Assessment of Microcystis growth rate potential and nutrient status across a trophic gradient in western Lake Erie

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Plankton tow samples collected from 2002 through 2009 indicate that Microcystis biovolume in western Lake Erie is often most dense in transition zone (TZ) waters between Maumee Bay and the center of the western basin. TZ waters are generally high in nutrients and turbidity, and concentrations of each decrease with distance from Maumee Bay. High Microcystis biovolume in the TZ suggests the possibility that the conditions in these waters support a greater Microcystis growth rate relative to the open lake. To test this hypothesis, during the 2008 bloom, Microcystis was collected from western Lake Erie for measurements of total protein content (TPC) as an indicator of growth rate potential and cellular nutrient content to indicate nutrient deficiencies. TPC results indicate that Microcystis in the TZ had a higher potential growth rate compared to offshore waters. TPC values in Maumee Bay were intermediate but not significantly different from the TZ and offshore. Nitrogen content of Microcystis remained high over the summer at all sites, despite very low dissolved nitrate concentrations and low total nitrogen-to-total phosphorus ratio in late summer in the lake. Ammonium level in the lake was constant during the summer, and likely provided the nitrogen source for Microcystis. Cellular phosphorus content varied between site and sample date suggesting that Microcystis was not deficient of micronutrients. Results of this study suggest the waters in and adjacent to Maumee Bay provide more favorable growth conditions for Microcystis than offshore waters.

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Introduction

In the mid-1900s Lake Erie experienced eutrophication and accompanying surface blooms of cyanobacteria (Davis, 1964; Matisoff and Ciborowski, 2005), leading to the passage of the Great Lakes Water Quality Agreement in 1972. The pollution control programs that followed succeeded in substantially reducing phosphorus (P) loading to the lake, resulting in a reduction of total phytoplankton biomass (DePinto et al., 1986; Matisoff and Ciborowski, 2005) with cyanobacteria a relatively small component of the phytoplankton community (Makarewicz, 1993). Since the mid 1990s however, total phytoplankton biomass and cyanobacterial biomass have increased in spite of nutrient control programs (Conroy et al., 2005b). Unlike the cyanobacterial blooms prior to nutrient controls, which were dominated by Anabaena spp. and Aphanizomenon spp. (nitrogen fixers), Microcystis aeruginosa (non-nitrogen fixer) dominates the current blooms of western Lake Erie (Brittain et al., 2000; Rinta-Kanto et al., 2005). The Microcystis biovolume recorded for the summer of 2009 was greater than any of the preceding seven years (Bridgeman et al., in review), but of greater concern is that blooms have become yearly occurrences in western Lake Erie over the last decade. The reoccurrence of Microcystis blooms, presence of large mats of the cyanobacterium Lyngbya wollei (Bridgeman and Penamnon, 2010), and discovery of the non-native cyanobacterium Cylindrospermopsis (Conroy et al., 2007) suggest that western Lake Erie may be again moving towards a more eutrophic state.

The Maumee River is the major source of nutrients and suspended sediments to Maumee Bay and western Lake Erie because of its large, highly agricultural watershed (Baker and Richards, 2002; Richards et al., 2008). The river produces enriched conditions in Maumee Bay with high concentrations of total phosphorus (TP) and total nitrogen (TN) (for example, 10.66 μM and 461.1 μM, respectively; Wang et al., 2009) and a river plume characterized by high nutrient concentrations and high turbidity (Moorhead et al., 2008) that extends into the western basin of the lake. The enriched conditions in the bay and river plume would be expected to promote cyanobacterial growth over the growth of eukaryotic algae (Tilman et al., 1986) and indeed, observations of previous Microcystis blooms in western Lake Erie suggest that the spatial extent of blooms often follows the spatial pattern of the suspended sediment plume (Chaffin, 2009). Internal P-loading in the bay and lake may also be significant due to the shallowness of Maumee Bay (<2.5 m), wind-driven resuspension...
of lake sediments (Søndergaard et al., 2003) and benthic invertebrate bioturbation (Chaffin and Kane, 2010). The outer boundary of the Maumee River plume is most often found in a region between 10 and 20 km from the mouth of the Maumee River referred to here as a “transition zone” where a distinct gradient in water quality parameters exists between waters characteristic of the Maumee River and Bay and offshore waters generally more influenced by inflow from Lake St. Clair and the upper Great Lakes via the Detroit River (Moorehead et al. 2008). Nutrient concentrations and turbidity quickly decline over the range of the transition zone with distance from the Maumee River mouth.

