Spatiotemporal Patterns and Ecophysiology of Toxigenic *Microcystis* Blooms in Lake Taihu, China: Implications for Water Quality Management

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ABSTRACT: Whole lake monitoring of hypertrophic Lake Taihu, China, was conducted during the summers of 2009–2010, with the intent of identifying environmental factors influencing *Microcystis* bloom formation and promoting the growth of toxigenic strains (*mcyE* possessing). Low N:P ratios (replete N & P) appeared to select for toxigenic populations of *Microcystis* spp., whereas nontoxic *Microcystis* spp. strains were dominant in more nutrient limited regions of the lake. Chlorophyll *a* (Adj. *R*² = 0.83, *p* < 0.0001) was equally predictive of microcystin variance across the lake as fluorescence based real-time quantitative PCR (qPCR) measurements of microcystin synthetase E (*mcyE*) gene equivalents (Adj. *R*² = 0.85, *p* < 0.0001). Interestingly, chlorophyll *a* was identified as a more robust and useful metric for predicting microcystin concentrations than qPCR measurements enumerating the total *Microcystis* population based on c-phycoerytranin (*α* subunit; *cpcA*) gene equivalents (Adj. *R*² = 0.61, *p* < 0.0001). Overall, the lakewide composition of *Microcystis* spp. was highly variable over time and space, and on average the population consisted of 36 ± 12% potentially toxic cells. On the basis of this study’s findings, a framework for the design and implementation of a water safety plan for Taihu water quality managers and public health officials is proposed.

INTRODUCTION

The unicellular, colony forming genus — *Microcystis* — is one of the most ubiquitous cyanobacterial harmful algal bloom (CHAB) formers in temperate and tropical freshwater systems. Blooms are promoted by nutrient replete, warm, and slow moving or stagnant waters, which allow *Microcystis* spp. to proliferate to the point that surface waters are covered with thick green scums. In lakes, rivers, and reservoirs worldwide, blooms of *Microcystis* spp. have steadily intensified in both duration and magnitude due to cultural eutrophication, rising water temperatures, and increasing frequency of extreme weather events.† Harmful algal blooms have major ecological (creation of bottom water hypoxia, disruption of food webs), economical (impact recreational, fishing, and drinking waters), and health repercussions (human and animal intoxication). Numerous strains of *Microcystis* spp. produce highly stable and potent polypeptides known as microcystins.‡–4 These metabolites are toxic to a range of eukaryotic organisms,§ and in humans acute doses can induce fatal liver hemorrhage, whereas chronic exposure is implicated in the formation of gastric and liver cancer.¶–‡ The majority of microcystins remain intracellular in healthy intact cells, which can lead to a large increase in dissolved microcystins if cells are lysed by herbicides or disinfection agents during drinking water treatment processes.¶,‡ Microcystins can accumulate in the environment due to their stability, with reported half-lives ranging from days to weeks.†‡ Additionally, the toxin is not destroyed by boiling.‖

Lake Taihu is the third largest freshwater lake in China, covering an area of 2338 km².‖ Its shallow average depth of two meters ensures it remains polymeric year round. In Mandarin, Taihu means Great Lake, and for many decades it was heralded for its scenic beauty and natural bounty. The lake, famed throughout China for its fish, shrimp, crabs and mollusks, is also flanked by rolling hills supporting numerous fruit nurseries and tea plantations. The natural resources afforded by Taihu, and its proximity to the Yangtze River, have led to rapid population growth around the lake, culminating in over 36 million inhabitants residing within its basin. Unfortunately, decades of wastewater discharge, industrial pollution, and overapplication of chemical fertilizers around the basin have transformed this once meso-oligotrophic lake in the 1950s into its present hypertrophic state.¶ Every spring, large areas of the...
lake turn green with dense *Microcystis* blooms that persist well into the fall. The blooms are comprised of multiple species and strains of *Microcystis* with varying degrees of toxicity. In addition to being a drinking water hazard, microcystins can bioaccumulate in the tissue and organs of fish and have been identified in the serum of a chronically exposed population of Chinese fishermen. Considering that fish from Taihu is a staple of the regional diet, and the personal observations of fishermen routinely harvesting fishes from areas fouled with dense *Microcystis* blooms, the potential human exposure risk (both acute and chronic) to microcystins through biomagnification up the food chain is a cause for concern. The goal of this study was to identify spatiotemporal patterns of toxic *Microcystis* blooms and the environmental factors favoring their formation. This information will be useful for water quality managers and/or drinking water utilities in developing water safety plans. The establishment of an action level that once reached would trigger additional water treatment processes, such as ozonation or filtration through activated carbon, would provide the greatest public health protection while limiting the cost of these treatment processes by employing them only as needed.

