Harmful Algal Blooms: A Prolific Issue in Urban Stormwater Ponds

Amy E. Grogan [1,2,*] , Catharina Alves-de-Souza [1] , Lawrence B. Cahoon [2] and Michael A. Mallin [1]

1   Center for Marine Sciences, University of North Carolina Wilmington, 5600 Marvin K. Moss Ln., Wilmington, NC 28409, USA
2   Department of Biology and Marine Biology, University of North Carolina Wilmington, 601 S. College Rd. Wilmington, NC 28403, USA
*   Correspondence: aeg7670@uncw.edu

Abstract: Nutrient-driven cyanobacteria blooms are an increasingly common issue in freshwater environments, particularly in anthropogenically altered landscapes. As stormwater runoff is one of the largest sources of nutrients for freshwater bodies, stormwater retention ponds in urban and sub-urban areas are likely environments for harmful cyanobacteria blooms and were thus targeted for an in-depth investigation assessing taxonomic composition, bloom morphological composition, toxicity, and impact of nutrients and other environmental drivers. Eighty-seven algal blooms were sampled from 2019 to 2022 in the greater Wilmington, North Carolina, area. Physicochemical parameters were recorded, and blooms were classified by type (defined as surface mat, surface scum, water column distribution, or benthic mat) and dominant taxa. Blooms of potentially toxic cyanobacteria genera in the water column of stormwater retention ponds were most prevalent. Dissolved inorganic phosphorus was significantly related to chlorophyll-α, *Microcystis* bloom formation, and the production of microcystin. Seventeen potentially toxic cyanobacteria genera were identified in retention ponds, some of whose blooms demonstrated detectable microcystin. Monoclonal cultures isolated from some blooms were found to produce anabaenopeptin and saxitoxin. The results demonstrate a higher incidence of potentially toxic cyanobacteria over other bloom-forming taxa (chlorophytes, euglenoids, chrysophytes, dinoflagellates, and diatoms) in the 39 water bodies sampled. The frequency of blooms occurring in stormwater ponds and the diversity of potentially toxic cyanobacteria identified suggest such harmful blooms are likely widespread in similar freshwater environments across multiple urbanizing areas. The blooms sampled in this study were all within residential, commercial, or recreational areas easily accessible to people, presenting serious hazards to both environmental and public health.

Keywords: cyanobacteria; cyanotoxin; microcystin; stormwater pollution; eutrophication

1. Introduction

Algal blooms are increasingly prevalent in marine and freshwater systems, raising concerns for public health and environmental welfare. Eutrophication [1–4] and temperature [5,6] are recognized as key factors driving algal bloom formation as well as toxin production in harmful algae taxa [7,8]. As such, bloom events occur most frequently in anthropogenically altered landscapes where urban development and nutrient-rich runoff are prevalent. The southeast and similar coastal regions of the United States (US) are rapidly developing, resulting in increased impervious surfaces and the construction of stormwater ponds [9]. These shallow and stagnant ponds are designed to capture and retain stormwater runoff and, over time, become increasingly eutrophic, providing an ideal environment for algal blooms, particularly those of cyanobacteria [10–13].

Harmful algal blooms (HABs) generate a number of problems, including fish kills, hypoxia, toxin production, and fisheries closures. However, unlike marine and estuarine

HABs, harmful cyanobacteria blooms (cyanoHABs) are not regionally limited by proximity to saltwater and occur in freshwaters, including those delegated for public and agricultural use. CyanoHABs are often associated with a suite of toxins, including over 300 congeners of the potent hepatotoxin microcystin as well as anatoxin, cylindrospermopsin, nodularin, saxitoxin, β-N-methylamino-L-alanine (BMAA), and several others [14–16].

