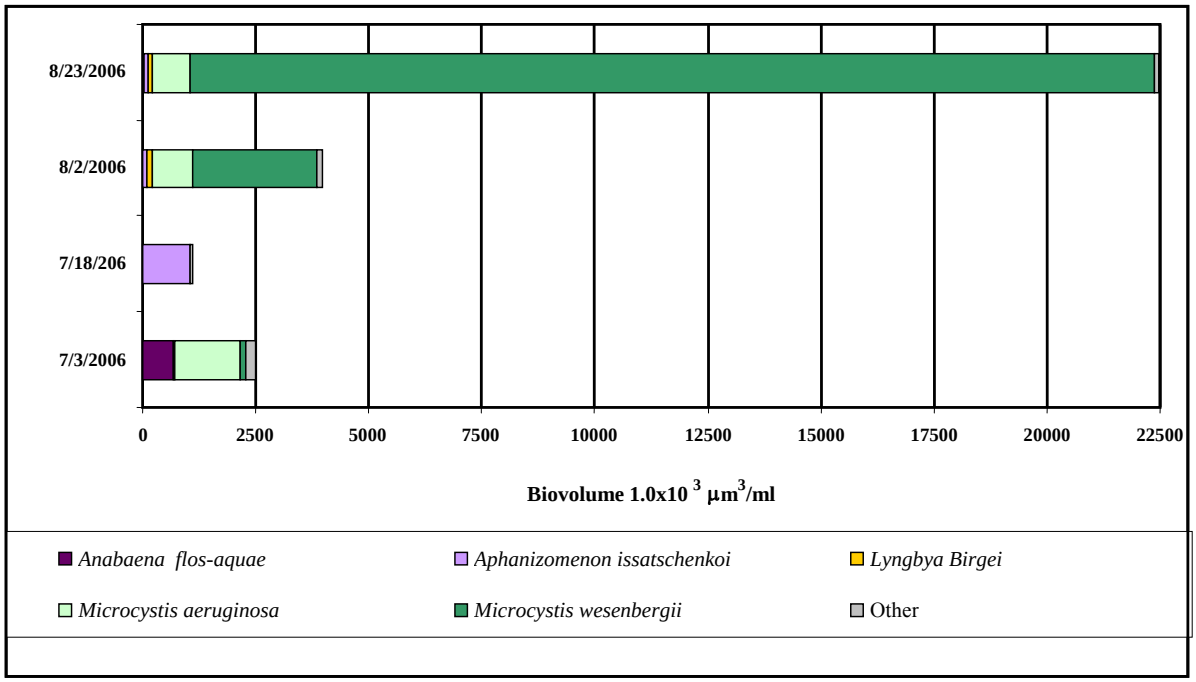


FIGURE 3.15. MEAN CYANOBACTERIA POPULATION COMPOSITION IN WHITE LAKE OPEN WATER LOCATIONS (2006).

The cyanotoxin and cyanobacteria results for White Lake are summarized in Table 3.10. Mean microcystin activity by PPIA was 0.11 µg/l and results ranged from <0.01-0.28 µg/l. The mean microcystin LR concentration was 0.061 µg/l with a range of 0.003-0.23 µg/l. There was no significant difference in microcystin LR concentrations between beach and open water samples (Mann-Whitney p=0.77). Mean microcystin LR equivalents by ELISA and mean total microcystins by HPLC/MS were 0.09 µg/l and 0.09 µg/l, respectively, indicating that both methods provided similar results. ELISA, however overestimated the LR concentration by factor of 1.5. Microcystin LR was the most abundant congener detected and LA, YR and RR were present in almost equal amounts. PPIA results were similar to ELISA and HPLC/MS concentrations, indicating that other congeners were not present. The maximum microcystin LR (0.28 µg/l) was less than the WHO moderate advisory level of 20 µg/l. Mean cyanobacteria cell counts (9.4×10^4) and chlorophyll-*a* concentration (19 µg/l) also were less than the moderate WHO advisory levels of 1.0×10^5, and 50 µg/l, respectively. Although some of the cyanobacteria numbers exceed the WHO guideline, the dominant organism, *Microcystis wesenbergii,* is not known for producing high levels of microcystins (Chorus et al. 2000). Anatoxin-a and cylindrospermopsin were not detected.

TABLE 3.10 CYANOTOXIN AND CYANOBACTERIA RESULTS FOR WHITE LAKE (2006).

Date	Event	Site	Anatoxin-a (ug/L)	Cylindrospermopsin (ug/L)	PPIA (ug/L)	ELISA Conc. (ug/L)	HPLC/MS Total Conc. (ug/L)	HPLC/MS RR (ug/L)	HPLC/MS YR (ug/L)	HPLC/MS LA (ug/L)	HPLC/MS LR (ug/L)	Cyanobac Total # Cells per mL	Cyanobac Biovolume $\mu m^3/ml$	Chl *a* (ug/L)
07/03/06	1	Open 1	<0.01	<0.01	< 0.01	0.020	0.017	0.003	0.003	< 0.001	0.011	8.70E+04	6.81E+06	18
07/03/06	1	Open 2	<0.01	<0.01	< 0.01	0.020	0.023	0.006	0.006	< 0.001	0.011	4.91E+03	1.83E+05	11
07/03/06	1	Open 3	<0.01	<0.01	0.07	0.020	0.033	0.009	0.008	< 0.001	0.016	8.12E+03	4.68E+05	13
07/18/06	2	Open 1	<0.01	<0.01	< 0.01	< 0.01	0.008	0.002	0.000	< 0.001	0.006	3.00E+02	1.85E+04	11
07/18/06	2	Open 1 D	<0.01	<0.01	< 0.01	< 0.01	0.006	0.002	0.000	< 0.001	0.004	-	-	12
07/18/06	2	Open 2	<0.01	<0.01	0.08	0.031	0.009	0.001	0.004	< 0.001	0.004	2.13E+03	9.58E+04	13
07/18/06	2	Open 3	<0.01	<0.01	0.10	0.023	0.013	0.003	0.005	< 0.001	0.005	7.47E+03	2.26E+05	13
08/02/06	3	Open 1	<0.01	<0.01	0.20	0.156	0.221	0.039	0.039	0.014	0.129	8.84E+04	6.48E+06	19
08/02/06	3	Open 2	<0.01	<0.01	0.23	0.150	0.211	0.021	0.056	0.017	0.117	4.07E+04	2.80E+06	16
08/02/06	3	Open 3	<0.01	<0.01	0.25	0.162	0.297	0.032	0.071	0.029	0.165	8.24E+04	2.67E+06	15
08/02/06	3	Open 2 D	<0.01	<0.01	0.28	0.126	0.201	0.018	0.061	0.012	0.110	-	-	15
08/23/06	4	Open 1	<0.01	<0.01	0.15	0.083	0.114	0.021	0.015	0.013	0.065	3.43E+05	2.53E+07	33
08/23/06	4	Open 1 D	<0.01	<0.01	0.13	0.079	0.075	0.019	0.004	0.010	0.042	-	-	32
08/23/06	4	Open 2	<0.01	<0.01	0.18	0.100	0.143	0.042	0.011	0.017	0.073	4.99E+05	3.42E+07	30
08/23/06	4	Open 3	<0.01	<0.01	0.22	0.130	0.165	0.047	0.033	0.014	0.071	1.09E+05	7.94E+06	27
07/03/06	1	Beach 1	<0.01	<0.01	< 0.01	0.059	0.052	0.018	0.005	< 0.001	0.029	2.73E+04	2.08E+06	15
07/03/06	1	Beach 2	<0.01	<0.01	< 0.01	0.021	0.015	< 0.001	0.006	< 0.001	0.009	2.12E+04	1.08E+06	13
07/03/06	1	Beach 3	<0.01	<0.01	< 0.01	0.035	0.026	0.005	0.005	< 0.001	0.016	1.41E+04	1.03E+06	13
07/03/06	1	Beach 3 D	<0.01	<0.01	0.09	0.029	0.035	0.003	0.007	< 0.001	0.025	-	-	12
07/19/06	2	Beach 1	<0.01	<0.01	0.10	< 0.01	0.007	0.001	< 0.001	< 0.001	0.006	1.02E+03	4.38E+04	11
07/19/06	2	Beach 2	<0.01	<0.01	< 0.01	< 0.01	0.004	0.001	< 0.001	< 0.001	0.003	4.13E+02	8.22E+03	10
07/19/06	2	Beach 3	<0.01	<0.01	< 0.01	< 0.01	0.003	< 0.001	< 0.001	< 0.001	0.003	3.51E+02	6.98E+03	8
08/07/06	3	Beach 1	<0.01	<0.01	0.23	0.337	0.359	0.050	0.066	0.055	0.188	8.86E+04	6.53E+06	20
08/07/06	3	Beach 2	<0.01	<0.01	0.26	0.302	0.389	0.039	0.064	0.055	0.231	1.37E+05	1.10E+07	29
08/07/06	3	Beach 3	<0.01	<0.01	< 0.01	0.233	0.260	0.021	0.042	0.044	0.153	7.02E+04	5.02E+06	22
08/18/06	4	Beach 1	<0.01	<0.01	0.15	0.120	0.131	0.015	0.015	0.026	0.075	2.83E+05	2.00E+07	35
08/18/06	4	Beach 2	<0.01	<0.01	0.15	0.119	0.102	0.009	0.010	0.016	0.067	1.32E+05	9.71E+06	30
08/18/06	4	Beach 3	<0.01	<0.01	0.15	0.182	0.148	0.012	0.031	0.024	0.081	1.41E+05	1.03E+07	31
Mean			<0.01	<0.01	0.110	0.091	0.110	0.016	0.020	0.013	0.061	9.12E+04	6.42E+06	19
Standard Error			<0.01	<0.01	0.02	0.017	0.022	0.003	0.004	0.003	0.012	2.53E+04	1.80E+06	2
Min			<0.01	<0.01	<0.01	<0.01	0.003	0.001	0.001	0.001	0.003	3.00E+02	6.98E+03	8
Max			<0.01	<0.01	0.28	0.337	0.389	0.050	0.071	0.055	0.231	4.99E+05	3.42E+07	35
World Health Organization Moderate Advisory Level											20	1.0E+05	-	50

3.6 Muskegon Lake

The results of water chemistry and cyanobacteria analyses for Muskegon Lake are summarized in Table 3.11. Mean summer nitrate and ammonia concentrations were 0.01 mg/l and 0.05 mg/l, respectively. Mean SRP was 0.006 mg/l and ranged from <0.005-0.007 mg/l. Mean TP and TKN concentrations were 0.020 mg/l and 0.66 mg/l, respectively. TP concentrations ranged from 0.01-0.0.05 mg/l while TKN results ranged from 0.28-1.93 mg/l. Mean molar TN:TP ratio for Muskegon Lake was 81, suggesting phosphorus limitation. The mean summer chlorophyll-*a* was 10 µg/l and ranged from 6-33 µg/l. Based on standard values for chlorophyll-*a* and TP used to assess lake trophic status (Cooke et al. 2003), concentrations in Muskegon Lake would indicate mesotrophic/eutrophic status. The summer mean Carlson TSI values for TP and chlorophyll-*a* were 49 and 53, respectively. These TSI values again indicate that Muskegon Lake is an upper mesotrophic to low eutrophic system.

TABLE 3.11. WATER CHEMISTRY RESULTS FOR MUSKEGON LAKE (2006). (TSI=CARLSON TROPHIC STATE INDEX).

