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Environmental factors influencing the quantitative distribution of microcystin and common potentially toxigenic cyanobacteria in U.S. lakes and reservoirs

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Abstract

Many species of cyanobacteria are capable of producing toxins and causing nuisance blooms, however response to environmental conditions is likely taxon-specific. Environmental factors influencing cyanobacterial composition and toxin production in lakes have been examined in many studies; yet are often confined to individual water bodies, or to a small number of systems within the same region. Here, data from the 2012 USEPA National Lakes Assessment are used to examine relationships between biovolume of common potentially-toxigenic cyanobacteria (*Aphanizomenon* spp., *Cylindrospermopsis* spp., *Dolichospermum* spp., *Microcystis* spp. and *Planktothrix* spp.) and environmental variables across the entire conterminous United States, and results are compared across nine distinct ecoregions. Total phosphorus and water clarity were identified as the most influential environmental factors correlated with phytoplankton community composition. The Northern, Southern and Temperate Plains ecoregions displayed the highest biovolumes of potentially toxigenic taxa on average, as well as highest mean concentrations of microcystin. In those three ecoregions, samples with microcystin concentrations greater than 1 ppb were primarily dominated by *Planktothrix* spp. while in all other ecoregions *Dolichospermum* spp. was the dominant genus. Canonical Correlation Analysis revealed a strong association between high microcystin concentrations and high nutrient concentrations (total nitrogen and total phosphorus), and between high microcystin concentrations and low percentage of watershed forest cover. Results from this study indicate that the likely occurrence of potentially toxigenic taxa in lakes and reservoirs is predictable on a biogeographical basis, depending on morphological and water quality characteristics. Data from this study may be useful to regional managers attempting to prevent or mitigate nuisance cyanobacterial blooms.

Introduction

Phytoplankton community composition in lakes and reservoirs (hereinafter, lakes) is strongly influenced both spatially and temporally by variability in water quality factors such as nutrient concentrations, hydrodynamics and water temperature (Tillman et al., 1982; Watson et al., 1997; de Souza Cardoso and da Motta Marques, 2009; Beaver et al., 2012, 2013). Generally, phytoplankton communities tend to be dominated by cyanobacteria in systems with high concentrations of nutrients, low water clarity and warmer surface temperatures – conditions that are characteristic of eutrophic water bodies (Smith, 1986; Downing et al., 2001; Kosten et al., 2012). Planktonic species of cyanobacteria can produce toxins as secondary metabolites, which can negatively impact lake ecosystems at higher trophic levels and cause adverse effects on human health and recreation. Both climate warming and eutrophication are predicted to increase the frequency and severity of toxic cyanobacterial blooms worldwide (Heisler et al., 2008; Paerl and Huisman, 2008; O’Neil et al., 2012; Paerl, 2017). Thus, determining which environmental conditions are most likely to lead to cyanobacterial blooms (and subsequent toxin production) is of great interest to lake managers and natural resource professionals (Paerl, 2017).

Most studies that monitor phytoplankton community structure and occurrence of toxins over space and time focus on a single lake or a small region, as large-scale and prolonged sampling can be difficult and costly. Consequently, conflicting results have been reported regarding which factors are most likely to lead to toxic bloom events. It is also likely that toxin production in specific species and strains of cyanobacteria is triggered by different environmental conditions (Marmen et al., 2016; Wood et al., 2017). The USEPA’s National Lakes Assessment (NLA), which surveys over 1000 randomly-selected lakes across the U.S. every five years is a relevant public resource for broad-scale limnological research (Pollard et al., 2018) and provides a unique opportunity to examine cyanobacterial communities over multiple ecoregions and a gradient of water quality conditions.

The current study seeks to determine which water quality variables are related to phytoplankton community composition and what environmental conditions promote the prevalence of potentially-toxigenic cyanobacteria in lakes in the conterminous U.S. Analysis of water quality and land use data from the 2007 NLA (Beaver et al., 2014) showed that microcystin concentrations >1 ppb (above the World Health Organization recommended threshold for safe drinking water) were primarily located in the upper Midwest region of the U.S., and were positively associated with agricultural land-use practices, high total nitrogen (TN) and high dissolved organic carbon (DOC). The current study utilizes data from the 2012 NLA and extends upon previous analyses to determine whether microcystin concentrations in those regions and elsewhere in the U.S. are associated with particular cyanobacterial taxa, and how those taxa are related to specific water quality variables. Based on the finding from Beaver et al. (2014) that similar land-use practices in different regions of the country did not yield similar toxicity, it is expected that high microcystin concentrations are correlated to high biovolumes of toxigenic taxa, and that those taxa are strongly influenced by water quality parameters associated with agriculture-dominated watersheds (high nutrients and DOC). Recent studies have used data from the NLA to examine the relationship between water quality variables and total cyanobacterial abundance (Beaver et al., 2014) or biomass (Beaulieu et al., 2013). This study expands on previous records by utilizing individually derived biovolumes to examine relationships between specific taxa and microcystin concentrations on a national scale.

Methods

Study sites

1038 lakes were comprehensively sampled throughout the conterminous U.S. from May through September of 2012 as part of the U.S. Environmental Protection Agency's 2012 National Lakes Assessment (USEPA, 2016). Lakes greater than 1 ha in area and 1 m in depth, with at least a quarter-acre of open water area were selected, using unbiased stratified random sampling, from the USGS/USEPA National Hydrography Dataset (NHDPlus) version 2 (see Simley and Carswell, 2009). Both natural and man-made lakes were included in the survey; however the Laurentian Great Lakes, the Great Salt Lake, commercial treatment/disposal ponds, brackish lakes and ephemeral lakes were excluded. A subset of lakes were sampled twice within the study period, and each sampling event was treated as an independent sample.

Sample collection

Each lake was sampled for water quality, biological condition, habitat conditions, and recreational suitability, however collection methodology described here applies only to the variables included in this study. Phytoplankton, microcystin and water quality samples were taken from an index site in each lake. The index site was considered an open water area up to 50 m deep or at the mid-point in reservoirs. Lake surface area was also recorded, and a ratio of area:depth was calculated using a modified version the equation for dynamic ratio (i.e. potential for disturbance) described in Håkansan (1982):

$$\sqrt{\mathrm{surfacearea}\left(\mathrm{km}^{2}\right)} \div \ \mathrm{index\ sitedepth\ (m)}$$

Vertical temperature profiles were conducted at the index site; however in this study only mean water temperatures from the upper 5 m of the water column were evaluated. Secchi depth was recorded using a standard Secchi disk on the shady side of the boat. An integrated sampler was used to collect whole water grab samples from within the euphotic zone, generally within the top 2 m of the water column. Water was transferred from the sampler into a rinsed 4 L cubitainer and this process was repeated until the cubitainer was filled. Subsamples were then taken from the cubitainer for nutrients, phytoplankton and microcystin. A 500 ml subsample for microcystin was immediately frozen following collection. A 1000 ml subsample for phytoplankton was preserved with Lugol's iodine solution. Microcystin and phytoplankton samples were then shipped to BSA Environmental Services, Inc. (Beachwood, OH) for analysis. A 250 ml subsample for nutrient analysis was acidified and shipped overnight to processing labs. Microcystin and phytoplankton were also sampled in the littoral zone of all lakes, however in this study only open-water samples were analyzed in order to be consistent with water quality variables. For more details on sample collection, see USEPA (2012a).