The pervasiveness of cyanobacteria blooms has created large-scale issues in the management of recreational waters and public drinking water globally [13]. A large-scale study of US lakes found potential microcystin-producing cyanobacteria in 95% of the samples [17]. In the US Great Lakes region, massive cyanoHABs have incited moratoria on public drinking water due to high concentrations of cyanotoxins [18,19]. The state of Florida (US) has experienced continuous intense and toxic cyanobacteria blooms in both fresh and estuarine waters [20], resulting in toxin exposure and illness in humans [21], drinking water contamination [22], and extensive seagrass loss in Florida Bay [23]. Anthropogenic land use and population growth in the Lake Victoria Basin (Africa) have increased the demand for freshwater while simultaneously endangering the water source, now plagued by cyanobacteria blooms [24]. Similar issues are pervasive in reservoirs and lakes used for drinking water, such as the Volga-Kama-Don reservoir system in Russia [25], Lake Vombsjön, Sweden [26], Lake Taihu, China [27], Lake Mannus, Australia [28], Lake Ypacara, Paraguay [29], Lake Eirdir, Turkey [30], Lake Latyan, Iran [31], and many more. Increases in harmful benthic mat-forming cyanobacteria in the orders Nostocales and Oscillatoriales have been identified globally [32], including widespread blooms of toxic *Lyngbya wollei* in the US Great Lakes region [33] and the proliferation of anatoxin-producing *Phormidium* in New Zealand [34]. Thus, the proximity of human populations to water sources creates a two-fold problem with cyanoHABs. Urban development and subsequent eutrophication spur algal blooms, and human interaction with freshwater where cyanoHABs occur is ubiquitous in developed regions.

The coastal city of Wilmington, North Carolina (NC), and surrounding New Hanover County exemplify rapid urbanization. Within the 41 mile^2 (~106 km^2) area of incorporated Wilmington, there are 41 city-owned stormwater ponds and many more privately owned ponds (S. Cohick, Wilmington Stormwater Services, personal communication). Though they are designed as stormwater control systems (SCM) to collect polluted runoff, stormwater ponds in parks and housing developments are often perceived and marketed as aesthetic water features. The misperception of stormwater ponds as potential recreation areas has promoted fishing, playing with pets, observing wildlife, and paddle sports in and around the water (Figure 1). Human interactions with these polluted waterbodies may be potentially dangerous, as demonstrated by the deaths of three dogs in Wilmington, North Carolina (NC), following contact with stormwater pond water containing potent cyanobacteria toxins [35].

Blooms in the Wilmington area have been anecdotally reported over the past several years, a few of which have been sampled and cataloged by the NC State Department of Environmental Quality (NCDHEQ). Additionally, for 25 years, the University of North Carolina Wilmington (UNCW) Aquatic Ecology Lab has kept records of blooms (designated as chlorophyll-a > 40 μg L^{-1}) occurring in the city-owned Greenfield Lake and local urban creeks based on a long-term monthly sampling program (https://uncw.edu/cms/aelab/). However, there has been no comprehensive examination to date of the frequency, distribution, and taxonomic diversity of algal blooms in Wilmington (and coastal North Carolina in general). Blooms in such urbanizing areas are frequently found in easily accessible freshwater bodies such as stormwater ponds, emphasizing the need for detailed investigation for public health. Evaluating the occurrence, conditions, toxin production, and taxa associated with freshwater cyanoHABs in local stormwater ponds and urban waterbodies can provide insight into the pervasiveness of blooms as well as the abiotic conditions facilitating bloom formation. This information can be used to assist in the mitigation of water quality issues and provide a baseline for future monitoring.

Figure 1. Human interactions with algal blooms and urban waterbodies; (**a**) rope swing over a blooming pond, (**b**) paddleboats in a marina with an active bloom, (**c**) kayak launch into residential stormwater pond (actively blooming), (**d**) people kayaking through an active bloom in a city park (Greenfield Lake), (**e**) bloom in a city park (Long Leaf Park) with a recreation walking/biking trail, (**f**) person fishing in a stormwater pond where regular blooms of harmful cyanobacteria have been documented.