High TP concentration is a predictor of high cyanobacteria biomass. Downing et al. (2001) predict that a lake with TP greater than 3.23 μM has an 80% chance that cyanobacteria will dominate the phytoplankton community. Offshore waters of Lake Erie’s three basins are generally considered to be P limited (Wilhelm et al., 2003). However, nitrogen (N) colimitation has been recently reported in the central (Moon and Carrick, 2007) and eastern basin (North et al., 2007) of Lake Erie. Nitrate concentrations decline to low levels during the late summer in western Lake Erie (Bridgeman unpublished data) and may become limiting to phytoplankton. Microcystis is a non-N fixing cyanobacterium; therefore it requires inorganic N to thrive, and can only sustain growth for one day following N deprivation (Baldrizzi et al., 2007). N-fixing cyanobacteria are favored at low ratios of TN to TP (Smith, 1983). Microcystis’ inability to store and fix N may result in lower cellular N content in the relatively-low N offshore region, and possibly limit its growth, therefore allowing N-fixing cyanobacteria, such as Anabaena spp. and Aphanizomenon spp., to become dominant. Metal ions and micronutrients are also of importance as cofactors for enzymes (Kim and Wyckoff, 1991; Canini et al., 2001) and for ionic balance across membranes (Wetzel, 2001), and can affect carbon (C), N, and P acquisition by phytoplankton (McKay et al., 2001). P deficiency has the potential to affect the cellular content of these nutrients (Ji and Sherrell, 2008).

During the summer of 2008 we measured Microcystis total protein and cellular nutrient content at sites ranging from near-shore Maumee Bay to the transition zone to offshore western basin waters over a period when bloom biomass was continually increasing. Total protein content is a good indicator of growth rate potential because protein content increased linearly with specific growth rate of Microcystis measured in laboratory studies (Oh et al., 2000; Long et al., 2001; Downing et al., 2005). Protein content of Microcystis is also affected by nutrients because Microcystis deficient in N and P (Oh et al., 2000; Chu et al., 2007) and iron (Amé and Wunderlin, 2005; Sevilla et al., 2008) will contain less protein. Microcystis blooms typically appear first and reach highest biovolumes in the transition zone; therefore we hypothesized that Microcystis in this region would have a higher protein content relative to Maumee Bay and offshore locations. Ratios of the cellular C (Cc) to N, and Cc to P, give an index of nutrient deficiency in phytoplankton (Healey and Hendzel, 1980). We hypothesized that Microcystis would be P deficient throughout the summer, and would become deficient in N as nutrient concentration and the ratio of TN:TP decline throughout summer.

Recent studies of Lake Erie phytoplankton (Moon and Carrick, 2007; North et al., 2007) have demonstrated limitation by nutrients other than P and N. Additionally, trace metal limitation has been documented in surface cyanobacterial blooms (Downs et al., 2008). Therefore, it is important to assess Microcystis micronutrient status in western Lake Erie. Ji and Sherrell (2008), using laboratory grown Microcystis isolated from Lake Erie, tested the hypothesis that P-deficient Microcystis would accumulate cellular metal ions. We expanded this hypothesis using Microcystis collected in western Lake Erie with a larger suite of cellular macronutrients and micronutrients to determine how cellular P contents affect the cellular quota of these essential nutrients.

Methods

Limnological methods

Six locations in western Lake Erie were sampled throughout the summer to determine water chemistry and light climate, and to collect Microcystis for measurements of protein and nutrient content (Fig. 1). These six sites were selected to span the turbidity and nutrient concentration gradients in western Lake Erie. Two sites (MB20, z = 1.9 m and MB18, z = 2.5 m) are located in the bay and are heavily influenced by the Maumee River flow. The two transition zone sites (8M, z = 5.7 m and 7M, z = 6.2 m) represent the intermediate zone between the bay and offshore. Finally, sites 4P (z = 10.0 m) and GR1 (z = 8.6 m) are in the center of the western basin (defined here as offshore) and are less likely to be affected by the Maumee River. Water was collected at 1 m depth by Van Dorn sampler to determine nutrient concentration of lake water. Water was filtered through a 0.45 μm membrane filter for analyses of dissolved nutrients (nitrate, nitrite, ammonium, and dissolved reactive phosphorus), while unfiltered water was used for total phosphorus and total Kjeldahl nitrogen analyses. Filtered and unfiltered samples were analyzed at the National Center for Water Quality Research at Heidelberg University. Secchi depth was measured as an index of turbidity. Vertical profiles of underwater photosynthetic active radiation (PAR) were measured at half meter intervals (or quarter meter if highly turbid) down to 5 m or to the depth when light was no longer measurable using a Li-Cor # LI-188B with spherical sensor. Light climate was calculated as average PAR (Guillford et al., 2005), substituting lake depth for mixing depth because western Lake Erie generally does not thermally stratify due to shallowness.