**MATERIALS AND METHODS**

**Sample Collection and Processing.** Twelve sampling transects encompassing the entire northern half of Lake Taihu were conducted between June 22 – July 27, 2009, and June 3 – June 21, 2010. Only the northern half of the lake was sampled because the southern half is dominated by submersed aquatic vegetation and rarely experiences *Microcystis* blooms. Water quality parameters (temperature, dissolved oxygen, pH, turbidity) were measured at each site by a YSI 6600 multiprobe sonde (OH, USA). Nutrients (total nitrogen (TN), dissolved total nitrogen (DTN), total phosphorus (TP), and dissolved total phosphorus (DTP)) were measured as previously described. During each transect, whole water column, depth integrated samples were collected by a 2 m long, 10 cm wide tube with a one-way valve from each of eight sample sites (n = 96 samples total). Samples were brought back to the laboratory and filtered onto Whatman (NJ, USA) GF/F filters (25 mm) for chlorophyll a (chl a) analysis by high performance liquid chromatography (HPLC) and onto Pall (MI, USA) Supor-200 membrane filters (47 mm, 0.2 μm) for subsequent DNA extraction. All samples were maintained at −20 °C until further processing.

**Creation of Plasmid Standards for Real-Time PCR.** DNA from a mid-exponential growth phase culture of *Microcystis aeruginosa* (UTEX 2667) grown in BG-11 media at 25 °C and under 30 μmol m−2 s−1 photons of cool white/grow lux fluorescent light with a 12:12 h light/dark cycle was extracted using a GeneRite RWo3C kit (NJ, USA) per the manufacturer’s instructions. Conventional PCR was performed in 50 μL volumes and consisted of: 25 μL Lucigen (WI, USA) EconoTaq Plus 2X Master Mix (400 μL dNTPs, 3 mM MgCl2, Reaction Buffer (pH 9.0), 0.1 units μL−1 DNA polymerase, 2 μL (10 μM) each of forward and reverse primers, ~10 ng genomic DNA, and DEPC H2O up to 50 μL. Two different primer sets were utilized. PC188F and PC254R, each of forward and reverse primers, ~10 ng genomic DNA, and DEPC H2O up to 50 μL. Two different primer sets were utilized. PC188F and PC254R produced amplicons within the α subunit of the c-phycocyanin gene (PC) and mcyE-F2 and MicmcyE-R8 within the microcystin synthetase E (mcyE) gene (Figure S1 of the Supporting Information). DNA folding structures of primers and probes were checked using the program Mfold. Primer and probe specificity to *Microcystis* spp. was verified experimentally by PCR screening cultures of various nontoxic and microcystin producing cyanobacterial genera. Reactions were carried out on a Techne TC-S12 thermocycler with the following conditions: initial denaturation at 94 °C for 2 min, followed by 35 cycles of 94 °C × 30 s, 58 °C × 30 s, 72 °C × 45 s and a final elongation step at 72 °C for 5 min. Twenty-five microliters of each PCR product was run on a Crystal Violet (2 mg mL−1) stained 1.2% agarose gel in 1× TAE buffer for 90 min at 75 V. Amplification products were extracted and purified using a Qiagen (CA, USA) Gel Extraction kit per the manufacturer’s instructions. Purified DNA was ligated into an Invitrogen (CA, USA) pCR2.1 TOPO vector and transformed into TOP10 Escherichia coli per the manufacturer’s instructions. Cells were grown overnight at 37 °C on Luria broth plates supplemented with kanamycin (50 μg mL−1). The correct sequence and orientation for each positive transformant was determined by sequencing on an ABI 3130 DNA sequencer. Gene target sequences from plasmid constructs can be viewed in Figure S2 of the Supporting Information. Plasmids from the *E. coli* transformants known to possess the target sequences were harvested using an Invitrogen Pure Link Plasmid Mini-prep kit. The isolated plasmids were then linearized by a single cut using Hind III restriction enzyme. Plasmid DNA concentration and purity was measured using a Thermo Scientific UV Nanodrop 1000 (DE, USA).

**Plasmid Standard Curves.** Amplification efficiencies (E = 10−(1/slope) − 1) for the reactions were determined empirically by creating standard curves using duplicate 10-fold serial dilutions (spanning 6 orders of magnitude ranging from 10 to 106 cell equivalents) of linearized plasmid DNA. Reaction efficiency was 98.2% (slope = −3.337, y-intercept = 44.9) for the total *Microcystis* assay based on c-phycocyanin gene equivalents (PC) and 96.6% (slope = −3.406, y-intercept = 44.6) for the toxigenic *Microcystis* assay based on microcystin synthetase E gene (mcyE) equivalents. The observed correlation coefficients (R2) for the standard curves were 0.963 for the PC assay and 0.995 for the mcyE assay.