Laboratory analyses

Total nitrogen (mg L^{-1}) and total phosphorus (μg L^{-1}) were determined using automated colorimetric analysis following persulfate digestion. Dissolved organic carbon (mg L^{-1}) was determined using UV promoted persulfate oxidation to CO_2 with infrared detection. Total microcystin concentrations (ppb) were determined using a Microcystins/Nodularins enzyme-linked immunosorbent assay (Abraxis, detection limit 0.1 μg L−1, -ADDA specific) following three freeze-thaw cycles to lyse cyanobacteria cells (Graham et al., 2010). Phytoplankton were examined under inverted light microscopes (Leica DMLB) at 400X using pre-concentrated Utermöhl sedimentation chambers that had been allowed to settle for a minimum of 8 h. Taxonomists identified organisms to the lowest possible taxonomic level, usually species, and enumerated at least 400 natural algal units (colonies, filaments and unicells). Up to 10 individual cells for each taxon were measured, and biovolume (μm3 L−1) was calculated using formulae for geometric shapes closely resembling specific taxa (Hillebrand et al., 1999). Total biovolume for each taxon was calculated by multiplying mean individual biovolume by cell abundance (# L−1). More information on laboratory analyses can be found in USEPA (2012b).

Five cyanobacterial genera including *Aphanizomenon* Morren, *Cylindrospermopsis* (Woloszynska) Seenayya and Subba Raju*, Dolichospermum* (Ralfs ex Bornet et Flahault), *Microcystis* Lemmermann, and *Planktothrix* (Gomont) constitute the focus of this study. *Aphanizomenon, Cylindrospermopsis, Dolichospermum* and *Planktothrix* were chosen because they were the most frequent potentially toxigenic taxa observed in the 2012 NLA survey. *Microcystis* was also included due to the extensive degree of previous scientific study regarding its occurrence and toxicity. While all five genera contain species and strains capable of cyanotoxin production, only *Dolichospermum*, *Microcystis*, and *Planktothrix* are capable of producing microcystins (Carmichael, 2001). As such, only those three genera are analyzed in the context of microcystin production. All species originally identified under the genus *Anabaena* are referred to and treated as *Dolichospermum* in this report, following recent nomenclatural changes that occurred between the time of initial identification and present data analysis (Komárek, 2016). Specimens identified as *Planktothrix* spp. may have been previously classified as *Oscillatoria* (Anagnostidis and Komárek, 1988).

Mapping, land use and trophic state determination

Using the latitude and longitude for all sample sites and the absolute value for each criterion (individual species biovolume, microcystin concentration), the data were plotted onto a map using Arc GIS® software. Quantitative data were overlaid onto a background map detailing 9 distinct ecoregions. For the purposes of the National Lakes Assessment and other components of the EPA’s National Aquatic Resource Surveys (NARS), the 85 Level III ecoregions of the conterminous U.S. (Omernik, 1987) were agglomerated into 9 broader ecoregions (Herlihy et al., 2008), which were assessed on the basis of uniformity in reference-site quality and naturally occurring variation in stream macroinvertebrate assemblages. Hereinafter the term “ecoregion” will refer to the 9 agglomerated ecoregions outlined by Herlihy et al. (2008). Percentage land use for each lake’s watershed was determined following methods outlined in Beaver et al. (2014). After all biological and chemical analyses were complete, trophic state was determined using chlorophyll-a, Secchi depth and total phosphorus data (USEPA, 2012c).

Niche centroids

Relationships between environmental variables and microcystin concentration and between environmental variables and five individual cyanobacteria genera (*Aphanizomenon, Cylindrospermopsis*, *Dolichospermum, Microcystis* and *Planktothrix*) were quantified using niche centroid analysis (ter Braak and Verdonschot, 1995). In this analysis, the weighted mean biovolume or microcystin concentration and the environmental variable of interest are used to determine the optimum value at which the maximal concentration/biovolume may be observed. Using biovolume of an individual species as an example, the niche centroid optima were calculated using:

$$u_k = \sum_{i=1}^{n} \frac{y_{ik}}{y_{+k}} X_i$$

where:

y_{ik} = the biovolume of species k in the ith sample,

y_{+k} = the summed biovolume of species k in all samples, and

x_i = the environmental variable in the ith sample.

1.1. Canonical correlation analysis

A canonical correlation analysis (CCA) was performed using the Canonical Analysis of Principal Coordinates (CAP) function of the PERMANOVA + add-on in PRIMER 6 (Clarke and Gorley, 2006) between standardized phytoplankton biovolume and seven normalized environmental variables (total nitrogen, total phosphorus, water temperature, Secchi depth, dissolved organic carbon, index site depth and area:depth ratio). Prior to analysis, all phytoplankton biovolumes were agglomerated by genus, or family when necessary. Taxa not contributing at least 5% biovolume to any sample were excluded from the analysis to reduce noise. Subsequent to agglomeration and noise reduction, a data matrix of 201 phytoplankton taxa from 1092 samples was prepared detailing the total biovolume for each taxon in every sample. A sample-matched matrix of water quality variables was also prepared. A Bray-Curtis resemblance matrix was computed for the standardized phytoplankton biovolume matrix, which was analyzed against the sample-matched, normalized water quality matrix using the CAP routine. A second CCA was performed between the standardized phytoplankton data and a sample-matched matrix of untransformed percentage land-use data (n = 1221) using the same process described above. Subsequent to running the CCAs, concentrations of microcystin were superimposed over the resultant ordination plots. Additional details on the statistical methodology are provided in Beaver et al. (2012, 2013).

Results

Ecoregion

Water quality factors varied by ecoregion, with the Temperate Plains, Northern Plains and Southern Plains tending to have poorer water quality (higher nutrients, lower water clarity) on average (Table 1). Secchi depth was lowest on average in the Coastal Plains (1.0 m), Temperate Plains (1.2 m), Northern Plains (1.2 m) and Southern Plains (1.0 m), indicating low water clarity and reduced photic zone; Secchi depth was highest on average (indicating high water clarity) in the Western Mountains region (3.6 m). Both mean total nitrogen and mean total phosphorus were highest in the Temperate Plains (1.64 mg L−1 and 173 μg L−1, respectively), Northern Plains (2.92 mg L−1 and 332 μg L−1, respectively) and Southern Plains (2.59 mg L−1 and 245 μg L−1, respectively), and lowest in the Northern Appalachians (0.41 mg L−1 and 25 μg L−1, respectively). Lakes in the three Plains ecoregions were also relatively shallow on average, with the Northern, Southern and Temperate Plains having mean index site depths of 5.55, 4.28 and 5.39 m respectfully. Dissolved organic carbon was highest on average in the Northern and Southern Plains (18.50 and 19.99 mg L−1 respectfully), although concentrations in the Southern Plains were highly variable. The Coastal Plains had the lowest average index site depth (3.92 m) and the highest average area:depth ratio (0.63), indicating that lakes in that region tended to be larger and shallower when compared to other ecoregions.