Microcystis biovolume in plankton tow samples was measured as a surrogate for abundance. Briefly, plankton was collected from lake bottom to surface using a 64 μm mesh net. Plankton was preserved in 4% buffered formalin. In the laboratory, samples were poured into a 11 l Imhoff cone and diluted to 1000 ml using tap water. After 24 h, most Microcystis colonies floated to the surface as negatively buoyant plankton settled to the bottom. The settled plankton was drained out through the bottom of the cone and the Microcystis layer drawn into the graduations. After another 24 h, Microcystis biovolume was read to the nearest 1 ml, and then converted to an areal unit (ml/m²). Microscopic examination of the separated plankton fractions showed that on average only about 15% of the Microcystis colonies remained entangled with other plankton in the non-buoyant fraction. The settled diatom proportion was then added to another Imhoff cone and diluted so that Microcystis would untagle and float, increasing the separation efficiency and give more accurate estimate of Microcystis biovolume.

Plankton tow samples from these six sites have been collected since 2002 for a total of 481 samples. To determine if a spatial pattern was present, the percentage of the annual bloom attributed to each site was calculated by dividing the total yearly biovolume of Microcystis at each site by the grand total of the six sites. Averaging over all years produced an average percentage of the annual bloom attributable to each site.

For physiological measurements to be made in the laboratory, Microcystis was collected over the entire water column from five sites in western Lake Erie using a 64 μm mesh plankton net on four dates (August 6, 12, 21 and September 1, 2008), when Microcystis biomass was rapidly increasing. Sample site MB20 was sampled to measure nutrients and turbidity, but Microcystis was not abundant enough there to collect for physiological measurements. Microcystis colonies retained in the net were concentrated and stored in dark polyethylene bottles on ice during transportation back to the laboratory. Upon arrival at the laboratory, the Microcystis samples were separated from other phytoplankton using Imhoff cones, concentrated on a 35 μm mesh, and stored at −80 °C in 1.5 ml microfuge tubes. After
separation, samples were viewed under a microscope and no other cyanobacteria were detected. Extraction of photosynthetic pigments using dimethyl sulfoxide indicated the lack of chlorophyll b or c, therefore samples did not contain green algae or diatoms. To determine how representative our net samples are of the Microcystis population, whole water samples were collected and strained through a series of mesh sizes (112 μm, 53 μm, and 30 μm) to fraction Microcystis colonies by size. Microcystis was boiled to break apart colonies into single cells (Joung et al., 2006a, 2006b) and cells were counted using a 0.1 ml palmer cell under a microscope at 200× or 400×.

Protein and nutrient content of Microcystis

Total protein content (TPC) is an indicator of potential growth rate increases as TPC increases (Oh et al., 2000; Long et al., 2001; Downing et al., 2005). Microcystis protein was extracted by grinding approximately 1 g of fresh weight tissue to a powder in liquid nitrogen using mortar and pestle, then transferring to a protein extraction buffer containing 1% sodium dodecyl sulfate (SDS) detergent, 0.1 M Tris buffer (pH=8.0), 10% glycerol, 0.1% bromophenol-blue, 1% sucrose, protease inhibitors (1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM EDTA, 1 mM benzamidine, and 10 μM leupeptin), a phenolic inhibitor (0.5% polyvinylpolypyrrolidone (PVPP)), and reductants (10 mM dithiothreitol (DTT) and 0.1 M ascorbic acid) (Mishra et al., 2008). Samples were then centrifuged at 15,000 g for 10 min at 4 °C. Supernatant containing soluble proteins was collected and total protein content was determined using the Folin reagent (Peterson 1977) and quantified using a standard curve of Bovine Serum Albumin (BSA) and normalized to gram dry weight.

For nutrient content, tissue was dried at 70 °C until a constant mass was reached. Cellular carbon (Cc) and Nc contents were determined on 3.1–3.7 mg of dried Microcystis tissue. Tissue was placed in foil and percentages of Cc and Nc were measured by gas-chromatography following combustion (HCN analyzer Perkin-Elmer 2400 series II). Acetanilide was used as a standard, with errors of −0.27% and 0.01% for Cc and Nc, respectively. To determine cellular phosphorus (Pc), metals, and other micronutrients, 100–200 mg of dried tissue was digested in a microwave digester (MARS; CEM Corp., Matthews, NC) using a modified EPA method (EPA method 3051; HNO3 digestion at 200 °C with an additional peroxide step). Nutrient content was then determined with inductively-coupled-plasma optical-emission spectrometry (ICP-OES; Model IRIS Intrepid II; Thermo Corp., Waltham, MA), as in Krug et al. (2009). Two samples (Aug. 6, 8M and 4P) did not have enough dried material for ICP-OES. For these samples, Pc was oxidized using persulfate (Menzel and Corwin, 1965), then Pc quantified after reacting with molybdate, as in Murphy and Riley (1962). Metals and micronutrients could not be determined for these two samples.