**Real-Time Quantitative PCR.** To determine the total *Microcystis* community in samples collected from Lake Taihu, the c-phycocyanin (PC) assay was utilized. To determine the total toxigenic *Microcystis* community the microcystin synthetase E (mcyE) assay was employed. In nondividing cells, both genes are predicted to exist as single copies (1:1 ratio). Amplification and quantification of all samples was carried out on a Cepheid Smart Cycler II (CA, USA) using the SmartCycler II software and fluorescence detection system. Each PCR reaction consisted of 5 μL DNA template, Cepheid OmniMix hyphalized beads (200 μM dNTPs, 4 mM MgCl2, 25 mM HEPES (pH 8.0), 1.5 U TaKaRa hot start Taq polymerase), 1 μM forward primer, 1 μM reverse primer, 0.2 μM probe, and sterile DEPC H2O to a volume of 25 μL. For optimal annealing temperatures and thermal cycling conditions, see Figure S1 of the Supporting Information. Prior to DNA extraction of environmental samples, 10 μL of a sample processing control (SPC) consisting of salmon (*Onchorhyncus keta*) sperm DNA (10 ng μL−1) was added as previously described. Each set of reactions included serial dilutions of DNA plasmid constructs for the standard curves, positive and negative extraction controls, and the salmon sperm external sample processing control for comparison of cycle threshold (Cq) values relative to SPC spiked environmental samples to assess PCR inhibition. Reaction inhibition was defined as Cq
differences greater than 3.0 between the SPC and the salmon sperm DNA spiked environmental sample. If sample DNA needed to be diluted due to inhibition of the SPC, the quantified gene copy number was corrected by multiplying by the dilution factor.

**RT-qPCR Detection Limits.** DNA extraction efficiency for the GeneRite Rwo3C kit was determined spectrophotometrically (A360) by comparing the total DNA extracted from *M. aeruginosa* PCC 7806 cultures (∼5 × 10^6 cells) maintained as previously described. Maximum extraction was determined as the DNA concentration obtained after boiling and pelleting the cells by centrifugation then collecting the supernatant. This value was compared to the DNA extracted by the kits following cell harvesting by centrifugation or by filtration onto membrane filters. The limit of detection (LOD), defined as the lowest template concentration at which 100% of positive samples are detected, was observed to be ∼10 gene copies for each assay when plasmid constructs were used. However, taking into account losses in DNA extraction efficiency after cells had been collected by membrane filtration, the LOD was empirically determined to be ∼75 copies ml^-1 for each assay. Finally, absolute quantification of the target DNA in each sample was determined following the delta Ct (ΔCt) method as previously described.29

**Toxin Analysis.** Total microcystins (i.e., all isoforms) were measured spectrophotometrically by an Abraxis (PA, USA) enzyme linked immunosorbent assay (ELISA) on a Thermo MultiSkan Spectrum plate reader (Vantaa, Finland). The Microcystins-ADDA ELISA microtiter plates utilized polyclonal antibodies allowing for congener-independent detection of all microcystins. The limit of detection for the assay was 0.1 μg l^-1 total microcystins. Samples below the detection limit were considered to be half the detection limit (0.05 μg l^-1) for statistical purposes.

**Statistical Analysis.** The data set was checked for collinearity using SAS v.9.2 (NC, USA) before performing multiple regression analyses. Variables with variance inflation factors (VIF) greater than 10 were considered to be autocorrelated. Total nitrogen (TN) and total phosphorus (TP) were removed from the multiple regression analysis due to their autocorrelation with dissolved total nitrogen (DTN) and dissolved total phosphorus (DTP), respectively. DTN and DTP were retained in place of TN and TP because the dissolved forms are more indicative of bioavailable nutrients. Multiple regression analysis with fully stepwise selection procedure was used to identify and model the strongest predictor variable(s) of microcystins. The model discarded several variables which had no effect or lowered the regression coefficient (R^2) and only retained those which could explain variance within the microcystin data.

### RESULTS AND DISCUSSION

**Amplification Efficiency and Quantification of Cell Equivalents.** Real-time quantitative PCR (qPCR) was used to enumerate total *Microcystis* cells based on c-phycocyanin (PC) gene equivalents. A second qPCR assay (mcyE) was required to enumerate potential microcystin-producing cells because not all strains produces toxins and the toxic and nontoxic genotypes are indistinguishable from each other based on morphology.30 Whereas the community of *Microcystis* cells is dynamic, with a portion likely undergoing binary fission at any given time, the fraction of toxicgenic cells (mcyE equivalents) only exceeded the total population (PC equivalents) on three occasions, even after accounting for the slightly lower amplification efficiency of the mcyE assay. In reality, it is impossible to have more toxic cells than total cells, but because the few exceedances were all within 5% of the total *Microcystis* population size, they were explained as interassay and natural variability of three particularly toxic blooms that were all ∼100% toxigenic.