Date	Event	Site	Water Temp °C	DO (mg/L)	pH	Turb (NTU)	Cl (mg/L)	SO4 (mg/L)	NO3-N (mg/L)	NH3-N (mg/L)	TKN-N (mg/L)	SRP-P (mg/L)	TP-P (mg/L)	TP-P (ug/L)	TSI TP	Ratio TN:TP	Chl *a* (ug/L)	TSI Chl *a*
07/06/06	1	Open 1	23.6	7.05	8.14	4	24	15	0.16	0.04	0.64	< 0.005	0.017	17	45	109	8	51
07/06/06	1	Open 2	23.6	7.00	8.27	5	48	15	0.12	0.03	0.64	< 0.005	0.018	18	46	97	8	51
07/06/06	1	Open 3	22.8	7.05	8.22	3	16	12	0.10	0.04	0.70	< 0.005	0.014	14	42	133	7	50
07/06/06	1	Open 1 D	23.6	7.05	8.14	3	23	16	0.19	0.03	0.70	< 0.005	0.014	14	42	146	8	51
07/25/06	2	Open 1	25.4	7.68	8.31	7	25	19	0.17	0.03	0.28	< 0.005	0.036	36	56	29	15	57
07/25/06	2	Open 2	25.3	7.32	8.34	5	25	19	0.16	0.02	0.55	< 0.005	0.034	34	55	47	9	52
07/25/06	2	Open 3	24.3	6.45	8.21	3	190	15	0.10	0.02	0.56	< 0.005	0.025	25	51	60	8	50
07/25/06	2	Open 2 D	25.3	7.32	8.34	5	53	20	0.15	0.02	0.63	< 0.005	0.032	32	54	56	10	53
08/04/06	3	Open 1	26.9	7.12	8.23	7	22	13	0.07	0.07	0.58	0.006	0.025	25	51	64	8	51
08/04/06	3	Open 2	27.2	7.45	8.28	7	28	17	0.09	0.05	0.50	< 0.005	0.027	27	52	53	8	51
08/04/06	3	Open 3	26.6	8.19	8.39	5	41	19	0.09	0.04	0.67	< 0.005	0.030	30	53	59	7	50
08/04/06	3	Open 3 D	26.6	8.19	8.39	5	38	19	0.08	0.05	0.74	< 0.005	0.034	34	55	57	6	49
08/24/06	4	Open 1	24.2	8.50	8.27	7	26	24	0.12	0.02	0.47	< 0.005	0.025	25	51	54	8	51
08/24/06	4	Open 1 D	24.2	8.50	8.27	6	26	24	0.12	0.43	0.92	< 0.005	0.024	24	50	135	8	51
08/24/06	4	Open 2	24.1	9.18	8.49	9	25	22	< 0.01	0.01	0.64	< 0.005	0.024	24	50	60	8	51
08/24/06	4	Open 3	23.9	8.77	8.56	8	26	22	< 0.01	0.01	0.60	< 0.005	0.018	18	46	75	10	53
07/07/06	1	Beach 1	23.4	7.51	8.33	3	24	17	0.10	0.03	0.62	< 0.005	0.016	16	44	104	7	49
07/07/06	1	Beach 2	23.5	7.22	8.36	3	23	17	0.10	0.04	0.60	< 0.005	0.015	15	43	108	8	50
07/07/06	1	Beach 3	23.2	6.88	8.30	3	24	17	0.10	0.03	0.54	< 0.005	0.014	14	42	105	7	49
07/31/06	2	Beach 1	27.2	8.07	8.40	3	24	16	0.09	0.03	0.51	< 0.005	0.022	22	49	63	14	57
07/31/06	2	Beach 2	26.9	7.96	8.38	3	23	18	0.10	0.02	0.70	0.005	0.021	21	48	87	9	53
07/31/06	2	Beach 3	27.0	7.77	8.39	2	36	21	0.13	0.03	0.54	0.005	0.020	20	47	77	9	52
08/10/06	3	Beach 1	25.5	8.52	8.39	7	28	19	0.05	0.03	0.62	< 0.005	0.023	23	49	67	10	54
08/10/06	3	Beach 2	25.5	8.11	8.43	14	27	18	0.05	0.04	0.53	< 0.005	0.020	20	47	69	9	52
08/10/06	3	Beach 3	25.5	7.79	8.42	5	26	17	0.04	0.05	0.59	< 0.005	0.022	22	49	68	9	53
08/22/06	4	Beach 1	24.8	8.81	8.55	7	27	22	< 0.01	< 0.01	0.63	< 0.005	0.015	15	43	93	15	57
08/22/06	4	Beach 2	25.3	8.72	8.58	8	30	22	< 0.01	< 0.01	0.58	< 0.005	0.016	16	44	80	9	52
08/22/06	4	Beach 3	25.0	8.78	8.59	7	27	20	< 0.01	< 0.01	0.61	< 0.005	0.018	18	46	75	9	52
08/04/06	3	Fish Land	27.2	5.69	7.96	5	23	13	0.08	0.12	1.93	0.007	0.053	53	61	89	33	65
08/24/06	4	AWRI	24.0	7.37	8.09	9	28	20	0.03	0.03	0.81	< 0.005	0.025	25	51	78	18	59
09/15/06	5	Channel	-	-	-	-	32	21	0.06	0.09	0.85	< 0.005	0.022	22	49	101	ND	ND
Mean			25.04	7.73	8.33	6	33	18	0.09	0.05	0.66	0.002	0.02	23	49	81	10	53
Standard Error			0.25	0.15	0.03	0.5	5	1	0.01	0.01	0.05	0.000	0.00	1	1	5	1	1
Min			22.76	5.69	7.96	2	16	12	0.01	0.01	0.28	0.002	0.01	14	42	29	6	49
Max			27.21	9.18	8.59	14	190	24	0.19	0.43	1.93	0.007	0.05	53	61	146	33	65

The distribution of phytoplankton organisms is shown in Figure 3.16. Muskegon Lake is dominated by cyanobacteria during the summer months with biovolumes for open water and beach samples of 2.6 x 10^6 μm^3/ml and 2.1 x 10^6 μm^3/ml, respectively. Dinoflagellates and diatoms also were components of the phytoplankton community, with green algae in lower abundance. Open water and beach samples contained similar cyanobacteria biovolumes. The composition of the cyanobacteria population for the beach and open water locations are given in Figures 3.17 and 3.18, respectively. *Microcystis aeruginosa* was the dominant organism in all but one of the sampling events. The beach samples on August 22, 2006 showed a shift to dominance by *Microcystis wesenbergii.* The maximum density of *Cylindrospermopsis* was 109 trichomes/ ml. *Cylindrospermopsis* densities in Muskegon Lake were100x greater than prior data from Muskegon Lake (Hong et al. 2006).

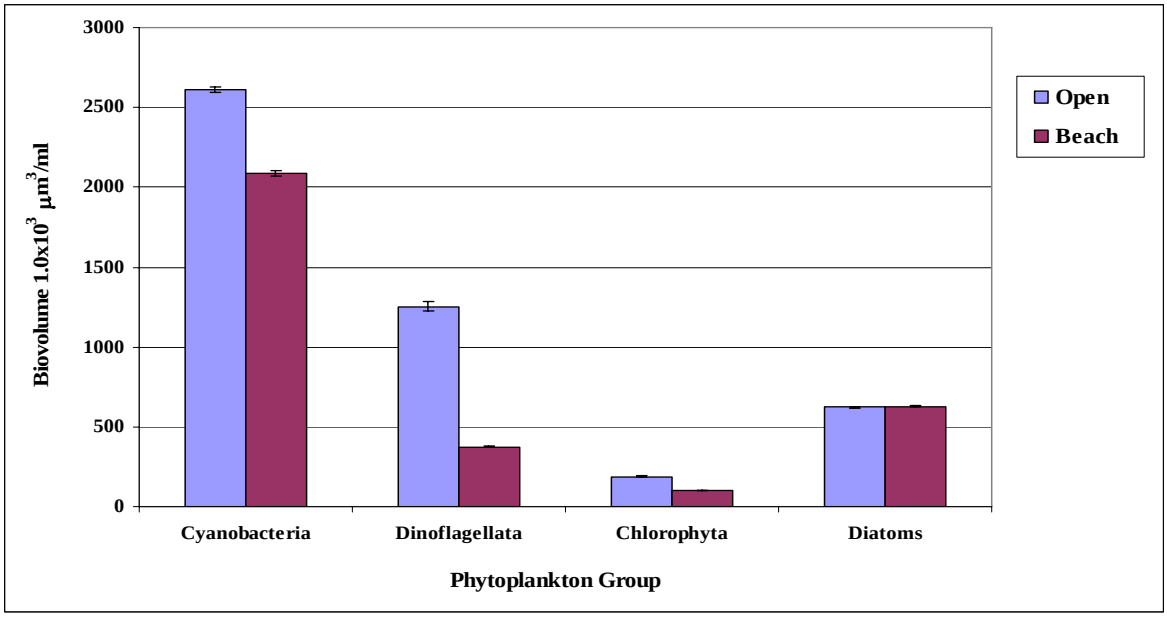


FIGURE 3.16. DISTRIBUTION OF PHYTOPLANKTON ORGANISMS IN MUSKEGON LAKE (2006).

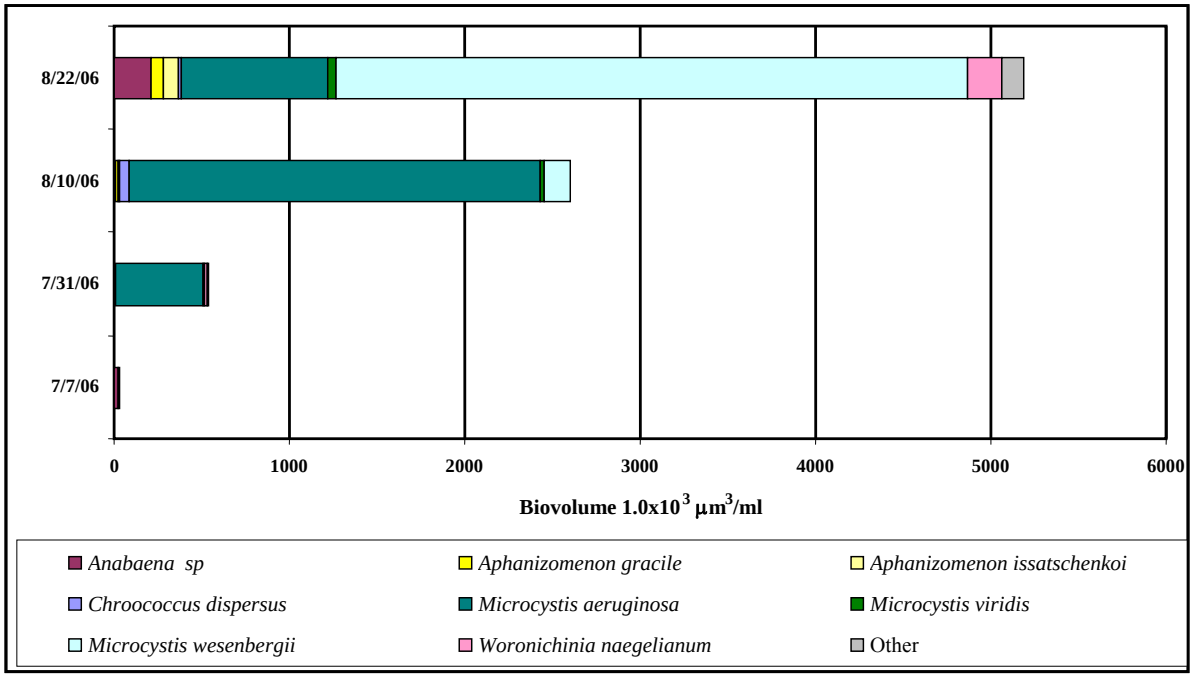


FIGURE 3.17. MEAN CYANOBACTERIA POPULATION COMPOSITION IN MUSKEGON LAKE BEACH LOCATIONS (2006).