Statistical analysis

TPC and nutrient concentrations were analyzed using repeated measures ANOVA with a mixed model using PROC MIXED of SAS (Statistical Analysis System; version 9.1), with sample date (F(3,12) = 3.49) as repeated variable and sample site (F(4,12) =3.26) as fixed effect. Tukey test was performed for difference among sites.

Ratios of Cc to Nc (Cc:Nc) and Cc to Pc (Cc:Pc) (C mol/mol) indicated the degree of N and/or P deficiency in Microcystis (Table 1, Healey and Hendzel, 1980). Nutrient concentrations for Microcystis obtained from literature are given in Table 2 to put our values into context. This data is from Microcystis collected from natural lakes [southern Wisconsin, USA (Gerloff and Skoog, 1957); Alabama and Mississippi, USA (Boyd, 1970); Lake Rostherne Mere, Cheshire, UK (Sigee and Levado, 2000)], or when no literature value for natural Microcystis (i.e. not-cultured in excessive nutrients) was available, concentrations were calculated

### Table 1

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Atomic ratio mol/mol</th>
<th>No deficiency</th>
<th>Moderate deficiency</th>
<th>Extreme deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>Cc:Nc</td>
<td>&lt;8.3</td>
<td>8.3–14.5</td>
<td>&gt;14.5</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>Cc:Pc</td>
<td>&lt;129</td>
<td>129–258</td>
<td>&gt;258</td>
</tr>
<tr>
<td>Phosphorus</td>
<td>Nc:Pc</td>
<td>&lt;22</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Levado (2000) reported Si ranges from 0.0 to 6800 mmol/kg. Lake properties

Results

Lake properties

Microcystis was most abundant at the transition zone sites 8M and 7M accounting for 33.8% and 27.3% (respectively) of the annual bloom biovolume from 2002 to 2009 (Fig. 2). The two offshore sites, GR1 and 4P, account for 14.8% and 15.2%, respectively. The two bay sites, MB20 and MB18, contain a small percentage of the total Microcystis biovolume. During 2008, Microcystis first appeared in mid July, remained at low levels until early August, then quickly increased in late August (Fig. 3). Early during the bloom, Microcystis biovolume was similar at all sites. By early September, Microcystis biovolume in the transition zones sites was nearly twice that of the offshore and bay sites.

Total phosphorus (TP) averaged over six sites ranged from 1.61 to 2.91 μM (0.50 to 0.90 mg P/l) indicating overall eutrophic conditions. TP concentration decreased with increasing distance from the river mouth (Fig. 4A,B). Dissolved reactive phosphorus (DRP) concentrations were 13% to 33% of the TP concentration and show similar patterns over space and time as did TP. Nitrate (NO₃⁻) was high during May through early July (lake mean 142.8 μM), then was nearly depleted by the end of the sampling season and also showed the same spatial pattern of decreasing with increasing distance from river mouth (Fig. 4). DRP (TN:TP) was not significantly different among sites (Fig. 4F). Secchi depth and average PAR were greatest in June, July and early August, then decreased in mid August and remained low for the rest of the season (Fig. 4G). Secchi depth increased with distance from Maumee Bay (Fig. 4H). Average PAR was very high at MB18 (Fig. 4I), despite low Secchi depths, because of shallow depth. All other sites had a much lower average PAR and were similar to each other.

Table 2

Elemental content of Microcystis collected on four dates at five sites in western Lake Erie during the 2008 bloom. Literature values for Microcystis collected from a natural lake environment (*Sigee and Levado, 2000; ^Boyd, 1970; *Gerloff and Skoog, 1957), or calculated from ratios to C (*Reynolds, 2006; ^Ji and Sherrell, 2008). NA, not available. *Sigee and Levado (2000) reported Si ranges from 0.0 to 6800 mmol/kg.

Macronutrients | C | H | N | P | K | Ca | Mg | S
---|---|---|---|---|---|---|---|---
No. of samples analyzed for element | 20 | 20 | 20 | 20 | 18 | 18 | 18 | 18
No. of samples within detection limit | 20 | 20 | 20 | 20 | 18 | 18 | 18 | 18
Average (mmol/kg dry wt.) | 39,779.8 | 66,210.3 | 5451.5 | 259.5 | 215.5 | 216.6 | 113.3 | 141.2
Standard deviation | 1150.11 | 1634.06 | 529.17 | 70.92 | 683 | 0.83 | 0.44 | 0.54
Redfield ratio relative to P | 1.93 | 21.01 | 1.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00
Literature concentration | 40,000 | NA | 5400 | 341 | 282 | 63 | 125 | 122