**Statistical Applications.** Multiple regression analyses indicated that total and toxigenic *Microcystis* cell equivalents were generally predictive of the microcystin content of a given sample (Table 1 and Figure 2). However, chl a (Adj. R^2 = 0.83, p < 0.0001) was a better predictor of microcystin variance than qPCR assays enumerating total *Microcystis* (PC) equivalents (Adj. R^2 = 0.61, p < 0.0001), and highly comparable to qPCR assays enumerating toxicogenic *Microcystis* (mcyE) equivalents (Adj. R^2 = 0.85, p < 0.0001). This finding may be explained by the fact that, even though *Microcystis* is the dominant phytoplankter comprising these blooms (up to 95% of total phytoplankton biomass), there are other potential microcystin producing cyanobacteria that co-occur in the lake during this period.31 Although site-specific measurements of wind speed and direction were unavailable, this information would have provided additional resolving power for the model by allowing the depth of mixing for each site to be calculated (the Wedderburn number), which would allow examination of the water column stability at a given depth.32 This is important because *Microcystis* spp. are well adapted to high light conditions33 and would be expected to occur in higher densities in protected regions of the lake where water column stability is greatest.

The presence of other microcystin producing CHAB genera should strengthen the relationship between chl a and total microcystins and weaken the relationship between total *Microcystis* and microcystins. Clusters of another potential microcystin producing cyanobacterium, *Anabaena* spp., were observed in 29% of samples collected (n = 96). The individual filaments occurred as either long and generally straight (relaxed coils) similar to *A. flos-aquae*, or as tightly coiled filaments similar to *A. circinalis* (Figure S3 of the Supporting Information). To a lesser extent, a third common microcystin producer, *Planktothrix* sp., has been reported at times to comprise a portion of the summer cyanobacterial community, although it was rarely observed in the present study. In addition to producing microcystins, some strains of *Anabaena* spp. also possess the genes to produce potent neurotoxins. The possibility exists that N-reduction strategies aimed at reducing eutrophication in Taihu could lead to a community shift from *Microcystis* spp. dominance to one of N₂-fixing *Anabaena* spp.

### Table 1. Multiple Regression Model with Stepwise Selection Procedure Displaying Variables Significantly Correlated with Measured Microcystin Concentrations in Lakewide Samples (n = 96) Collected 2009–2010^a^

<table>
<thead>
<tr>
<th>variable</th>
<th>Individual Adj. R^2</th>
<th>model R^2</th>
<th>Adj. R^2</th>
</tr>
</thead>
<tbody>
<tr>
<td>toxic <em>Microcystis</em> cell eqmr. (mcyE)</td>
<td>0.8499</td>
<td>0.85</td>
<td>0.8483</td>
</tr>
<tr>
<td>chlorophyll a</td>
<td>0.8338</td>
<td>0.8566</td>
<td>0.8332</td>
</tr>
<tr>
<td>total <em>Microcystis</em> cell eqmr. (cpcA)</td>
<td>0.6118</td>
<td>0.8592</td>
<td>0.8542</td>
</tr>
<tr>
<td>dissolved oxygen</td>
<td>0.0189</td>
<td>0.8619</td>
<td>0.8552</td>
</tr>
<tr>
<td>pH</td>
<td>0.0187</td>
<td>0.8647</td>
<td>0.8565</td>
</tr>
</tbody>
</table>

^a^Model p-value <0.0001. Dragged from model: temperature, dissolved total nitrogen, dissolved total phosphorus, turbidity, wind direction, and wind speed.


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Previous work on Taihu31 suggested that *Anabaena* would likely be more plentiful in the lake center relative to *Microcystis* due to its diazotrophic capability. However, the samples collected in this study indicated that the *Anabaena* distribution and abundance in Taihu is generally limited to nutrient replete areas of the lake. The absence of requisite heterocysts for N2-fixation in all *Anabaena* observed suggests that these filaments were using available combined nitrogen, as opposed to N2, to support their growth. Similar to *Microcystis*, *Anabaena* filaments are positively buoyant, and therefore light limitation is not likely a factor controlling its growth.

