Final Report
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Diversity of *Microcystis* across trophic gradients

Dr. Thomas B. Bridgeman Ph.D.
Justin D. Chaffin

Lake Erie Center and Department of Environmental Sciences
University of Toledo
6200 Bayshore Rd.
Oregon Ohio, 43616

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Abstract

The *Microcystis* bloom during 2011 was very intense covering the majority of the western basin in August and reached the central basin in October. Molecular fingerprinting revealed that the *Microcystis* blooms that occurred in the central basin during October and the western basin in July and August were very similar. This indicates that Lake Erie *Microcystis* blooms are actually one bloom, rather than separated blooms. The bloom that develops near Maumee Bay spreads eastward. Further, cluster analysis revealed that lake sediments are a likely population source for summer blooms. Tributaries are not likely sources, although similar *Microcystis* was found in the Maumee River in August after the summer bloom began. Genetic diversity of *Microcystis* was consistent throughout the summer and among the basins, despite the large difference of dissolved nutrient concentrations. This indicates that the *Microcystis* that occurs in July during high nutrient concentration can survive (and even thrive) in waters low in nutrients typical of the central basin. Microcystin concentration often exceeded the WHO guidelines in 2011 and was positively correlated with *Microcystis* biovolume and ammonium. Partitioning of the total nitrogen pool indicates that dissolved organic nitrogen can be a major factor in contributing to the blooms.

Activities

J. Chaffin is a Ph.D. student at University of Toledo in the Department of Environmental Sciences. Funding from this project (in part) supported his dissertation research. All data presented in this report will be included in his dissertation. J. Chaffin is graduating in May 2013 and will begin a career with Ohio Sea Grant at Stone Laboratory. Funding from this project also supported a summer undergraduate laboratory technician, Courtney Mobilian, who aided in collecting and processing samples collected during this project. C. Mobilian is senior working on her honors thesis in Dr. Thomas Bridgeman's laboratory.

Timelines

Our timeline was slightly adjusted when we applied for a one-month extension to complete the report. All work has been completed.

Outcomes and technical reports

The results presented in the technical report will likely be divided into two separate reports and submitted to two peer-reviewed journals for publication.

Changes in project activities and hurdles experienced.

In the initial proposal, we indicated we would sample three tributaries of Lake Erie: Maumee, Sandusky, and Vermilion. However, logistical constraints prevented sampling of the Vermilion, therefore only the Maumee and Sandusky were sampled.

Additional sampling was conducted in October in response to the *Microcytis* bloom that spread into the central basin. Only two sites were sampled in October and both were in the bloom.

Lessons learned.

A main result of this project indicates that the *Microcystis* bloom of 2011 began in Maumee Bay during July and spread eastward into the central basin by October. Stronger nutrient management is suggested for the Maumee River watershed. Further, the results point to the lake sediments as being the source population for the summer blooms, rather than the tributaries.

Concentrations of the toxin microcystin were correlated with *Microcystis* biomass and ammonium. Therefore, minimizing ammonium loading into the lake, along with P, will help prevent toxic blooms.

Dissolved organic nitrogen was a large percentage of the total nitrogen pool. Further research in understanding dissolved organic nitrogen effects on cyanobacterial blooms is warranted.

Introduction

Lake Erie, specifically the western basin of the lake, has been plagued by the harmful cyanobacterium *Microcystis aeruginosa* since the mid 1990s (Brittian et al. 2000; Millie et al. 2009; Bridgeman et al. in revision). Other cyanobacteria also occur in western Lake Erie, but are in lower densities than *Microcystis* (Millie et al. 2009) or occur in other locations of the lake (Conroy et al. 2007). *Microcystis* is of concern because of the potential to produce the toxin microcystin. Microcystin concentrations in western Lake Erie often exceed the World Health Organization's limit for drinking water (1 μg/L) and recreational water (20 μg/L) (Rinta-Kanto et al. 2005; Dyble et al. 2008; Wilson et al. 2008). Western Lake Erie is the source of drinking water for millions people, and the city of Toledo, Ohio spends an extra $3000 to $4,000 per day during a bloom to remove microcystin from the water (Bridgeman et al. in revision). Further, microcystin has been recorded in the fillets of the Lake Erie fishes yellow perch (Dyble et al. 2011) and walleye (Poste et al. 2011). However, the yellow perch excrete the toxin within a few hours of ingestion (Dyble et al. 2011).

The return of *Microcystis* blooms in Lake Erie is likely due to the increase load of dissolved reactive phosphorus (P) from the Maumee River (Richards et al. 2010). The general paradigm of freshwater ecology is that the phytoplankton biomass of freshwater lakes is regulated by the availability of P (Wetzel 2001; Reynolds 2008). Therefore, increasing P loads will increase total phytoplankton biomass. Furthermore, lakes with very high P concentrations will have higher amounts of cyanobacteria (Downing et al. 2001). Recently, it was determined that high P levels (> 3 μmol/L) in Maumee Bay of Lake Erie during the early summer result in phytoplankton growth that is not constrained

by P (Chaffin and Bridgeman 2012). Thus, it is very critical to reduce P in Lake Erie to prevent blooms. Reducing P levels has been shown to reduce the amount of cyanobacteria and total phytoplankton biomass in many other eutrophic lakes (Edmondson 1970; Schindler 1974,1977).

However, recent data suggests that nitrogen (N) is equally important and that reducing N along with P is required to improve water quality (Conley et al. 2009; Scott and McCarthy 2010; 2011). Further, it was recently shown that cyanobacterial blooms of Lake Erie are stimulated by the addition of N, while no response was seen with P addition (Chaffin and Bridgeman 2012). N additions have stimulated the growth of phytoplankton elsewhere (Elser et al. 2007; Piehler et al. 2008; Paerl et al. 2011). In spite of the data supporting N-limitation of freshwater lakes, there is a debate among limnologists regarding the need to control N loadings to constrain cyanobacterial blooms (for example see Paterson et al. [2011] and Scott and McCarthy [2011]). Therefore more data is needed. Understanding the differences of how low-N and high-N affect cyanobacteria ecology will be important in resolving the N debate.

If lake managers decide that N inputs to lakes need to be regulated to control cyanobacterial blooms, they need to be aware of the different forms of bioavailable N, and how each form affects cyanobacteria. Unlike bioavailable P that mostly occurs as phosphate, bioavailable N occurs as organic (urea, amino acids, ect.) or inorganic and reduced (ammonium [NH_4^+]) or oxidized (nitrate [NO_3^-]) forms. Therefore, targeting the form of N that drives cyanobacteria abundance, diversity, and toxin production will be most beneficial.

Western Lake Erie offers the ideal location to study the effects of low-N concentration *vs*. high-N concentration on phytoplankton dynamics for several reasons. First, a spatial gradient of NO_3^- exists from high in Maumee Bay to lower offshore (Moorhead et al. 2008; Chaffin et al. 2011). Combined with the spatial gradient, NO_3^- has a temporal gradient from high levels in May (250 μmol/L) to levels below detection limit in late summer (Chaffin et al. 2011). Further, western Lake Erie has relatively high levels of NH_4^+, the preferred N source for phytoplankton (Dugdale and Goering 1967). NH_4^+ does not follow the same spatial and temporal pattern that NO_3^- does, and values are typically between 2 μmol/L and 5 μmol/L (Chaffin et al. 2011). The constant presence of NH_4^+ (although much lower concentration than early summer NO_3^-) allows studying the effects of decreased NO_3^- in the presence of NH_4^+. As a result of decreasing NO_3^-, the total N-to-total P-ratio shows a steady decline throughout the season (Chaffin et al. 2011), which would indicate a shift from P-limitation to N-limitation (Downing et al. 1992; Guildford and Hecky 2000). These annual temporal patterns of in-lake nitrate concentrations and TN:TP follow the loading rate of the Maumee River, which shows steady declines from early summer to late summer. The decreasing concentrations and loading of NO_3^- to Lake Erie sets up a lake-scale experiment to study how the cyanobacteria population will be affected decreases in NO_3^-. Studying this phenomenon allows to make comparisons to other systems that have high N loadings year round.

The overall goal of this project was to determine how a wide range of nutrient concentration affects cyanobacteria ecology in Lake Erie. In accomplishing this goal, we first partition the total P and N pools into dissolved and particulate, and organic and inorganic forms. Secondly, we measured the concentration of the cyanotoxin microcystin

and the abundance of the most dominant microcystin-producer *Microcystis* to determine how nutrients affects both. Thirdly, we use molecular fingerprinting methods to access the diversity of cyanobacteria, and specifically *Microcystis*, to determine how nutrients affect diversity, and in turn how diversity affects microcystin concentrations. Finally, using the molecular fingerprints, we determine the source population of the *Microcystis* blooms - either the lake sediments or tributaries that drain into the lake. This study takes place in the western basin and in the Sandusky sub-basin of Lake Erie.

Methods

Sample collection

Ten sample locations (Fig. 1, Table 1) were selected to cover a large spatial area in Lake Erie and tributaries. These sites included two rivers (Maumee, site MR, and Sandusky site SR), two bay sites (Maumee, site MBay, and Sandusky, site SBay), three western basin sites (WB west, center, east), two Sandusky sub-basin sites (SSB west and east), and one site in the central basin site (CB).

Samples from MR and SR were collected by wading to a depth of 1.3 meters (top of the waders). Bay and lake sites were visited aboard the University of Toledo Lake Erie Center's research boat, Ohio State Stone Laboratory's *RV* Erie Monitor, or Cranberry Creek Marina boats (Huron, Ohio). At all sample locations the following data was recorded. Vertical profiles of water temperature, pH, specific conductivity, and dissolved oxygen were recorded from lake surface to sediments at one-meter intervals using a calibrated multiprobe YSI sonde (Yellow Springs Instruments, Yellow Springs, Ohio). Secchi disk depth was measured. Water samples from MR and SR were collected from

the surface, and an integrated tube-sampler was used at the bay and lakes sites to collect water from the surface to near sediments (Table 1). Water samples for chlorophyll (chl) *a*, nutrients, and DNA were stored in acid-washed polyethylene bottles and kept on ice during transportation back to laboratory. Water samples for phytoplankton identification were preserved using Lugol's solution. Water samples for the toxin microcystin (MC) were collected at all sites and dates from July to end of the season. Water for MC analysis was poured into 20 mL amber glass jars and kept on ice. WB_C was not analyzed for MC.

Upon arriving at the laboratory, lake water containing phytoplankton was filtered onto GF/F filters for analysis of chl *a*, and then filters where stored on silica gel at -80°C until analysis. Water for dissolved nutrient analysis [nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), dissolved kjeldahl N (DKN), dissolved reactive phosphorus (DRP), and total dissolved P (TDP)] was filtered through 0.45 μm membrane filter. Unfiltered water was used for analysis of total P (TP) and total kjeldahl N (TKN). Nutrient and MC samples were kept at -20°C until analysis. For DNA analysis, water was collected on a 0.45 μm polyvinylidene fluoride (PVDF) filter. Forceps to handle the filters were dipped in ethanol then exposed to flame to sterilized between filters. The filters were placed in sterile tubes and stored at -80°C until analysis.

To determine if the lake bottom is a source of the cyanobacterial blooms, five sites (MBay, WB_W, WB_E, SBay, and SSB_W) were visited on 8 June 2011 to collect sediment samples for DNA analysis. After all the above data and samples were collected, sediments were collected using petite-ponar. Sediment collected using ponar was deposited into a bin and surface sediments (top 3 cm) were collected in sterile 50 mL tubes. Three ponar samples were collected at each site and pooled into the same 50 mL

tube. Sediment samples were placed on ice while in transportation. The ponar was cleaned between each sample location, first by scrubbing with a brush with phosphate-free detergent, then placed in 0.33% chlorix bleach solution for at least 20 minutes, then in sodium thiosulfate for five minutes, then finally rinsed in DI water. Upon arriving at the laboratory the 50 mL tubes with sediments were stored at -80°C.