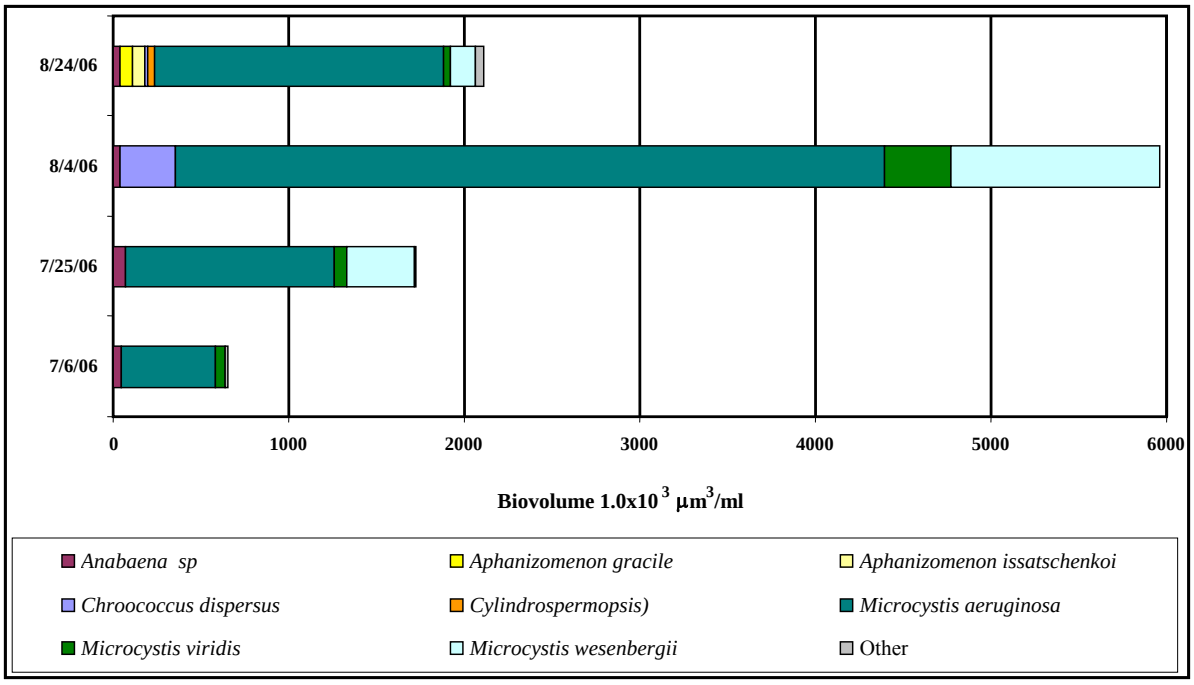


FIGURE 3.18. MEAN CYANOBACTERIA POPULATION COMPOSITION IN MUSKEGON LAKE OPEN WATER LOCATIONS (2006).

The cyanotoxin and cyanobacteria results for Muskegon Lake are summarized in Table 3.12. Mean microcystin activity by PPIA was 0.89 µg/l and results ranged from 0.08-8.0 µg/l. The mean microcystin LR concentration was 0.90 µg/l with a range of 0.010-12 µg/l. There was no significant difference in microcystin LR concentrations between beach and open water samples (Mann-Whitney p=0.52). Mean microcystin LR equivalents by ELISA and mean total microcystins by HPLC/MS were 0.99 µg/l and 1.09 µg/l, respectively. These data indicate that both methods provided similar results. ELISA and HPLC/MS results for microcystin LR also were similar. The low percentage of congeners other than LR would reduce the opportunity for cross reactivity in the ELISA test. Microcystin LR was the most abundant congener detected and LA, YR and RR were present at concentrations less than 10% of the LR value. PPIA results were similar to ELISA and HPLC/MS concentrations. The dominance microcystin LR in Muskegon Lake resulted in good agreement between the three methods. The maximum microcystin LR (12.7 µg/l) was less than the WHO moderate advisory level of 20 µg/l. Mean cyanobacteria cell counts (3.5 x 10^4/ml) and chlorophyll-*a* concentration (10 µg/l) also were less the moderate WHO advisory levels of 1.0 x 10^5/ml, and 50 µg/l, respectively. The bloom sample collected at Fisherman's Landing on 8/04/06 contained the highest level of microcystin LR in the 2006 data set. This sample was surface scum that appeared to be >90% *Microcystis aeruginosa*. Muskegon Lake also had the second highest mean microcystin LR concentration in the data set. Anatoxin-a and cylindrospermopsin were not detected.

TABLE 3.12 CYANOTOXIN AND CYANOBACTERIA RESULTS FOR MUSKEGON LAKE (2006).

Date	Event	Site	Anatoxin-a (ug/L)	Cylindrospermopsin (ug/L)	PPIA (ug/L)	ELISA Conc. (ug/L)	HPLC/MS Total Conc. (ug/L)	HPLC/MS RR (ug/L)	HPLC/MS YR (ug/L)	HPLC/MS LA (ug/L)	HPLC/MS LR (ug/L)	Cyanobac Total # Cells per mL	Cyanobac Biovolume $\mu m^3/ml$	Chl *a* (ug/L)
07/06/06	1	Open 1	<0.01	<0.01	0.081	0.016	0.007	< 0.001	< 0.001	< 0.001	0.007	1.97E+04	1.37E+06	8
07/06/06	1	Open 2	<0.01	<0.01	< 0.01	0.022	0.010	0.003	< 0.001	< 0.001	0.007	5.68E+03	1.88E+05	8
07/06/06	1	Open 3	<0.01	<0.01	0.078	0.022	0.024	0.006	< 0.001	< 0.001	0.018	8.37E+03	4.05E+05	7
07/06/06	1	Open 1 D	<0.01	<0.01	<0.01	0.020	0.018	0.002	< 0.001	< 0.001	0.016	-	-	8
07/25/06	2	Open 1	<0.01	<0.01	0.228	0.161	0.273	0.060	0.015	0.020	0.178	2.44E+04	1.81E+06	15
07/25/06	2	Open 2	<0.01	<0.01	0.336	0.231	0.640	0.085	0.032	0.050	0.473	3.67E+04	2.62E+06	9
07/25/06	2	Open 3	<0.01	<0.01	0.169	0.124	0.192	0.050	0.011	0.013	0.118	1.17E+04	7.49E+05	8
07/25/06	2	Open 2 D	<0.01	<0.01	0.283	0.228	0.603	0.119	0.024	0.017	0.443	-	-	10
08/04/06	3	Open 1	<0.01	<0.01	0.398	0.410	0.634	0.081	< 0.001	< 0.001	0.553	1.71E+05	1.02E+07	8
08/04/06	3	Open 2	<0.01	<0.01	0.367	0.451	0.718	0.100	0.022	< 0.001	0.596	8.77E+04	6.08E+06	8
08/04/06	3	Open 3	<0.01	<0.01	0.252	0.204	0.341	0.061	0.007	< 0.001	0.273	2.71E+04	1.62E+06	7
08/04/06	3	Open 3 D	<0.01	<0.01	1.415	1.150	0.836	0.112	0.043	< 0.001	0.681	-	-	6
08/24/06	4	Open 1	<0.01	<0.01	0.401	0.255	0.317	0.055	0.010	0.030	0.222	9.11E+03	5.29E+05	8
08/24/06	4	Open 1 D	<0.01	<0.01	0.459	0.243	0.342	0.056	0.013	< 0.001	0.273	-	-	8
08/24/06	4	Open 2	<0.01	<0.01	0.942	0.685	0.763	0.101	0.051	< 0.001	0.611	5.28E+04	3.44E+06	8
08/24/06	4	Open 3	<0.01	<0.01	1.420	0.965	1.208	0.172	0.147	< 0.001	0.889	3.55E+04	2.36E+06	10
07/07/06	1	Beach 1	<0.01	<0.01	0.150	0.061	0.056	0.004	0.006	< 0.001	0.046	2.33E+02	1.08E+04	7
07/07/06	1	Beach 2	<0.01	<0.01	0.102	0.048	0.043	0.007	0.006	< 0.001	0.030	5.40E+03	3.11E+04	8
07/07/06	1	Beach 3	<0.01	<0.01	0.079	0.033	0.032	0.004	0.012	< 0.001	0.016	1.73E+03	4.75E+04	7
07/31/06	2	Beach 1	<0.01	<0.01	<0.6	0.020	0.010	0.004	< 0.001	< 0.001	0.006	2.38E+03	7.79E+04	14
07/31/06	2	Beach 2	<0.01	<0.01	0.100	0.056	0.061	0.028	< 0.001	< 0.001	0.033	1.83E+04	1.31E+06	9
07/31/06	2	Beach 3	<0.01	<0.01	<0.6	0.032	0.034	0.010	< 0.001	< 0.001	0.024	3.13E+03	2.21E+05	9
08/10/06	3	Beach 1	<0.01	<0.01	1.972	1.289	1.661	0.204	0.097	0.075	1.285	6.11E+04	4.12E+06	10
08/10/06	3	Beach 2	<0.01	<0.01	1.300	1.017	1.328	0.264	0.055	0.038	0.971	3.42E+04	2.33E+06	9
08/10/06	3	Beach 3	<0.01	<0.01	1.227	0.859	1.332	0.231	0.061	0.030	1.010	2.00E+04	1.36E+06	9
08/22/06	4	Beach 1	<0.01	<0.01	1.282	1.198	1.016	0.083	0.082	0.042	0.809	1.35E+05	9.81E+06	15
08/22/06	4	Beach 2	<0.01	<0.01	1.047	0.779	0.821	0.071	0.071	0.041	0.638	5.27E+04	2.95E+06	9
08/22/06	4	Beach 3	<0.01	<0.01	1.271	1.039	1.049	0.107	0.082	0.049	0.811	3.52E+04	2.80E+06	9
08/04/06	3	Fish Land	<0.01	<0.01	8.039	14.826	14.175	0.965	0.326	0.157	12.727	-	-	33
08/24/06	4	AWRI	<0.01	<0.01	ND	2.897	4.375	0.535	0.325	0.140	3.375	-	-	18
09/15/06	5	Channel	<0.01	<0.01	1.560	1.313	0.814	0.084	0.052	0.042	0.636	-	-	ND
Mean			<0.01	<0.01	0.89	0.99	1.09	0.12	0.05	0.02	0.90	3.58E+04	2.35E+06	10
Standard Error			<0.01	<0.01	0.29	0.47	0.46	0.03	0.01	0.01	0.41	8.67E+03	5.71E+05	1
Min			<0.01	<0.01	0.01	0.02	0.01	0.00	0.00	0.00	0.01	2.33E+02	1.08E+04	6
Max			<0.01	<0.01	8.04	14.83	14.18	0.97	0.33	0.16	12.73	1.71E+05	1.02E+07	33
World Health Organization Moderate Advisory Level											20	1.0E+05	-	50

3.7 Bear Lake

The results of water chemistry and cyanobacteria analyses for Bear Lake are summarized in Table 3.13. Mean summer nitrate and ammonia concentrations were 0.01 mg/l and 0.02 mg/l, respectively. Mean SRP was <0.005 mg/l and ranged from <0.005-0.008 mg/l. Mean TP and TKN concentrations were 0.064 mg/l and 1.41 mg/l, respectively. TP concentrations ranged from 0.021-0.0.54 mg/l while TKN results ranged from 0.76-6.1 mg/l. The maximum TP and TKN values were collected from a location with a heavy surface cyanobacteria scum. Mean molar TN:TP ratio for Bear Lake was 59, suggesting phosphorus limitation. The mean summer chlorophyll-*a* was 49 µg/l and ranged from 32-100 µg/l. Based on standard values for chlorophyll-*a* and TP used to assess lake trophic status (Cooke et al. 2003), concentrations in Bear Lake would indicate upper eutrophic status. The summer mean Carlson TSI values for TP and chlorophyll-*a* were 60 and 68, respectively. These TSI values indicate that Bear Lake is an upper eutrophic system that borders on hypereutrophic conditions.

TABLE 3.13. WATER CHEMISTRY RESULTS FOR BEAR LAKE (2006). (TSI=CARLSON TROPHIC STATE INDEX).