Micronutrients | B | Cu | Fe | Mn | Zn | Mo | Ni | Si
---|---|---|---|---|---|---|---|---
No. of samples analyzed for element | 18 | 18 | 18 | 18 | 18 | 18 | 18 | 18
No. of samples within detection limit | 2 | 18 | 18 | 18 | 18 | 18 | 18 | 18
Average (mmol/kg dry wt.) | 0.15023 | 0.19902 | 3.87006 | 0.21763 | 0.57823 | 0.01193 | 0.29282 | 5.09626
Standard deviation | 0.023 | 0.042 | 0.899 | 0.054 | 0.288 | 0.006 | 0.077 | 1.525
Redfield ratio relative to P (×1000) | 0.58 | 0.77 | 14.91 | 0.84 | 2.23 | 0.05 | 1.13 | 19.64
Literature concentration | 0.333 | 0.350 | 6.13 | 0.183 | 0.011 | 0.008 | 0.097 | 1767

Fig. 2. Percentage of the total Microcystis bloom biovolume measured at each of our six sites. Values are mean (±standard error) of data collected from 2002 to 2009. Sites are arranged in increasing distance from the mouth of the Maumee River.

Fig. 3. Microcystis abundance (ml/m²) in western Lake Erie during 2008. Microcystis biovolume was greater at transition zone sites (8M and 7M) than at near-shore (MB18) or offshore sites (GR1 and 4P).
Protein and nutrient content of Microcystis

In trials, Microcystis cells retained on the 112 μm and 53 μm mesh accounted for 93% to 99% respectively, of the total sum of all fractions. The plankton net used to collect Microcystis for analysis of protein and nutrient content had a mesh size of 64 μm and therefore would have retained a high percentage (~93%) of Microcystis.

Total protein content (TPC), an indicator growth rate potential, was significantly affected by sample site (F*=5.01, p=0.0131) and sample date (F*=4.66, p=0.0222). The transition zone sites (8M and 7M) had higher TPC than the offshore sites (GR1 and 4P) on each sampling date (Fig. 5). Tukey test indicated that Microcystis collected from the transition zone had significantly greater TPC than Microcystis from the offshore. The bay site (MB18) had mean protein content that was on average intermediate between the transition zone and offshore sites, but the Tukey test indicated that the bay site was not significantly different from the transition zone or offshore.

Complete results for macronutrients and micronutrients are presented in Table 3. Cellular carbon (Cc) of Microcystis was relatively constant, ranging from 38,010 to 41,557 mmol/kg and was statistically unaffected by both site and date (p>0.05). Similarly, Nc ranged from 4503.9 to 6538.2 mmol/kg, which was only affected by date (F*=5.56; p=0.0126). Pc content was much more variable, ranging from 171.1 to 403.5 mmol/kg. Date had a significant effect of P (F*=14.17; p=0.0003); site did not significantly affect P (F*=2.36; p=0.1133).

The atomic ratios of Cc to Nc (Cc:Nc), Cc to Pc (Cc:Pc), and Nc to Pc (Nc:Pc) were used to indicate nutrient deficiencies. Sample date had a statistically significant effect on both Cc:Nc and Cc:Pc (F*=8.29, 3.15, respectively; p=0.0030, 0.0494, respectively), while sample location

Table 3
Western Lake Erie Microcystis average nutrient quota (μmol/mol C), standard error, and statistical results of regressions of normalized nutrient quota vs. P quota (μmol P/mol C). Positive slopes indicate that the nutrient is increasing as P increases, therefore a possible co-limitation with P at low P quota. B was not included in regression because B was only detected twice. Bold values indicate significance.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Quota</th>
<th>SE</th>
<th>P value</th>
<th>Slopea</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mg</td>
<td>2844.44</td>
<td>151.00</td>
<td>-0.0001</td>
<td>8.23</td>
<td>0.941</td>
</tr>
<tr>
<td>Ca</td>
<td>5442.42</td>
<td>243.30</td>
<td>0.0078</td>
<td>4.49</td>
<td>0.366</td>
</tr>
<tr>
<td>K</td>
<td>5410.25</td>
<td>140.08</td>
<td>-0.0001</td>
<td>4.46</td>
<td>0.773</td>
</tr>
<tr>
<td>Fe</td>
<td>97.12</td>
<td>5.37</td>
<td>0.0236</td>
<td>4.35</td>
<td>0.281</td>
</tr>
<tr>
<td>S</td>
<td>3540.87</td>
<td>82.73</td>
<td>-0.0001</td>
<td>3.83</td>
<td>0.652</td>
</tr>
<tr>
<td>Cu</td>
<td>4.99</td>
<td>0.25</td>
<td>0.8867</td>
<td>NS</td>
<td>0.001</td>
</tr>
<tr>
<td>Mn</td>
<td>5.48</td>
<td>0.38</td>
<td>0.6770</td>
<td>NS</td>
<td>0.153</td>
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<tr>
<td>Zn</td>
<td>14.59</td>
<td>1.77</td>
<td>0.5167</td>
<td>NS</td>
<td>0.027</td>
</tr>
<tr>
<td>Ni</td>
<td>7.34</td>
<td>0.45</td>
<td>0.7581</td>
<td>NS</td>
<td>0.006</td>
</tr>
<tr>
<td>Si</td>
<td>127.83</td>
<td>9.02</td>
<td>0.8463</td>
<td>NS</td>
<td>0.002</td>
</tr>
<tr>
<td>Mo</td>
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<td>0.07</td>
<td>0.1763</td>
<td>NS</td>
<td>0.402</td>
</tr>
<tr>
<td>B</td>
<td>3.89</td>
<td>0.46</td>
<td>x</td>
<td>x</td>
<td>x</td>
</tr>
</tbody>
</table>