This study represents the first targeted assessment of the frequency, distribution, and taxonomic diversity of microalgae blooms in the waterbodies around Wilmington, NC. Water samples were collected from active blooms from 2019–2022. The primary goals were to assess the relative importance of physicochemical parameters (particularly temperature and nutrient concentrations) on (i) bloom occurrence and intensity (defined as chlorophyll-α concentration), (ii) predominance of diazotroph cyanobacteria blooming taxa, and (iii) frequency of microcystin detection.

2. Methods

2.1. Sampling and Water Analysis

Locations known to host regular algal blooms, such as Greenfield Lake and the Burnt Mill Creek watershed, were visually inspected periodically for water discoloration, indicating high algal biomass, every 7–10 days during the months with higher bloom frequency (May–September). Most other sampling sites were visible from high-traffic roadways and blooms were sampled incidentally when observed by authors or reported by citizens or government groups. Blooms were sampled from 39 waterbodies (Figure 2) distributed in three types (pond, creek, lake) located in the greater Wilmington area (NC). Physicochemical parameters (temperature, pH, dissolved oxygen, turbidity, and salinity) were recorded on-site using a Xylem Pro DSS YSI (Professional Designs Digital) multi-meter (Yellow Springs, OH, USA). Whole water samples were collected from water bodies with ongoing

algal blooms. Chlorophyll-α (µg L^{-1}) was determined in the UNCW Aquatic Ecology Lab via NC state-certified grinding method (Method S1) followed by fluorometry [36]. Total nitrogen and phosphorous samples were processed using American Public Health Association Method 4500-P J. [37], and inorganic nutrient samples were processed using US Environmental Protection Agency Method 365.5 [38]. Total nitrogen, total phosphorous, and dissolved inorganic nutrients (orthophosphate, ammonium, and nitrate) were determined using an SPXFLOW Bran and Luebbe AutoAnalyzer 3 (Charlotte, NC, USA). Algal bloom taxa were identified by light microscopy to the genus level. All cyanobacteria bloom samples were brought to the UNCW Algal Resources Collection for strain isolation and identification to the species level whenever possible. Species-level identifications were confirmed only when species-specific characteristics such as heterocyst and akinete morphology or placement were present. Samples from blooms composed of cyanobacteria genera recorded in the literature as potential microcystin producers were assessed for the presence of broad-spectrum microcystin congeners (semi-quantitative results in the range of 0–10 ppb) using Gold Standard Diagnostics Abraxis Microcystins (0–10 ppb) Recreational Water with QuikLyse® test strips (Warminster, PA, USA).
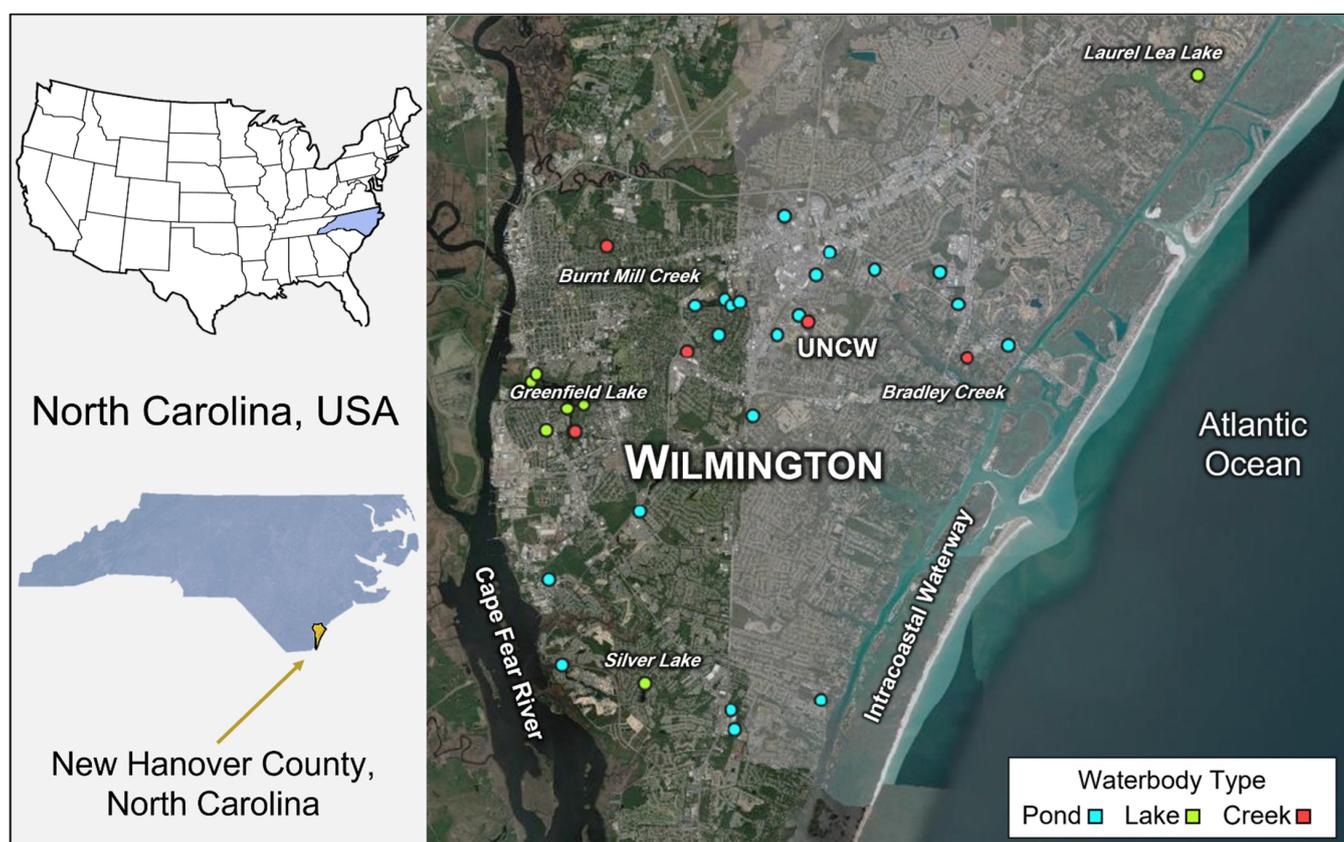