Date	Event	Site	Water Temp °C	DO (mg/L)	pH	Turb (NTU)	Cl (mg/L)	SO4 (mg/L)	NO3-N (mg/L)	NH3-N (mg/L)	TKN-N (mg/L)	SRP-P (mg/L)	TP-P (mg/L)	TP-P (ug/L)	TSI TP	Ratio TN:TP	Chl *a* (ug/L)	TSI Chl *a*
07/06/06	1	Open 1	24.2	7.40	8.70	37	57	15	< 0.01	0.02	1.84	< 0.005	0.066	66	65	62	54	70
07/06/06	1	Open 2	24.2	6.65	8.61	36	51	14	< 0.01	0.02	1.72	< 0.005	0.070	70	65	55	39	66
07/06/06	1	Open 3	23.2	6.19	8.25	29	40	16	0.05	0.05	2.18	< 0.005	0.039	39	57	127	47	68
07/06/06	1	Open 2 D	24.2	6.65	8.61	32	58	16	< 0.01	0.02	1.60	< 0.005	0.059	59	63	61	32	65
07/25/06	2	Open 1	26.0	8.51	8.94	38	61	17	< 0.01	< 0.01	1.31	< 0.005	0.073	73	66	40	78	73
07/25/06	2	Open 2	26.1	7.63	8.99	42	58	16	< 0.01	< 0.01	1.27	< 0.005	0.070	70	65	40	38	66
07/25/06	2	Open 3	25.2	6.75	8.70	47	54	16	< 0.01	< 0.01	1.22	< 0.005	0.079	79	67	34	46	68
07/25/06	2	Open 2 D	26.1	7.63	8.99	42	59	16	< 0.01	< 0.01	1.04	< 0.005	0.069	69	65	33	32	65
08/04/06	3	Open 1	27.8	8.25	8.92	25	60	14	< 0.01	0.04	0.76	< 0.005	0.035	35	55	50	38	66
08/04/06	3	Open 2	28.0	8.47	8.89	29	69	14	< 0.01	0.02	0.92	0.005	0.037	37	56	56	41	67
08/04/06	3	Open 3	26.9	7.35	8.51	36	50	13	0.02	0.02	0.90	0.008	0.042	42	58	49	45	68
08/04/06	3	Open 2 D	28.0	8.47	8.89	31	68	15	< 0.01	0.02	1.03	0.005	0.041	41	58	57	45	68
08/24/06	4	Open 1	24.4	8.69	8.91	31	66	19	< 0.01	< 0.01	0.78	< 0.005	0.026	26	51	66	41	67
08/24/06	4	Open 1 D	24.4	8.69	8.91	33	63	18	< 0.01	0.01	0.76	< 0.005	0.028	28	52	61	46	68
08/24/06	4	Open 2	24.1	8.84	8.90	29	55	17	< 0.01	< 0.01	0.93	< 0.005	0.023	23	49	90	55	70
08/24/06	4	Open 3	23.7	8.29	8.71	23	49	19	< 0.01	< 0.01	0.86	< 0.005	0.021	21	48	91	54	70
07/07/06	1	Beach 1	24.2	8.15	8.84	36	54	16	< 0.01	0.03	1.04	< 0.005	0.071	71	66	33	63	71
07/07/06	1	Beach 2	24.2	7.90	8.85	35	55	17	< 0.01	0.05	2.94	< 0.005	0.071	71	66	93	38	66
07/07/06	1	Beach 3	24.1	7.93	8.86	41	56	17	0.01	0.02	< 0.1	< 0.005	0.057	57	62	1	65	72
07/07/06	1	Beach 4	-	-	-	32	140	16	< 0.01	0.03	6.12	< 0.005	0.514	514	94	26	100	76
07/31/06	2	Beach 1	31.8	11.21	9.15	51	64	17	0.02	< 0.01	1.08	< 0.005	0.049	49	60	49	41	67
07/31/06	2	Beach 2	31.9	10.80	9.17	48	345	15	0.05	< 0.01	1.34	0.006	0.048	48	60	62	40	67
07/31/06	2	Beach 3	31.8	10.90	9.16	48	69	19	0.04	< 0.01	1.07	< 0.005	0.048	48	60	49	44	68
08/10/06	3	Beach 1	26.5	9.75	9.01	39	61	16	0.02	0.01	0.97	< 0.005	0.035	35	55	64	40	67
08/10/06	3	Beach 2	26.4	9.75	9.12	37	47	13	0.01	0.02	1.03	< 0.005	0.034	34	55	69	49	69
08/10/06	3	Beach 3	23.4	9.68	9.11	36	54	14	0.01	0.08	1.57	< 0.005	0.038	38	57	97	39	67
08/22/06	4	Beach 1	26.2	10.30	9.09	60	68	19	< 0.01	0.01	1.24	< 0.005	0.044	44	59	62	54	70
08/22/06	4	Beach 2	26.1	10.44	9.08	50	80	21	< 0.01	0.02	0.97	< 0.005	0.035	35	55	62	38	66
08/22/06	4	Beach 3	26.5	10.25	9.12	44	58	17	< 0.01	0.02	0.99	< 0.005	0.039	39	57	58	66	72
Mean			26.06	8.63	8.89	38	71	16	0.01	0.02	1.40	0.003	0.064	64	60	59	49	68
Standard Error			0.47	0.26	0.04	2	10	0.4	0.002	0.003	0.19	0.0003	0.016	16	2	5	3	0.5
Min			23.23	6.19	8.25	23	40	13	0.01	0.01	0.76	0.002	0.021	21	48	1	32	65
Max			31.90	11.21	9.17	60	345	21	0.05	0.08	6.12	0.008	0.514	514	94	127	100	76

The distribution of phytoplankton organisms is shown in Figure 3.19. Bear Lake is dominated by cyanobacteria during the summer months with biovolumes for open water and beach samples of 1.8 x 10^7 μm^3/ml and 1.9 x 10^7 μm^3/ml, respectively. Dinoflagellates, diatoms, and green algae, were found at lower abundances. Beach samples contained similar cyanobacteria biovolumes as the open water samples. The composition of the cyanobacteria population for the beach and open water locations are given in Figures 3.20 and 3.21, respectively. *Aphanizomenon gracile, Microcystis aeruginosa*, *Microcystis botrys, Microcystis viridis,* and *Microcystis wesenbergii* are the dominant organisms in Bear Lake. *Cylindrospermopsis sp.* was present during the August samples. The maximum density of *Cylindrospermopsis* was 1,628 trichomes/ml. *Cylindrospermopsis* densities in Bear Lake were similar to populations reported in Florida lakes (Chapman and Schelske 1997).

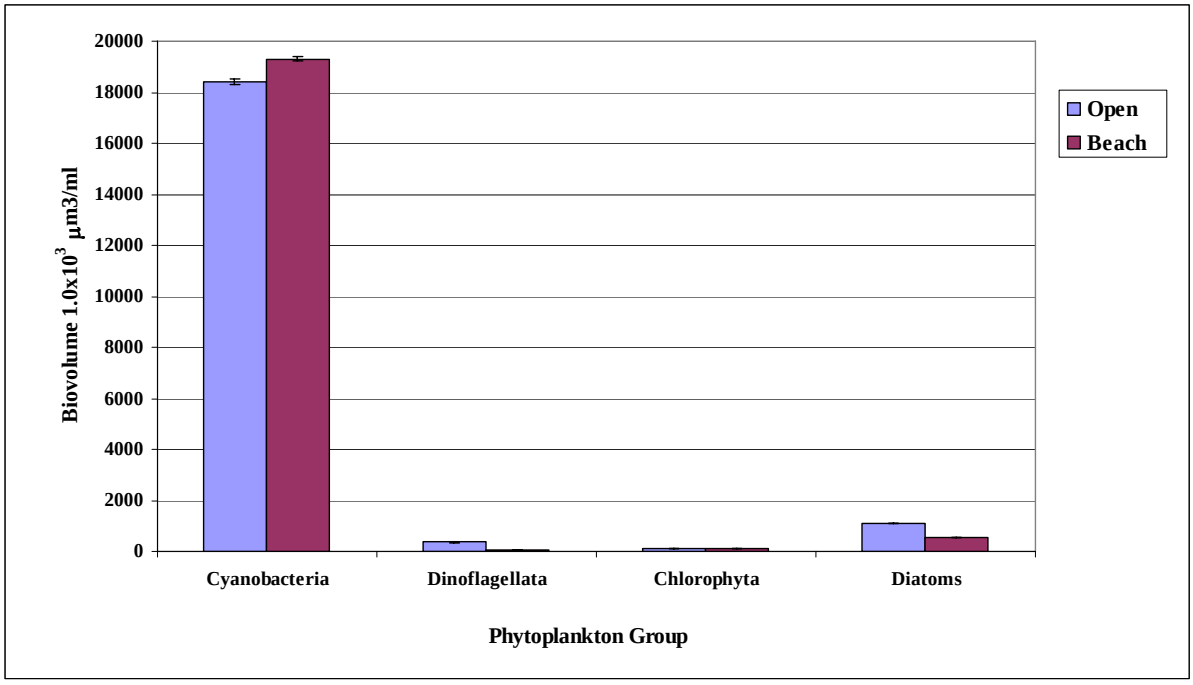


FIGURE 3.19. DISTRIBUTION OF PHYTOPLANKTON ORGANISMS IN BEAR LAKE (2006).

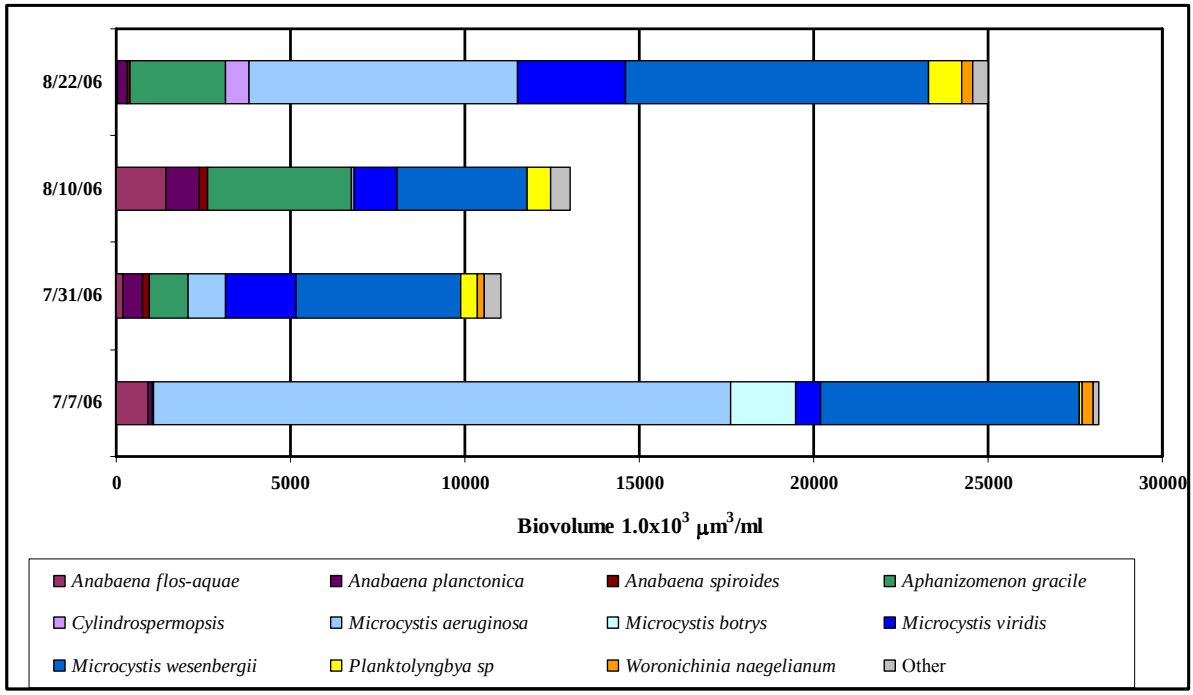


FIGURE 3.20. MEAN CYANOBACTERIA POPULATION COMPOSITION IN BEAR LAKE BEACH LOCATIONS (2006).

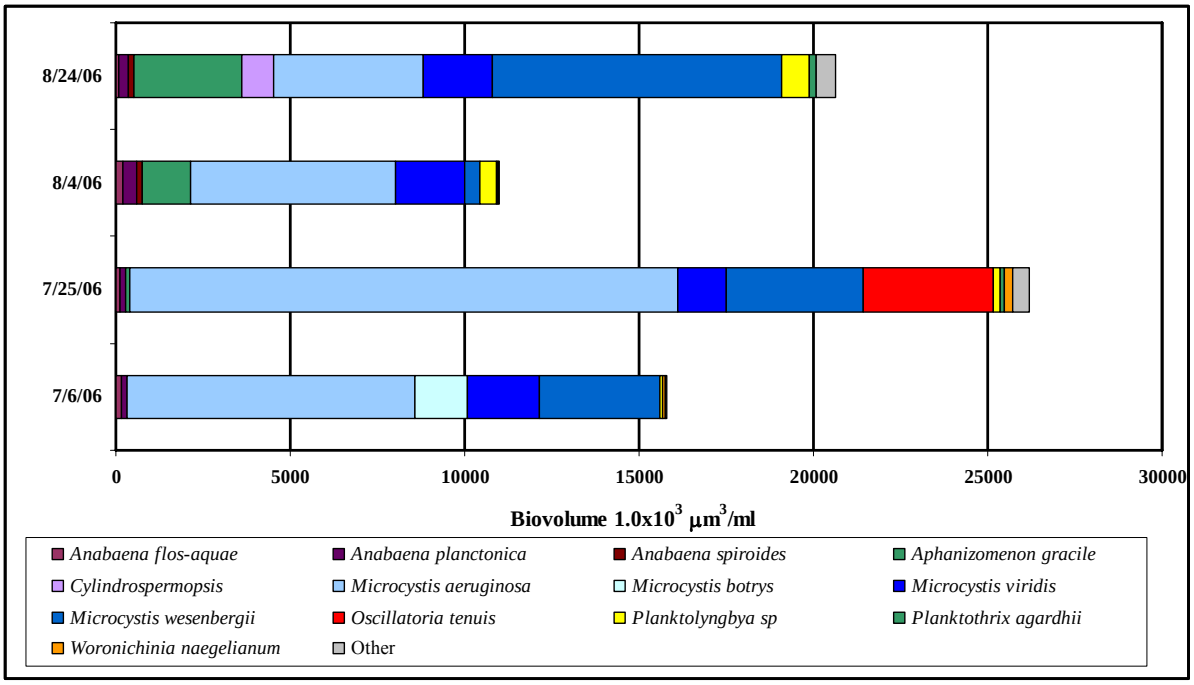


FIGURE 3.21. MEAN CYANOBACTERIA POPULATION COMPOSITION IN BEAR LAKE OPEN WATER LOCATIONS (2006).