Out of the 1038 lakes sampled for microcystin, only 95 had concentrations >1 ppb in the open-water zone. Within that group of 95 lakes, 72% were located in the Northern, Temperate and Southern Plains (Table 2). The Northern, Temperate and Southern Plains ecoregions had higher microcystin on average (2.05, 1.74 and 1.06 ppb) than the other six ecoregions (all <0.6 ppb, Table 2, Fig. 1). Notably, there were no samples containing >1 ppb microcystin from the Western Mountains ecoregion.

Relative cyanobacterial taxa biovolume also showed distinct ecoregional patterns. Highest mean biovolumes of *Aphanizomenon* spp. were observed in the Northern and Temperate Plains (1.64 * 106 and 6.67 * 105 μm3 L−1), as well as in the Xeric region (6.53 * 105 μm3 L−1) (Fig. 2a). High biovolumes of *Cylindrospermopsis* spp. occurred in a few instances in the Southern and Temperate Plains (means of 1.14 * 106 and 1.12 * 106 μm3 L−1), but were notably higher in the Coastal Plains (mean of 1.47 * 106 μm3 L−1) compared to other cyanobacterial taxa (Fig. 2b). Of the five taxa examined, *Dolichospermum* spp. had the least clear ecoregional distribution, with relatively similar biovolumes across all ecoregions (Fig. 2c). Occurrences of *Microcystis* spp. were observed primarily in lakes in the eastern portion of the U.S., with few observations in the Northern Plains, Southern Plains, Southern Appalachians or Xeric ecoregions (Fig. 2d). No instances of *Microcystis* spp. were observed in open-water regions in lakes of the Western Mountains ecoregion. Biovolume was lowest overall for *Microcystis* spp. compared to other common toxigenic taxa. Highest biovolumes for *Planktothrix* spp. were found in the Northern, Southern and Temperate Plains ecoregions (1.67 * 106, 3.15 * 106 and 9.11 * 105 μm3 L−1 respectfully, Fig. 2e).

Amongst genera capable of producing microcystin, *Planktothrix* spp. had higher mean biovolumes in ecoregions where microcystin was highest (Northern, Southern and Temperate Plains, Fig. 3a), while *Dolichospermum* spp. was the dominant microcystin-producing taxa in all other ecoregions. A similar pattern was observed in lakes with >1 ppb microcystin (Fig. 3b). In the Northern and Southern Plains, *Planktothrix* spp. contributed the majority of the biovolume amongst potentially-toxigenic taxa. In the Temperate Plains, both *Planktothrix* spp. and *Dolichospermum* spp. contributed appreciably to total biovolume of potential microcystin producers. In all other ecoregions *Dolichospermum* spp. tended to be dominant in samples >1 ppb microcystin, while *Microcystis* spp. contributed less to total biovolume in those samples. Samples in which microcystin concentrations were >1 ppb but no specimens of *Microcystis* spp., *Dolichospermum* spp. or *Planktothrix* spp. were observed were excluded from this analysis. As such, it is possible that high concentrations of microcystin in lakes in the Plains ecoregions and elsewhere in the U.S. could be caused by other potentially-toxigenic taxa (see Paerl, 2018), or may have been sampled after bloom die-off.

A subset of lakes in the 2012 NLA (n = 1030) for which key chemical and biological data were collected were classed into four trophic states: hypereutrophic, eutrophic, mesotrophic or oligotrophic. Mean biovolume was determined for common potentially toxigenic taxa for each trophic state (Fig. 4). For both mesotrophic and oligotrophic lakes, mean biovolumes for all potentially toxigenic cyanobacteria were low (>1.31 * 105 µm3 L−1). In eutrophic lakes, *Dolichospermum* spp. (8.88 *105 µm3 L−1) and *Aphanizomenon* spp. (5.19 * 105 µm3 L−1) had highest biovolume, while biovolume for other potentially toxigenic taxa was comparatively low on average. Mean biovolumes for both *Dolichospermum* spp. and *Aphanizomenon* spp. were higher in hypereutrophic lakes than in eutrophic lakes (2.30 * 106 µm3 L−1 and 1.07 * 106 µm3 L−1 respectfully). Mean biovolumes for *Microcystis* spp. and *Planktothrix* spp. were highest in hypereutrophic lakes (2.01 * 105 and 2.71 * 106 µm3 L−1 respectfully) and constituted over an order of magnitude increase from mean biovolumes in eutrophic lakes (4.15 * 104 and 6.67 * 104 µm3 L−1 respectively).

Niche centroids

Niche centroid optima for the five common toxigenic taxa were determined for Secchi depth, TN, TP, water temperature, DOC, N:P, index site depth and area:depth (Table 3). Results indicate highest *Dolichospermum* spp. biovolume would be found in lakes with relatively higher surface temperatures and lower relative concentrations of DOC, while *Microcystis* spp. biovolume is predicted to be highest at relatively lower temperatures. Lakes with higher N:P values would favor *Aphanizomenon* spp. over other potentially toxigenic taxa, but *Cylindrospermopsis* spp. and *Planktothrix* spp. would have higher biovolumes at higher TP concentrations. Optima for Secchi depth show that *Planktothrix* spp. biovolume tends to be highest in lakes with lower water clarity and low area:depth ratios. Niche centroid optima for microcystin indicate that higher concentrations tend to occur under conditions of warmer temperatures and low area:depth ratios.

Canonical correlation analyses

The CCA comparing phytoplankton taxa and environmental variables produced two pairs of canonical axes explaining 49% (CAP1, first axis) and 30% (CAP2, second axis) of the variance amongst correlations between phytoplankton biovolume and environmental variables. Secchi depth (water clarity) displayed a strong negative correlation with the first axis (−0.874), while total phosphorus displayed a strong positive correlation (0.810) with the second axis. All other environmental variables were weakly correlated with phytoplankton biovolume. Secchi depth was inversely correlated with area:depth. High biovolumes of *Aphanizomenon* spp. appeared on the positive side of the second axis, implying an association with higher nutrients (Fig. 5a). High biovolumes of *Cylindrospermopsis* spp.

showed a positive correlation with the first axis, indicating that highest biovolumes occurred in sites with low water clarity and higher area:depth (Fig. 5b). Both *Dolichospermum* spp. and *Microcystis* spp. did not show a clear association with any water quality variables (Figs. 5c, d), while for *Planktothrix* spp. there was a strong, positive correlation with the positive sides of the first and second axes, implying a strong correlation with higher nutrients (Fig. 5e). Superimposition of microcystin concentrations showed an association between higher microcystin concentrations and higher nutrients (TN, TP and DOC), and between higher microcystin concentrations and lower Secchi depths (reduced water clarity) (Fig. 6a).

The CCA comparing phytoplankton taxa and land-use categories produced two pairs of canonical axes explaining 47% (CAP1, first axis) and 38% (CAP2, second axis) of the variance amongst correlations between phytoplankton biovolume and percentage land-use. Superimposition of microcystin concentrations showed a negative relationship between microcystin and forested area (Fig. 6b), indicating that lakes with low forest cover in their watersheds tended to have increased concentrations of microcystin.