Figure 2. Location of the 39 sampled sites denoted by waterbody type. Two locations (Miller Park Pond in Rocky Point, NC and Shark's Den Creek in Emerald Isle, NC, USA) fall outside the map area. Map created with ESRI ArcGIS online.

2.2. Statistical Analyses

All statistical analyses were performed using R software 4.2.0 (R Core Team, 2013), with packages freely available from the CRAN repository (https://cran.r-project.org/). A Principal Components Analysis (PCA) was performed using a dataset removing samples missing nutrient data (*n* = 72) to assess if physicochemical parameters were associated with the grouping of samples related to three criteria: waterbody type (as previously defined), bloom type (benthic mats, surface mats, surface scum, water column distribution), and dominant taxa (cyanobacteria, chlorophytes, euglenoids, and others). For that, all variables

were previously centered and scaled by subtracting the mean and dividing by the standard deviation, and the PCA was performed using the *rda* function of the "vegan" package in R software (Oksanen 2007). A multiple correspondence analysis (MCA; *n* = 72) was then performed using the MCA function of the package "FactoMineR" in R software to look for relationships between the presence of the main potentially toxic cyanobacteria taxa related to the waterbody types and bloom types (both converted to categorical variables). The detection of microcystin was included as an additional categorical variable with three levels: non-determined, below detection, and detected (>5 ppb). The envfit function in the package "vegan" was used to fit the environmental variables to the MCA scores. Linear regressions were used to assess trends between LOG_{10}-transformed physio-chemical conditions and bloom intensity (defined by chlorophyll-α concentration).