The cyanotoxin and cyanobacteria results for Bear Lake are summarized in Table 3.14. Mean microcystin activity by PPIA was 4.59 µg/l and results ranged from 1.36-48 µg/l. The sample with the 48 µg/l PPIA result was from a surface scum collected at near the shore of the beach. The mean microcystin LR concentration was 1.0 µg/l with a range of 0.23-8.7 µg/l. There was no significant difference in microcystin LR concentrations between beach and open water samples (Mann-Whitney p=0.80). The maximum LR concentration was from the same beach sample with the elevated PPI value. Mean microcystin LR equivalents by ELISA and mean total microcystins by HPLC/MS were 2.06 µg/l and 2.14 µg/l, respectively. These data indicate that both methods provided similar results. ELISA and HPLC/MS results for microcystin LR were 2.06 and 1.00, respectively. Bear Lake contained the highest levels of microcystin RR (mean 0.98 µg/l) a LR:RR ratio of 0.89. Microcystin RR also reacts to the ELISA reagents and can produce a false positive for LR (Envirologics 2006). PPIA results were higher than ELISA and total HPLC/MS concentrations, indicating the presence of other congeners. The high PPIA result may be related to the diverse cyanobacteria assemblage in Bear Lake. The maximum microcystin LR (8.7 µg/l) was less than the WHO moderate advisory level of 20 µg/l. Mean cyanobacteria cell counts (5.1 x 10^5) exceeded the moderate WHO advisory levels of 1.0 x 10^5. The mean chlorophyll-*a* concentration (49 µg/l) was close to 50 µg/l WHO advisory guideline. Anatoxin-a and cylindrospermopsin were not detected.

TABLE 3.14 CYANOTOXIN AND CYANOBACTERIA RESULTS FOR BEAR LAKE (2006).

Date	Event	Site	Anatoxin-a (ug/L)	Cylindrospermopsin (ug/L)	PPIA (ug/L)	ELISA Conc. (ug/L)	HPLC/MS Total Conc. (ug/L)	HPLC/MS RR (ug/L)	HPLC/MS YR (ug/L)	HPLC/MS LA (ug/L)	HPLC/MS LR (ug/L)	Cyanobac Total # Cells per mL	Cyanobac Biovolume μm^3/ml	Chl *a* (ug/L)
07/06/06	1	Open 1	<0.01	<0.01	1.987	1.58	1.10	0.59	0.086	< 0.001	0.42	3.78E+05	2.56E+07	54
07/06/06	1	Open 2	<0.01	<0.01	2.405	1.04	0.98	0.55	0.056	< 0.001	0.38	1.16E+05	7.20E+06	39
07/06/06	1	Open 3	<0.01	<0.01	ND	0.78	0.59	0.31	0.047	< 0.001	0.23	2.86E+05	1.46E+07	47
07/06/06	1	Open 2 D	<0.01	<0.01	1.971	1.05	1.12	0.58	0.078	< 0.001	0.47	-	-	32
07/25/06	2	Open 1	<0.01	<0.01	3.752	1.97	3.34	1.79	0.238	< 0.001	1.31	9.12E+05	5.67E+07	78
07/25/06	2	Open 2	<0.01	<0.01	4.343	3.07	2.99	1.52	0.257	< 0.001	1.21	1.43E+05	6.04E+06	38
07/25/06	2	Open 3	<0.01	<0.01	6.131	3.21	3.63	1.77	0.302	< 0.001	1.56	1.98E+05	1.59E+07	46
07/25/06	2	Open 2 D	<0.01	<0.01	4.323	4.78	3.22	1.72	0.234	< 0.001	1.27	-	-	32
08/04/06	3	Open 1	<0.01	<0.01	1.379	1.25	1.23	0.66	0.073	< 0.001	0.50	2.79E+05	7.04E+06	38
08/04/06	3	Open 2	<0.01	<0.01	1.665	1.51	1.97	1.08	0.109	< 0.001	0.78	2.51E+05	9.80E+06	41
08/04/06	3	Open 3	<0.01	<0.01	1.597	1.44	1.31	0.65	0.077	< 0.001	0.59	4.59E+05	1.62E+07	45
08/04/06	3	Open 2 D	<0.01	<0.01	1.750	1.97	1.29	0.65	0.089	< 0.001	0.56	-	-	45
08/24/06	4	Open 1	<0.01	<0.01	2.061	1.64	1.64	0.88	0.112	< 0.001	0.65	2.22E+06	9.99E+06	41
08/24/06	4	Open 1 D	<0.01	<0.01	1.902	1.18	1.32	0.70	0.088	< 0.001	0.53	-	-	46
08/24/06	4	Open 2	<0.01	<0.01	2.120	1.59	1.41	0.77	0.091	0.010	0.54	6.01E+05	2.75E+07	55
08/24/06	4	Open 3	<0.01	<0.01	2.286	1.54	1.62	0.75	0.125	0.009	0.74	5.15E+05	2.44E+07	54
07/07/06	1	Beach 1	<0.01	<0.01	4.569	1.45	1.60	0.70	0.118	< 0.001	0.78	6.03E+05	3.77E+07	63
07/07/06	1	Beach 2	<0.01	<0.01	6.124	1.97	1.95	0.86	0.152	< 0.001	0.94	1.23E+05	6.57E+06	38
07/07/06	1	Beach 3	<0.01	<0.01	8.404	1.46	1.40	0.61	0.109	< 0.001	0.69	6.22E+05	4.03E+07	65
07/07/06	1	Beach 4	<0.01	<0.01	48.131	9.08	15.15	5.16	1.125	< 0.001	8.87	-	-	100
07/31/06	2	Beach 1	<0.01	<0.01	1.362	1.38	1.23	0.71	0.087	< 0.001	0.43	3.46E+05	1.01E+07	41
07/31/06	2	Beach 2	<0.01	<0.01	1.582	1.67	1.19	0.68	0.081	< 0.001	0.43	2.00E+05	9.73E+06	40
07/31/06	2	Beach 3	<0.01	<0.01	1.468	1.42	1.17	0.69	0.083	< 0.001	0.40	4.72E+05	1.33E+07	44
08/10/06	3	Beach 1	<0.01	<0.01	1.921	1.28	0.88	0.38	0.079	< 0.001	0.43	3.56E+05	9.57E+06	40
08/10/06	3	Beach 2	<0.01	<0.01	2.191	1.56	1.07	0.45	0.103	< 0.001	0.52	6.56E+05	2.05E+07	49
08/10/06	3	Beach 3	<0.01	<0.01	1.360	1.33	0.70	0.35	0.052	< 0.001	0.30	3.71E+05	8.95E+06	39
08/22/06	4	Beach 1	<0.01	<0.01	4.613	2.79	2.69	1.20	0.185	< 0.001	1.31	8.23E+05	2.68E+07	54
08/22/06	4	Beach 2	<0.01	<0.01	4.222	2.91	2.22	0.94	0.162	< 0.001	1.12	2.84E+05	6.23E+06	38
08/22/06	4	Beach 3	<0.01	<0.01	2.948	1.94	1.90	0.87	0.124	< 0.001	0.91	9.50E+05	4.19E+07	66
Mean			<0.01	<0.01	4.59	2.06	2.14	0.98	0.156	0.002	1.00	5.07E+05	1.89E+07	49
Standard Error			<0.01	<0.01	1.65	0.29	0.49	0.17	0.037	0.000	0.29	8.90E+04	2.80E+06	3
Min			<0.01	<0.01	1.36	0.78	0.59	0.31	0.047	0.001	0.23	1.16E+05	6.04E+06	32
Max			<0.01	<0.01	48.13	9.08	15.15	5.16	1.125	0.010	8.87	2.22E+06	5.67E+07	100
World Health Organization Moderate Advisory Level											20	1.0E+05	-	50

4.0 Evaluation of World Health Organization Guidelines and Microcystin Methods

The WHO has established a recommended guideline for recreational water exposure to microcystin LR at 20 µg/l (WHO 1999). In addition, secondary guidelines were established for chlorophyll-*a* (50 µg/l) and cyanobacteria density (100,000/ml). A comparison of microsystin LR data for all lakes is shown in Table 4.1. All of the lakes were below the 20

TABLE 4.1. COMPARISON OF MICROCYSTIN LR CONCENTRATIONS FOR ALL DROWNED RIVER MOUTH LAKES (2006). (WHO MODERATE ADVISORY LEVEL = 20 µg/l)

Microcystin LR by HPLC/MS	Bear Lake	Duck Lake	Lake Macatawa	Mona Lake	Muskegon Lake	Spring Lake	White Lake
# Analyzed	29	29	30	28	31	28	28
> 0.01 µg/l	29	0	30	24	31	28	20
> 0.1 µg/l	29	0	20	0	21	0	7
> 1 µg/l	7	0	0	0	3	0	0
>20 µg/l	0	0	0	0	0	0	0

µg/l guideline for recreational exposure. Muskegon Lake (3 of 31 samples) and Bear Lake (7 of 29 samples) were the only lakes with microcystin LR concentrations > 1 µg/l. Microcystin LR was not found at concentrations of > 0.01 µg/l in Duck Lake. With data from Duck Lake excluded, 93% of all samples contained microcystin LR at concentrations > 0.01 µg/l.

A comparison of microcystin LR equivalents by ELISA and total microcystin activity by PPIA for all lakes is shown in Tables 4.2 and 4.3, respectively. The ELISA test kits exhibit cross reactivity between certain congeners and cannot be used to provide a precise level of microcystin LR. The cross reactivity of congeners is illustrated by the fact that 38 samples exceeded 1 µg/l with ELISA compared to 10 samples with HPLC/MS. The PPIA test measures all microcystin congeners and the results show one sample above 20 µg/l in Bear Lake and 41 samples > 1 µg/l. While both methods overestimate the concentration of microcystin LR, the ELISA test exhibits less bias. Using the entire data set, ELISA, PPIA, and HPLC/MS methods produced significantly different results when paired with each other (wilcoxon; $p<0.01$).

