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Spatial and Temporal Dynamics of Microcystin in a Missouri Reservoir

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Abstract

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Environmental factors associated with spatiotemporal variation of microcystin (MC) in Mozingo Lake, a Missouri reservoir, were studied during summer 2001, and annual MC trends were characterized from May 2001-May 2002. MC increased during summer, ranging from 20 to1220 ng/L. Seasonal patterns in MC corresponded with chlorophyll >35 µm (Net Chl) and cyanobacterial biovolume associated with increased dominance by *Microcystis* and *Anabaena*. MC showed strong negative correlations with dissolved nitrogen (r=-0.95) and cations (r=-0.98), and strong positive correlations with Net Chl (r=0.91). Once the lake stratified, MC and Net Chl remained uniform throughout the photic zone and decreased significantly ($p<0.05$) in the aphotic zone. Field experiments indicated MC did not change independently of Net Chl in response to decreased light or increased nutrients; however, enclosure effects may have substantially influenced experimental results. Mozingo Lake MC was tightly coupled with seasonal lake processes, including stratification and nutrient loss from the epilimnion, and cyanobacterial community composition, abundance and distribution in the water column. MC was detected in all monthly samples suggesting the potential for problems associated with MC exists year round, but peaks in early fall presented the greatest concern in Mozingo Lake.

Key Words: microcystin, cyanotoxin, cyanobacteria, vertical distribution, field experiments, Midwest, ELISA

Microcystin (MC), a cyanobacterial hepatotoxin, is a potential health risk in Midwestern water resources (Graham *et al.* 2004). Produced by strains in at least 13 cyanobacterial genera, including *Anabaena*, *Microcystis* and *Oscillatoria*, MC has been implicated in incidents of human and animal illness and death in over twenty countries worldwide (Chorus and Bartram 1999). Understanding environmental factors associated with high MC is critical to lake management decisions and minimization of health risks associated with the toxin.

Spatiotemporal heterogeneity is characteristic of MC, and in extreme cases concentrations vary by several orders of magnitude among sites, depths and seasons (Lindholm 1991, Kotak *et al.* 1995, Welker *et al.* 2003). Spatiotemporal variation in MC is generally associated with changes in cyanobac-

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terial abundance or strain composition, both influenced by environmental factors (Reynolds 1998); thus, spatiotemporal variation in MC results from complex interactions among cyanobacterial community structure, MC production and the environment. Empirical studies indicate MC levels are affected by factors limiting cyanobacterial growth, such as phosphorus (Jacoby *et al.* 2000), nitrogen (Jones and Jones 2002) and light (Wicks and Thiel 1990); however, few field experiments involving MC have been conducted, and environmental factors that influence MC concentrations in natural phytoplankton assemblages are not well understood.

Several surveys have documented MC occurrence in the Midwest (McDermott *et al.* 1995, Dodds 1996, Graham *et al.* 2004); however, its spatiotemporal variability has not been described. Therefore, during summer 2001 a Missouri reservoir was studied to describe the spatiotemporal variability in MC with respect to the physicochemical environment. Field experiments directly assessed the influence of light and nutrients on MC. In addition, monthly surface samples were collected from May 2001-May 2002 to describe annual MC trends.

Methods

Site Description

Mozingo Lake, located in the Dissected Till Plains of northwestern Missouri (Fig. 1), serves as both a drinking water supply and a recreational resource. Like most Dissected Till Plain lakes (Jones and Knowlton 1993), Mozingo is eutrophic with summer mean total phosphorus = 35 µg/L, total nitrogen = 915 µg/L,and chlorophyll = 18 µg/L. The 8-km-long reservoir has a surface area of 407 ha and a volume of 1489 ha · m, with mean and maximum depths of 3.7 m and 12.0 m, respectively. The northern end of the lake remains well mixed throughout the year while the southern end stratifies in late spring, with anoxia rapidly developing in the hypolimnion.