a Slope×10^-5.
did not have an effect \( (F^* = 2.22, 0.88, \text{respectively}; \ p = 0.1285, 0.5033, \text{respectively}) \). In Fig. 6, the bold lines represent the boundary between no deficiency and moderate deficiency, while the dotted lines indicate the boundary between moderate and extreme deficiencies \((\text{Healey and Hendzel, 1980})\). In terms of N, 95% of samples were N-replete \((C_N:C_C > 8.3 \text{ mol/mol})\), and Microcystis had a slight moderate N-deficiency on only one occasion \((\text{August 12 at MB18})\). Moderate P-deficiency was measured in 70% of the Microcystis samples \((C_P:C_C = 129-258 \text{ mol/mol})\); no deficiency in P was detected in the remaining samples \((C_P:C_C < 129 \text{ mol/mol})\). N:P gave a very similar pattern as C:P, but only 50% of the samples would be considered P-deficient using this index.

Potassium \((\text{K})\), magnesium \((\text{Mg})\), sulfur \((\text{S})\), and iron \((\text{Fe})\), were all within range of the values obtained from literature \((\text{Table 2})\). Calcium in our Microcystis samples was nearly 3.5 times greater than in Microcystis from Lake Rostherine Mere \((\text{UK})\), but this difference can most likely be attributed to differences in hardness between the two lakes. Molybdenum \((\text{Mo})\) was detectable using ICP for only 6 of 18 samples; Mo was detected on all four dates at MB18 and at the two offshore sites on August 21. Mo was near the literature value for these samples. Boron \((\text{B})\) was only detected twice, consistent with the fact that only heterocystous cyanobacteria require B \((\text{Bonilla et al., 1990})\), but B has been detected in Microcystis previously \((\text{Boyd, 1970})\). Manganese \((\text{Mn})\) was not detected in any sample from the bay site \((\text{MB18})\) even though Mn was detected in all other samples near the literature value concentration. The majority of the elements had similar concentrations across both site and date, however, P, Fe, Zn, and nickel \((\text{Ni})\) were highly variable during the month of sampling. For example, zinc \((\text{Zn})\) ranged from 0.23 to 1.28 mmol/kg. Ni content in lake Microcystis was 3 times greater than in Microcystis grown in the laboratory \((\text{Ji and Sherrell, 2008})\). Silica \((\text{Si})\) content was 5.10 mmol/kg, however no reliable reference for Si could be found for Microcystis, most likely because phytoplankton without a Si cell wall require very small amounts of Si \((\text{Reynolds, 2006})\). Krivtsov et al. \((2005)\) reported an average Si for Microcystis of 51 mmol/kg, while Sige and Levado \((2000)\) reported 1767 mmol/kg.

To determine the relationship between P-deficiency and other important nutrients, the ratio of the cellular quota of these nutrients to C\(_5\) \((\text{Nut.c:C_C} \mu\text{mol/mol})\) was regressed against P\(_5\):C\(_5\) \((\mu\text{mol/mol})\). No negative slopes were observed for Nut\(_5\):C\(_5\) vs. P\(_5\):C\(_5\) \((\text{Table 3})\), which indicates that these nutrients were not accumulating as Microcystis became P-deficient in western Lake Erie. Macronutrients \((\text{K, Ca, Mg, and S})\) and Fe had significant positive slopes, which indicates that the quota of these nutrients increased as P\(_5\) increased. Mg had a slope nearly twice that of K, Ca, S, and Fe, and was highly correlated which indicates a stronger relationship. Micronutrients \((\text{Cu, Mn, Zn, Ni, and Si})\) had no significant relationship with P\(_5\):C\(_5\).