For DNA and MC analysis, the bay and lake sites were sampled once a month in July, August, and September during the cyanobacterial bloom. Because of the great distance between sites, all sites could not be sampled on the same day, but they were sampled within two days of each other. Sites MBay and WB_W were sampled on 22 July, 12 August, and 14 September. Sites WB_E, SBay, and $SSB_{E\ and\ W}$ were sampled on 20 July, 11 August, and 12 September. The river sites, MR and SR, were sampled approximately one week prior to lake sampling to determine if the rivers are a source population on 11 July, 4 August, and 6 September. MR and SR were also sampled on 23 June before the formation of the cyanobacterial bloom. SSB_E and CB were sampled on 10 October in response to a cyanobacterial bloom seen in the central basin by MODIS satellite. WB_C was not sampled for DNA and MC analysis.

Laboratory methods

All nutrient analyzes were preformed by the National Center for Water Quality Research at Heidelberg University. Dissolved organic N (DON) was calculated by DKN minus NH_4^+. Particulate organic N (PON) was calculated as TKN minus DON. Dissolved organic P (DOP) was calculated as TDP minus DRP. Particulate P (PP) was calculated as TP minus TDP. Total N was calculated as the sum of TKN, NO_3^-, and NO_2^-

(if detected). Total inorganic N (TIN) was calculated as the sum of NH_4^+, NO_3^-, and NO_2^-. Total dissolved N (TDN) was calculated as the sum of TIN and DON.

Chl *a* from GF/F filters was extracted in dimethylformamide (DMF) and quantified by fluorometry as in Speziale et al. 1984.

MC samples were thawed to room temperature before analysis. Cell lysis was preformed using the Abraxis Quik-lyse kit (Abraxis LLC, Warminster, PA, USA; Abraxis product number 529911QL) following the manufacture protocol. MC was determined using enzyme-linked immunosorbent assay (ELISA) (product number 520011). This MC analysis is congener-independent; therefore total MC concentration was determined.

Phytoplankton in the Lugol's fixed samples were concentrated to 10-30 mL by allowing phytoplankton to settle to the bottom in a 500 mL graduated cylinder for at least 48 hours. A one mL subsample from the concentrated sample was pipetted on a gridded Sedgewick-Rafter counting chamber and viewed at 100X or 200X using a Leica microscope. Cells of *Microcystis* colonies were determined using Spot Advanced software, as in Davis et al. (2012). At least 50 *Microcystis* colonies were measured until a constant average was obtained, and all colonies were counted in the 1 mL subsample. When present, 100 *Planktothrix* filaments' length and width were measured using Spot and biovolume calculated assuming cylindrical shape. Cell density was converted to biovolume, as in Wetzel and Likens (2000), except *Planktothrix*, which was determined directly.

Molecular methods

DNA from the PVDF filters and sediments was extracted using MoBio Power Water kit (MoBio Laboratories, Inc. Carlsbad, CA. USA #14900) and MoBio PowerMax Soil DNA kit (#12988), following manufacturing protocol and stored at -20°C until PCR amplification. Cyanobacteria specific primers were used to PCR amplify the 16S-23S rRNA internal transcribed spacer (ITS) (16S CSIF with a GC clamp, 23S ULR; Janse et al. 2003). PCR was performed following the cycles outlined in Janse et al. (2003). PCR products were separated on a 2% agarose gel with ethidium bromide to strain the DNA. Gels were visualized and photographed under UV transillumination using Kodak Gel Logic 200 (New Haven, CT. USA) imaging system in combination with Kodak 1D image analysis software. To obtain information specific to the *Microcystis* population, the *Microcystis* 550 bp band (Janse et al. 2003) was excised and cleaned using Promega PCR clean-up system (Madison, WI. USA #A9281). The excised sample was reamplified, ran on a 2% gel, and the 550 bp band was excised to ensure only the 550 bp band was used. The twice-excised sample was reamplifed.

Both the total cyanobacteria 16S-23S rRNA ITS and *Microcystis* 16S-23S rRNA ITS (from the excised 550 bp band) was subject to denaturing gradient gel electrophoresis (DGGE) profiling to generate fingerprints. Sample collected from SSB_W in August (non-excised) was used as the marker because of high number of bands that spanned the denaturing gradient. To allow comparison within and among gels, the marker was used every fifth lane - each gel had 12 samples and 4 markers. DGGE profiling followed the methods of Janse et al. (2003). Bands on the DGGE of the *Microcystis* 16S-23S rRNA ITS were excised, PCR amplified, and confirmed on agarose gel to be 550 bp.

To determine similarities among blooms across time and space in Lake Erie, a gel was ran with peak biovolumes of each location. MBay and WB_W July and August, WB_E and SSB_W August and September, SSB_E September, and CB October were loaded into the same gel. To determine possible source populations of blooms (lake bottom or rivers), samples in the gel were arranged as follows. For the western basin bloom, the gel included: sediment samples from MBay, WB_W, and WB_E collected on 8 June and MR, MBay, and WB_E samples collected in July, August, and September. For Sandusky sub-basin bloom the gel included: sediment samples from SBay and SSB_W collected on 8 June and SR, SBay, and SSB_W collected in July, August, and September. The Sandusky gel included a WB_W sample collected in August to determine if the western basin is a possible source for the Sandusky sub-basin bloom. The remaining samples were included on another gel.

Image Analysis

Fingerprints from DGGE were analyzed using GelComparII software (Version 4.5, Applied Maths, Austin, TX. USA). Gels were aligned using 11 bands in the marker as external references. Similar bands among samples were used as internal references. Bands were identified with a minimum profiling of 5%. Dice index (Dice 1945) was used to calculate similarity matrices. Cluster analysis was used to produce dendrograms using unweighted pair group method with arithmetic means algorithm to illustrate similarity among samples. The number of bands in each sample was used as an index of the number of different cyanobacteria and *Microcystis* strains present. The Shannon-Wiener Index ($H´$) was used as an index of diversity and calculated as:

$$H´ = \sum (n_i/N) \ln (n_i/N)$$

where n_i is the volume of each band and N is total volume of all bands in the sample (Tan et al. 2009).

Data analysis

Correlations matrixes were generated for diversity indexes, microcystin concentration, and *Microcystis* biovolume among each other and among environmental parameters measured (nutrients, temperature, secchi depth, ect.). Variables that had a Pearson's coefficient (R) of greater than 0.10 were loaded into a stepwise multiple regression models. Because *Microcystis* biovolume is low in Sandusky Bay, a subset of data excluded site SBay. All statistical tests were preformed in SPSS.

Results

Nutrient Concentrations

Total N concentrations measured in June were twice the total N measured in any other month (Fig. 2A). Total N decreased throughout the summer at most sites. Within a given month, total N was greatest in the river and the bays and lake basins were similar. The bays and lake basins sites were below 100 μmol/L (1.4 mg N/L) from July through September.

Total P concentrations were also greatest during June at most sites (Fig. 2B), but the difference among other months was not as pronounced as total N. The bays and lake basins sites were near or below 2 μmol/L (0.062 mg P/L) from July through September. Sites SR and SBay generally had higher total P than MR and MBay. However, the western basin sites had higher total P than the Sandusky sub-basin sites.

The ratio of total N to total P (TN:TP) was highest in June and July, and then decreased during August and September at all sites except WB_E (Fig. 2C). No spatial patterns are apparent.

Figure 3 presents N concentrations partitioned among nitrate (NO_3^-), ammonium (NH_4^+), dissolved organic N (DON), and particulate organic N (PON) arranged by month and sample site from West to East. NO_3^- decreased throughout the year and decreased from highest levels in the river to lower levels the lake basins. Highest NO_3^- was detected at SR on June 24 at 563.17 μmol/L (7.89 mg N/L). NO_3^- as below detectable levels at SBay in July, August, and September. NO_3^- as also below detection in August at MBay, WB_W, and WB_C. NO_3^- increased slightly in September. NH_4^+ was low in all samples and ranged from 1.93 to 9.42 μmol/L (0.027 to 0.13 mg N/L) and did not show any spatial or temporal patterns. DON was highest in the rivers and decreased into lake basins and did not show any temporal patterns. During August and September DON at the bay and lakes sites was greater than NO_3^-. PON did not have any apparent spatial or temporal patterns. PON concentrations ranged from 3.78 to 71.23 μmol/L (0.053 to 0.998 mg N/L). NO_2^- was below 4.3 μmol/L in all samples and below 1 μmol/L for 74% of the samples (not shown).

P partitioning is presented in Figure 4. Both MR and SR had particulate P (PP) as the highest proportion of TP. SR had dissolved reactive P (DRP) greater than 2 μmol/L (0.062 mg P/L) in June and August while the other six river samples had relatively much lower DRP. All forms of P decreased from rivers into the bays and eastward into the basins. Highest DRP of 3.01 μmol/L (0.0932 mg P/L) was measured in June at MBay and WB_W (2.32 μmol/L), and in fact DRP made of the highest proportion of TP in these two

samples. High DRP values were accompanied by high DOP values. Unlike NO_3^-, there was no seasonal pattern of P values.

Chlorophyll a

The greatest chlorophyll *a* (chl *a*) concentration was measured at MR during June at 210.1 μg/L (Fig. 5). MR chl *a* was greater than 35 μg/L during the other three months. MBay and the WB sites had chl *a* levels that exceed 50 μg/L in August, which indicates the peak of the bloom. SR had relatively low chl *a* in June (10.3 μg/L), unlike MR, then SR had relatively high chl *a*. SBay had chl *a* greater than 50 μg/L each month. The two SSB sites had low chl *a* compared to the WB. SSB_E and CB were sampled on October 10 when MODIS satellite images showed a large bloom in the central basin. SSB_E and CB had chl *a* values of 31.3 and 13.9 μg/L, respectively. It should be reminded and emphasized here that all samples were integrated over the entire water column, thus a surface scum sample would contain much higher chl *a*.

Secchi disk depth

Secchi disk depths show a distinct spatial and temporal pattern (Fig. 6). During a given month, secchi depth was lowest in the river and increased in the bay and eastward into each basin. Highest secchi depths were recorded during July, and the secchi depth were reduced each month after as the lake became more turbid due to increases of suspended sediments.

Microcystin

Twenty-eight water column samples were analyzed for the toxin microcystin (MC). MC ranged from 0.07 μg/L to 8.67 μg/L (Fig. 7). MC in July were below 1.0 μg/L at all sites, except SBay. Highest MC concentrations were measured in August in MBay

and WB_W, which corresponded to the peak of the bloom. In September, MC concentrations were high (>2.5 μg/L) at both MBay and SBay, while the other sites were below 0.7 μg/L. The river sites, MR and SR, had low MC on each sample trip. The October central basin bloom had low MC concentration.

MC was positively correlated with *Microcystis* biovolume, chl *a*, NH_4^+, DON, and TKN, and was negatively correlated with secchi disk depth (Table 2). Stepwise multiple regression generated four models to predict MC (Table 3). Parameters included DON (models 1-4), *Microcytis* biovolume (models 2-4), NH_4^+ (models 3 and 4), and chl *a* (model 4).

In the subset of data that excluded site SBay, MC was positively correlated with *Microcystis* biovolume, chl *a*, NH_4^+, DON, PON, and TP (Table 4). Stepwise multiple regressions generated two models (Table 5). The first model included NH_4^+ and explained 56% of the variation. The second model included NH_4^+ and chl *a*.

Microcystis biovolume

In July, MBay was the only site with relatively high *Microcystis* biovolume (Fig. 8). As the bloom progressed, biovolume increased in the western basin with greatest values recorded in August. In September the Sandusky sub-basin had higher *Microcystis* biovolume than the western basin. Only two sites were visited in October, and SSB_E had higher biovolume than any values recorded in September.

SBay was dominated by the filamentous cyanobacterium *Planktothrix* (Fig. 8). Very little *Microcystis* was present. The biovolume of *Planktothrix* as SBay during August and September exceeded the greatest recorded *Microcystis* biovolumes.