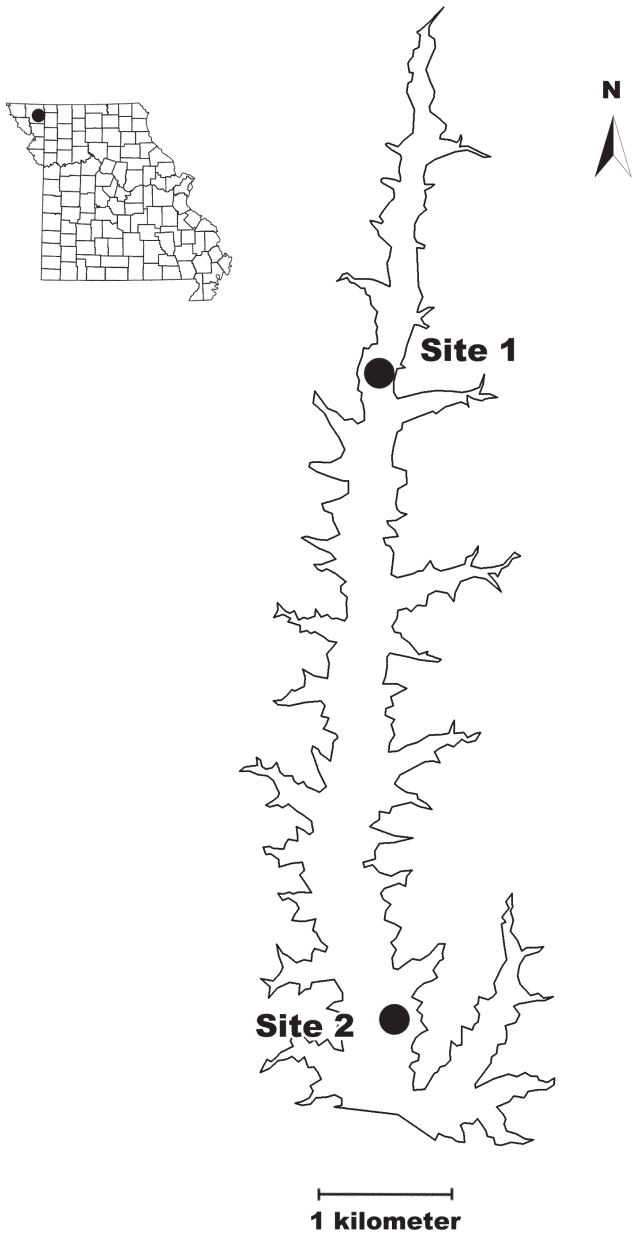

Figure 1.-Mozingo Lake, Nodaway County, Missouri. Circles indicate sampling sites.

Descriptive Limnology

Sample design, collection and analysis

Two sites were sampled monthly during May-September 2001. Site 1 was 5.5 km north of the dam (Z_{max} = 3.5 m) and Site 2 was 0.5 km north of the dam (Z_{max} = 12 m; Fig. 1). Ideally, during May-August sites were to be sampled at dawn, mid-day and dusk during three consecutive days each month (n=9) to describe daily and seasonal variation, but weather and equipment trouble prevented adhering to this schedule. August, when sites were sampled for two full days (n=6), came closest to the ideal. In May n=5; in June Site 1 n=3 and Site 2 n=4; Site 1 was not sampled in mid-July, while Site 2 n=7. Both sites were sampled once mid-day in September. During early July diel trends were studied by collecting samples every 4 h over a 24 h period (n=6). To describe annual MC trends, monthly surface samples were collected at Site 2 from May 2001-May 2002.

During each sampling event Secchi transparency and surface pH were measured and light, temperature, dissolved oxygen and conductivity profiles were conducted. Photic and epilimnetic depths were determined from profiles. Samples were collected from the surface and the middle and bottom of both the photic zone and the epilimnion; meta- and hypo-

limnetic samples were also collected. Water was collected from discrete depths using a submersible pump, and samples were analyzed for particulate MC, chlorophyll (Chl), total and dissolved phosphorus (TP and DP, respectively), total and dissolved nitrogen (TN and DN), and cations (Na^+, K^+, Ca^{2+} and Mg^{2+}). Phytoplankton samples were also collected from each depth. Alkalinity was measured in surface samples. Discrete depth samples collected during the diel study in early July were analyzed for MC and Chl.

MC-producing cyanobacteria tend to form large filaments and colonies (Chorus 2001); thus, Chl size fractions were used as a general assessment of phytoplankton community structure. Whole water from each depth was passed through a 35-mm screen, with Chl measured on the filtrate (Nano Chl, as defined by Lane and Goldman 1984). The difference between Chl and Nano Chl represents phytoplankton >35-µm (Net Chl).

Seston (20 L) was concentrated for particulate MC analysis using a 20-µm plankton net. The 20-µm mesh maximized concentration of algal material without net clogging. Samples were frozen, lyophilized and stored at -80°C. The mass of seston >20-µm (µg/L, dry weight) was measured and MC was extracted using deionized water. MC was measured using Envirogard® ELISA kits (detection limit: 0.1 µg/L; includes the variants -LR, -YR, -RR and nodularin). MC was expressed volumetrically by multiplying seston concentration (µg/L, dw) by MC content of the seston (µg/g seston dw, Chorus 2001); values were re-expressed as ng/L.

Chl, nutrients and suspended solids were analyzed after Graham *et al.* (2004). Cations were determined using Sequential ICP-AES (O'Dell *et al.* 1993). Cation and dissolved nutrient analyses were conducted on 0.45-µm membrane filtrate. Alkalinity was measured on whole water by sulfuric acid titration (Eaton *et al.* 1995). Monthly surface samples from each site were sent to PhycoTech, St. Joseph, MI, for phytoplankton identification and biovolume analysis.