3. Results

A total of 87 algal blooms were sampled across 39 individual sites (Table S1) from 2019 to 2022. Algal blooms were observed in urban areas, particularly near residential and commercial development (Figure 2). Blooms were categorized into four types: benthic mat, surface mat, surface scum, and water column distribution (Figure 3). Mats were defined as collections of filamentous microalgae forming dense masses. Surface scum was defined as microalgae aggregating primarily in thick streaks in surface waters, and water column-distributed blooms were those where microalgae were distributed uniformly throughout the photic zone. Most blooms (61%) occurred in constructed stormwater ponds or other waterbodies directly receiving stormwater runoff (Figure 4B). The majority of pond blooms concentrated in the upper center of the map (Figure 2) represent an area near the University of North Carolina Wilmington (UNCW), which hosts twenty-plus housing complexes and several shopping plazas (within 2.5 km² of campus). Two of the ponds in this area are located within university grounds and hosted multiple blooms caused by potentially toxic cyanobacteria over the course of this study.

Figure 3. Bloom types observed in this study: (**A**) benthic mat, (**B**) surface mat, (**C**) surface scum, and (**D**) water column distributed.

Most cyanobacteria blooms in ponds were column-water distributed (60% of the blooms; Figures 3D and 4A). While blooms dominated by other taxonomic groups were observed (i.e., chlorophytes, euglenoids, chrysophytes, dinoflagellates, and diatoms), cyanobacteria blooms occurred with much greater frequency (68% of the sampled blooms; Figure 4C) demonstrating that varied physicochemical conditions supported the growth of this microalgae group. N-fixing cyanobacteria of the order Nostocales, accounted for the greatest number of blooms both overall and within cyanobacteria-specific blooms (68% of cyanobacteria blooms).
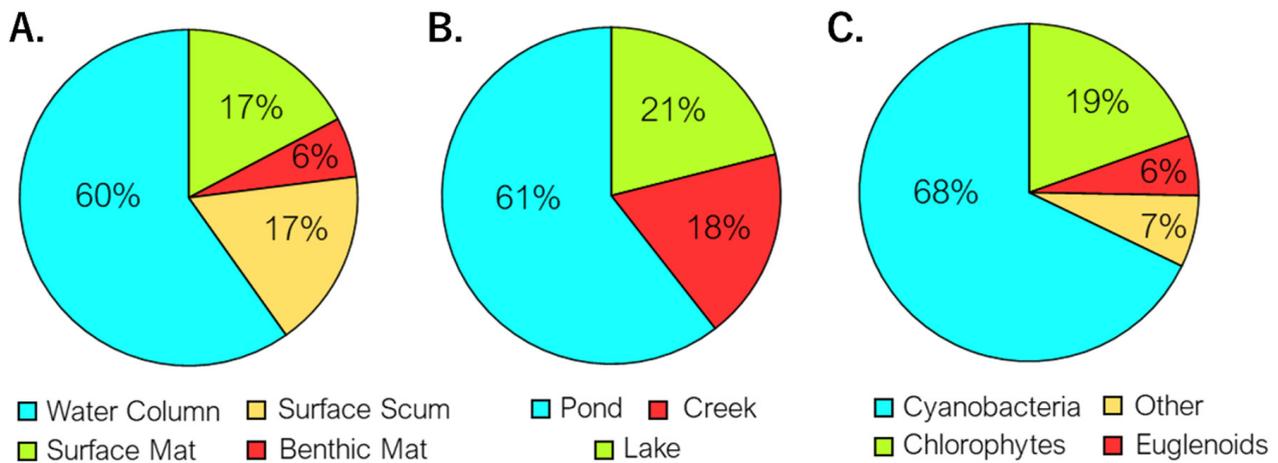
Figure 4. Distribution of blooms according to (**A**) bloom type (*n* = 87), (**B**) waterbody types of sampled locations (*n* = 39), and (**C**) dominant microalgal group (*n* = 87).