Microcystis biovolume was positively correlated with MC, chl *a*, PON, TKN, and PP, while negatively correlated with DRP (Table 4). Because chl and particulate matter will naturally increase with increasing *Microcystis* biomass, multiple regressions was not preformed for *Microcystis* biovolume.

Diversity indexes

The number of cyanobacterial bands ranged from 4 to 21 (Fig. 9A). *H´* gave similar results as the number of bands (Fig. 9B). The number of band and *H´* were highly correlated (R = 0.959); therefore only correlations with the number of bands are presented to avoid redundancy. The number of cyanobacterial bands positively correlated with *Microcystis* bands, chl *a*, DON, PON, TKN, PP, and TP, and negatively correlated with NO_3^-, TIN, and TN:TP (Table 2). Among those variables, only TKN was selected by the stepwise multiple regression model (Table 3). In the data subset excluding site SBay the number of cyanobacterial bands was correlated with DON and PP (Table 4). The multiple regression model was not significant (Table 5).

The number of *Microcystis* bands ranged from 3 to 17, but most samples contained 5 to 8 bands (Fig. 10A). There was no spatial or temporal pattern of the number of bands. The Shannon-Wiener Index (*H´*) gave very similar results as the number of bands and no trend is evident (Fig. 10B). The number of bands and *H´* were highly correlated (R = 0.918); therefore only correlations with the number of bands are presented. The number of *Microcystis* bands positively correlated with cyanobacterial bands, chl *a*, PON, and PP, and negatively with NO_3^- (Table 2). Stepwise multiple regression only selected PON, however, the model was not significant (Table 3). In the data subset excluding site SBay, the number of *Microcystis* bands was negatively

correlated with NO_3^-, NH_4^+, TIN, TN, DRP, and TDP (Table 4). Multiple regression generated two models. Model 1 included DRP, and model 2 included DRP and NH_4^+ (Table 5). The coefficients for DRP and NH_4^+ were negative.

Microcystis *cluster analysis*

Ten samples were selected to generate a dendrogram for the entire lake from July to October (Fig. 11). There are several ubiquitous bands that occur in all samples, and all samples showed greater than 43% similarity. The first branch off the tree was the July samples from MBay and WB_W. The remaining cluster had greater than 52% similarity.

Gels with samples confined to either the western basin or the Sandusky sub-basin and the associated tributaries were used to indicate source populations. In the western basin gel (Fig. 12A), the MR samples in July and September do not cluster with the bay or western basin samples. Among the remaining samples, there were two main clusters. One cluster contained the June sediment samples and the July MBay and WB_E samples. The other cluster contained the August and September MBay and WB_E samples and the August MR sample. Temporal patterns were more prominent than spatial patterns. Excluding the MR samples, samples collected during the same month clustered together. For example, the August MBay sample was more similar to August WB_E than July or September MBay.

The Sandusky gel also contained two main clusters (Fig. 12B). One cluster contained all SR samples, July SBay and SSB_W, and August SBay. The other cluster contained the sediment samples, SSB_W August and September, WB_W August, and SBay September. The temporal patterns were not as apparent as compared to the western basin gel. The WB_W August sample included on this gel clustered with SSB_W September.

The final gel (Fig. 12C) supports the previous gel results. The June SR and MR samples are only 9% similar to the lake samples. Temporal patterns were more prominent than site patterns. The two October blooms were similar, as were the two September samples.

Discussion

In this study, we partitioned the pools of total N and P into dissolved and particulate, and organic and inorganic fractions for two major tributary-bay-lake systems in Lake Erie throughout the summer. Then knowing how nutrients are partitioned, we attempted to find relationships between those nutrients and microcystin, the diversity of cyanobacteria, and *Microcystis* in order to determine how diversity will be affected by altering nutrients. Further, because diversity of *Microcystis* was determined over time and space, source populations (*i.e*. sediments or river inputs) of the blooms and how blooms from different basins are related can be determined.

Nutrients

The seasonal decline of NO_3^- throughout the growing season is typical for Lake Erie (Moorhead et al. 2008; Chaffin et al. 2011). The decline of NO_3^- can be attributed to several factors. First, the loading rate of NO_3^- from the Maumee and Sandusky rivers to Lake Erie decreases throughout the summer (Richards et al. 2010), thus NO_3^- consumption must be greater than the loading rate from the rivers. Secondly, the N cycle is very complex and mediated by microbes (Wetzel 2001). N is rapidly cycled between organic and inorganic, and exists with oxidation states from +5 to -3 (Wetzel 2001). As phytoplankton and bacteria grow they assimilate NO_3^- and convert the N into PON. That

N will be lost from the system if those phytoplankton and bacteria are moved out of the system by currents before reconverted back to NO_3^-. Lake sediments and anoxic waters provide a location for anaerobic bacteria to use NO_3^- as the electron donor, termed denitrification (Seitzinger 1988). Denitrification plays a key role in the N cycle and can drive water bodies towards N-limitation because it results in N being lost from the system as N_2 gas (Seitzinger 1988). The final process that can deplete NO_3^- is disassimilatory NO_3^- reduction to NH_4^+ (DNRA). DNRA occurs in the sediments and results in NO_3^- being converted to NH_4^+ (Burgin and Hamilton 2007). In the hypereutrophic Lake Taihu of China (McCarthy et al. 2007a) and wetlands in Texas (Scott et al. 2008) DNRA was an overall minor player in N removal in comparison to sediment denitrification. Near tributaries of Lake Taihu DNRA removed nearly as much N as denitrification (McCarthy et al. 2007a). In Lake Erie, N removal by DNRA in the wetland Old Woman's Creek was one-fifth that of sediment denitrification, and nearshore Lake Erie sediment denitrification rates about one-third that of the wetland (McCarthy et al. 2007b). Maumee and Sandusky Bays are the only likely locations were DNRA may have a significant role in NO_3^- removal. NO_3^- assimilation, out flow, denitrification, and the lack of NO_3^- supply from rivers results in NO_3^- concentrations to decrease throughout the summer.

NH_4^+ was detected in all samples, but was in lowest concentrations (except when NO_3^- was below detectable levels). NH_4^+ is the preferred N source among phytoplankton because of its reduced inorganic state, and assimilation of non-NH_4^+ N is much decreased in the presence of 1 μmol/L NH_4^+ (Dortch 1990). Hence, assimilation is a major sink for NH_4^+. Nitrification (NH_4^+ to NO_3^-) has also been shown to be an important sink of NH_4^+ by ammonium-oxidizing microbes in Lake Erie (Darren Bade, Kent State University,

unpublished data). Regeneration of NH_4^+ by microbes in the water column is greater than that of the sediments and DNRA (McCarthy et al. 2007b). NH_4^+ excretion for *Dreissena* mussels is also an important N source to the water column (Conroy et al. 2005). The concentrations of NH_4^+ reported in this study do not vary spatially or temporally. Therefore sources of NH_4^+ (regeneration, DNRA, excretion) and sinks of NH_4^+ (assimilation, nitrification) must be occurring at similar rates.

Limnologists often overlook DON, while oceanographers have long understood the role of DON in marine systems. This is most likely due to the general paradigms that P limits freshwaters (Schindler 1974) while N limits marine waters (Seitzinger 1988). Recently the P-limitation of freshwater has been challenged (Conley et al. 2009), and the importance of DON in freshwater is beginning to be recognized (Finlay et al. 2010; Bogard et al. 2012). In Lake Erie DON contained the largest pool of N during August and September. DON can contain numerous numbers of organic N compounds that are available for assimilation or refractory (Lewis et al. 2011). Urea is likely a major proportion of the DON because of agricultural and city wastewater sources (Finlay et al. 2010). Inputs of urea into N-stressed lakes have resulted in *Microcystis* blooms (Finlay et al. 2010). Lake Erie is N-stressed during times of low NO_3^- availability (Chaffin and Bridgeman 2012 IAGLR presentation). Therefore, efforts to minimize urea inputs from agriculture and wastewater treatment would help control cyanobacterial blooms. Further research into identifying the DON compounds is warranted.

P is generally the limiting nutrient for freshwater phytoplankton growth and abundance (Schindler 1974). Lakes with higher P concentrations have higher biomasses of cyanobacteria (Downing et al. 2001). A 2009 study in the Maumee River and western

Lake Erie Bridgeman et al. (2012) partitioned total P into DRP, DOP, and PP and discussed the implications. The results between Bridgeman et al. (2012) and the data presented in this report generally agree. All P forms are highest in the rivers and decrease in concentration with increasing distance from the river. The only disagreement between the two data sets is the PP and DOP concentrations measured in June. Bridgeman et al. (2012) reported that DOP was more than half of the total P while PP was a relatively minor proportion. In this report, PP was had high proportions in most samples collected during June. Two samples in June, MBay and WB_W, had large proportions of DRP. The high concentrations of DRP in these two samples are likely the result of heavy rainstorms that occurred in the Maumee River watershed during late May 2011. The increase of DRP at most sites in September is likely do to N-limitation as a result of low NO_3^-. Bridgeman et al. (2012) also partitioned the PP by size and buoyancy, and found that more than 90% of the PP is less than 112 μm. *Microcystis* blooms are in the fraction that would be larger than 112 μm because of large colonies. The 2009 *Microcystis* bloom was very dense in the western basin, but only contained up to 10% of the entire water column P (Bridgeman et al. 2012). However, the 2011 bloom was nearly 3x as dense than the 2009 bloom (Bridgeman et al. in revision). Assuming all other things are equal between years, the 2011 bloom could have contained 3x more of the water column P than 2009.

Microcystin

The World Health Organizations for acceptable levels for MC is 20 μg/L for recreational waters and 1 μg/L for drinking water. Nine samples had MC greater than the WHO limit for drinking water, but none were above the recreational waters limit. We measured MC concentrations from samples integrated over the water column.

Microcystis can form scums that are just millimeters thick at the water's surface (Ibelings 1996). *Microcystis* was concentrated at the surface on most sampling days; therefore we can assume that MC was also concentrated. If we assume that all the *Microcystis* is contained in the top 10 cm of the water column, we can estimate the surface scum MC concentrations. For example, on 12 August the MC concentrations at MBay and WB_W were 8.66 and 6.78 μg/L, respectively. Multiplying by the depth of the tube sampler used at each site will give an areal measurement (μg/m^2). Then multiplying by 10 cm will give the surface scum concentration. Thus, the surface scum concentrations of MC could have been as high as 1,732 μg/L at MBay and 4,603 μg/L at WB_W on 12 August. Water samples collected in western Lake Erie during late July 2011 by NOAA GLERL had MC concentrations greater than 1,180 μg/L (glerl.noaa.gov/res/centers/HABS/western_lake_erie_archive.html). Our calculated surface MC concentration and NOAA data clearly indicate levels that are well above WHO recommended guidelines.

MC concentration was correlated with *Microcystis* biovolume, which indicates that the amount of MC in the water column is limited by *Microcystis* biomass. Previous work has indicated a positive correlation between *Microcystis* biomass and MC concentration in Lake Erie (Millie et al. 2009; Rinta-Kanto et al. 2009b). Although the proportion of potentially toxic *Microcystis* colonies is about one-third (Dyble et al. 2008) and percentage of toxin-producing cells is about 8% (Rinta-Kanto et al. 2009b), those toxic strains are producing MC at a rate that matches the total *Microcystis* population growth.