Data analysis

Seasonal trends, site comparisons and empirical relationships were based on monthly epilimnetic mean values. Relationships between particulate MC and environmental variables were developed using Pearson correlation analysis. To meet the assumption of normality, data were $\log_{10}$ transformed for correlation analysis; correlations were considered significant at $p \leq 0.05$. Distinct daily patterns in MC and chlorophyll were not evident; therefore, depth data were combined into a single monthly profile for each site. Changes with depth were determined using randomized block two-way ANOVA and Tukey's paired comparisons (α=0.05). Depth was the treatment effect and each profile was a block. Data were $\log_{10}$ transformed when the assumptions of normality and heteroscedasticity were not met.

Field Experiments

Two field experiments were conducted at Site 2 to assess: (1) change in particulate MC, Net Chl and Nano Chl along the photic gradient and (2) influence of nutrient enrichment on MC, Net Chl and Nano Chl. For both experiments, surface water was incubated *in situ* for 72 h using 9-L cubitainers. Water used to fill the cubitainers was analyzed for initial particulate MC, Net Chl and Nano Chl. The proportional change in each variable was calculated as final value/initial value, referred to as yield, and used in statistical analyses (Knowlton and Jones 1996). Both experiments employed a randomized block design. Three anchored floats, representing blocks, suspended one replicate of each treatment (all n=3). Results were analyzed using randomized block two-way ANOVA and Tukey's pair-wise comparisons (α=0.05).

Experiment 1 (July 17-20, 2001) determined if MC yield changed along the photic gradient. Photic depth was 5 m during experimental set-up; thus, cubitainers were suspended every meter from the surface to 5 m. The influence of macronutrient addition on MC yield was assessed in Experiment 2 (August 6-9, 2001). The four experimental treatments were: (1) added nitrogen (+N); (2) added phosphorus (+P); (3) added nitrogen and phosphorus (+NP); and (4) no addition (control). Experiment 2 was incubated at 0.5 m. Nutrient additions were calculated as 60% increases in July TP and TN levels to ensure stimulation of algal growth; +P additions (14 µg/L as sodium phosphate) represented ~40% increases over ambient August surface values, while +N additions (420 µg/L as ammonium nitrate) represented ~60% increases.

Results

Descriptive Limnology

Mozingo Lake was well buffered, with pH $\geq$8.4, alkalinity >80 mg/L, specific conductivity >220 µS, and total cations >40 mg/L. Ca^{2+} was the dominant cation, constituting ~60% of total cations. Mg^{2+} and K^+ each comprised ~15% of total cations and Na^+ ~10%. Epilimnetic alkalinity, conductivity and total cations decreased by 15-30% during summer, but relative cation proportions remained unchanged.

Site 1 was mixed throughout summer, while Site 2 was stably stratified with an anoxic hypolimnion by June and remained stratified through September. Secchi depths (0.6-2.1 m) at Site 1 were shallower than Site 2 (0.8-3.2 m), but seasonal trends were similar, with maxima in early July and minima in September. Photic depth ranged between 2 and 3.5 m at Site 1, and between 2.5 and 4.8 m at Site 2, with minima and maxima concurrent with Secchi values.

Table 1.-Phytoplankton and Cyanophyta community structure in Mozingo Lake during summer 2001. Values represent the % contribution of each group to the total phytoplankton biovolume (Division) or cyanobacterial biovolume (Cyanophyta genera). Data points are based on one surface sample per month. Percentages may not add to 100 due to rounding errors.

	Site 1				Site 2				
	May	June	Aug	Sept	May	June	Mid July	Aug	Sept
Division									
Bacillariophyta	0.8	0.3	3.2	29.2	0.1	0.4	0.0	1.1	22.3
Chlorophyta	2.0	21.9	1.5	0.4	0.3	11.1	5.9	1.2	5.9
Cryptophyta	22.6	21.7	0.6	0.0	1.5	23.0	1.4	0.8	0.4
Cyanophyta	74.2	54.6	91.1	66.3	97.5	62.3	92.0	97.0	65.8
Other	0.3	1.5	3.7	4.1	0.6	3.2	0.7	0.0	5.6
Cyanophyta Genera									
Anabaena	0.0	0.0	36.6	58.0	0.0	0.0	0.0	21.5	47.2
Aphanizominon	98.0	20.9	1.3	3.5	96.0	9.9	23.9	1.1	9.2
Coelosphaerium	1.4	69.3	0.9	6.9	2.5	72.8	48.1	2.6	5.7
Microcystis	0.4	9.7	58.5	30.2	1.3	16.5	27.2	72.1	37.6
Other	0.2	0.1	2.7	0.4	0.2	0.7	0.7	2.7	0.2

Epilimnetic TP and TN were consistently greater at Site 1 (36-62 and 951-994 µg/L, respectively) than Site 2 (22-33 and 746-945 µg/L), while dissolved nutrients were occasionally within 1-3 µg/L. Nutrients did not show strong seasonal trends, although DN declined by ~300 µg/L between June and September. TN:TP ratios declined during summer, but were always ≥ 17, indicating potential phosphorus limitation.