TABLE 4.2. COMPARISON OF MICROCYSTIN LR EQUIVALENTS BY ELISA FOR ALL DROWNED RIVER MOUTH LAKES (2006). (WHO MODERATE ADVISORY LEVEL = 20 µG/L FOR MICROCYSTIN LR)

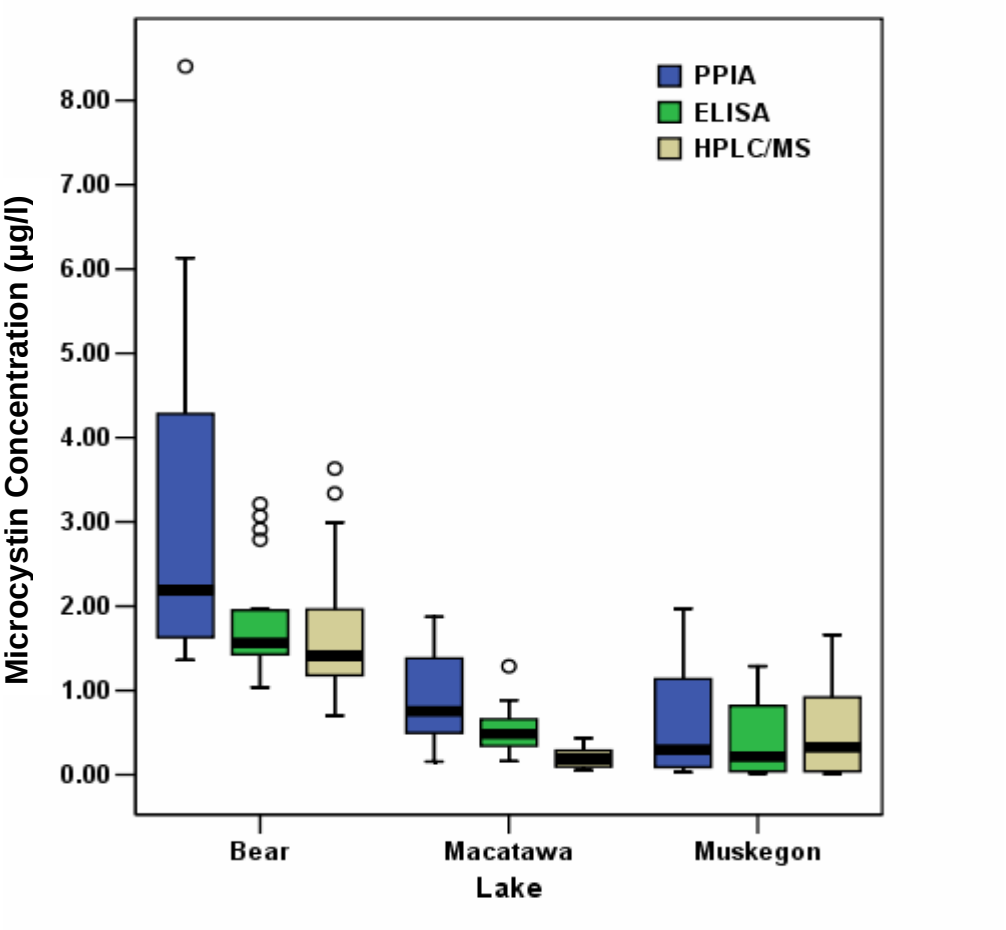
Microcystin ELISA	Bear Lake	Duck Lake	Lake Macatawa	Mona Lake	Muskegon Lake	Spring Lake	White Lake
# Analyzed	29	29	30	28	31	29	28
> 0.01 µg/l	29	3	30	28	31	29	23
> 0.1 µg/l	29	0	30	10	21	5	13
> 1 µg/l	28	0	2	0	8	0	0
>20 µg/l	0	0	0	0	0	0	0

TABLE 4.3. COMPARISON OF MICROCYSTIN ACTIVITY BY PPIA FOR ALL DROWNED RIVER MOUTH LAKES (2006). (WHO MODERATE ADVISORY LEVEL = 20 µg/l)

Microcystin PPIA	Bear Lake	Duck Lake	Lake Macatawa	Mona Lake	Muskegon Lake	Spring Lake	White Lake
# Analyzed	29	29	30	28	30	29	28
> 0.01 µg/l	29	11	30	23	26	23	18
> 0.1 µg/l	29	1	30	18	22	16	13
> 1 µg/l	29	0	12	0	10	0	0
>20 µg/l	1	0	0	0	0	0	0

Comparisons of the methods for the individual lakes are shown in Figures 4.1 and 4.2. Box plots (box-and-whisker diagram) of PPIA, ELISA, and Total HPLC/MS data for Bear Lake, Muskegon Lake, and Lake Macatawa are shown in Figure 4.1. The box represents the middle 50% of the data around the median. The whiskers represent the lower 25% and upper 75% quartile. For Bear Lake, PPIA results were significantly different from ELISA and HPLC/MS (wilcoxon; $p<0.01$). ELISA and Total HPLC/MS results also were significantly different (wilcoxon; $p<0.05$). Microcystin data from Lake Macatawa showed a similar lack of agreement as the results for ELISA, Total HPLC/MS, and PPIA all were significantly different from each other (wilcoxon; $p<0.01$). In contrast, the results for PPIA and Total HPLC/MS and ELISA and Total HPLC/MS were similar (Mann-Whitney; $p=0.49$ and 0.22, respectively). Samples from Muskegon Lake were 90+% microcystin LR and consequently, bias from other congeners would not influence the methods. Muskegon Lake was the only system where ELISA and microcystin LR data were not significantly different (wilcoxon; $p=0.22$).

FIGURE 4.1. BOX PLOTS OF PPIA, ELISA, AND HPLC/MS DATA FOR BEAR LAKE, MUSKEGON LAKE, AND LAKE MACATAWA (2006).



Box plots of PPIA, ELISA, and Total HPLC/MS data for Duck Lake, Mona Lake, Spring Lake, and White Lake are shown in Figure 4.2. For Mona Lake, Spring Lake, and Duck Lake, PPIA results were significantly different from ELISA and HPLC/MS (wilcoxon; $p<0.01$). ELISA and HPLC/MS results also were significantly different (wilcoxon; $p<0.01$). In contrast, the results for PPIA and ELISA, PPIA and HPLC/MS and ELISA and HPLC/MS were similar (Mann-Whitney; $p=0.06$, $p=0.08$, and $p=0.09$, respectively) for White Lake. These data suggest that the microcystin activity by PPIA was mostly due to the 4 congeners analyzed by HPLC/MS.

Figures 4.3, 4.4, 4.5, and 4.6 illustrate the positive bias of ELISA and PPIA when compared to HPLC/MS. A comparison of ELISA and HPLC/MS microcystin LR is shown in Figure 4.3. With the exception of data from Muskegon Lake, most of the data shows a positive bias in the ELISA test due to the cross reactivity of other congeners present in the sample. A comparison of ELISA and total microcystin congeners by HPLC/MS is shown in Figure 4.4.

FIGURE 4.2. BOX PLOTS OF PPIA, ELISA, AND HPLC/MS DATA FOR DUCK LAKE, MONA LAKE, SPRING LAKE, AND WHITE LAKE (2006).

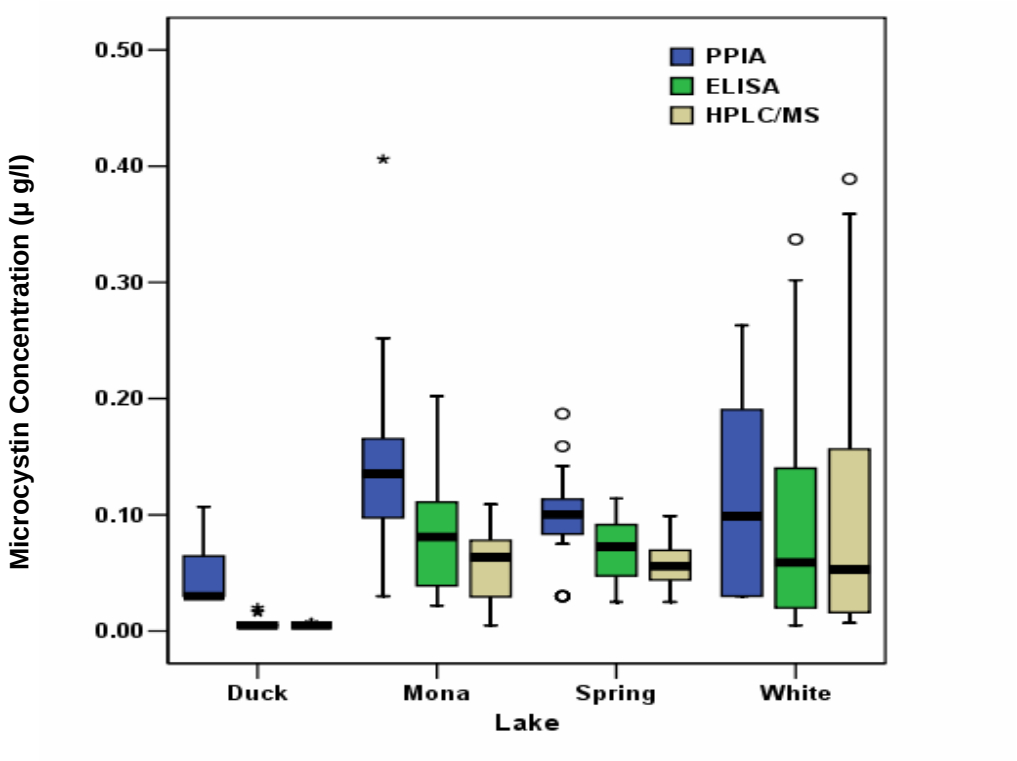


FIGURE 4.3. COMPARISON OF ELISA AND MICROCYSTIN LR HPLC/MS RESULTS FOR WEST MICHIGAN DROWNED RIVER MOUTH LAKES (2006). (DASHED LINE REPRESENTS A 1:1 RELATIONSHIP.)

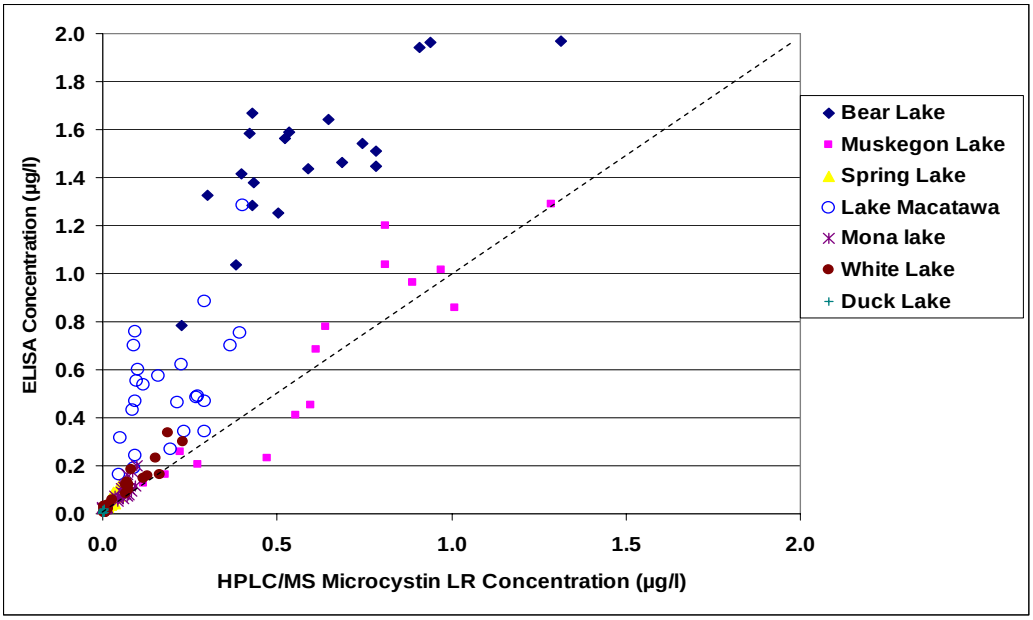
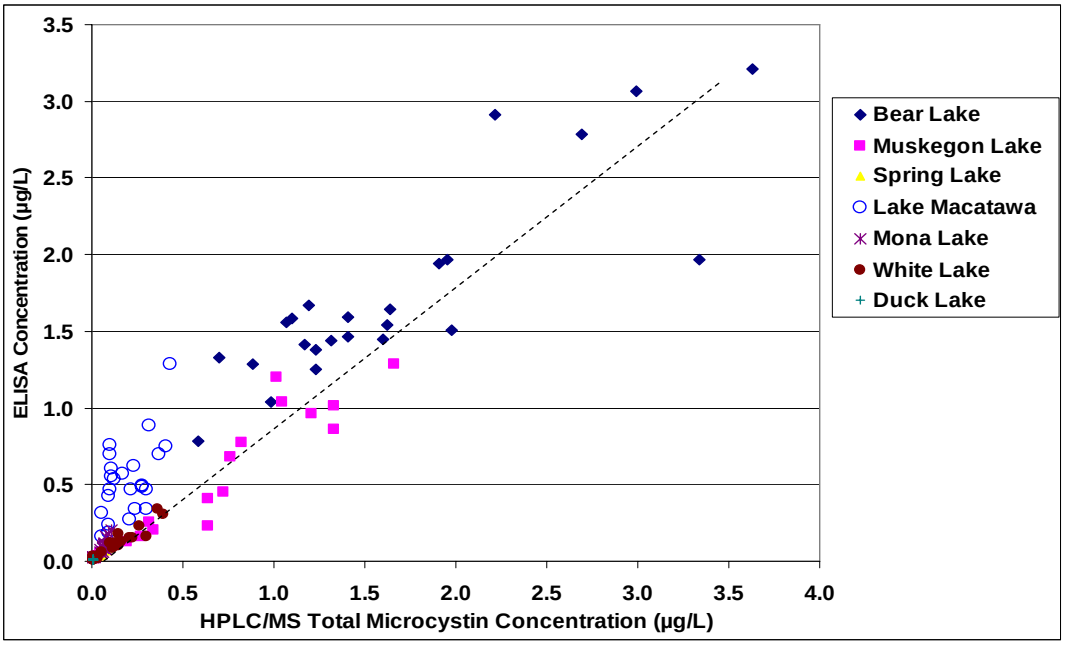


FIGURE 4.4. COMPARISON OF ELISA AND TOTAL MICROCYSTINS BY HPLC/MS RESULTS FOR WEST MICHIGAN DROWNED RIVER MOUTH LAKES (2006). (DASHED LINE REPRESENTS A 1:1 RELATIONSHIP.)