MC concentration was significantly correlated with NH_4^+ and DON of both data sets (Tables 2, 4). In previous years, MC concentration was shown to positively correlate with P forms including DRP, TP, and TDP (Rinta-Kanto et al. 2009b). The likely difference between our study and Rinta-Kanto et al. (2009b) is that Lake Erie was N-limited in 2011, rather than P-limited (Chaffin and Bridgeman 2012). Lake Erie water incubated with enriched N resulted in additional phytoplankton growth compared to lake water enriched with P (Chaffin and Bridgeman 2012). Overall, it appears that whatever environmental factors limits *Microcystis* growth (*i.e*. P or N), that parameter will show positive correlations with MC concentrations. Millie et al. (2009) concluded that environmental factors indirectly regulate MC concentration in Lake Erie through regulating toxin-producers biomass, which have stronger correlations with MC than nutrients. In several laboratory experiments MC production by *Microcystis* was shown to increase with growth rate (Orr and Jones 1998; Downing et al. 2005; Rueckett and Cary 2009). During N-limitation, an increase of N will result in *Microcystis* to produce more MC per cell (Orr and Jones 1998). Further, Downing et al. (2005) reported that *Microcystis* produces more MC with increased growth rate only when N is abundant. Therefore, during N-limitation (Chaffin and Bridgeman 2012), increases of NH_4^+, the favored N source for phytoplankton (Dortch 1990), will increase growth rate and favor the production of MC. However, predicting MC concentration from environmental parameters can be spurious. Other factors such as competition for light among toxic and non-toxic strains will affect those relationships (Kardinaal et al. 2007a). Lag effects between MC and nutrients or biomass will further complicate the relationships between

MC and biomass or environmental parameters (Vaitomaa et al. 2003; Kardinaal et al. 2007b; Ye et al. 2009).

Diversity of cyanobacteria and Microcystis

The number of *Microcystis* bands positively correlated with cyanobacterial bands. This indicates that conditions that promote diversity among all cyanobacteria will also promote diversity among *Microcystis*. However, it should be reminded that the methods used in this study (PCR and DGGE) are not qualitative for each band. Therefore, the presence of a certain band does not imply that that band (strain of cyanobacteria) is in equal abundance as other bands. Which particular strains are present in a given sample is likely due to an interaction among the parameters measured here and parameters not quantified in this study, such as grazing pressure and vertical mixing of the water column.

The number of cyanobacteria and *Microcystis* bands was negatively correlated with many forms of N. This finding goes against our hypothesis that diversity would decrease with decreased N. In the subset of data, NH_4^+ was included in multiple regression models for the number of *Microcystis* bands and microcystin concentration. Samples with higher levels of NH_4^+ had lower diversity of *Microcystis* and higher microcystin. Therefore, it is possible that higher levels of NH_4^+ selects for toxic strains of *Microcystis* in Lake Erie.

Overall, DGGE indicated that the *Microcystis* bloom is highly dynamic with strains appearing and disappearing throughout the lake and over time, but strains are present in all samples. Cluster analysis indicates that temporal and spatial patterns are present, which allows for tracking of the bloom. Separate populations of *Microcystis* are present in Lake Erie system. In the Maumee River to western basin continuum, there are

two separate *Microcystis* populations. The Maumee River is separate from the Maumee Bay and the rest of the western basin. In terms of *Microcystis,* Maumee Bay is very similar to the rest of the western basin (sites WB_W and WB_E) and Maumee Bay can be included as a part of the western basin. In the Sandusky River to Sandusky sub-basin continuum, there are three separate *Microcystis* populations. The Sandusky River, Bay, and sub-basin (sites SSB_W and SSB_E) are separate from each other. Sample locations that usually have low biomass of *Microcystis,* such as the rivers and Sandusky Bay, have separate *Microcystis* populations from the rest of the lake. Although the rivers and Sandusky Bay has high levels of nutrients, *Microcystis* does not bloom in these waters. The lack of blooms may be due to genetic limitations that prevent *Microcystis* from reaching high biomasses, or physical factors such as turbulent water prevent rapid growth.

There was high similarity among the peak bloom samples (Fig. 11). It is apparent that the intense Lake Erie bloom of 2011 was one bloom, and not several separate blooms. The large October central basin bloom can be tracked back to the Sandusky sub-basin in September and to the western basin (including Maumee Bay) in August. The initiation of the bloom during July was less similar compared to August through October, although similar bands were present in all samples. Therefore, preventing the bloom in Maumee Bay will also prevent the blooms in Sandusky sub-basin and central basin.

The *Microcystis* bloom was very similar among basins despite a wide range of nutrient concentrations (Figs. 2, 3, and 4). Two possible explanations are likely as to why the peak blooms were similar in spite of different nutrient concentrations. First, the common *Microcystis* strains that are present during the bloom are adapted to very low

nutrient concentrations. Secondly, these strains could be utilizing an internal supply of stored nutrients. *Microcystis* is capable of maintain growth for several cellular divisions without an external supply of P (Nalewajko and Murphy 2001; Tsukada et al. 2006). When external nutrients are in excess more than demanded by growth, *Microcystis* will take up more and store additional nutrients to support growth later (Baldia et al. 2007). A combination of low nutrient adaption and using internal storage is also likely.

The clustering of the lake sediments with the early western basin bloom indicates that the sediments are the more likely source of the bloom. The surface sediments at site MBay had 13,400 *Microcystis* cells per gram prior to bloom formation in 2009 (Gruden 2010). Moreover, it was shown that *mcyA* (one of the genes required for MC production) sequences found in lake sediments one-month prior the bloom were similar to *mcyA* sequences found in the water column during the bloom (Rinto-Kinta et al. 2009a). Further, Rinto-Kinta et al. (2009a) was able to grow *Microcystis* from lake sediments in the laboratory. The Maumee River does not appear to be a significant contributor of *Microcystis* to Lake Erie because the June and July samples are not similar with the lake samples. This agrees with a recent study by Kutovaya et al. (2012) who showed that Maumee River *mcyA* genes were associated with *Plankthotrix* and not *Microcystis*, and that *mcyA* sequences from lake samples associated with *Microcystis*. Our August Maumee River was 47% similar with the August and September lake samples. Because the bloom was already growing in the lake by the time of the August river sampling, the similarity between the lake and river samples is a likely indicator of reverse flow of the lake into the river. As suggested by Kutovaya et al. (2012) and Rinto-Kinta et al. (2009a),

our data suggests that the sediments are a more likely source for the *Microcystis* blooms than the Maumee River.

Like the Maumee River and western basin, the Sandusky River samples were separate from the lake samples. The Sandusky Bay samples of July and August clustered with the Sandusky River samples. The Sandusky River may be a potential source of *Microcystis* to the Sandusky Bay, but not likely to the lake. However, the similarity in *Microcystis* populations between the Sandusky River and Bay is a moot point because the filamentous cyanobacteria *Planktothrix* dominates the Sandusky Bay while *Microcystis* is sparse in the bay. The shallow, turbid, well-mixed conditions of Sandusky Bay favor filamentous cyanobacteria over colonial cyanobacteria (like *Microcystis*) and eukaryotic algae (Scheffer 1998).

Insights regarding the need to control nitrogen

The need to regulate N to control cyanobacterial blooms has been supported by many researchers lately (*i.e*. Conley et al. 2009; Scott and McCarthy 2010; 2011; Wihelm et al. 2011). In shallow European lakes a total N threshold of 143 μmol/L (2 mg N/L) exists irrespective of the total P concentration (Gonzalez Sagrario et al. 2005). Lakes with total N greater than 143 μmol/L are turbid and dominated by cyanobacteria, while lakes with less total N are clear and contain many macrophytes (Gonzalez Sagrario et al. 2005). The majority of the samples analyzed during this study collected during July and after had total N well below 143 μmol/L (Fig. 2A). Western Lake Erie phytoplankton growth in late summer is stimulated by the addition of N and not P (Chaffin and Bridgeman 2012), which suggests that regulating external N loading would minimize the cyanobacterial blooms. However, N-limitation alone does not entirely indicate that

regulating N loading will be effective at improving water quality because of the complexity of the N biogeochemical cycle in lakes.

NO_3^- concentrations decreased from high levels in June to below detection in August in the western basin, and yet high biovolumes of *Microcystis* were recorded. Past years have similar patterns of NO_3^- depletion (Bridgeman unpublished data), and yet the intensity of *Microcystis* blooms is extremely variable among years (Bridgeman et al. in revision). Therefore, reducing the amount of NO_3^- that enters Lake Erie may not be worthwhile, due to the disconnect between NO_3^- and bloom intensity. Further, eukaryotic algae are more competitive for NO_3^- than cyanobacteria (Blomqvist et al. 1994; Hyenstrand et al. 1998); thus, quicker depletion of NO_3^- may shift phytoplankton community to one dominated by cyanobacteria earlier in the year. However, we believe increasing NO_3^- loading will exacerbate the blooms.

NH_4^+ is the favored N source for phytoplankton (Dortch 1990). In this study, NH_4^+ concentration explained 56% of the variation in microcystin concentration. Decreasing NH_4^+ in Lake Erie would be beneficial. Further, cyanobacteria are more competitive for NH_4^+ than eukaryotic algae (Blomqvist et al. 1994; Hyenstrand et al. 1998). However, regulating external NH_4^+ loading may not be effective at decreasing lake concentrations. N is recycled as NH_4^+ by sediment microbes (McCarthy et al. 2007a) and *Dreissena* mussels (Conroy et al. 2005). NH_4^+ regeneration is responsible for maintaining *Microcystis* blooms in Lake Taihu in China (Paerl et al. 2011). Although we did not measure NH_4^+ regeneration rates, it is likely that those rates are high enough to support *Microcystis* blooms because NH_4^+ concentrations are similar across time and space in Lake Erie.

An important finding generated from this report is that DON makes up a majority of the total N pool. Although we did not identify the N compounds that make up DON in Lake Erie, urea is a likely component (Bogard et al. 2012). The importance of urea to eutrophication of freshwater lakes is beginning to be noticed by researchers. Phytoplankton growth on urea requires less energy than NO_3^- or N_2 by N-fixation (Flores and Herrero 2005). For each urea molecule taken in by the cell, two ammonia ions are generated, and further, one CO_2 molecule is produced (Flores and Herrero 2005). The CO_2 released from urea can be incorporated by the alga cell (Finlay et al. 2010). Additions to urea to hypereutrophic lakes resulted in an increased growth of non-N-fixing cyanobacteria (Donald et al. 2011) and increased concentrations of microcystin (Finlay et al. 2010). Agricultural and urban wastewater inputs to lakes affect urea concentration in lakes, although in-lake cycling of PON, DON, and urea is equally as important (Bogard et al. 2012). Minimizing the amount of urea entering N-limited Lake Erie would be suggested for the above reasons. However, this would be difficult because tertiary treatment does not affect urea concentration in the effluent (Bogard et al. 2012), and the use of urea as an N fertilizer is expected to increase in the future (Finlay et al. 2010). During the summer of 2011 we are determining the concentration of urea in Lake Erie and if urea can stimulate phytoplankton growth.

In this report, we focused on the N question strictly from a biological standpoint. Although in this report we show that low N did not affect the overall diversity of *Microcystis* and sample concentrations of N did not correlate with *Microcystis* biovolume, we believe that minimizing N inputs during late summer will bolster P constraints. Concentrations of the toxin microcystin were positively correlated with NH_4^+

and the low N concentrations in August in September indicate widespread N-limitation in Lake Erie. It is likely that blooms would be more severe if not for the low N concentrations. Thus, supplying N-limited cyanobacterial bloom with N will result in higher growth rates and more toxin production. However, a separate economic analysis is required to determine if the cost of N regulation is matched by economic gain in better water quality.

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Table 1. Sample locations with site depth and sample depth of water collected. Notation is as follows: M = Maumee, WB = western basin, S =Sandusky, SSB = Sandusky sub-basin, R = River, CB = central basin. Subscripts: W = west, C = center, E = East. CB was only sampled once on October 10.