Like nutrients, Chl and Nano Chl at Site 1 were consistently greater (8-57 and 3-29 µg/L, respectively) than Site 2 (5-38 and 2-15 µg/L), with minima during June and early July and maxima in September (Fig. 2b-c). Net Chl (2-28 µg/L) was similar between sites, with minima in June (Fig. 2d). Although seasonal patterns in cyanobacterial biovolume correspond with chlorophyll trends, the biovolume of potential MC producers (*Anabaena*, *Microcystis* and *Oscillatoria*) had minima in May rather than June-early July (Fig. 2b-f).

Cyanobacterial community structure was similar between sites, and during summer dominance shifted from *Aphanizominon* to *Microcystis* and *Anabaena* (Table 1). Cyanophyta consistently dominated (>50%) phytoplankton biovolume at both sites, but MC producers did not become dominant until August (Table 1). While cyanobacterial communities were similar between sites, Chlorophyta and Cryptophyta comprised a greater portion of the phytoplankton biovolume at Site 1 (Table 1), suggesting Site 1 supported more small algae than Site 2.

Microcystin

Epilimnetic MC ranged from 20 to1219 ng/L at Site 1 and 20 to 837 ng/L at Site 2. MC minima occurred in May, and values increased to maxima in September (Fig. 2a). MC was similar between sites, except in early July when MC at Site 1 was 3-4 times greater than Site 2. Seasonally, MC corresponded with patterns in the biovolume of potential producers, and peak MC was concurrent with peak chlorophyll and cyanobacterial biovolume (Fig. 2). Distinct daily trends in MC were not discernable, even during the diel study in early July.

Surface MC at Site 2 increased from May (20 ng/L) to October (2460 ng/L); by November values decreased by two orders of magnitude (27 ng/L). Low levels of MC (<10 ng/L) were detected from December 2001 to April 2002. May 2002 MC was similar to that observed in May 2001 (28 ng/L). The biovolume of potential MC producers, dominated by *Anabaena* (24% of total) and *Microcystis* (58%), also peaked in October 2001 (Fig. 3).

Microcystin Depth Distribution

Vertical gradients in particulate MC and Net Chl were consistently observed at Site 2 (Fig. 4). In May, when Site 2 was mixed, MC and Net Chl were 2-3 times greater at the surface than the bottom (Fig. 4). During summer stratification MC and Net Chl were generally uniform throughout the photic zone at Site 2, regardless of mixing depth, and values declined significantly at depths below the photic zone (Fig. 4). Similar patterns were observed at Site 1 (data not shown).