*Microcystis* and *Anabaena* are capable of luxury P uptake and storage, and both possess alkaline phosphatases enabling the additional utilization of organic P, and therefore phosphorus limitation is unlikely to promote the growth of one genus over the other.35 Additionally, blooms of either filamentous or colonial cyanobacteria are not usually controlled by zooplankton because they are poor sources of nutrition and their large sizes make them resilient to heavy population losses by grazing.33,36 One possibility for the inability of *Anabaena* spp. to establish dominance in the lake is that *Microcystis* spp. may secrete allelopathic compounds which lower the fitness of nearby *Anabaena* populations. For instance, microcystin-LR has been shown to inhibit photosynthesis in a variety of phytoplankton,37 and *Microcystis* also produces many other potentially inhibitory secondary metabolites, such as aeruginosins and microviridins, which have unclear ecological functions. Overall, it remains unclear why *Anabaena* did not grow well in the center of the lake, where it would have had little competition with *Microcystis*, because both DTN and DTP were on average only present in low concentrations (2.64 mg l\(^{-1}\) and 0.02 mg l\(^{-1}\), respectively) throughout the study period. Currently, work is underway to determine the cyanotoxic potential of other cyanobacteria populations in Taihu.
From these data, a regression equation to estimate the microcystin concentration of a given sample based on chl $a$ concentration was empirically derived as:

$$MC_s = 0.01021 + 0.07965(\text{Chl}_a)$$

(1)

Where: $MC_s =$ total microcystins ($\mu$g l$^{-1}$) and Chl $a =$ total chlorophyll $a$ ($\mu$g l$^{-1}$).

Figure 2 contains individual linear regressions displaying the strength of associations between total microcystins with chl $a$, total *Microcystis* cell equivalents (PC assay), and toxigenic *Microcystis* cell equivalents (mCyE assay).

**Microcystis Toxigenicity.** On a lake-wide scale, the relative proportion of mCyE possessing *Microcystis* declined as water temperature increased ($r = -0.25$, $p = 0.018$). Likewise, total microcystins decreased, even though the total *Microcystis* abundance was not necessarily reduced, as high cell concentrations were found at all water temperatures ($21-31$ °C). Although temperature was not a primary driver of biomass, it still played a role in niche partitioning of the different *Microcystis* morphospecies. The summer blooms were made up of at least four different *Microcystis* morphospecies (i.e., unique colony morphologies). These co-occurred throughout the study period, with two having toxigenic potential (*M. aeruginosa* and *M. flos-aquae*) and two always being nontoxic (*M. ichthyoblabe* and *M. wesenbergii*) based on whole-cell PCR genotyping of individual colony isolates. Levels of microcystin expression are highly variable according to environmental conditions and individual genotype, and therefore it is possible for a site to have a relatively low concentration of *Microcystis* yet produce significant amounts of microcystins, or have a high concentration of *Microcystis* but be nontoxic.

**Microcystis Distribution Patterns.** Figure 1 displays the relative proportions of *Microcystis* abundances across the lake based on qPCR derived total and toxigenic *Microcystis* cell equivalents from all sampling sites and sampling transects. The figure illustrates that *Microcystis* abundance was not uniformly distributed across the lake. For instance, the northwestern sample site (Zhushan Bay) contributed 41.4% of the total *Microcystis* biomass relative to all sampling sites, whereas the lake center and the drinking water intake sites only accounted for 4.4% and 2.9% of the total *Microcystis* biomass, respectively. Fortunately, accumulation of *Microcystis* in the northwestern region of the lake (Gonghu Bay), where the drinking water intake for the city of Wuxi is located, is generally restricted by the bay’s short hydraulic residence time owing to a Yangtze River diversion (Wangyu River) flowing into the lake. However, the high proportion of toxigenic *Microcystis* cells detected in this region of the lake highlights the potential for the reoccurrence of toxic *Microcystis* blooms, such as those that occurred during the 2007 drinking water crisis if the hydrology is changed and the water is allowed to stagnate (e.g., droughts). Comparisons of the physical and chemical properties inherent to different sites enables the assessment of the factors promoting *Microcystis* growth, as well as those variables selecting for toxigenic strains over nontoxic ones. For instance, the high *Microcystis* biomass observed in Zhushan Bay (Northwest) can be explained by the fact that this area is fed by four nutrient replete rivers ensuring the growing bloom receives adequate nutrient replenishment. Relative to lakewide averages, Zhushan Bay had 17% greater DTN and 100% greater DTP concentrations. The prevailing dogma for many years was that P availability was the major driver of freshwater cyanobacterial growth because N$_2$-fixers would provide the lake’s nitrogen needs if concentrations were too low. However, there are exceptions to the rule as N availability, particularly in the summer, frequently limits or colimits phytoplankton production and bloom formation in many freshwater systems.