Twenty-five cyanobacteria genera were observed, including seventeen genera for which toxin production has been previously reported (Figure 5). However, of all the cyanobacteria genera recorded, only fifteen were identified as the dominant taxa in the sampled blooms. All fifteen dominant cyanobacteria are potential toxin producers (Table 1).

Table 1. Cyanobacteria genera observed as dominant in the sampled blooms (identified species included when applicable). Asterisks denote taxa associated to toxic production in this study. Five blooms were confirmed (via Abraxis test strips) as actively producing microcystin at the time of sampling. Potential toxin production [1] obtained from [15,16,39,40].

Genera	Identified Species in This Study	Potential Toxin Production
Anabaena		ATX, CYN, MC, STX, Guanitoxin
Anabaenopsis		ATX, MC, STX
Aphanizomenon	*A. flos-aqua*	ATX, CYN, MC, STX, Nodularins
Cuspidothrix *	*C. issatschenkoi*	ATX, STX
Chrysoporum		CYN
Cylindrospermum		ATX
Dolichospermum *	*D. affine, D. circinales, D. crassum, D.* cf. *flos-aqua, D. perturbatum, D. planctonicum, D. smithii, D. spiroides*	ATX, CYN, MC, STX, Guanitoxin
Lyngbya		CYN, MC, STX, Lyngbyatoxin
Microcystis *	*M. aeruginosa, M. flos-aquae, M. ichthioblable, M. novacekii, M. protocystis*	ATX, MC
Nodularia		Nodularins
Oscillatoria		ATX, CYN, MC, Lyngbyatoxin
Planktothrix	*P. agarhii*	ATX, CYN, MC, STX, Lyngbyatoxin
Phormidium		ATX, MC
Sphaerospermopsis	*S. reniformis, S. aphanizomenoides*	ATX, CYN, Guanitoxin
Synechococcus		ATX, CYN, MC

Note(s): [1] ATX, CYN, MC, and STX, represent anatoxin, cylindrospermopsin, microcystin, and saxitoxin, respectively.

Microcystin (MC) congeners (identified with Abraxis strips) were present in five of the sampled blooms dominated by *Microcystis* spp. and/or *Dolichospermum* spp. Though the presence of MC detected in blooms was not remarkable, the five blooms found to be positive for MC occurred across five unique locations (Table S1), demonstrating toxin production is not isolated to specific waterbodies. Additionally, several strains of cyanobacteria were successfully isolated in culture and found to produce other toxins. Preliminary liquid chromatography coupled to mass spectrometry (LC-MS/MS) analysis of these strains detected anabenopeptin-B and saxitoxin in *Microcystis* and *Cuspidothrix* strains, respectively. MC producing *Microcystis* as well as strains previously reported as producers of other