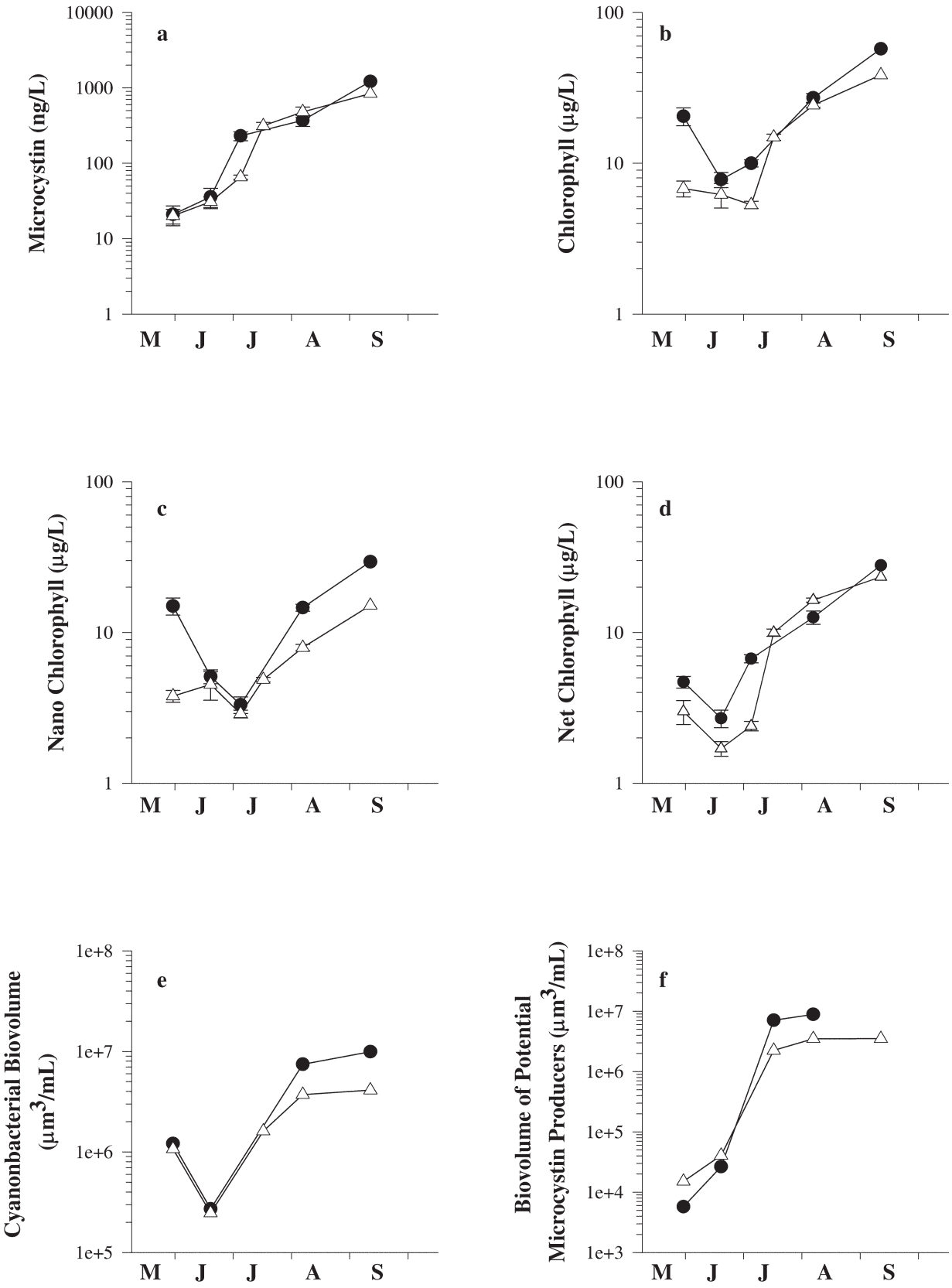

Figure 2.-Seasonal dynamics in (a) microcystin, (b) chlorophyll, (c) nano chlorophyll, (d) net chlorophyll, (e) cyanobacterial biovolume, and (f) biovolume of potential microcystin producers at Sites 1 (closed circles) and 2 (open triangles) during summer 2001. For MC and chlorophyll – monthly means (± 1 SE), with the exception of September (n=1). Biovolumes are based one surface sample per month.

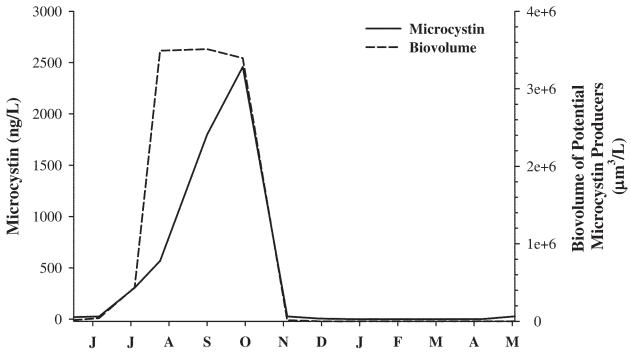

Figure 3.-Annual trends in microcystin and the biovolume of potential microcystin producers.

Microcystin and Environmental Variables

During May-September MC (n=9-11) showed a strong negative correlation with DN (r=-0.95) and a strong positive correlation with Net Chl (r=0.91, Table 2). MC also showed strong negative correlations with total cations (r=-0.98), each cation (Ca^{2+}: r=-0.96; Mg^{2+}: r=-0.94; K^{+}: r=-0.88; Na^{+}: r=-0.81) and conductivity (r=-0.91, Table 2). Several other environmental variables, including mixed depth and temperature, were also significantly and relatively strongly (r >0.60), correlated with MC (Table 2). Many variables were colinear. For example, MC, DN, total cations and Net Chl were strongly correlated (all r >0.85), likely due to similar increasing or decreasing seasonal trends (Fig. 2).

Table 2.-Pearson correlations (r) between microcystin and environmental variables based on May–September, 2001 data. All data were $\log_{10}$ transformed. Bold p-values indicate significance at α=0.05.

Variable	n	r	p
Total cations (mg/L)	9	-0.98	**<0.01**
Ca^{2+} (mg/L)	9	-0.96	**<0.01**
DN (µg/L)	9	-0.95	**<0.01**
Mg^{2+} (mg/L)	9	-0.94	**<0.01**
Net Chl (µg/L)	11	0.91	**<0.01**
Conductivity (µS)	11	-0.91	**<0.01**
K^{+} (mg/L)	9	-0.88	**<0.01**
Na^{+} (mg/L)	9	-0.81	**<0.01**
Chl (µg/L)	11	0.78	**<0.01**
Mixed depth (m)	11	-0.68	**0.03**
Temperature (°C)	11	0.65	**0.03**
Alkalinity (mg/L)	9	-0.65	0.06
Nano Chl	11	0.53	0.10
DP (µg/L)	9	-0.45	0.06
Secchi (m)	11	-0.42	0.22
TN:TP	9	-0.35	0.20
Photic Depth (m)	11	-0.34	0.35
TN (µg/L)	9	-0.22	0.45
TP (µg/L)	9	0.19	0.55

Field Experiments

Experiment 1 – Light

Experiment 1 cubitainers at 4 m and above were always in the photic zone, whereas those at 5 m rarely were. MC yield did not differ significantly across this photic gradient, and clear chlorophyll responses were not discernable (Table 3). In contrast, MC and chlorophyll values at 4-5 m in the lake were two times lower than at depths receiving more light (Fig. 4, mid-July).