**Discussion**

Sizable Microcystis blooms have been documented in western Lake Erie nearly every year since 1995 \((\text{Brittain et al., 2000; Vincent et al., 2004; Millie et al., 2009})\). In the year of this study the bloom appeared to progress in a typical manner, eventually growing to proportions such that surface scums of Microcystis were apparent to boaters and were visible in satellite imagery \((\text{MODIS})\). Microcystis biovolume measured in the two transition zone sites was nearly twice that of the offshore and near shore sites during the peak of the bloom in September of 2008 \((\text{Fig. 3})\), which corresponds to the spatial pattern indicated by biovolume data collected in western Lake Erie from 2002 to 2009 \((\text{Fig. 2})\).

Total protein content \((\text{TPC})\) was greater at the two transition zone sites than the two offshore sites \((\text{Fig. 5})\), but not significantly greater than the bay site. Greater TPC indicates a greater growth rate potential \((\text{Oh et al., 2000; Long et al., 2001; Downing et al., 2005})\). High growth rates in the transition zone would be supported by the high concentration of dissolved nutrients; especially P. The concentrations of dissolved nutrients measured during 2008 were typical for western Lake Erie \((\text{Moorhead et al., 2008; Wang et al., 2009})\). Turbidity was also very high in the transition zone \((\text{Fig. 4})\), which may favor buoyant Microcystis and affect protein content. The suspended sediments may decrease light intensity and result in a greater percentage of fixed C incorporated into protein \((\text{Morris et al., 1974})\). Further, the light climate \((\text{average PAR})\) was similar between the transition zone and offshore sites, which suggests that higher TPC of the transition zone is not the result of light climate \((\text{Morris et al., 1974})\), but rather greater potential growth rate. The high average PAR at the bay site would suggest that the TPC indicates a similar potential growth rate of Microcystis in the bay and transition zone, but less C would need to be incorporated into protein in the bay because of less light limitation \((\text{Morris et al., 1974})\).

We found that Microcystis biovolume was greatest in the transition zone of western Lake Erie where nutrient concentrations and turbidity is high. TPC of Microcystis was higher in the transition zone than the offshore region, which would support our hypothesis that high TPC would occur in areas where Microcystis is most abundant. However,
TPC was also high in Maumee Bay where *Microcystis* biovolume is much lower, a finding which did not support our hypothesis that TPC in the transition zone would be greater than both bay and offshore locations. High TPC in the bay suggests high growth potential in the bay as well as in the transition zone. The low *Microcystis* biomass in the bay might be explained by several factors. First, the flushing rate of Maumee Bay may be sufficiently high to transport *Microcystis* to the transition zone before high biomasses can be accumulated in the bay. Secondly, Maumee Bay is very shallow (~2.5 m) and our biovolume is expressed as an areal unit because the plankton net samples the entire water column. The concentration of *Microcystis* in the bay per volume can be, at times, as high as the transition zone, but biovolume per square meter is lower in the bay due to the shallow depth.

Cellular C of *Microcystis* was very stable (Table 2), which indicates that *Microcystis* was not accumulating and storing excess C. Further, changes in nutrient to C ratios are due to fluctuations in the nutrient rather than changes in C. Additionally, the C:N ratios greater than 6 indicate that C was not limiting (Reynolds, 2006).

The ratios of C:P indicate that *Microcystis* in our samples was generally moderately P-deficient (Fig. 6). Results of analyses of alkaline phosphatase activity (APA) in *Microcystis* from a separate study conducted in 2008 in the same region of western Lake Erie (Horst and Sarnelle, 2009) showed elevated APA in *Microcystis*, also suggesting P-limitation in *Microcystis*. Although not statistically significant, the transition zone sites usually had a slightly greater P content on each sample trip (Fig. 6). Nutrient concentrations at the bay and transition zone sites were two to three-fold greater than at the offshore sites (Fig. 4), however P of *Microcystis* did not follow the spatial pattern of available nutrients. On each sample date, P of *Microcystis* from offshore was only slightly less than that of the bay and transition zone sites and was very similar at all 5 sites on September 1 and 4 of the 5 on August 21 (Fig. 6). Low C:P ratios of *Microcystis* at sites that have a wide range of P concentration would suggest that *Microcystis* is possibly accumulating and storing excess P in the near-shore to support growth in the relatively low-P offshore. However, our data is not sufficient to fully support a hypothesis of luxury uptake in Maumee Bay. *Microcystis* is capable of luxury uptake when TP exceeds 7.10 μM (Baldia et al., 2007) that can support growth for several days (Nalewajko and Murphy, 2001; Tsukada et al., 2006). In the year prior to this study, Wang et al. (2009) measured TP of 10.66 μM in Maumee Bay, which is high enough to support luxury uptake. Although we did not observe TP concentrations greater than 7.10 μM on our sampling trips during 2008 it is possible that we may have missed an episodic event of high TP and DRP. TP of near-shore environments near the mouth of the Maumee River are extremely variable on the temporal scale and can be 100× greater than the rest of the lake (Schwab et al., 2009). If future research is able to show that *Microcystis* demonstrates luxury uptake of P in Maumee Bay, then the reduction of P concentration of near-shore environments can be, at times, as high as the transition zone, but biovolume per square meter is lower in the bay due to the shallow depth.