Site	Latitude	Longitude	Depth (m)	Sample depth (m)
MR	41.56020	-83.64260	1.3	0-0.25
MBay	41.74250	-83.40090	2.3	0-2
WB_W	41.78890	-83.35630	5.8	0-5
WB_C	41.75020	-83.10360	9.9	0-8
WB_E	41.63918	-82.88988	8.5	0-7
SR	41.33440	-83.11570	1.3	0-0.25
SBay	41.47590	-82.76404	2.7	0-2
SSB_W	41.50828	-82.58571	13.1	0-8
SSB_E	41.48500	-82.35920	12.7	0-8
CB	41.56037	-82.12592	13.9	0-8

Table 2. Correlations between cyanobacteria and *Microcystis* diversity, *Microcystis* biovolume, and microcystin concentration between environmental parameters measured during this study. River samples were not included in the analysis. Values are Pearson correlation coefficient (R). All R values are $p < 0.10$. NS indicates $p > 0.10$.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	Cyanobacteria bands number	*Microcystis* bands number	*Microcystis* biovolume $\mu m^3/mL$	Microcystin $\mu g/L$
Cyano. bands	1	0.425*	NS	NS
Micro. bands	0.425*	1	NS	NS
Micro. biovol.	NS	NS	1	0.471**
Microcystin	NS	NS	0.471**	1
Chlorophyll *a*	0.568**	0.359	0.468*	0.575*
Temperature	NS	NS	NS	NS
Secchi depth	NS	NS	NS	-0.375
Nitrate	-0.430*	-0.465*	NS	NS
Ammonium	NS	NS	NS	0.636**
TIN	-0.430*	0.426	NS	NS
DON	0.422	NS	NS	0.672**
PON	0.621**	0.382	NS	NS
TKN	0.678**	NS	NS	0.502*
TDN	NS	NS	NS	NS
Total N	NS	NS	NS	NS
DRP	NS	NS	NS	NS
TDP	NS	NS	NS	NS
DOP	NS	NS	NS	NS
PP	0.731***	0.461*	NS	NS
Total P	0.672**	NS	NS	NS
TN:TP	-0.384	NS	NS	NS

Table 3. Multiple regression results for the number of cyanobacteria and *Microcystis* bands, and microcystin. See Table 2 for variable included into the models. Variables excluded from the models are not listed.

Cyanobacteria bands	Variables included	Coefficient	p value
Model 1	Constant	4.389	
$p < 0.001$; $R^2 = 0.534$	TKN	0.155	< 0.001

Microcystis bands	Variables included	Coefficient	p value
Model 1	Constant	6.678	
$p = 0.089$; $R^2 = 0.152$	PON	0.025	0.089

Microcystin (μg/L)	Variables included	Coefficient	p value
Model 1	Constant	-2.250	
$p = 0.001$; $R^2 = 0.457$	DON	0.127	0.001
Model 2	Constant	-2.251	
$p < 0.001$; $R^2 = 0.592$	DON	0.116	0.001
	Biovolume	5.86×10^{-7}	0.030
Model 3	Constant	-3.269	
$p < 0.001$; $R^2 = 0.676$	DON	0.078	0.029
	Biovolume	5.50×10^{-7}	0.028
	NH_4^+	0.381	0.058
Model 4	Constant	-3.283	
$p < 0.001$; $R^2 = 0.753$	DON	0.059	0.029
	Biovolume	4.15×10^{-7}	0.028
	NH_4^+	0.398	0.058
	Chlorphyll a	0.023	0.047

Table 4. Correlations among parameters measured for a sub-set of data excluding site SBay. Values are Pearson correlation coefficient (R). All R values are $p < 0.10$. NS indicates $p > 0.10$.

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

	Cyanobacteria bands number	*Microcystis* bands number	*Microcystis* biovolume $\mu m^3/mL$	Microcystin µg/L
Cyano. bands	1	NS	NS	NS
Micro. bands	NS	1	NS	NS
Micro. biovol.	NS	NS	1	05471**
Microcystin/L	NS	NS	0.571**	1
Chlorophyll a	NS	NS	0.848***	0.691*
Temperature	NS	NS	NS	NS
Secchi depth	NS	NS	NS	NS
Nitrate	NS	-0.425	NS	NS
Ammonium	NS	-0.410	NS	0.731**
TIN	NS	-0.454	NS	NS
DON	0.452	NS	NS	0.674*
PON	NS	NS	0.750***	0.620**
TKN	NS	NS	0.531**	NS
TDN	NS	NS	NS	NS
Total N	NS	-0.449*	NS	NS
DRP	NS	-0.558*	-0.349	NS
TDP	NS	-0.538*	NS	NS
DOP	NS	NS	NS	NS
PP	0.452*	NS	0.475*	NS
Total P	NS	NS	NS	0.436
TN:TP	NS	NS	NS	NS

Table 5. Multiple regression results for the number of cyanobacteria and *Microcystis* bands, and microcystin. See Table 4 for variable included into the models. Variables excluded from the models are not listed.

Cyanobacteria bands	Variables included	Coefficient	p value
Model 1	Constant	6.194	
$p = 0.060$; $R^2 = 0.205$	DON	0.142	0.060

Microcystis bands	Variables included	Coefficient	p value
Model 1	Constant	7.397	
$p = 0.013$; $R^2 = 0.311$	DRP	-1.936	0.013
Model 2	Constant	8.802	
$p = 0.007$; $R^2 = 0.458$	DRP	-1.874	0.010
	NH_4^+	-0.273	0.053

Microcystin (µg/L)	Variables included	Coefficient	p value
Model 1	Constant	-3.097	
$p = 0.001$; $R^2 = 0.562$	NH_4^+	0.883	0.001
Model 2	Constant	-3.097	
$p < 0.001$; $R^2 = 0.883$	NH_4^+	0.691	<0.001
	Chl *a*	0.065	0.001

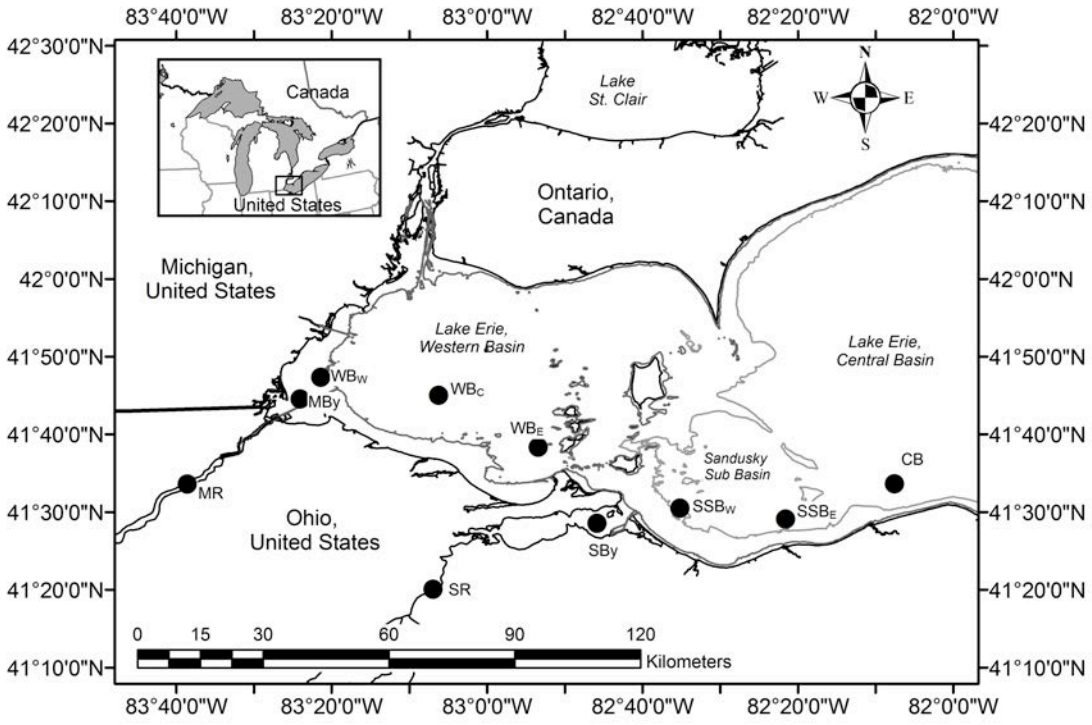

Figure 1. Sample sites in Lake Erie and tributaries. Contour lines are 5 meters and 12 meters.

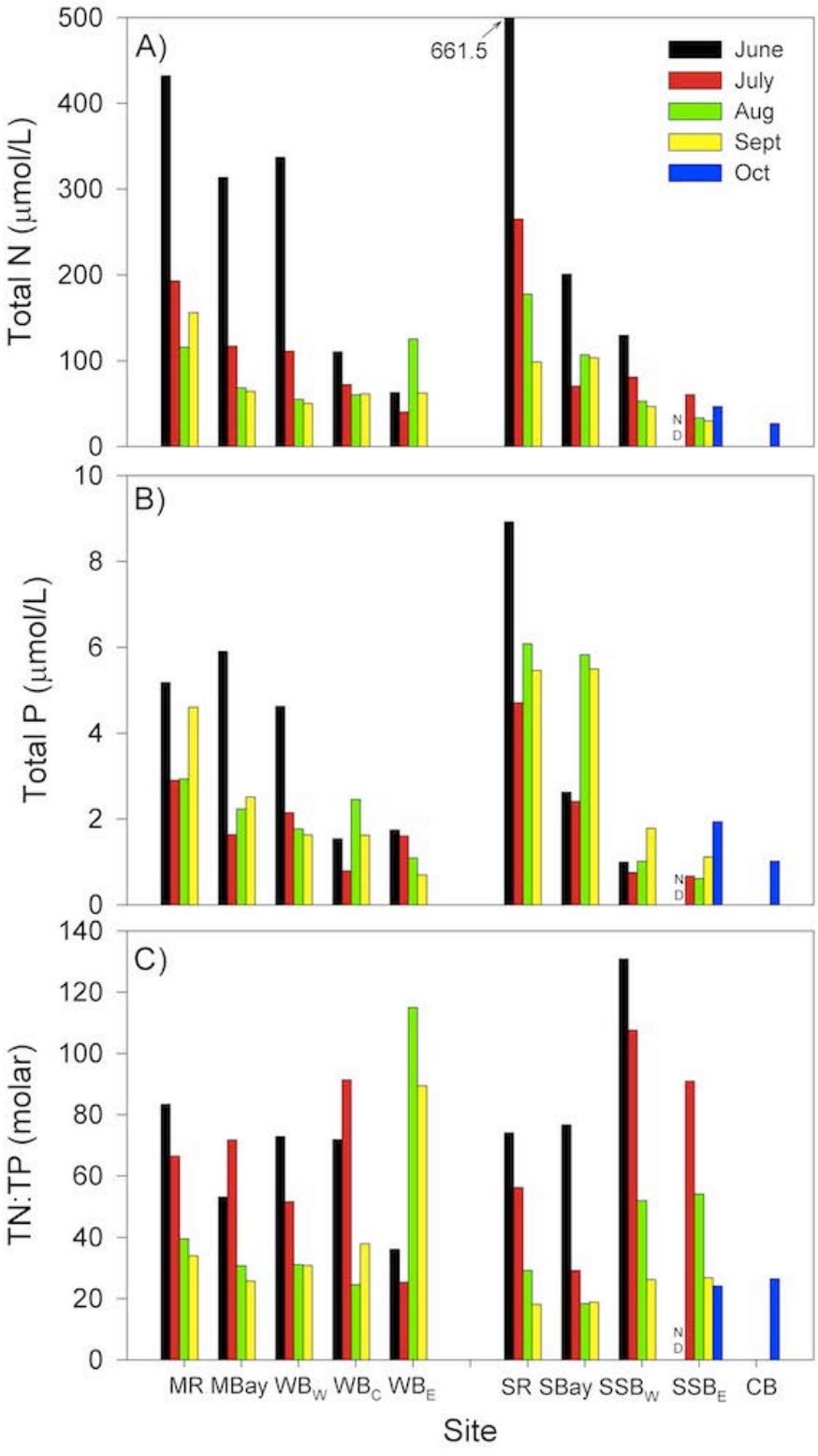

Figure 2. Total nitrogen (A), total phosphorus (B), and the ratio of total N to total P (C).