Table 3.-Experiment 1 (light) results expressed as the proportion of the initial value (final/initial, f/i). Mean ± 1 SE, all n=3. For each variable, proportions were used in randomized block two-way ANOVA and Tukey's comparisons (α=0.05). Letters indicate significant differences among depths.

Depth (m)	Microcystin (f/i)	Chlorophyll > 35-µm (f/i)	Nano Chlorophyll (f/i)
0.0	1.26 ± 0.18	1.07 ± 0.01$^{a, b}$	0.64 ± 0.05^{a}
1.0	1.55 ± 0.13	1.00 ± 0.06^{b}	0.89 ± 0.03$^{a, b}$
2.0	1.45 ± 0.13	1.27 ± 0.06$^{a, b}$	1.05 ± 0.15^{b}
3.0	1.44 ± 0.13	1.23 ± 0.09$^{a, b}$	1.00 ± 0.04^{b}
4.0	1.42 ± 0.19	1.34 ± 0.03^{a}	0.86 ± 0.03$^{a, b}$
5.0	1.22 ± 0.06	1.17 ± 0.08$^{a, b}$	0.88 ± 0.05$^{a, b}$
	F=0.76, p=0.60	**F=3.57, p=0.04**	**F=4.36, p=0.02**

Experiment 2 – Nutrient addition

Nutrient addition significantly influenced MC and chlorophyll yields. MC and Net Chl yields in the +N and +NP treatments were double those in the control and +P treatments (Fig. 5). Similarly, +NP doubled Nano Chl yields relative to the control (Fig. 5). Although nutrients significantly influenced MC and Net Chl yields, overall values generally remained unchanged in the +N and +NP treatments and decreased by ~50% in the control and +P treatment (Fig. 5). In contrast, Nano Chl values increased (Fig. 5), suggesting a shift in