Although the data points are closer to the 1:1 ratio line, there still is a positive deviation to the data. Microcystin congeners, other than LR, YR, RR, and LA, are cross reacting with the antibodies in the ELISA test and producing higher results. While ELISA overestimates the concentration of microcystin LR, it is a good screening tool and provides an indication of the potential for other congeners to be present. Lake Macatawa and Bear Lake show the greatest positive deviation and potential for the presence of additional congeners. The comparison of PPIA and total microcystin congeners by HPLC/MS (Figure 4.5) follows a similar trend. A greater positive deviation however is noted due to broad reactivity of the PPIA test. Lake Macatawa and Bear Lake again show a larger positive deviation due to greater enzyme inhibition activity from congeners not included in the HPLC/MS method. The PPIA method also exhibits a positive deviation from ELISA (Figure 4.6). This was expected due to the ability of PPIA to react to all microcystin congeners in an equal manner. In contrast to the previous comparisons, about 50% of the data for Bear Lake and Lake Macatawa are close to the 1:1 line, showing good agreement between the two methods. Given the changes in the phytoplankton communities observed in the two lakes, it is possible that a congener group is produced by certain organisms that does not react with the ELISA antibodies. These data illustrate some of the analytical difficulties inherent in the assessment of the microcystin group of cyanotoxins. While it is important to focus on the accurate measurement of microcystin LR for public health assessments, each lake exhibits a unique pattern of congeners that react differently to the analytical methods. One end of the spectrum, Muskegon Lake contained primarily microcystin LR and showed good agreement between PPIA, ELISA, and HPLC/MS. Bear Lake was at the opposite end and significant deviations

FIGURE 4.5. COMPARISON OF PPIA AND TOTAL MICROCYSTINS BY HPLC/MS RESULTS FOR WEST MICHIGAN DROWNED RIVER MOUTH LAKES (2006). (DASHED LINE REPRESENTS A 1:1 RELATIONSHIP.)

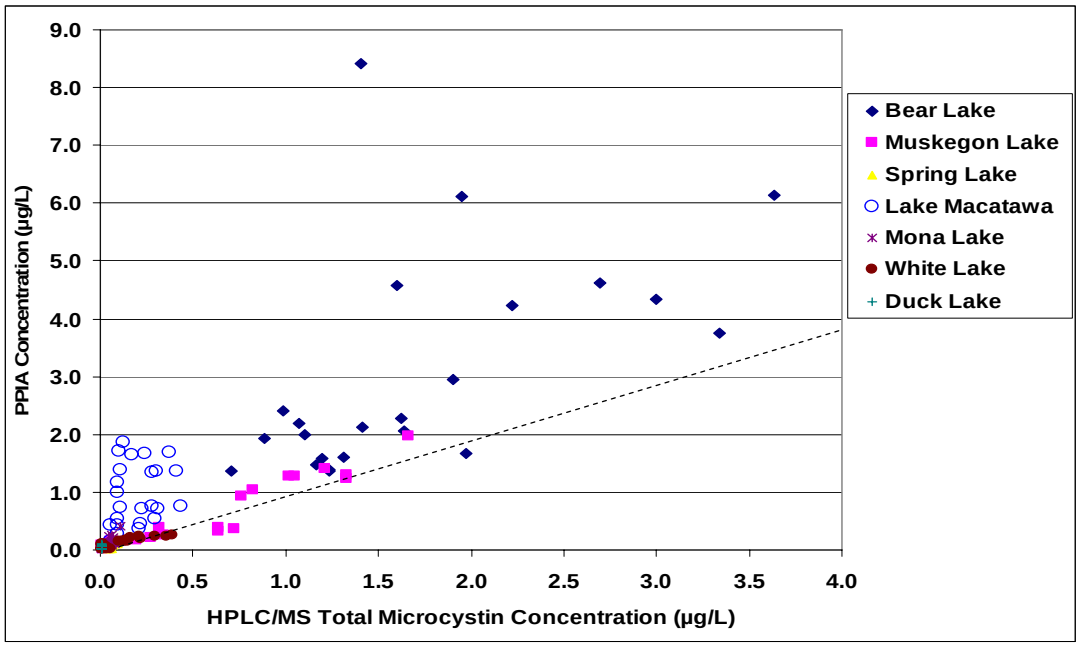
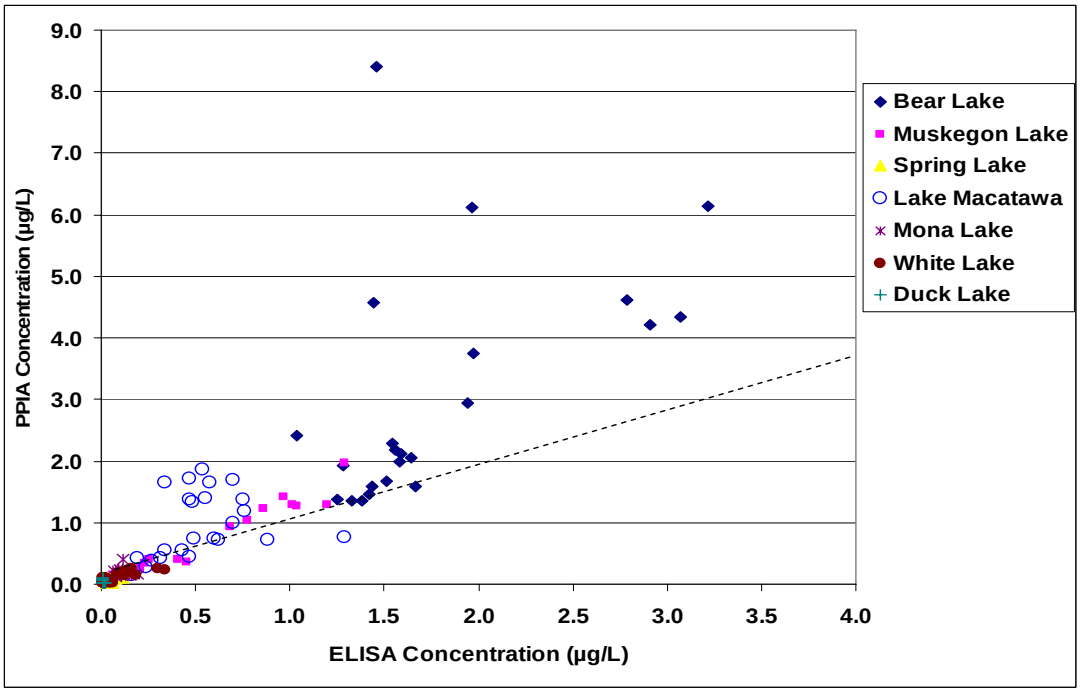


FIGURE 4.6. COMPARISON OF PPIA AND ELISA RESULTS FOR WEST MICHIGAN DROWNED RIVER MOUTH LAKES (2006). (DASHED LINE REPRESENTS A 1:1 RELATIONSHIP.)



between all methods were noted. There also appeared to be internal variations within two of the lakes that may be related to shifts in cyanobacteria communities or spatial/temporal variability. A combination of methods may be necessary to provide a more complete understanding of the nature and extent of microcystins in a given lake.

In addition to guidelines for microcystins, the WHO lists secondary guidelines for chlorophyll-*a* and cyanobacteria cell counts. A comparison of chlorophyll-*a* and cyanobacteria cell numbers for all lakes are shown in Tables 4.4 and 4.5, respectively. The lakes in the upper eutrophic to hypereutrophic TSI classification (Bear Lake, Mona Lake, Spring Lake, and Lake Macatawa) had a majority of their cyanobacteria cell counts (93 of 97 samples) and chlorophyll-*a* measurements (74 of 113 samples) over the WHO guideline. When all the lakes are examined, 60% of the samples exceed the cyanobacteria cell counts guideline and 27% exceed the chlorophyll-*a* guideline.

TABLE 4.4. COMPARISON OF CYANOBACTERIA CELL NUMBERS FOR ALL DROWNED RIVER MOUTH LAKES (2006). (WHO MODERATE ADVISORY LEVEL = 100,000/ ML)

Cyanobacteria Cell #/ml	Bear Lake	Duck Lake	Lake Macatawa	Mona Lake	Muskegon Lake	Spring Lake	White Lake
# Analyzed	25	24	24	24	24	24	24
>10000	25	0	24	24	16	24	16
>100000	25	0	22	24	2	22	7
>1000000	1	0	3	9	0	10	0
>10,000,00	0	0	0	0	0	0	0

TABLE 4.5. COMPARISON OF CHLOROPHYLL-*a* CONCENTRATIONS FOR ALL DROWNED RIVER MOUTH LAKES (2006). (WHO MODERATE ADVISORY LEVEL = 50 µG/L)

Chlorophyll a	Bear Lake	Duck Lake	Lake Macatawa	Mona Lake	Muskegon Lake	Spring Lake	White Lake
# Analyzed	29	28	28	28	30	28	28
> 1 ug/l	29	28	28	28	30	28	28
> 10 ug/l	29	0	28	28	5	28	27
> 50 ug/l	9	0	27	21	0	17	0
>100 ug/l	0	0	0	0	0	0	0

Based on the 2006 results from the drowned river mouth lakes, elevated cyanobacteria cell counts and chlorophyll-*a* are not reliable indicators of the presence of microcystin LR. Because of the presence of other congeners and their cross reactivity in the ELISA test, HPLC/MS appears to be the best analytical method to provide an accurate assessment of microcystin LR concentrations.

5.0 Summary and Conclusions

Cyanobacteria populations and their associated toxins were investigated in seven drowned river mouth lakes in west Michigan during the summer of 2006. A gradient of low mesotrophic to hypereutrophic systems were examined to determine if concentrations of cyanotoxins exceeded the WHO guidelines and to evaluate the performance of three analytical methods. Bear Lake, Spring Lake, Mona Lake, and Lake Macatawa are hypereutrophic systems with extensive histories of cyanobacteria blooms. Muskegon Lake, White Lake, and Duck Lake are mesotrophic/eutrophic systems with increasing reports of algal bloom corresponding to the invasion of zebra mussels. These seven lakes are connected either directly or indirectly to Lake Michigan, and used extensively for boating, skiing, fishing, and swimming.

Six of the seven lakes were found to have summer cyanobacteria blooms and contained low levels of cyanotoxins throughout July and August (2006). Duck Lake, a mesotrophic system, had no samples with microcystin LR above the detection limit (0.001 µg/l). None of the lakes had microcystin LR concentrations above the WHO recreational water guideline of 20 µg/l and only two of the seven lakes had concentrations > 1 µg/l. A summary of the data is shown below:

Microcystin LR by HPLC/MS	Bear Lake	Duck Lake	Lake Macatawa	Mona Lake	Muskegon Lake	Spring Lake	White Lake
# Analyzed	29	29	30	28	31	28	28
> 0.01 µg/l	29	0	30	24	31	28	20
> 0.1 µg/l	29	0	20	0	21	0	7
> 1 µg/l	7	0	0	0	3	0	0
>20 µg/l	0	0	0	0	0	0	0

The WHO secondary guidelines for chlorophyll-*a* and cyanobacteria cell counts were found to be unreliable indicators of cyanotoxin concentrations as 60% of the samples exceeded the cyanobacteria cell counts guideline of > 100,000/ ml and 27% exceeded the chlorophyll-*a* guideline of 50 µg/l. Diverse populations of cyanobacteria were found in each lake and seasonal changes in species and abundance were observed. A significant difference in cyanotoxin levels was not observed between beach and open water samples.