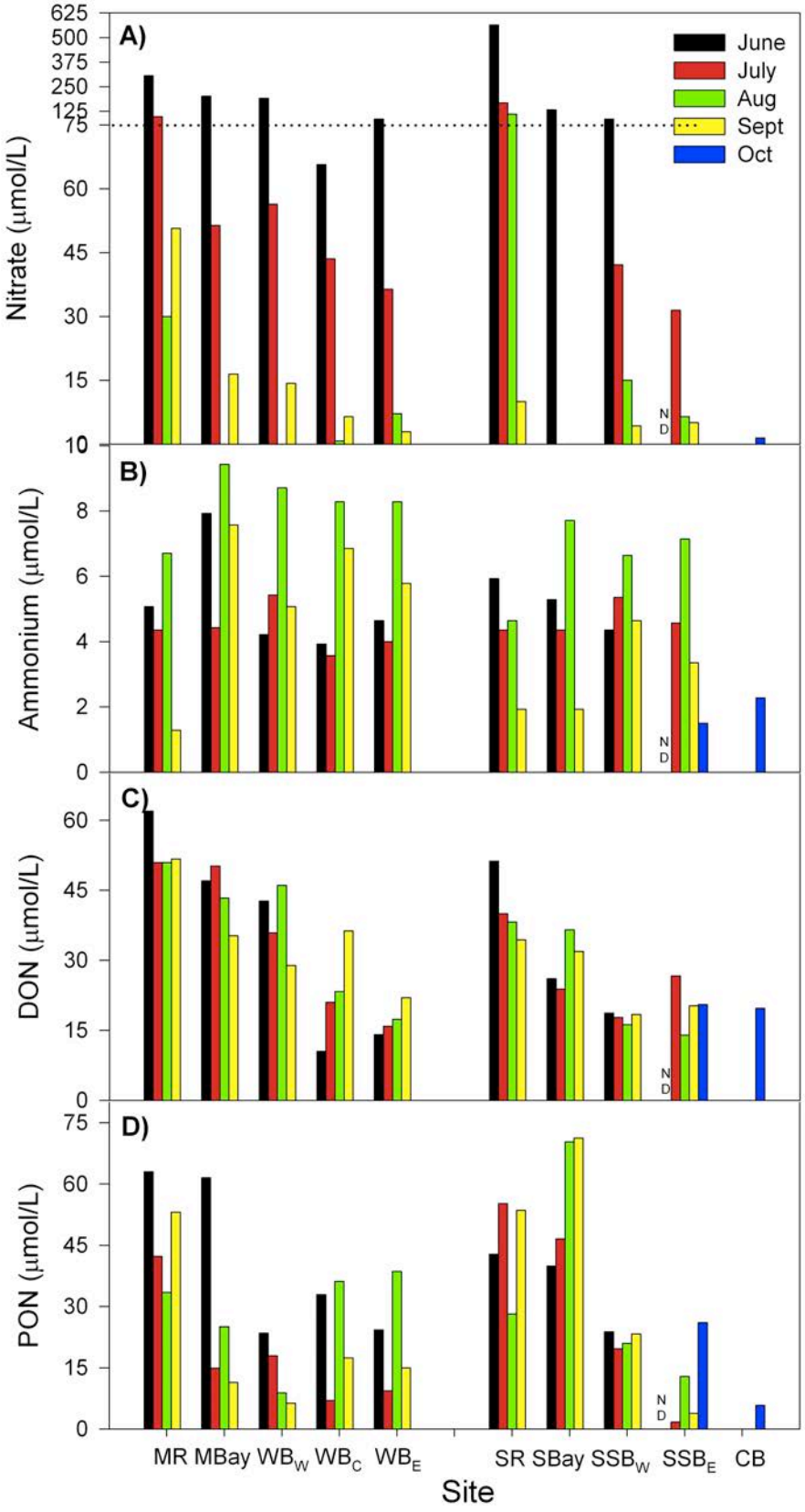
A)
B)
C)
D)
June
July
Aug
Sept
Oct
Nitrate (µmol/L)
Ammonium (µmol/L)
DON (µmol/L)
PON (µmol/L)
ND
MR
MBay
WB$_W$
WB$_C$
WB$_E$
SR
SBay
SSB$_W$
SSB$_E$
CB
Site

Figure 3. Total nitrogen concentration partitioned among nitrate (A), ammonium (B), dissolved organic N (C) and particulate organic N (D) measured in June, July, August, September, and October. SSB_E was not sampled in June (ND = no data). Only SSB_E and CB were sampled in October. Note difference of NO_3^- y-axis values greater then 75 μmol/L (the dotted line). Note the difference of scales on each axis.

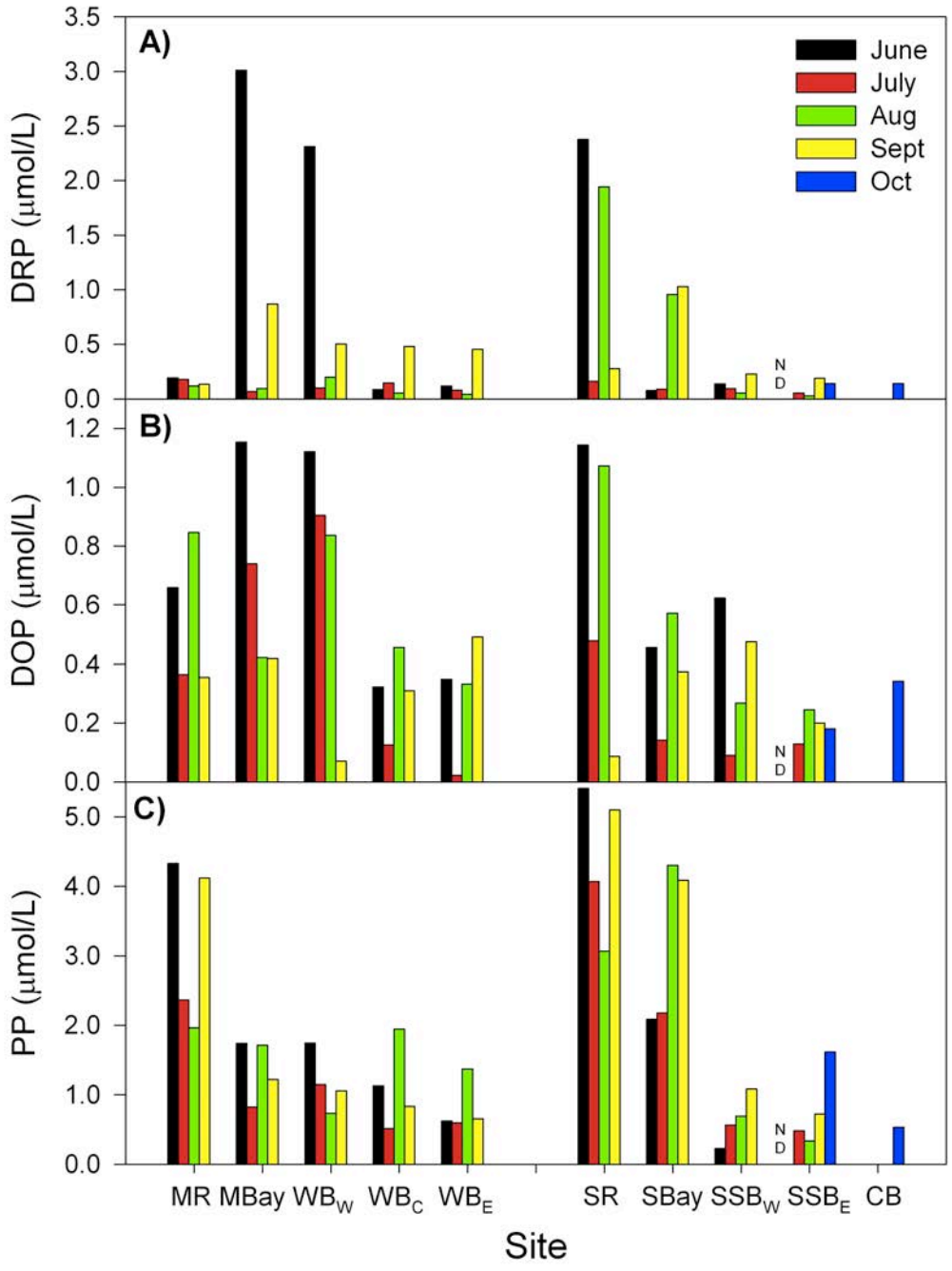

Figure 4. Total phosphorus concentration partitioned among dissolved reactive P (A), dissolved organic P (B), and particulate P (C) measured in June, July, August, September, and October. SSB_E was not sampled in June (ND = no data). Only SSB_E and CB were sampled in October. Note the difference of scales on each axis.

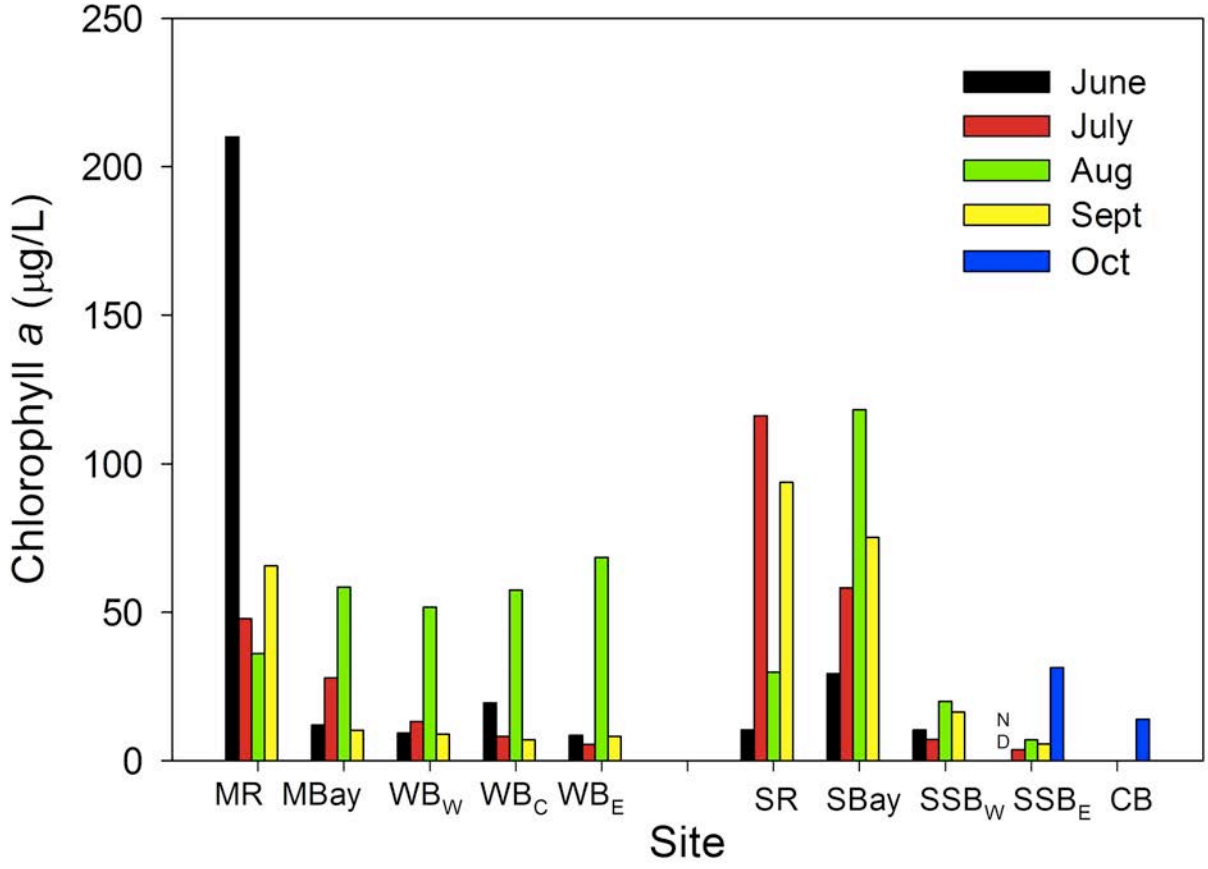

Figure 5. Chlorophyll *a* measured during this study. Water samples were collected over the entire water column. Chlorophyll *a* would be much higher if samples were surface-only.