Discussion

Studies focusing on cyanobacterial biogeography have found conflicting evidence for the ubiquitous distribution of potentially toxigenic species, indicating that some species may be truly cosmopolitan and equally likely to appear anywhere on earth if conditions are suitable (e.g. *Microcystis aeruginosa*, Neilan et al., 1997; van Gremberghe et al., 2011; Harke et al., 2016) while others have biogeographically distinct populations (e.g. *Cylindrospermopsis raciborskii*, Moreira et al., 2011). Strain-specific identification of cyanobacteria was beyond the scope of this study, and as such it is difficult to draw definitive conclusions about the distribution of cyanobacteria species within the U.S. Nevertheless, the observed regional trends may prove valuable for environmental managers interested in prevention of harmful algal blooms in specific systems. Differential distributions of potentially toxigenic cyanobacterial taxa were observed within the U.S., which seems to be driven primarily by regional trends in water quality, particularly in terms of water clarity and total phosphorus.

A recent study using the 2012 NLA data found that nation-wide assessments of cyanobacteria and microcystin did not show clear trends, and more-focused, regional assessments were necessary to draw significant conclusions (Marion et al., 2017). Likewise, similar findings that the Plains ecoregions showed poorest water quality as assessed during the 2012 NLA (Marion et al., 2017) agreed with both the findings of this study and with findings from the 2007 NLA (Beaver et al., 2014; Doubek et al., 2015; Taranu et al., 2017). Previous studies have also found that individual cyanobacteria taxa are differentially influenced by fluctuations in nutrient concentrations and temperature (Rigosi et al., 2014). The findings from this study add to a growing body of evidence (Brookes and Carey, 2011; Carey et al., 2012; Beaver et al., 2012, 2014; Doubek et al., 2015; Mantzouki et al., 2016; Paerl et al., 2016; Marion et al., 2017; Taranu et al., 2017) suggesting that the long-term control and mitigation of harmful cyanobacteria blooms must be tailored to meet specific water quality challenges that vary by region and by dominant taxa.

Since the cyanobacteria are a group that varies greatly in terms of morphology, habitat-preference and ecophysiology (Chorus and Bartram, 1999; Carey et al., 2012; Mantzouki et al., 2016), different mitigation strategies may be more or less effective depending on the dominant taxa and hydrological features of individual lakes. Many cyanobacteria are able to form gas vesicles, allowing cells and colonies to migrate vertically through the water column. Those taxa that form large, floating colonies, such as *Microcystis* spp. and *Dolichospermum* spp., possess superior vertical migration velocities which may lend an advantage in highly stratified lakes where nutrients are often trapped in the darker layers of the hypolimnion (Wagner and Adrian, 2009; Carey et al., 2012). Nitrogen-fixation capability, exhibited by *Dolichospermum* spp., *Aphanizomenon* spp. and *Cylindrospermopsis* spp. is advantageous under nitrogen-deplete conditions (Wood et al., 2010), while non-N-fixing cyanobacteria such as *Microcystis* spp. and *Planktothrix* spp. are generally considered indicators of nitrogen enrichment (Paerl, 2018). In systems where N-fixers and non-N-fixing cyanobacteria occur together, mitigation may be especially challenging and reduction of both nitrogen and other nutrients (i.e. phosphorus, iron) may be necessary to deter nuisance blooms. Low N:P ratios (<23) have been found in association with a greater potential for microcystin production (Orihel et al., 2012); results from this study support this idea, as niche centroid analysis showed an N:P optima of 19.78 for microcystin.

The genus *Cylindrospermopsis* is historically a tropical taxon that has slowly expanded its range into the northern latitudes of North America (Padisák, 1997; Sinha et al., 2012), and is generally thought to be adapted to warmer systems; results of this study support this notion and show a strong association between *Cylindrospermopsis* spp. biovolume and warmer surface temperatures. Many species of *Microcystis*, particularly toxic strains (Davis et al., 2009), have also been shown to exhibit relatively higher growth rates at warmer temperatures and to be limited in growth rate at lower temperatures, compared to other cyanobacteria (Robarts and Zohary, 1987). In contrast, niche centroid analysis predicted that *Microcystis* spp. biovolume would be highest at relatively lower temperatures compared to other potentially toxigenic taxa. Likewise, a toxic bloom of *Microcystis* recently appeared in Lake Mead (Beaver et al., 2018) when surface temperatures were sub-maximal (approximately 22 °C). Despite an association between high growth rates and warmer temperatures, *Cylindrospermopsis* spp. blooms rarely produce toxins (saxitoxin and cylindrospermopsin) in northern latitudes (Sinha et al., 2012; Antunes et al., 2015). It is important to note that while higher growth rate for all potentially toxigenic taxa is often an indication of excess nutrients and eutrophic conditions, toxin production is still poorly understood and is not always correlated with high cyanobacterial biovolume (Hollister and Kreakie, 2016).

Several studies (Post et al., 1985; Scheffer et al., 1997) have shown that *Planktothrix* spp. has a high tolerance for reduced light availability, likely allowing it to outcompete other cyanobacteria in shallow lakes prone to frequent mixing. The fact that lakes in the Plains ecoregions have, on average, lower Secchi depths (reduced visibility and shallower photic zone depths) may be linked to the increased prevalence of *Planktothrix* spp. in those regions. In this study, niche-centroid analysis predicted highest biovolumes of *Planktothrix* spp. at relatively low visibilities; however other common toxigenic taxa also showed visibility optima within a similar range, suggesting that *Planktothrix* spp. would not necessarily have an advantage under low visibility conditions. The CCA showed that *Planktothrix* spp. biovolume in this study was more strongly associated with increased nutrient concentrations than reduced water clarity. Similar results showing that environmental factors other than light availability (i.e. nutrients, temperature) have a stronger influence on *Planktothrix* spp. have been seen in Lake Okeechobee (Florida, U.S.A.) – a shallow, well-mixed lake where one would expect to find low-light adapted species positively associated with turbid conditions (Havens et al., 1998). These results indicate that compounding factors likely influence the dominance of certain cyanobacterial taxa, and that ecophysiological adaptations may be speciesor strain-specific.

Hypereutrophic lakes in the Plains that experience extraordinarily high biovolumes of *Planktothrix* spp. on an internannual basis (Buckeye Lake and Grand Lake Saint Mary's, Ohio, USA), *Planktothrix* spp. biovolume is strongly associated with reduced light availability and high area:depth ratio (J. Beaver, unpublished data). In contrast, niche centroid optima in this study indicated that highest biovolumes of *Planktothrix* spp. were observed in lakes that had comparatively low area:depth ratios, which lends further evidence that other environmental factors (likely nutrient concentrations) may be better predictors for the occurrence of *Planktothrix* spp. Although the Coastal Plains had highest area:depth ratios on average, *Planktothrix* spp. were rarely seen there, and were more commonly observed at high biovolumes in lakes in the Northern, Southern and Temperate Plains. Kokociński et al. (2010) found that *Planktothrix* spp. and *Cylindrospermopsis* spp. can co-occur in eutrophic lakes, and may compete with each other for dominance based on shifting environmental conditions. When light availability is low and phosphorus is high*, Planktothrix* spp. tends to be dominant, while *Cylindrospermopsis* spp. may have a competitive advantage at higher temperatures and lower phosphorus concentrations due to its ability to store phosphorus intracellularly (Isvánovics et al., 2000). Warmer temperatures and lower total phosphorus concentrations likely favor *Cylindrospermopsis* spp. in the Coastal Plains ecoregion, which may interfere with potential dominance by *Planktothrix* spp. – despite the abundance of large, shallow and turbid lakes in that region. The fact that *Planktothrix* spp. biovolume was over an order of magnitude higher in hypereutrophic lakes regardless of ecoregion implies that certain environmental conditions (namely low area:depth combined with high nutrient concentrations) could lead to dominance by *Planktothrix* spp., and associated toxin risk, anywhere in the country. Notably, however, 67% of hypereutrophic lakes were reported from the Northern, Southern and Temperate Plains, which makes it difficult to separate ecoregion patterns with overall water quality trends. The results of this study imply that the prevalence of *Planktothrix* spp. may be predictable on a spatial (ecoregion) scale within the U.S.; however spatial trends in distribution and relative magnitude of this taxon are also closely tied to water quality.