Three methods were used to measure cyanotoxins in the investigation. ELISA, PPIA, and HPLC/MS were used to evaluate microcystins. In addition, HPLC/MS was used to measure anatoxin-a and cylindrospermopsin. The latter two toxins were not detected in the 2006 samples. PPIA is an inexpensive screening method ($20-$30/test) that measures total microcystin activity and all congeners respond in a 1:1 ratio. ELISA also is an inexpensive screening tool ($10-$20/test) that is more focused on microcystin LR. Other congeners can cross react with the method and consequently, the data cannot be used as an absolute indicator of LR concentration. HPLC/MS is a more accurate analytical method that identifies individual microcystin congeners based on retention time and molecular weight. While HPLC/MS has clear advantages with respect to accuracy and sensitivity, it requires expensive analytical equipment ($150,000) and only a few microcystin standards are

commercially available. PPIA and ELISA were found to significantly overestimate the concentration of microcystin LR in most of the samples. Muskegon Lake was an exception as it contained mostly microcystin LR and the three methods yielded relatively similar results. ELISA and PPIA results were similar for three lakes, however Bear Lake and Lake Macatawa had PPIA concentrations significantly greater than ELISA. These data suggest that the congener composition for each lake is unique and related to community composition and/or limnological characteristics of each lake. Only one sample from a cyanobacteria bloom in Bear Lake had PPIA results of >20 µg/l.

The diversity of cyanobacteria communities and analytical results suggests that no single analytical method can be used to assess cyanotoxin levels. In Muskegon Lake, conditions in 2006 showed that the three methods produced comparable results. Based on the variability of cyanobacteria communities observed in the other lakes, this relationship needs to be evaluated with several years of data to determine if it is consistent over time. Data from the other lakes suggest that a combination of HPLC/MS and a broad screening method such as PPIA may be necessary to accurately measure LR concentrations and evaluate the total amount of cyanotoxins present. Since PPIA and ELISA consistently overpredicted the level of microcystin LR, these methods appear to be good conservative screening tools for the cost effective evaluation of large numbers of samples. If levels of cyanotoxins above 20 µg/l are measured by PPIA or ELISA, analysis by HPLC/MS is recommended to provide an accurate determination of the microcystin LR concentration.

All of the lakes studied in 2006 had cyanotoxin levels below the WHO guidelines for microcystin LR. Although toxin producing organisms were present in most of lakes at cell counts above the WHO guideline, the recreational value of the water was not impacted by elevated cyanotoxin concentrations. Since cyanobacteria blooms and toxin production are influenced by a variety of local and regional factors, the use of single year of data may not be representative of future conditions.

6.0 References

APHA (American Public Health Association). 1998. Standard Methods for the Evaluation of Water and Wastewater. 20th edition. APHA, Washington, DC.

Bennett E, Carpenter SR. 2002. P Soup. World Watch. 15:24-32.

Bogialli S, Bruno M, Curini R, Corcia AD, Lagana A, Mari B. 2005. Simple Assay for Analyzing Five Microcystins and Nodularin in Fish Muscle Tissue: Hot Water Extraction Followed by Liquid Chromatography – Tandem Mass Spectrometry. Journal of Agricultural and Food Chemistry. 53:6586-6592.

Briand JF, Jacquet S, Bernard C, Humbert JF. 2003. Health hazards for terrestrial vertebrates from toxic cyanobacteria in surface water. Veterinary Research. 34:361-377.

Carlson RE. 1977. A trophic state index for lakes. Limnology. and Oceanography. 22: 361-369.

Carmichael WW. 1992. Cyanobacterial secondary metabolites – the cyanotoxins. Journal of Applied Bacteriology. 72:445-459.

Carmichael WW, An J. 1999. Using an Enzyme Linked Immunosorbent Assay (ELISA) and a Protein Phosphatase Inhibition Assay (PPIA) for the Detection of Microcystins and Nodularins. Natural Toxins. 7:377-385.

Chorus I, Falconer I, Salas H, Bartram J. 2000. Health Risks Caused by Freshwater Cyanobacteria in Recreational Waters. Journal of Toxicology and Environmental Health, Part B. 3:323-347.

Chapman AD, Schelske CL. 1997. Recent appearance of Cylindrospermopsis (Cyanobacteria) in five hypereutrophic Florida lakes. Journal of Phycology. 33:191–195.

Cooke GD, Welch EB, Peterson SA, Newroth PR. 1993. Restoration and management of lakes and reservoirs. Lewis Publishing, Boca Raton, FL.

Crayton MA. 1993. Toxic Cyanobacteria Blooms: A Field/Laboratory Guide. Biology Department Pacific Lutheran University. 19pp.

Dittmann E, Neilan BA, Bornter T. 2001. Molecular biology of peptide a polyketide biosynthesis in cyanobacteria. Applied Microbiology and Biotechnology. 57:467-473.

Downing JA, McCauley E. 1992. The nitrogen:phosphorus relationship in lakes. Limnology and Oceanography 37: 936-945.

EnviroLogix. 2005. Quantiplate Kit for Microcystins. EnviroLogix Inc. 7 pp.

Falconer IR. 1999. An Overview of Problems Caused by Toxic Blue-Green Algae (Cyanobacteria) in Drinking and Recreational Water. Environmental Toxicology. 14:5-12.

Fastner J, Flieger I, Neumann U. 1998. Optimized extraction of microcystins from field samples – a comparison of different solvents and procedures. Water Research. 10: 3177-3181.

Fastner J, Codd GA, Metcalf JS, Woitke P, Wiedner C, Utkilen H. 2002. An international intercomparison exercise for the determination of purified microcystin-LR and microcystins in cyanobacterial field material. Analytical and Bioanalytical Chemistry. 374:437-444.

GRERL. 2002. The Zebra Mussel Connection: Nuisance Algal Blooms, Lake Eire Anoxia, and other Water Quality Problems in the Great Lakes. Great Lakes Environmental Research Laboratory. 4pp.

Griffiths D, Saker M. 2003. The Palm Island Mystery Disease 20 Years on: A Review of Research on the Cyanotoxin Cylindrospermopsin. Environmental Toxicology. 18:78-93.

Heinze R. 1999. Toxicity of the Cyanobacterial Toxin Microcystin LR to Rats after 28 days Intake with Drinking Water. Environmental Toxicology. 14:57-60.

Hong Y, Steinman A, Biddanda B, Rediske R, Fahnenstiel G. 2006. Occurrence of the Toxin-producing Cyanobacterium *Cylindrospermopsis raciborskii* in Mona and Muskegon Lakes, Michigan. Journal of Great Lakes Research. 32:645-652.

Johnston B, Jacoby J. 2003. Cyanobacterial toxicity and migration in a mesotrophic lake in western Washington, USA. Hydrobiologia. 495:79-91.

Juhel G, Davenport J, O'Halloran J, Culloty S, Ramsay R, James K, Furey A, Allis O. 2006. Pseudodiarrhea in zebra mussels *Dreissena polymorpha* (Pallas) exposed to microcystins. The Journal of experimental biology. 209(Pt 5):810-6.

Kaebernick M, Neilan, BA, Borner T, Dittmann, E, 2000. Light and the transcriptional response of the microcystin biosynthesis gene cluster. Applied Environmental Microbiology. 66:3387-3392.

Li CM, Chu RY, Hsientang Hsieh DP. 2006. An enhanced LC-MS/MS method for microcystin-LR in lake water. Journal of Mass Spectrometry. 41(2):169 – 174.

Long BM, Jones GJ, Orr PT. 2001. Cellular Microcystin Content in N-Limited *Microcystis aeruginosa* Can be Predicted from Growth Rate. Applied and Environmental Microbiology. 67:278-283.

Mathys W, Surholt, B. 2004. Analysis of microcystins in freshwater samples using high performance liquid chromatography and an enzyme-linked immunosorbent assay. International Journal Hygiene Environmental Health. 207:601-605.

McElhiney J, Lawton LA. 2005. Detection of the cyanobacterial hepatotoxins microcystins. Toxicology and Applied Pharmacology. 203:219-230.

Metcalf JS, Hyenstrand P, Beattie KA, Codd GA. 2000. Effects of physiochemical variables and cyanobacterial extracts on the immunoassay of microcystin-LR by two ELISA kits. Journal of Applied Microbiology. 89:532-538.

Oberholster PJ, Botha A-M, Grobbelaar JU. 2004. *Microcystis aeruginosa*: source of toxic microcystins in drinking water. African Journal of Biotechnology. 3:159-168.

Orr P.T, Jones GJ. 1998. Relationship between microcystin production and cell division rates in nitrogen-limited Microcystis aeruginosa cultures. Limnology and Oceanography. 43:1604-1614.

Osswald J, Rellán S, Gago A, Vasconcelos V. 2007. Toxicology and detection methods of the alkaloid neurotoxin produced by cyanobacteria, anatoxin-a. Environment international. 33:1070-1089.

Paerl HW, Ustach J. 1982. Blue-green algal scums: An explanation for their occurrence during freshwater blooms. Limnology and Oceanograpy 27:212-217.

Paerl HW. 1988. Nuisance phytoplankton blooms in coastal, estuarine and inland waters. Limnology and Oceanography. 33:823-847.

Paerl HW. 1996. Microscale physiological and ecological studies of aquatic cyanobacteria: Macroscale implications. Microscopy Research and Technique. 33:47-72.

Paerl HW, Fulton R, Moisander PH, Dyble J. 2001. Harmful freshwater algal blooms, with an emphasis on cyanobacteria. The Scientific World 1: 76-113.

Pitois S, Jackson MH, Wood BJB. 2001. Sources of the Eutrophication Problems Associated with Toxic Algae: An Overview. Journal of Environmental Health. 64:25-32.

Rogers EH, Hunter ES, Moser VC, Phillips PM, Herkovits J, Munoz L, Hall LL, Chernoff N. 2005. Potential developmental toxicity of anatoxin-a, a cyanobacterial toxin. Journal of Applied Toxicology. 25:527-534.

Rolland A, Bird DF, Giani A. 2005. Seasonal changes in composition of the cyanobacterial community and the occurrence of hepatotoxic blooms in the eastern townships, Quebec, Canada. Journal of Plankton Research. 27:683-694.

Sakamoto, M. 1966. Primary production by phytoplankton community in some Japanese lakes and its dependence on lake depth. Archiv für Hydrobiologie 62: 1-28.

Smith, V.H. 1982. Phosphorus versus nitrogen limitation in the marine environment. Limnology and Oceanograpy 27: 1101-1112.

Smith, V.H. 1983. Low nitrogen to phosphorus ratios favor dominance by blue-green algae in lake phytoplankton. Science 221: 669-671.

Steinman AD, Rediske R, Reddy KR. 2004. The reduction of internal phosphorus loading using alum in Spring Lake, Michigan. Journal of Environmental Quality. 33: 2040-2048.

Steinman AD, and MA Ogdahl. 2007. Ecological effects following an alum treatment in Spring Lake, Michigan. Journal of Environmental Quality. In press.

Sutherland TF, Leonard C, Taylor FJR. 1992. A segmented integrating pipe sampler for profiling the upper water column. Journal of Plankton Research. 14: 915-923.

USEPA. 1997a. Lake Michigan Mass Balance Method 086. Chlorophyll – Spectrophotometric Method. Great Lakes National Program Office. United States Environmental Protection Agency.

USEPA. 1997b. Lake Michigan Mass Balance Method 087. Standard Operating Procedure for Phytoplankton Analysis. Great Lakes National Program Office. United States Environmental Protection Agency.

USEPA, 2001. Creating a Cyanotoxin Target List for the Unregulated Contaminant Monitoring Rule. Technical Service Center. United States Environmental Agency.

Vanderploeg HA, Leibig JR, Carmichael WW, Agy MA, Johengen TH, Fahnenstiel GL, Nalepa TF. 2001. Zebra mussel (*Dreissena polymorpha*) selective filtration promoted toxic *Microcystis* blooms in Saginaw Bay (Lake Huron) and Lake Erie. Canadian Journal of Fisheries and Aquatic Science. 58: 1208-1221.

WDHHS. 2004. Guidance for Local Health Departments: Cyanobacteria and Human Health. Wisconsin Department of Health and Human Services. 4pp.

WHO. 1999. Toxic Cyanobacteria in Water: A guide to their public health consequences, monitoring and management. World Health Organization. 455 pp.

WHO. 2003. Guidelines for safe recreational water environments. Volume 1, Coastal and fresh waters. World Health Organization. 253pp.

Zurawell RW, Chen H, Bruke JM, Prepas EE. 2005. Hepatotoxic Cyanobacteria: A Review of the Biological Importance of Microcystins in Freshwater Environments. Journal of Toxicology and Environmental Health, Part B. 8:1-37.