In Lake Taihu, the overabundance of P relative to N during the summer months in the regions of the lake with the greatest *Microcystis* concentrations indicates that nitrogen availability primarily controls phytoplankton growth. In addition to nitrogen replenishment from river inputs, *Microcystis* assemblages in highly productive Zhushan Bay benefit from a relatively stable water column due to wind buffering by the adjacent shoreline. Zhushan Bay also has a longer hydraulic residence time than most parts of the lake, owing to a weak counter clockwise current that entraps the water and facilitates accumulation of the otherwise slow growing, buoyant *Microcystis* colonies within the bay. Even though *Microcystis* biomass was higher in Zhushan Bay compared to the rest of the lake, the average percentage of toxigenic cell equivalents there (41.4%) was comparable to the lakewide average (36.2% ± 12.3%). In contrast, the low *Microcystis* biomass near the drinking water intake throughout the duration of this study was encouraging from an environmental health perspective, although the average proportion of toxigenic to nontoxic *Microcystis* (60.8%) was higher than at all but one other site. Even in samples with high chl $a$ concentrations, the percentage of toxigenic *Microcystis* was generally observed to be ~40%, with the occasional possibility for blooms to be ~100% toxic, as well as instances in which they were completely nontoxic.

**Factors Controlling Toxigenicity.** Turbidity and the depth of light penetration are predicted to exert a controlling effect on microcystin production, as cells exposed to high light intensities have been shown to upregulate microcystin biosynthesis. The rationale is that buoyant *Microcystis* cells will undergo photodestructive damage from prolonged exposure to high irradiance. As a result, microcystin transcription will be upregulated because they are theorized to provide intracellular protection of phycobilisome proteins against reactive oxygen species generated by photodegradative processes. In contrast, regions of the lake such as the lake center, which are continually exposed to wind induced mixing should be composed of less toxic strains of *Microcystis* because the cells are unable to maintain their position at the water’s surface and therefore are not exposed to high irradiance. This hypothesis is further supported by the finding that nontoxic strains of *Microcystis* are able to routinely outcompete toxic strains of *Microcystis* when light is a limiting factor. The well mixed lake center was therefore predicted to be dominated by diatoms and green algae due to their lower critical light intensities required for growth relative to *Microcystis*. Under this light-limitation scenario, diatoms and/or green algae dominance is predicted; even under low (nonsaturating) N and P concentrations which generally favor cyanobacteria, and particularly those capable of N$_2$-fixation. This hypothesis was refuted; however, based on HPLC analyses of photopigments diagnostic of major algal groups present in samples collected at the lake center. These data indicated that diatoms (fucoxanthin) and green algae (chlorophyll b) were on average ~15X and ~8X less abundant than their lakewide averages. The low *Microcystis* biomass in the lake center, as well as the high ratio of nontoxic to toxigenic cells, is inadequately explained solely by physical processes such as wind induced mixing and the cells’ inability to maintain their position at the air–water interface.
interface. The other critical variable potentially exerting community level control at this site was the low average TP and DTP concentrations; 0.051 mg l\(^{-1}\)P and 0.016 mg l\(^{-1}\)P, respectively. Microcystis is a strong competitor under low P conditions due to its high \(V_{\text{max}}\) for phosphorus uptake and capacity to accumulate and store excess P in the form of polyphosphates.\(^54\) Its presence in such low cell densities at the lake center suggests that P concentrations were too low to sustain high amounts of any phytoplankton group.

Even though nitrogen deficiency has been shown to incite a greater stimulatory effect on microcystin expression than phosphorus deficiency,\(^55\) nutrient conditions were expected to be replete and not play a role in selecting toxigenic strains over nontoxic Microcystis populations in a hypertrophic lake such as Taihu. However, not all regions of the lake were equally nutrient replete, which may explain the observed differences in phytoplankton community composition, densities, and distributions across the lake. Previous experiments using batch cultures have demonstrated that nontoxic strains of Microcystis tend to out-compete toxigenic strains under low nutrient or low light conditions.\(^49,56,60\) To our knowledge, these findings have yet to be corroborated in Microcystis blooms occurring in natural lake settings. In Lake Taihu, nutrient concentrations were observed to not only impact microcystin production but also the Microcystis community structure with regard to toxigenicity. Greater N and P availability, both as absolute concentrations and lower N:P ratios, favored growth of toxigenic Microcystis strains. Regions of the lake with high N:P ratios and low DTP concentrations were considerably less toxic in regard to both measured total microcystins, as well as the relative proportions of toxigenic to nontoxic cells.

With regard to microcystin biosynthesis, maximal rates tend to coincide with periods when cell growth rates are maximized. In this case, the ratio of N:P may be more important than the absolute concentration of either N or P because maximum microcystin production frequently occurs when N:P ratios are approximately 16:1.\(^57\) This N:P ratio, commonly termed the Redfield Ratio, reflects the ratio needed for balanced growth of photoautotrophs.\(^58,59\) This may explain why blooms tend to be more toxic at their onset, when nutrients are replete, and become progressively less toxic over time as nutrients are depleted. This would be expected if microcystin production is metabolically expensive and does not play a critical cellular role. Although an alternative hypothesis explaining this trend could be that, as the spring transitions into summer, the cells become photoaclimatized by adjusting their pigment contents, and, as a result, photoinhibition is limited thereby reducing the need for protective intracellular microcystins.