Much attention has been focused on determining the environmental factors that influence bloom formation and toxicity of *Microcystis* spp., and in particular *Microcystis aeruginosa* (e.g. Watanabe and Oishi, 1985; Jiang et al., 2008; Jähnichen et al., 2011; Harke et al., 2016; Geada et al., 2017). This may be due to the fact that *Microcystis* spp. is one of the most common producers of microcystin worldwide (Harke et al., 2016), and blooms regularly in many high-profile, well-studied eutrophic lakes including Lake Taihu (China) (Paerl et al., 2011), Lake Okeechobee (Florida, USA) (Havens et al., 1998) and Lake Erie (Ohio, USA) (Michalak et al., 2013). Interestingly, in this study, *Microcystis* spp. was found at much lower biovolumes than either *Dolichospermum* spp. or *Planktothrix* spp., particularly in the Plains ecoregions, which saw highest mean microcystin concentrations. While this does not necessarily indicate lower potential for toxicity in *Microcystis*-dominated lakes, it does suggest that future studies should focus on determining environmental factors more likely to influence *Dolichospermum* spp. or *Planktothrix* spp. in order to better understand how lakes respond to eutrophication and the development of chronic toxicity.

It is widely agreed-upon that warming climate scenarios are likely to favor the frequency and duration of harmful cyanobacteria blooms (Paerl and Huisman, 2008, 2009; Wagner and Adrian, 2009; Carey et al., 2012; O’Neil et al., 2012; Paerl and Paul, 2012). Climatically induced short term warming of the water column has been shown to lead to toxic bloom events in lakes that are considered meso-oligotrophic (Beaver et al., 2018), while steady increases in ambient air and water temperatures over the course of decades may lead to the establishment of persistent and annually recurring blooms (Wagner and Adrian, 2009). Unfortunately, there is likely not a “one size fits all” solution to effectively control cyanobacterial growth or prevent toxic bloom events (Paerl, 2018). This is especially true given that the degree and extent of toxicity from harmful algal blooms has historically been difficult to predict, even when high biovolumes of potential toxin producers are present.

Knowledge of region-specific patterns may allow for wide-scale collaborations amongst environmental and water-quality management agencies in certain areas. Of particular interest is high frequency of toxicity, high average nutrient concentrations and low average light availability in lakes of the Plains ecoregions. Although certainly not all lakes in the Plains experience these conditions, it appears that lakes in these ecoregions are more susceptible to such characteristics than lakes in other ecoregions of the U.S. The analysis relating land-use practices to phytoplankton biovolume suggests that lack of forest cover is associated with increased risk of cyanobacterial blooms, especially involving *Planktothrix* spp. A similar spatial trend showing increased risk of cyanobacteria blooms in the Plains ecoregions was observed by Marion et al., (2017) using county-level land use data. That study found that low deciduous forest cover and higher cultivated crop cover were associated with increased cyanobacteria bloom area, and speculated that denitrification in forest ecosystems could play a role in slowing or preventing eutrophication. Over half of the samples with microcystin concentrations >1 ppb fell within the boundaries of the most recent North American glaciated extent (data not shown), which includes large portions of the Northern Plains, Temperate Plains and Upper Midwest ecoregions. Soils in those regions are mineraland nutrientrich, and are prone to high rates of erosion (Karlen et al., 2010). Those properties, along with high agricultural activity, likely make lakes in those ecoregions especially susceptible to nutrient runoff and eutrophication. Further research that aims at determining specific landscape and/or anthropogenic factors that influence the trend of poor water quality in those ecoregions is needed.

Analysis of the 2012 NLA data is limited by the fact that plankton samples were collected only once or twice over a relatively short time period, which does not capture the full range of environmental conditions that any particular lake may experience over the course of a year (Hayes and Vanni, 2018). It is likely that some lakes were sampled during peak productivity, while other systems were sampled during periods of relatively low productivity. Additionally, environmental factors that could impact cyanobacterial growth and toxicity such as fish abundance and presence of cyanophages were not collected. Despite these limitations, the NLA provides a comprehensive picture of regional trends in water quality in the U.S. due to the broad spatial extent of sampled lakes and the non-targeted nature of site selection. For researchers wishing to investigate drivers of toxic blooms on a site-specific or regional scale, the NLA has identified particular lakes in which blooms are known to occur, and this study outlines a framework of environmental factors likely affecting particular taxa. Future studies using comparable data from the 2017 NLA will help to put this study and others into the context of environmental change over time and may highlight the potential impacts of climate warming on lake water quality.

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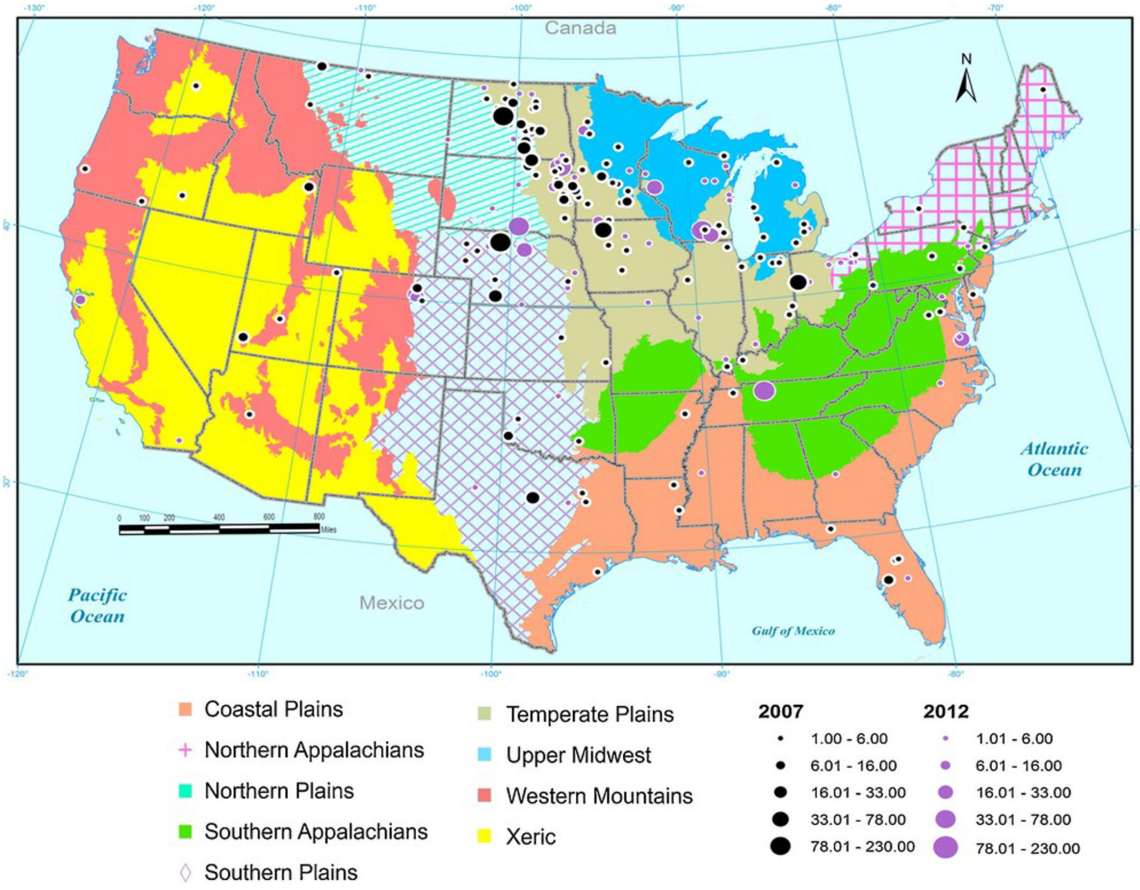