The importance of co-limitation of lake phytoplankton by micronutrients, especially Fe, has been recently highlighted (Sterner, 2008). In our study, *Microcystis* was not deficient in other macronutrients or micronutrients because the concentration of these essential nutrients was in agreement with several published studies (Table 2). In a laboratory experiment, Ji and Sherrell (2008) tested the hypothesis that P-deficient *Microcystis* will accumulate metal ions as P limits growth but metal ion uptake proceeds. Our data did not suggest this is occurring in western Lake Erie with any of the nutrients we measured (Table 3), however our data is not sufficient to fully support a hypothesis of luxury uptake in Maumee Bay. *Microcystis* is capable of luxury uptake when TP exceeds 7.10 μM (Baldia et al., 2007) that can support growth for several days (Nalewajko and Murphy, 2001; Tsukada et al., 2006). In the year prior to this study, Wang et al. (2009) measured TP of 10.66 μM in Maumee Bay, which is high enough to support luxury uptake. Although we did not observe TP concentrations greater than 7.10 μM on our sampling trips during 2008 it is possible that we may have missed an episodic event of high TP and DRP. TP of near-shore environments near the mouth of the Maumee River are extremely variable on the temporal scale and can be 100× greater than the rest of the lake (Schwab et al., 2009). If future research is able to show that *Microcystis* demonstrates luxury uptake of P in Maumee Bay, then the reduction of P concentration of near-shore environments should be considered as a possible management priority.

N content was similar at all sites on each date. No N-deficiency was detected in *Microcystis* during 2008 (Fig. 5), despite the low TN:TP ratios in the lake water (Fig. 4). In a concurrent study (Chaffin 2009), chlorophyll α and phycocyanin content was determined in these *Microcystis* samples and the nitrogen content of these pigments explains 70% of the variation in N. The levels of NO₃ in western Lake Erie decreased nearly 50-fold throughout the summer, but NH₄ remained fairly stable and was above 1.80 μM for the entire year. The stability of NH₄ concentration over space and time (Fig. 4) would likely be the result of a continuous internal cycling of NH₄ due to Dreissena mussel excretion (Arnott and Vanni, 1996; Conroy et al. 2005a). Dreissena mussels are found across the sediments of the western basin of Lake Erie at densities approaching 10,000 individuals m⁻² (Patterson et al., 2005). Non-N-fixing cyanobacteria, such as *Microcystis*, are superior competitors over eukaryotic phytoplankton for NH₄ in lakes where NO₃ has been depleted (Blomqvist et al., 1994). In the late summer, eukaryotic algae would have been severely N-limited if they were out-competed for NH₄ by *Microcystis*. Without a source of nitrate, green algae and diatoms would have not been able to compete with *Microcystis*. The high *Microcystis* biovolumes attained in 2008 suggest that NH₄⁺ of 1.80 μM is adequate to support a *Microcystis* bloom in Lake Erie. This follows the observations of Jacoby et al. (2000), who reported that an NH₄⁺ level of 0.50 μM was able to support a *Microcystis* bloom in Steilacoom Lake (Washington, USA), while NO₃ had become depleted. Because *Microcystis* does not fix nitrogen, traditional TN:TP models suggest that *Microcystis* should not be able to persist at low TN:TP and that N-fixing cyanobacteria should become dominant (Smith, 1983). During 2008, the NH₄⁺ proportion was 2.4% to 4.7% of the TN. Thus, TN:TP ratios that are thought to favor N-fixing cyanobacteria may not apply in lakes with adequate NH₄⁺.
approach of altering the conditions of Maumee Bay and the transition zone of western Lake Erie to be more like that of the offshore, where turbidity and nutrient concentration are low. Best-management land-use practices for agriculture in the Maumee River watershed can target the loading of suspended sediments and nutrients from terrestrial sources. Although practices will be beneficial in the long term for reducing Microcystis blooms, there may be a lag-time before improvements are observed because there may be a high rate of internal loading of suspended sediments and nutrients from the lake bottom (Jacoby and Frazer, 2009). Further, future research should address the roles of nitrogen in promoting Microcystis blooms in western Lake Erie, specifically focusing on loading and internal cycling of NH$_4^+$, and the difference between NH$_4^+$ and NO$_3^-$ as nitrogen sources of phytoplankton.

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