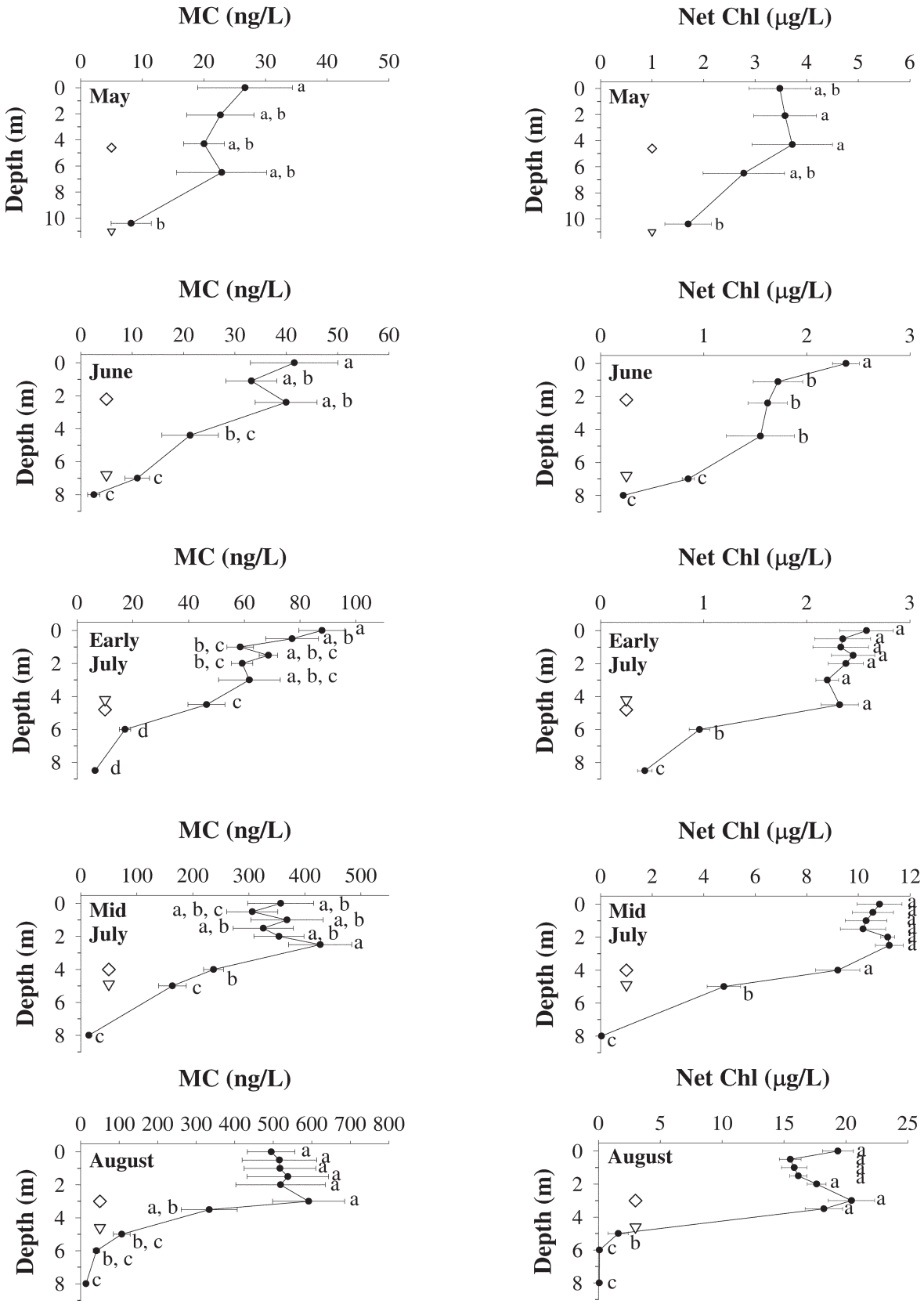

Figure 4.-Site 2 microcystin (MC) and chlorophyll >35 μm (Net Chl) vertical distribution during summer 2001. Mean ± 1 SE. Significant differences among depths, indicated by different letters, were determined using randomized block ANOVA ($\alpha = 0.05$). Diamonds indicate monthly mean photic depth and triangles indicate monthly mean mixed depth. Note the differences in scale among months.

community structure toward small algae. Decreases in MC and Net Chl and increases in Nano Chl were not measured in the lake, indicating changes were effects of treatment and enclosure.

Discussion

Particulate MC in Mozingo Lake was associated with cyanobacterial community dynamics and seasonal lake processes. *Microcystis*, capable of exploiting water column structure, was not abundant until after stable stratification in June, and *Anabaena*, a diazotroph, was not present until DN values reached seasonal lows in August. Both *Microcystis* and *Anabaena* have MC-producing strains (Chorus 2001), and increased MC corresponded with increased dominance by these genera (Fig. 2; Table 1). Net Chl, an indirect measure of the abundance of large colonial and filamentous taxa such as *Microcystis* and *Anabaena*, reflected seasonal patterns in cyanobacterial biovolume and was strongly correlated with MC (Fig. 2; Table 3). Additionally, MC never responded independently of Net Chl during the field experiments (Table 3; Fig. 5), reinforcing laboratory studies that indicate light and nutrients influence MC indirectly by influencing cyanobacterial biomass, rather than having a direct effect on intracellular MC production (Orr and Jones 1998, Long *et al.* 2001).

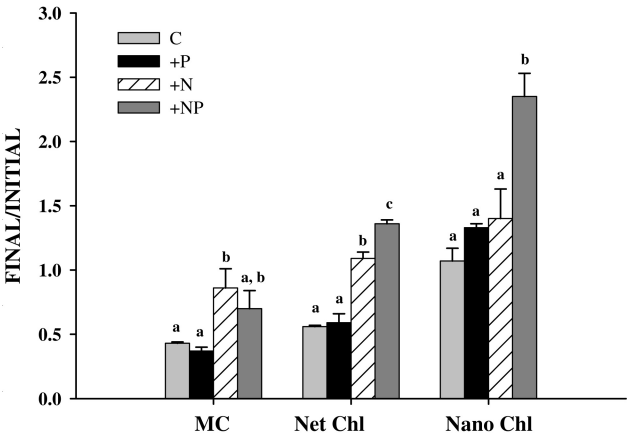

Figure 5.-Macronutrient addition (Experiment 2) results for microcystin (MC), chlorophyll >35 µm, and nano chlorophyll expressed as the proportion of the initial value of the variable (final/initial). Mean ± 1 SE. Treatments were: (1) added nitrogen (+N), (2) added phosphorus (+P), (3) added nitrogen and phosphorus (+NP), and (4) no addition (control, C; all n=3). For each variable, significant differences among treatments, indicated by different letters, were determined using randomized block ANOVA (MC: F=5.19, p=0.04; Net Chl: F=73.39, p<0.01; Nano Chl: F=9.29, p=0.01).

Many environmental variables that reflect seasonal patterns were significantly correlated with MC, but total cations and DN were most strongly correlated (Table 2). Epilimnetic cations and DN declined during summer, while MC increased. Although correlation does not imply causality, these strong associations reflect seasonal trends. Cation decline (~25% after stratification) likely resulted from epilimnetic dilution by rainfall events (accounting for ~50% of the measured decline, data not shown) and precipitation as carbonates. Despite summer decline, none of the cation constituents reached levels considered limiting to algal growth (Wetzel 2001). By comparison, DN decline (~40% after stratification) was likely due to a combination of algal uptake and epilimnetic dilution. Unlike cations, DN may have limited algal growth during late summer. TN:TP ratios indicated potential phosphorus limitation (≥ 17) but may not have reflected nutrient bioavailability (Reynolds 1998). The appearance of *Anabaena* in August suggests nitrogen limitation and limited data suggest nitrate was not detectable and ammonia was <20 µg/L during late summer (data not shown). DN constituents can strongly influence cyanobacterial dynamics (Blomqvist *et al.* 1994) and may have been important in structuring the Mozingo Lake cyanobacterial community, thereby influencing MC.