Fig. 1.

Map of the U.S. showing 9 ecoregions and distribution and relative concentrations (ppb) of microcystin in samples containing at least 1 ppb from the 2007 (black) and 2012 (purple) NLA. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

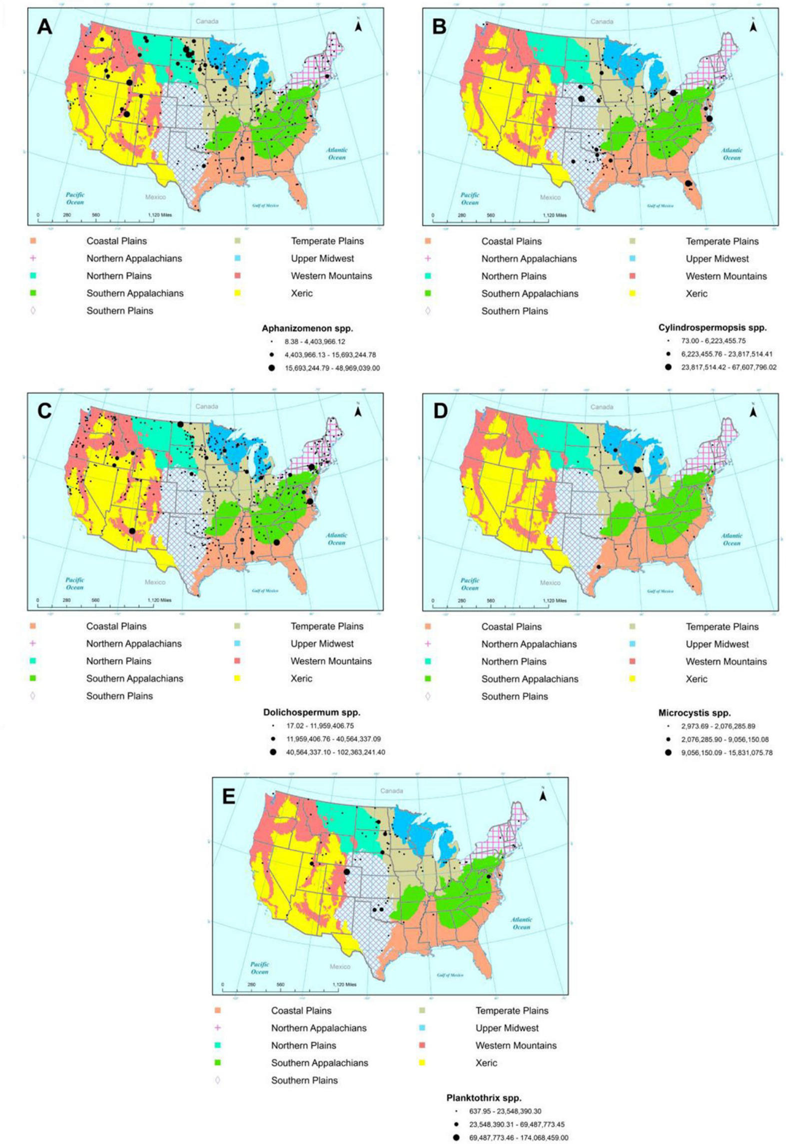

Fig. 2.

Map of the U.S. showing 9 ecoregions and distribution and relative biovolume (μm3 L−1) of common potentially toxigenic cyanobacterial genera in samples from the 2012 NLA. A)*Aphanizomenon* spp. B) *Cylindrospermopsis* spp. C) *Dolichospermum* spp. D) *Microcystis* spp. E) *Planktothrix* spp

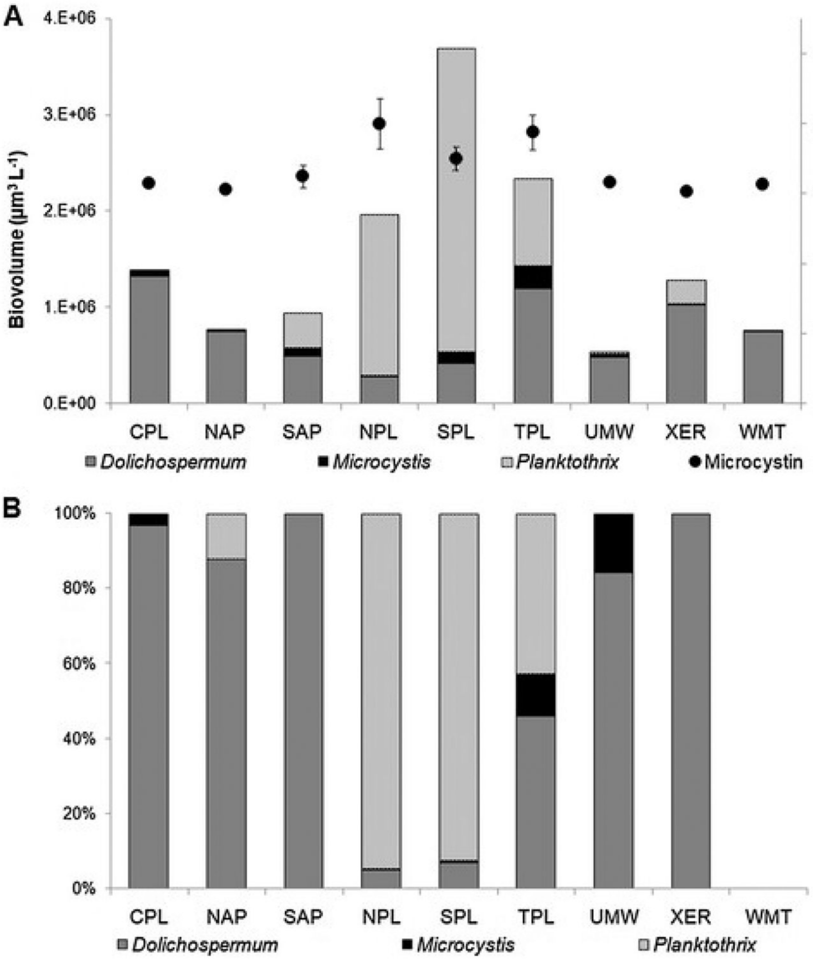

Fig. 3.