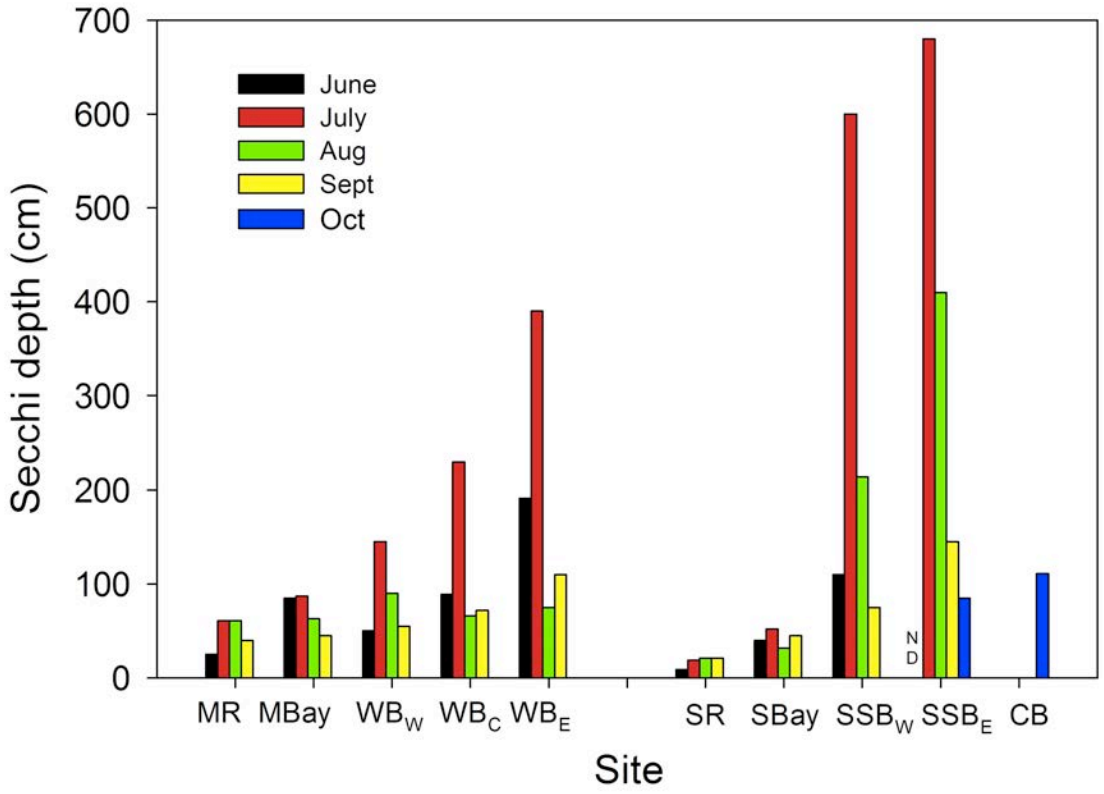

Figure 6. Secchi disk depths recorded during this study.

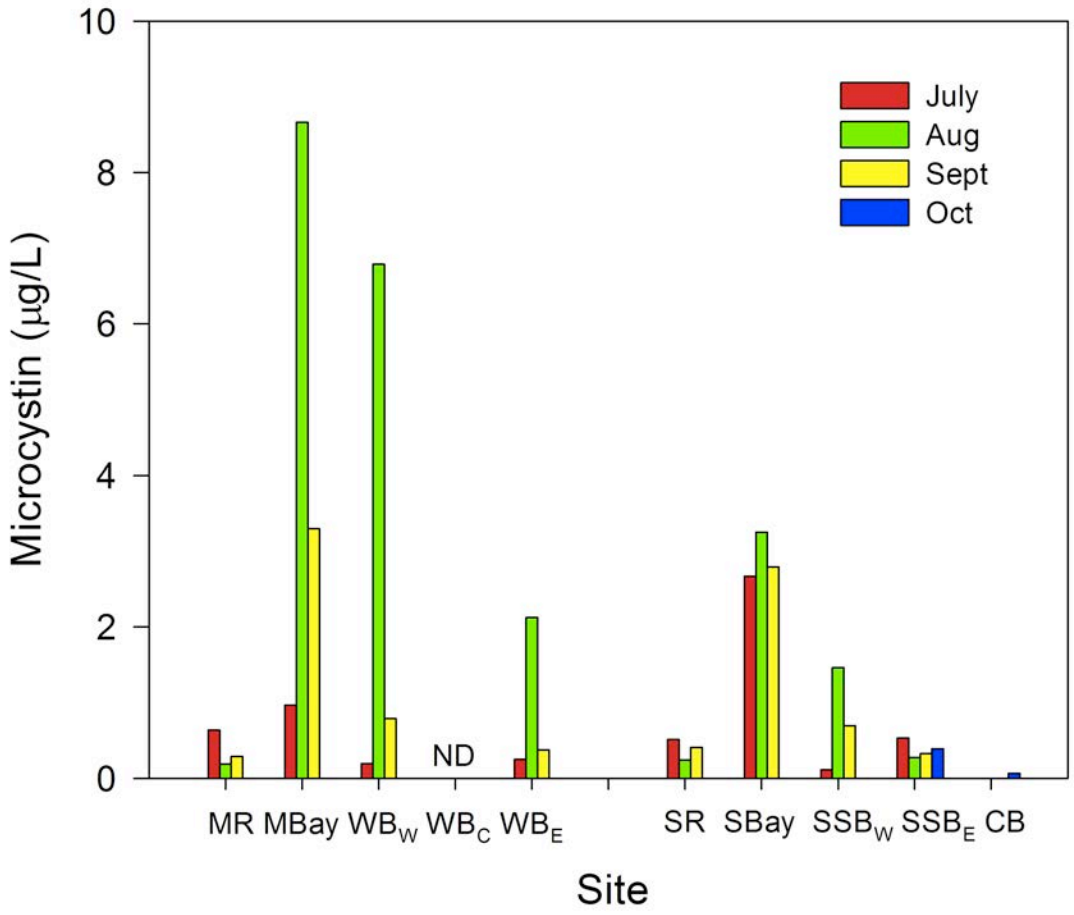

Figure 7. Microcystin measured during this study. Water samples were collected over the entire water column. Microcystin would be much higher if samples were surface-only. WB_C was not analyzed for microcystin (ND = no data).

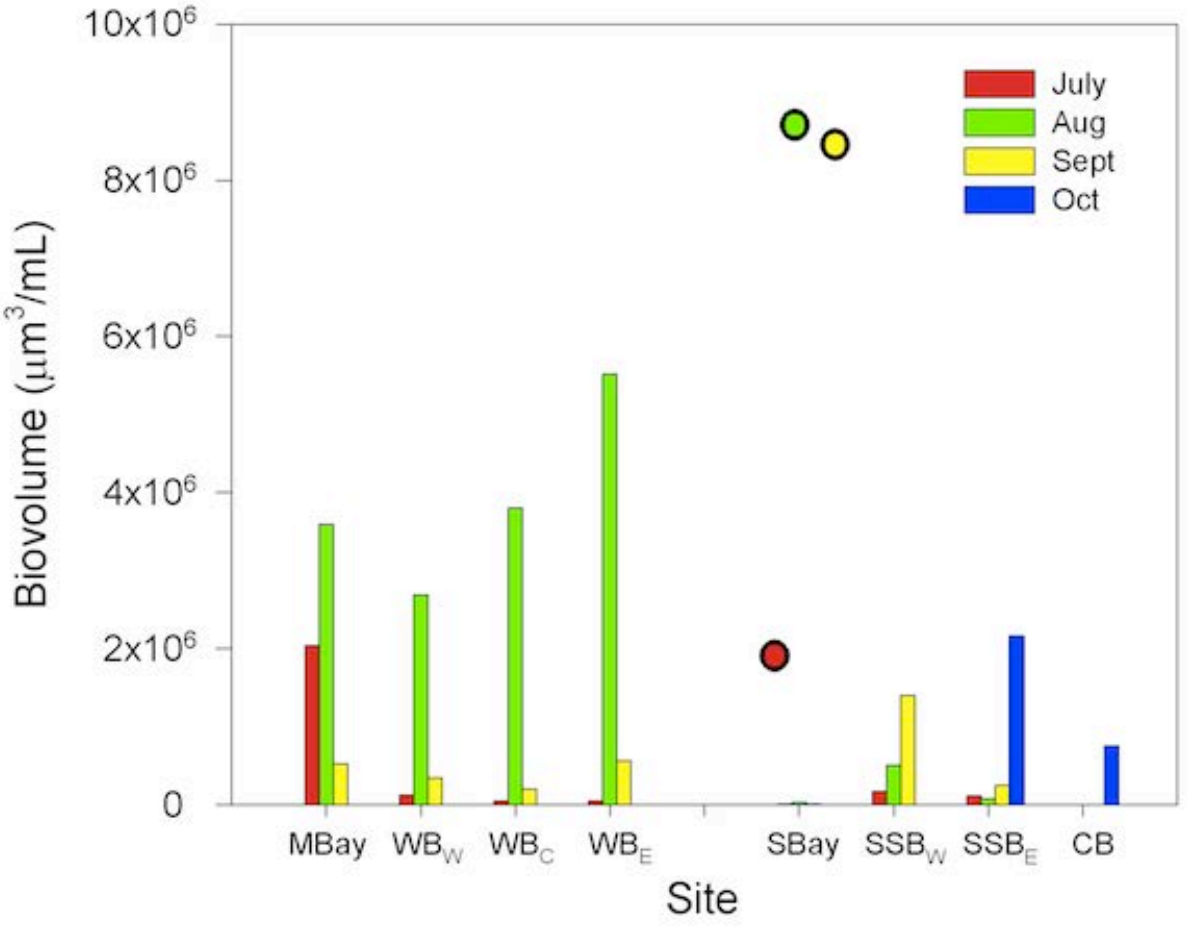

Figure 8. Biovolume of *Microcystis* (Bars) and *Planktothrix* (Circles).

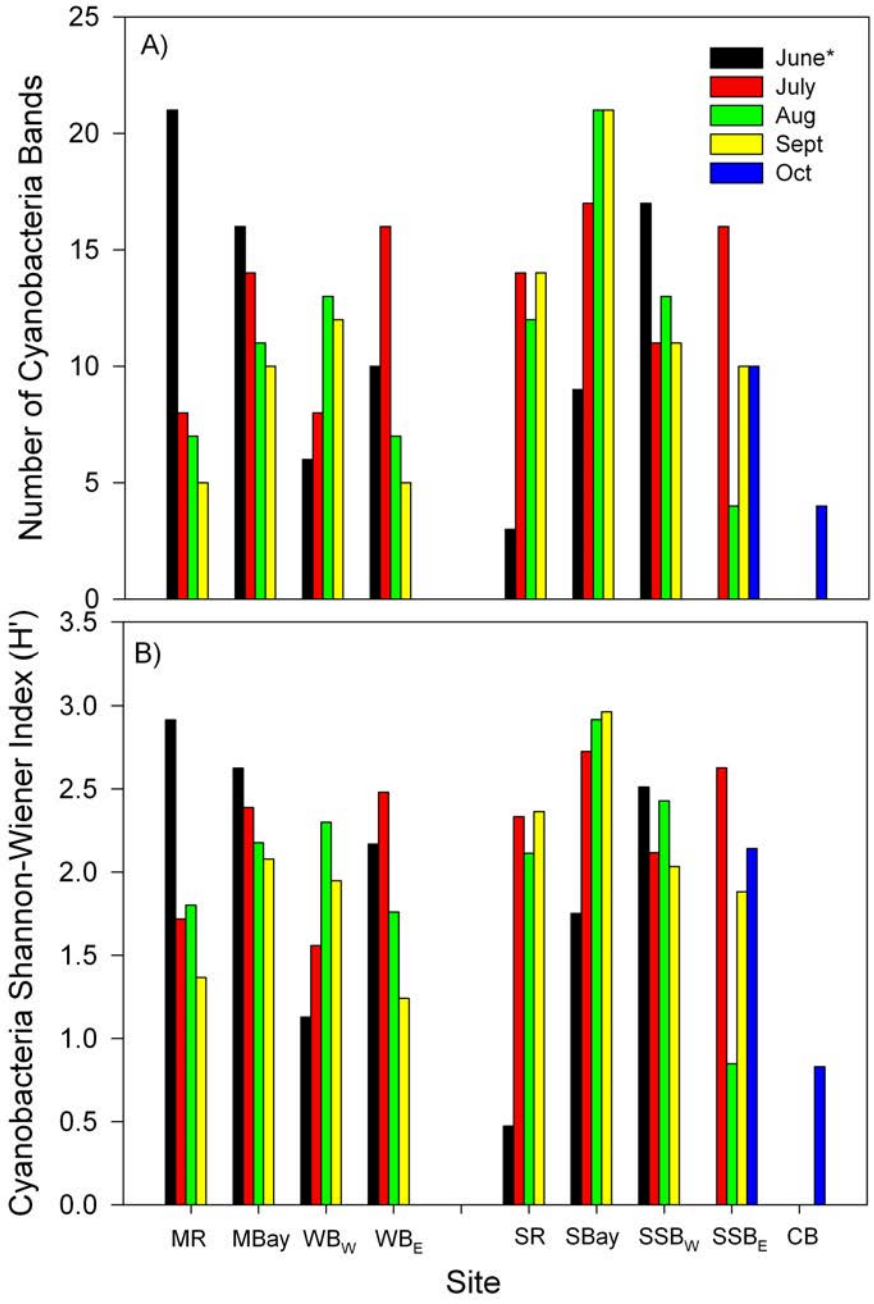

Figure 9. Number of cyanobacteria bands detected by DGGE (A) and the Shannon-Wiener Index of cyanobacteria (B). *June samples indicate sediment samples while other months are water column measurements. June river samples were water samples.