A closer look at the average nutrient ratios for the three stations along the western shore and the lake center provides a comparison between two highly toxic sites with two generally nontoxic sites (Figure 1 and Table 2). One difference between these two groups was that water temperatures were on average 1–2 °C warmer at the high Microcystis biomass sites (1 and 2). These sites were also characterized by their abundant N and P relative to lake-wide averages. The higher average total microcystins measured at these two sites is predicted by eq 1 based on their higher average chl \(a\) content. This is in stark contrast to sites 3 and 4, which were predominantly nontoxic with below average Microcystis biomasses and were characterized by low P concentrations relative to the lake average. On the basis of these differences in nutrient ratios, it is proposed that, at sites 1 and 2, a stable water column and nutrient replete conditions stimulated and maintained the Microcystis blooms, and these same conditions promoted the growth of the otherwise less competitive toxigenic strains. At sites 3 and 4, for the reasons stated above, Microcystis growth was limited and consisted almost entirely of nontoxic strains. In fact, the most toxic Microcystis morphospecies, \(M.\ aeruginosa\), was never observed in the lake center and was only detected once at site 3.\(^17\) For the Microcystis cells, which were able to survive in these locations, they did so under both low light and low nutrient conditions. Although the mechanistic explanation is unclear, these findings corroborate previous experimental manipulations that found nontoxic Microcystis strains better competitors under both light-limited and nutrient-depleted conditions.\(^9,56,60\) The most plausible physiological explanation is that microcystin biosynthesis is metabolically costly for the cells; therefore, toxigenic strains are limited to nutrient replete regions of the lake.

**Application of Study Findings to Development of a Water Safety Plan in Taihu.** Direct measurement of cyanotoxins (e.g., ELISA or HPLC) remains the gold standard for assessing the impact of CHABs on drinking water supplies. The widespread use of PCR technologies allows researchers to rapidly and unequivocally identify bloom forming genera before they reach problematic concentrations. In this study, it was shown that enumeration of total and toxigenic Microcystis cells by qPCR can be used to predict the total microcystin content of water samples in Lake Taihu. However, an equally sensitive (relative to the mcyE assay) and much simpler metric, chl \(a\), was identified as a useful predictor of microcystin variance in the lake. Considering that drinking water utilities often lack the resources and expertise to conduct biochemical and molecular analyses on raw waters, it is recommended that chl \(a\) measurements be routinely taken to estimate microcystin concentrations as the first step (hazard identification) in a comprehensive water safety plan for Lake Taihu.\(^3\) A pilot study will need to be conducted to determine what the action limit should be for the water utility based on the percentage of microcystins that are

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**Table 2. Comparison of Site-Specific Average Water Quality Parameters Measured during the Summers of 2009 and 2010**

<table>
<thead>
<tr>
<th>sample site</th>
<th>temperature (°C)</th>
<th>ratio TN:TP</th>
<th>ratio DTP:DTN</th>
<th>avg. DTN (mg l(^{-1}))</th>
<th>avg. DTP (mg l(^{-1}))</th>
<th>chl (a) (μg l(^{-1}))</th>
<th>avg. MCs (μg l(^{-1}))</th>
<th>% toxic (mcyE possessing)</th>
<th>turbidity (NTUs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1) Northwest (n = 12)</td>
<td>26.4 ± 3.1</td>
<td>17:1</td>
<td>29:1</td>
<td>3.62 ± 0.91</td>
<td>0.12 ± 0.10</td>
<td>192.1 ± 359.5</td>
<td>287 ± 6.6</td>
<td>42.6</td>
<td>26.4 ± 9.6</td>
</tr>
<tr>
<td>(2) West (n = 12)</td>
<td>26.8 ± 3.0</td>
<td>18:1</td>
<td>40:1</td>
<td>3.62 ± 0.86</td>
<td>0.09 ± 0.03</td>
<td>97.1 ± 92.3</td>
<td>8.2 ± 0.8</td>
<td>44.6</td>
<td>47.6 ± 23.3</td>
</tr>
<tr>
<td>(3) Southwest (n = 12)</td>
<td>25.7 ± 3.0</td>
<td>50:1</td>
<td>144:1</td>
<td>25.6 ± 1.00</td>
<td>0.02 ± 0.01</td>
<td>8.6 ± 8.9</td>
<td>1.1 ± 0.1</td>
<td>11.1</td>
<td>34.8 ± 18.0</td>
</tr>
<tr>
<td>(4) Lake Center (n = 12)</td>
<td>25.1 ± 3.1</td>
<td>58:1</td>
<td>132:1</td>
<td>2.64 ± 0.79</td>
<td>0.02 ± 0.01</td>
<td>7.1 ± 3.9</td>
<td>0.4 ± 0.1</td>
<td>15.5</td>
<td>23.9 ± 12.6</td>
</tr>
<tr>
<td>All Sites (n = 96)</td>
<td>26.3 ± 3.0</td>
<td>23:1</td>
<td>49:1</td>
<td>3.09 ± 1.06</td>
<td>0.06 ± 0.06</td>
<td>56.1 ± 147.9</td>
<td>6.4 ± 1.2</td>
<td>36.2</td>
<td>32.7 ± 20.3</td>
</tr>
</tbody>
</table>