A) Mean biovolume (µm3 L−1) by ecoregion for three common, potential microcystin-producing taxa, and mean microcystin (ppb) by ecoregion. Error bars represent standard error. B) relative biovolume of potential microcystin-producing taxa in samples containing >1 ppb microcystin by ecoregion. No samples in the Western Mountains ecoregion contained >1 ppb microcystin. Abbreviations for ecoregions are as follows: NAP = Northern Appalachians, SAP = Southern Appalachians, CPL = Coastal Plains, TPL = Temperate Plains, NPL = Northern Plains, SPL = Southern Plains, UMW = Upper Midwest, WMT = Western Mountains, XER = Xeric

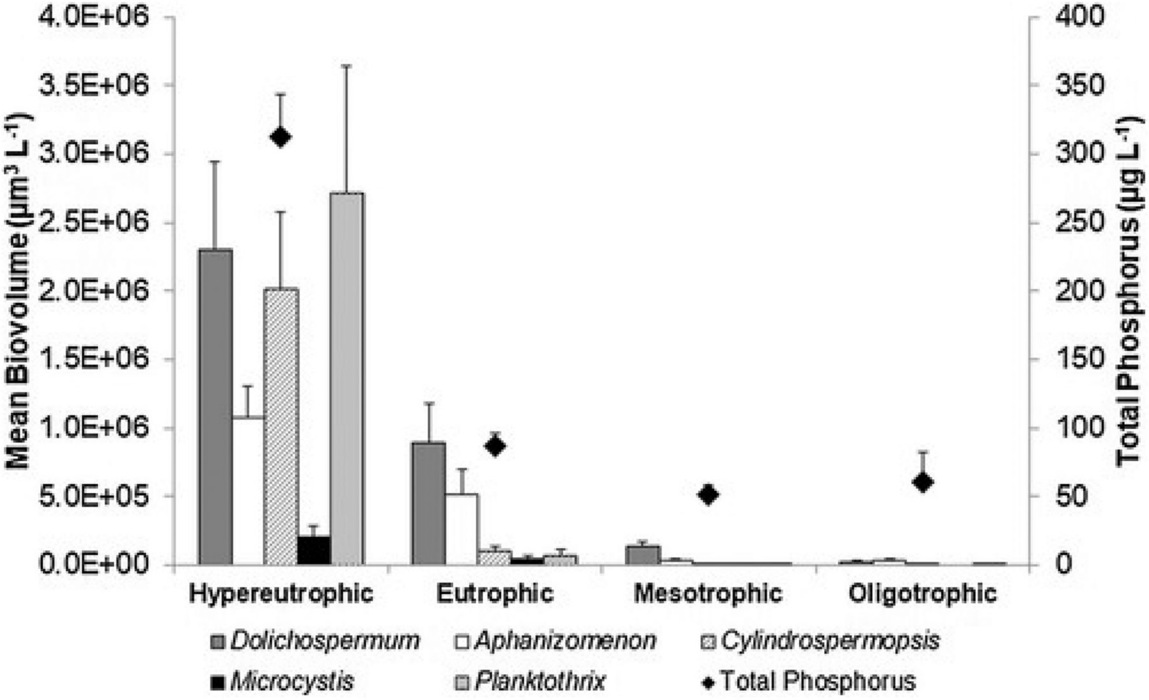

Fig. 4.

Mean biovolume of common potentially toxigenic taxa in lakes in four trophic categories: Hypereutrophic (n = 236), Eutrophic (n = 306), Mesotrophic (n = 325) and Oligotrophic (n = 163). Error bars represent standard error.

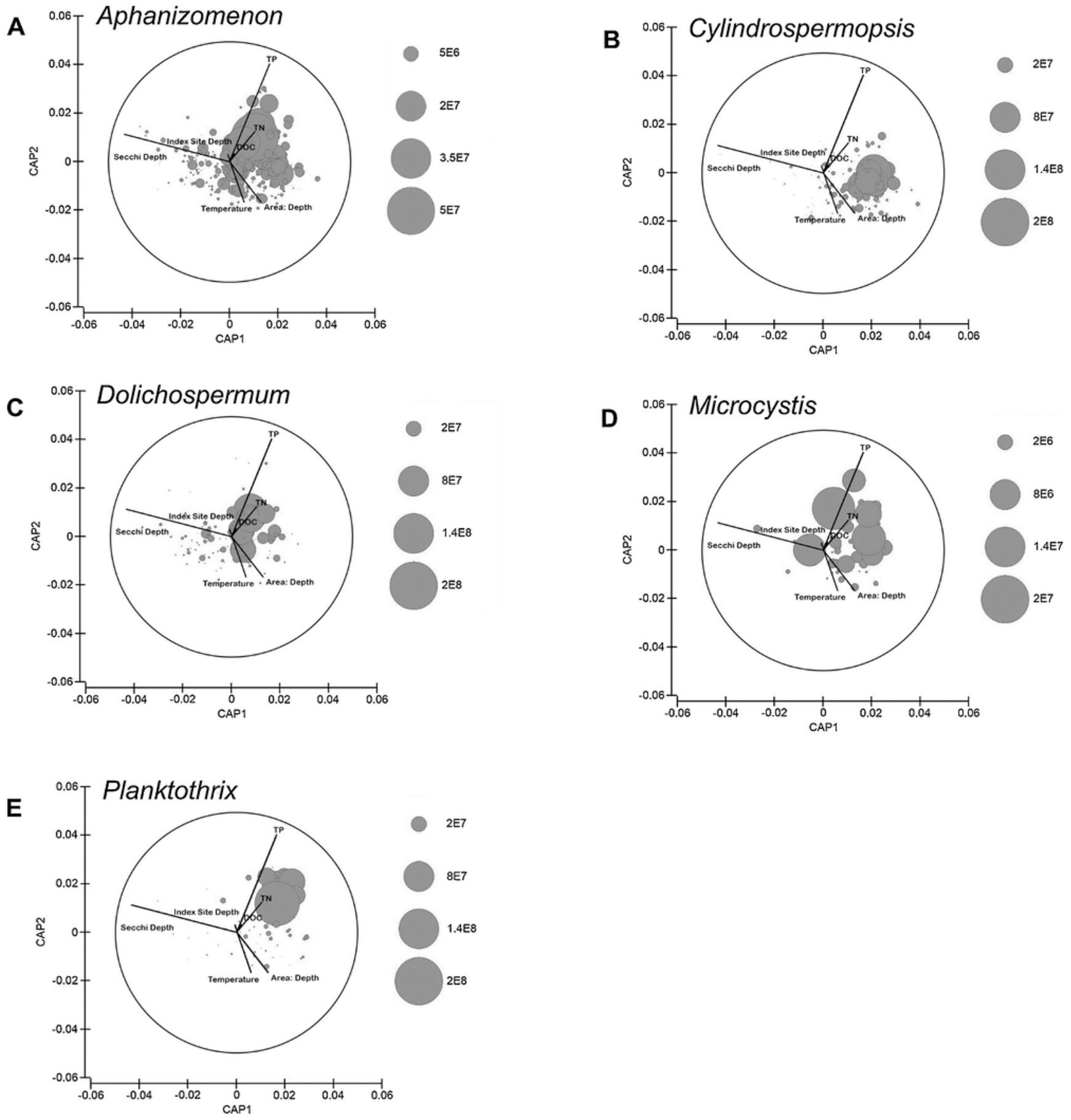

Fig. 5.