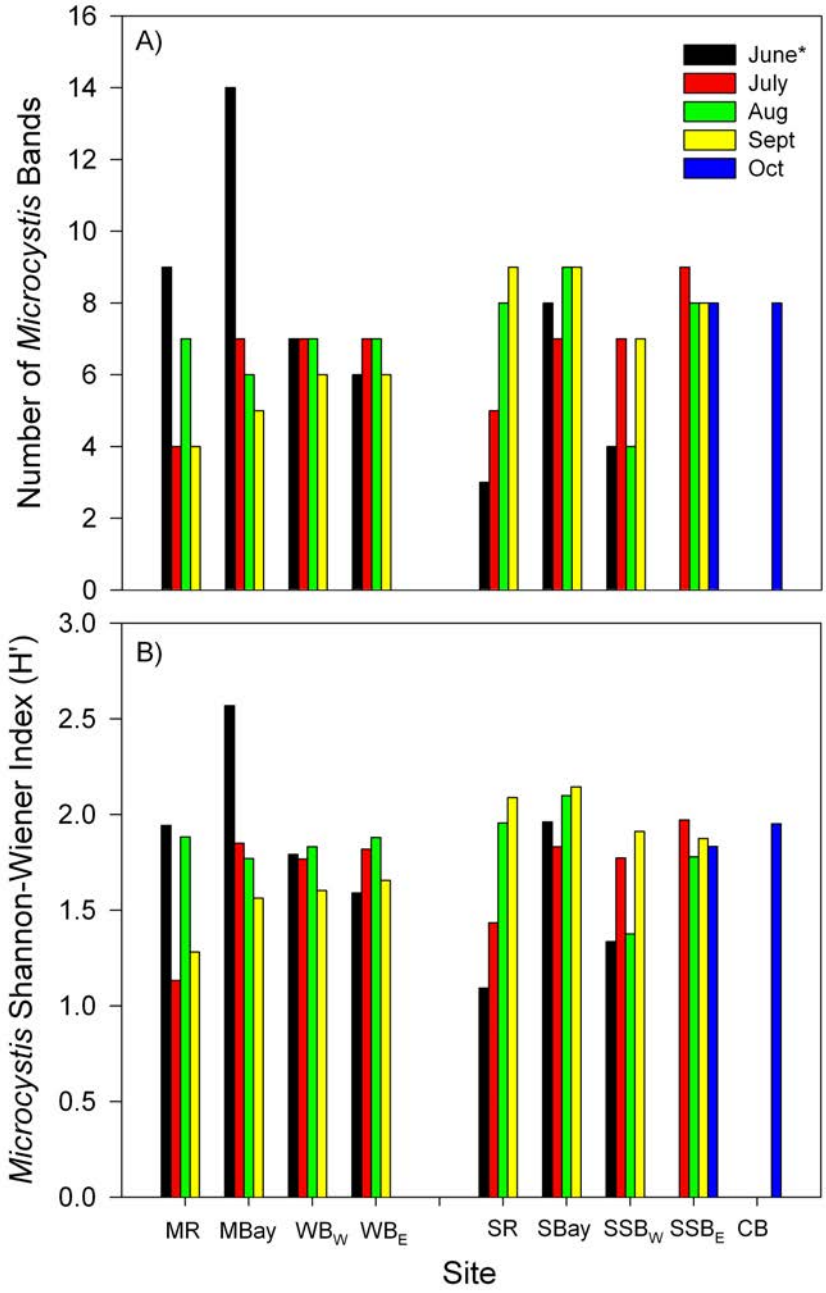

Figure 10. Number of *Microcystis* bands detected by DGGE (A) and the Shannon-Wiener Index of *Microcystis* (B). *June samples indicate sediment samples while other months are water column measurements. June river samples were water samples.

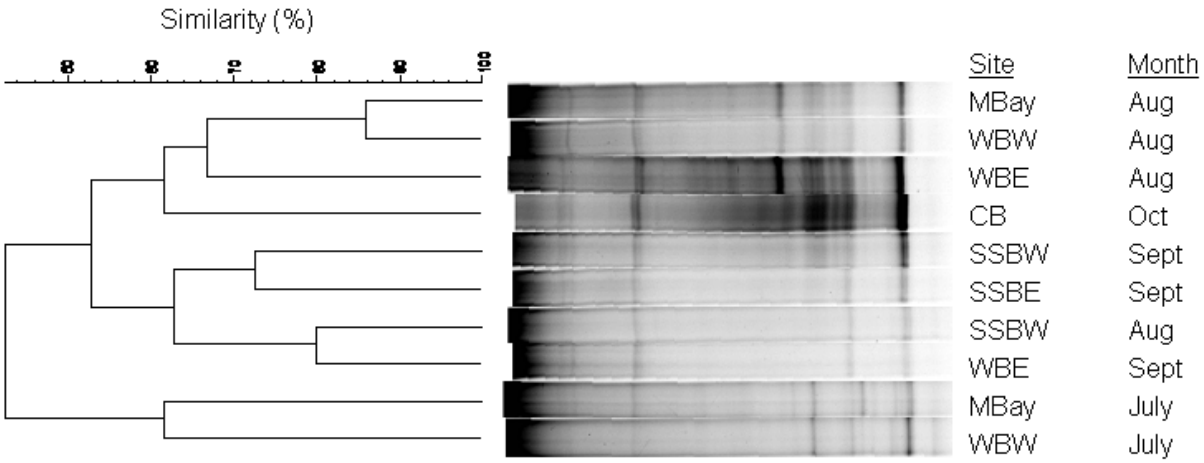

Figure 11. Dendrogram of peak *Microcystis* bloom samples across Lake Erie throughout the summer of 2011.

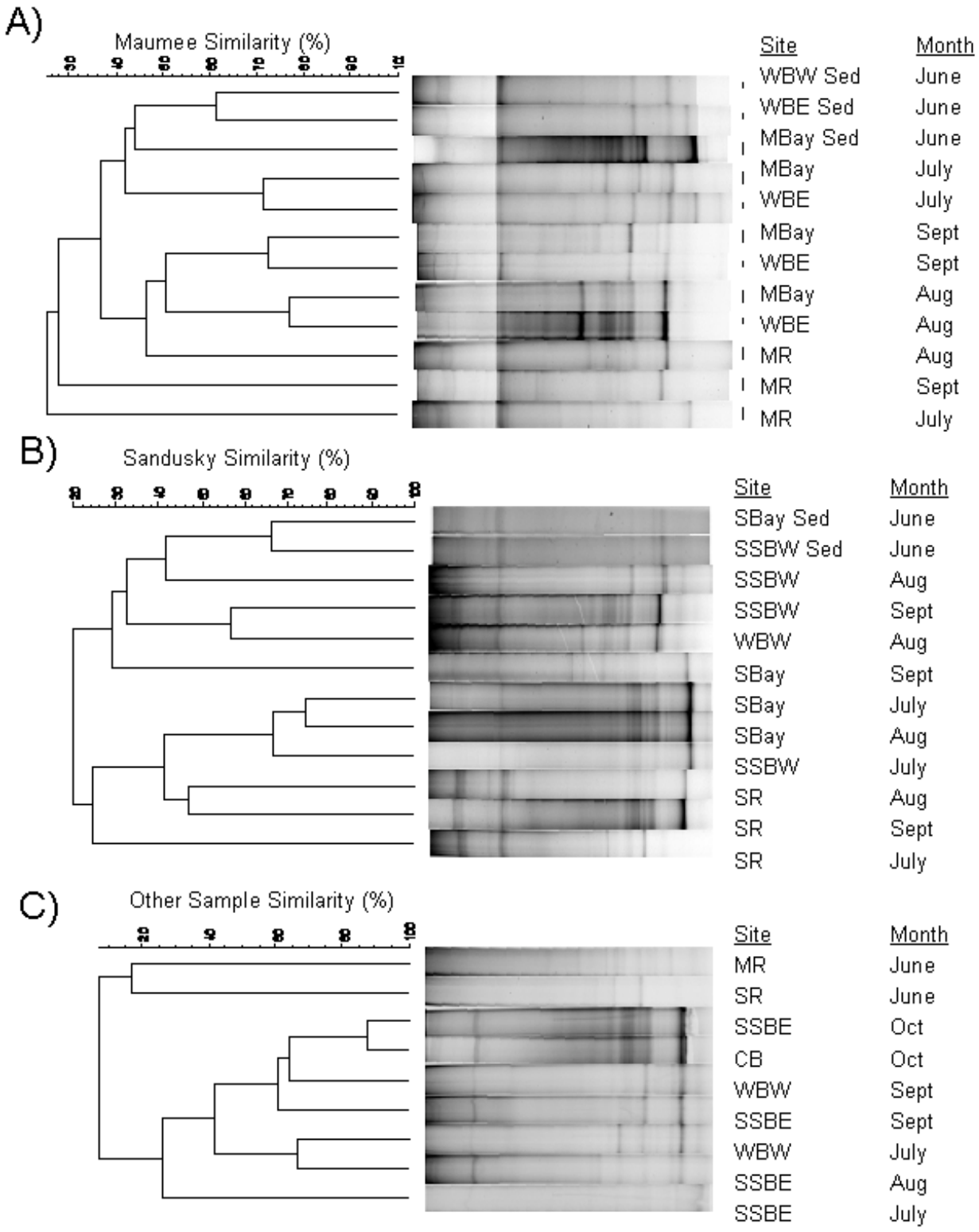
A)
Maumee Similarity (%)
Site
Month
WBW Sed June
WBE Sed June
MBay Sed June
MBay July
WBE July
MBay Sept
WBE Sept
MBay Aug
WBE Aug
MR Aug
MR Sept
MR July
B)
Sandusky Similarity (%)
Site
Month
SBay Sed June
SSBW Sed June
SSBW Aug
SSBW Sept
WBW Aug
SBay Sept
SBay July
SBay Aug
SSBW July
SR Aug
SR Sept
SR July
C)
Other Sample Similarity (%)
Site
Month
MR June
SR June
SSBE Oct
CB Oct
WBW Sept
SSBE Sept
WBW July
SSBE Aug
SSBE July

Figure 12. Dendrograms of the Maumee River-western basin system (A), the Sandusky River-subbasin system (B), and remaining samples (C). Note the difference in similarity scale.