“Sites (1) and (2) = high Microcystis biomass and toxigenicity (mcyE possessing). Sites (3) and (4) = low Microcystis biomass and toxigenicity (mcyE possessing)."
removed through their normal treatment process, with an end goal of less than 1.0 µg L⁻¹ total microcystins in the finished drinking water. If this target is unachievable based on standard treatment procedures, targeting the removal of intact Microcystis cells would be the next logical step because intracellular microcystins will account for the bulk of total microcystins in intact cells. Physical removal of Microcystis prior to disinfection has been shown to be particularly effective at removing much of the toxin load.⁶²,⁶³ However, because Microcystis is positively buoyant, standard coagulation, flocculation, and sedimentation techniques are only mildly effective. When dealing with buoyant algae, instead of adding coagulants to increase flocculation and sedimentation, it may be easier and more effective if the floc is maintained at the surface using dissolved air flotation techniques and cropped from the top.⁶⁴ Whichever methods are utilized, the goal should be to minimize cell lysis while maximizing cell removal. Throughout this process, the extracellular portion of microcystins will generally not be reduced and may actually increase depending on the mechanical forces exerted on the cells and how long they are handled during removal. The dissolved extracellular toxins may be present in low enough concentrations not to warrant any additional treatment processes, as chlorination can achieve moderate microcystin deactivation of the remaining microcystins depending on the water temperature, pH, concentration of hypochlorite and contact time.⁶⁵ However, since all water safety plans are specific to the water body and the treatment processes utilized, if the microcystin concentration in the finished drinking water exceeds 1.0 µg L⁻¹, then it becomes necessary to remove them via advanced treatment processes, such as ozonation or filtration through powdered activated carbon.⁶⁶,⁶⁷ These advanced treatment processes can be ceased once the microcystin concentrations are again below the tolerable daily intake level (TDI). This approach limits the cost of these more expensive treatment processes, while minimizing public health risk from microcystins in drinking waters. It is unclear to what extent microcystins may be entering the drinking water distribution system. Only one peer-reviewed study was identified that measured microcystins in finished drinking water from Lake Taihu, and it came from a period spanning June—November, 2005.¹⁹ The reported microcystin concentrations ranged from 0.059 – 0.657 µg L⁻¹. Although these values were below the World Health Organization’s TDI limit, specific such as sampling frequency, location, and how the samples were collected and processed were not detailed. In any event, the combination of chronic low level exposure to microcystins in drinking water, and the potential for supplemental dietary intake of additional microcystins through the ingestion of seafood, raises the public health risk for the general population, and especially for infants and individuals with pre-existing kidney conditions (e.g., infection with Hepatitis B or exposure to aflatoxins).⁶⁸

Toxicogenic Microcystis was detected at all sampling sites using quantitative PCR, although the density and composition of the phytoplankton assemblages were heterogeneous in time and space. The identification of a simple metric, such as chl a concentration, is an easily executed and rapid way for Taihu water managers to estimate the microcystin content of lake samples collected during the problematic summer months. Moreover, the implementation of a water safety plan is recommended due to the abnormally high prevalence of microcystin associated diseases endemic to the area. Finally, nutrient availability and ratios appeared to control bloom densities, as well as Microcystis toxicity, with replete conditions favoring toxicogenic strains. The lake center was anomalous to other more productive sites sampled, as low P availability limited all phytoplankton growth there. Additionally, the high N:P ratio and low light availability at this site appeared to select for a predominantly nontoxic Microcystis population. Therefore, nutrient reduction strategies aimed at lowering total nitrogen inputs to the lake should also include phosphorus input reductions due to their colimiting roles and the finding that low N:P ratios (i.e., sufficient DTN and DTP) were more likely to promote toxicogenic Microcystis strains.

### ASSOCIATED CONTENT

#### Supporting Information

Plasmid construct DNA sequences for QPCR standard curves. This material is available free of charge via the Internet at http://pubs.acs.org.

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#### Notes

The authors declare no competing financial interest.

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