Although clear associations between particulate MC, cyanobacteria and the environment were evident in Mozingo Lake (Fig 2.; Table 2), such relationships are not consistent among lake studies. For example, in a hypereutrophic German reservoir, MC was not correlated with any measured variables, including the relative biomass of potential MC producers (Jungmann *et al.* 1996). Even when strong correlations are found, the factors associated with MC vary from lake to lake (Wicks and Thiel 1990, Kotak *et al.* 1995, Vézie *et al.* 1998, Jacoby *et al.* 2000, Jones and Jones 2002), likely due to the broad range of variables that may limit cyanobacterial growth (Reynolds 1998) and the co-occurrence of both toxic and nontoxic strains (Vézie *et al.* 1998).

Longitudinal gradients are characteristic of reservoirs, and environmental factors may vary by orders of magnitude between the dam and up-lake sites (Jones and Novak 1981, Knowlton and Jones 1989). In Mozingo Lake, the up-lake site (Site 1) was expected to have elevated nutrients and reduced light relative to the dam site (Site 2). Because elevated nutrients and reduced light favor cyanobacteria (Reynolds 1998), MC, Net Chl and cyanobacterial biovolume were expected to be greater at Site 1 than Site 2. While nutrients were consistently greater and Secchi depths were consistently shallower at Site 1 than Site 2, values varied by less than two-fold between sites. Likewise, MC, Net Chl and cyanobacterial biovolume were generally similar between sites (Fig. 2). Physicochemical differences between dam and up-lake sites in reservoirs are related to riverine influence, which varies depending on rainfall and hydrology (Jones and Novak 1981). While reservoir hydrodynamics likely influence MC values, longitudinal gradients in Mozingo Lake during summer 2001 did not result in marked longitudinal variability of MC.

Many MC-producing cyanobacteria have the ability to control water column position, allowing the optimization of light resources during the day and nutrient resources at night. Cyanobacterial distribution in the water column depends on community composition and the physicochemical environment; thus, MC distribution in the water column may vary on a diel basis and will be unique to individual lakes (Reynolds 1987). MC producers in Mozingo Lake likely maintained a position in the photic zone. When Mozingo Lake was stably stratified, MC and Net Chl were consistently greater in the photic zone than the aphotic zone despite deeper mixing and, during the light experiment, MC and Net Chl in movement-restricted populations did not decline in the aphotic zone as observed in the lake (Table 3; Fig. 4). Diel changes in MC distribution and concentration in the water column were not detected, although fluctuations may have occurred. By comparison, in a shallow Alberta, Canada lake, MC at 0-2 m decreased by more than six-fold at night, likely due to vertical migration by *Microcystis aeruginosa* (Kotak *et al.* 1995).

Few studies have addressed vertical MC distribution, but all those that have found different trends. MC was uniformly distributed throughout the water column in a stably stratified Washington, U.S.A. lake where *Microcystis aeruginosa* colonies regularly migrated from the sediments into the water column (Johnston and Jacoby 2003). In contrast, MC values were highest at the surface and had declined substantially by 2 m in a shallow Turkish lake where *M. aeruginosa* concentrated near the surface (Albay *et al.* 2003). *Oscillatoria aghardii*, a species known to form metalimnetic peaks, was present in a stratified Finnish lake where MC was undetectable in the epilimnion but had a sharp metalimnetic peak (Lindholm 1991).

During Experiment 2 the nanoplankton responded more strongly to nutrient addition than MC and Net Chl, which declined in the control and +P treatments (Fig. 5). Small enclosures may favor non-cyanobacterial genera (Paerl and Bowles 1987), while nutrient enrichment may favor nanoplankton (Lane and Goldman 1984). Therefore, Experiment 2 results likely reflect enclosure effects more than nutrient influence on MC and Net Chl. Larger-scale field experiments conducted for a longer period of time may provide more insight into environmental influence on MC in mixed phytoplankton assemblages.

Mozingo Lake particulate MC values were within the range encountered in Dissected Till Plain lakes during a Midwestern survey (Graham *et al.* 2004). Site 2 surface samples indicate MC in Mozingo Lake peaked in October (Fig. 3). MC maxima have been observed in lake studies anytime from early spring to late fall (Kotak *et al.* 1995, Vézie *et al.* 1998, Jacoby *et al.* 2000, Jones and Jones 2002), but the majority of problems associated with cyanotoxins are reported in late summer and early fall (Chorus and Bartram 1999). MC was detected throughout the year in surface samples, indicating MC can be present and may potentially cause health concerns year round.

The current study contributes to understanding of the forcing factors driving MC spatiotemporal dynamics in single systems. Mozingo Lake MC values were tightly coupled with seasonal lake processes, including stratification and nutrient loss from the epilimnion, and cyanobacterial community composition, abundance and distribution in the water column as controlled by reservoir hydrodynamics. MC may vary by orders of magnitude both spatially and temporally due to changes in both cyanobacterial community structure and the physicochemical/hydrological environment causing spatiotemporal patterns and empirical relationships between MC, cyanobacteria and the environment to vary among lake studies. Knowledge of the environmental factors influencing spatiotemporal variation in MC is critical to understanding regulatory factors which will lead to effective lake management and minimization of human health risks.

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