Ordination diagrams from the CCA comparing phytoplankton biovolume with environmental variables. Each bubble represents one sample, while bubble size represents relative biovolume (μm3 L−1) for A) *Aphanizomenon* spp., B) *Cylindrospermopsis* spp., C) *Dolichospermum* spp., D) *Microcystis* spp. and E) *Planktothrix* spp.

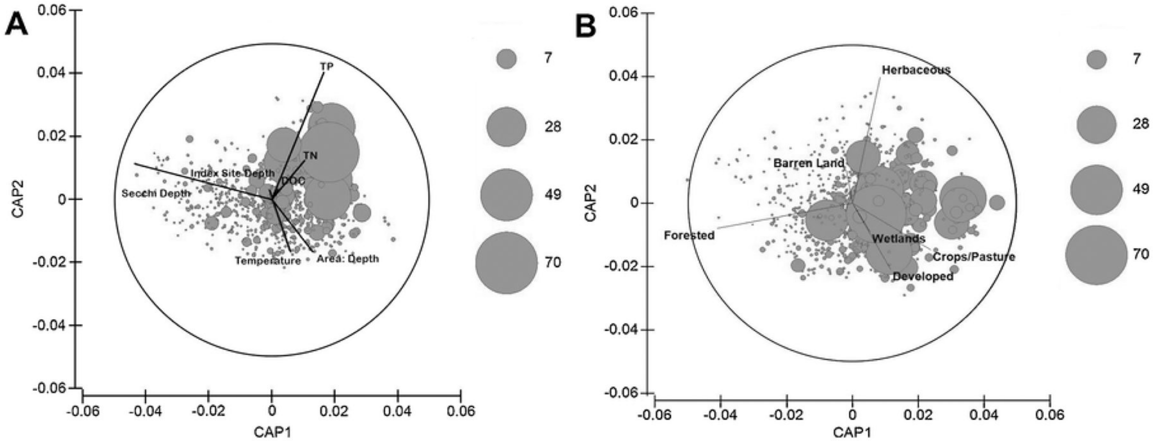

Fig. 6.

Ordination diagrams from A) the CCA comparing phytoplankton biovolume with environmental variables and B) the CCA comparing phytoplankton biovolume with percentage land-use. Each bubble represents one sample, while bubble size represents relative microcystin concentration (ppb).

Table 1.

Mean values for water quality parameters by ecoregion (±SE). Abbreviations for ecoregions are as follows: NAP = Northern Appalachians, SAP = Southern Appalachians, CPL = Coastal Plains, TPL = Temperate Plains, NPL = Northern Plains, SPL = Southern Plains, UMW = Upper Midwest, WMT = Western Mountains, XER = Xeric.

	Secchi Depth (m)	**TN (mg L−1)**	**TP (μg L−1)**	**Temp (°C)**	**DOC (mg L−1)**	**Index Site Depth (m)**	**Area:Depth**
NAP	2.9 *(± 0.2)*	0.41 *(± 0.03)*	25 *(± 3)*	23.2 *(± 0.6)*	4.58 *(± 0.21)*	9.57 *(± 0.98)*	0.15 *(± 0.02)*
SAP	2.0 *(± 0.1)*	0.57 *(± 0.05)*	58 *(± 9)*	25.1 *(± 0.3)*	3.69 *(± 0.21)*	8.88 *(± 0.73)*	0.17 *(± 0.02)*
CPL	1.0 *(± 0.1)*	1.24 *(± 0.12)*	125 *(± 24)*	28.1 *(± 0.2)*	8.41 *(± 0.65)*	3.92 *(± 0.29)*	0.63 *(± 0.13)*
TPL	1.2 *(± 0.1)*	1.64 *(± 0.10)*	173 *(± 19)*	24.8 *(± 0.5)*	9.43 *(± 0.48)*	5.39 *(± 0.35)*	0.27 *(± 0.03)*
NPL	1.2 *(± 0.1)*	2.92 *(± 0.69)*	332 *(± 68)*	20.4 *(± 0.4)*	18.50 *(± 1.50)*	5.55 *(± 0.90)*	0.33 *(± 0.05)*
SPL	1.0 *(± 0.3)*	2.59 *(± 0.58)*	245 *(± 47)*	25.2 *(± 0.5)*	19.99 *(± 6.20)*	4.28 *(± 0.34)*	0.38 *(± 0.04)*
UMW	2.3 *(± 0.1)*	0.87 *(± 0.06)*	42 *(± 4)*	22.9 *(± 0.3)*	8.66 *(± 0.38)*	8.25 *(± 0.51)*	0.14 *(± 0.01)*
WMT	3.6 *(± 0.3)*	0.42 *(± 0.04)*	52 *(± 7)*	17.8 *(± 0.3)*	4.09 *(± 0.31)*	13.41 *(± 1.08)*	0.12 *(± 0.01)*
XER	1.9 *(± 0.2)*	0.77 *(± 0.10)*	130 *(± 18)*	20.4 *(± 0.4)*	5.91 *(± 0.53)*	9.66 *(± 1.08)*	0.25 *(± 0.03)*

Table 2.

Mean microcystin concentrations (ppb) (±SE) and number of lakes with microcystin concentrations greater than 1 ppb by ecoregion. Abbreviations for ecoregions are as follows: NAP = Northern Appalachians, SAP = Southern Appalachians, CPL = Coastal Plains, TPL = Temperate Plains, NPL = Northern Plains, SPL = Southern Plains, UMW = Upper Midwest, WMT = Western Mountains, XER = Xeric.

n		**Microcystin (ppb)**	**# Lakes >1 ppb**
120	NAP	0.12 (*±0.04*)	3
103	SAP	0.57 (*±0.38*)	4
137	CPL	0.33 (*±0.14*)	8
156	TPL	1.74 (*±0.53*)	39
78	NPL	2.05 (*±0.72*)	17
93	SPL	1.06 (*±0.36*)	12
156	UMW	0.32 (*±0.12*)	9
187	WMT	0.06 (*±0.01*)	0
100	XER	0.31 (*±0.15*)	3

Table 3.

Locations of niche centroids (ter Braak and Verdonschot, 1995) showing the relationship between toxigenic taxa and microcystin to water quality variables.

	Dolichospermum	***Aphanizomenon***	***Cylindrospermopsis***	***Microcystis***	***Planktothrix***	**Microcystin**
Secchi Depth (m)	1.6	2.1	1.7	1.6	1.7	1.9
Total Nitrogen (mg L−1)	1.17	0.98	1.68	1.30	1.24	1.26
Total Phosphorus (μg L−1)	127	104	182	113	182	79
Surface Temp (°C)	24.0	22.8	23.5	18.6	22.9	23.6
Dissolved Organic Carbon (mg L−1)	7.00	7.43	10.74	8.82	9.69	9.87
N:P	18.17	20.32	15.37	13.36	11.67	19.78
Index Site Depth (m)	6.66	5.41	5.73	6.80	5.76	7.84
Area:Depth	0.24	0.41	0.28	0.48	0.18	0.16