cyanotoxins were collected from Greenfield Lake where regular cyanobacteria blooms occurred (44 blooms sampled from 2019 to 2022).
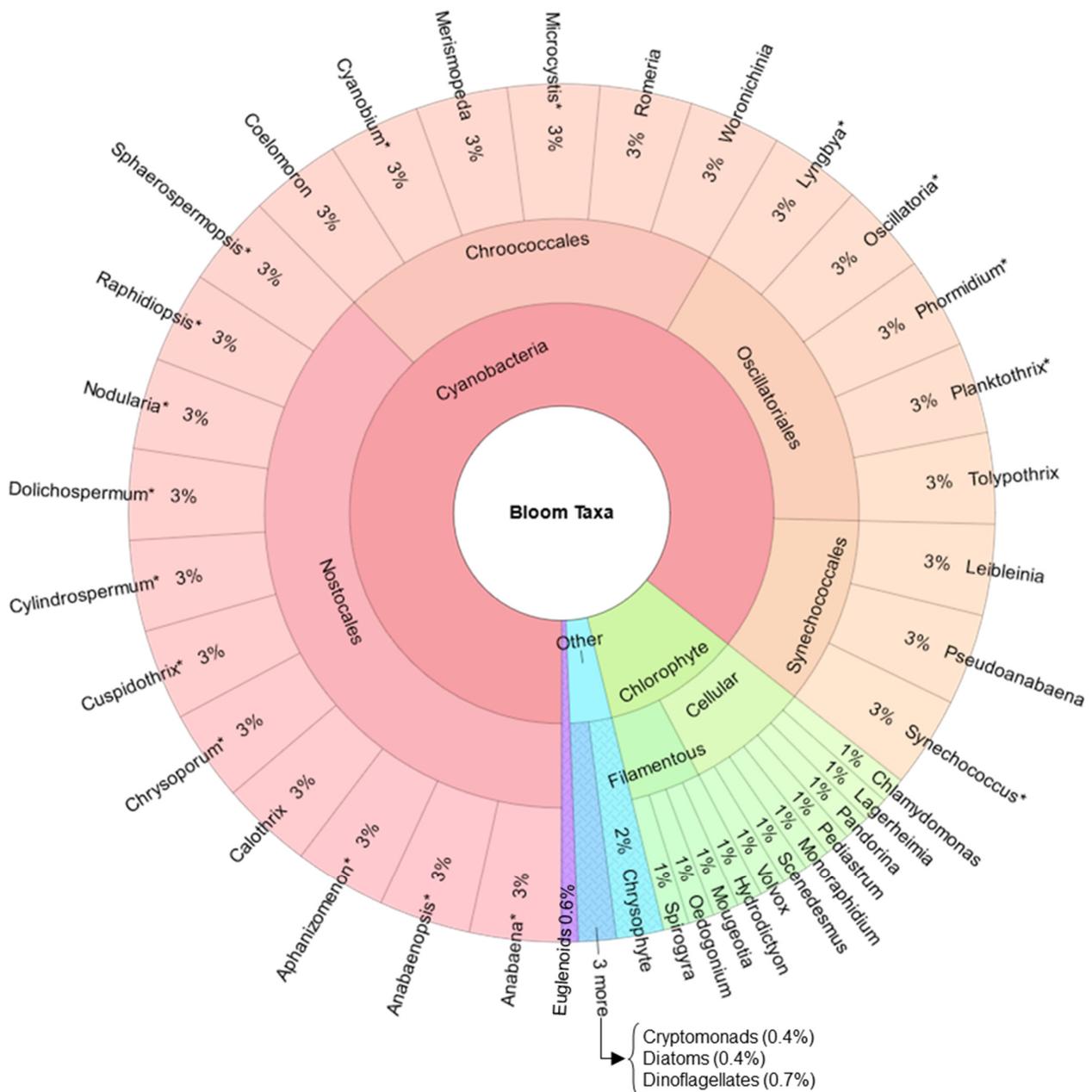

Figure 5. Krona chart of taxa observed across all sampled blooms. Asterisks denote potentially toxic genera. Genera percent values are weighted by higher order contribution to the total, i.e., Chlorophytes account for 11% of the total number of identified genera. Therefore, each of the eleven individual chlorophyte genera is 1%.

This is the first report of the genus *Cuspidothrix* in the US. The only confirmed saxitoxin producing *Cuspidothrix* strain was collected from a bloom in Laurel Lea Lake dominated by *Microcystis*. However, *Cuspidothrix* has been identified in samples from nine blooms across five distinct locations including Greenfield Lake. A *Synechococcus/Cyanobium* strain was also isolated from Laurel Lea Lake and was demonstrated to be toxic using a brine shrimp assay, although its bioactive compound is yet to be identified. Similar to other isolated potentially toxic species, *Synechococcus/Cyanobium* spp. has been observed in three other locations beyond Laurel Lea Lake.

Production of several other toxins and bioactive compounds (not mentioned in Table 1) has been previously reported in the dominant genera identified in this study including, anabaenopeptin, antillatoxin, aplysiatoxin, cyanopeptolin, jamaicamides, kalkitoxin, lipopolysaccharides (LPS), and β-N-methylamino-L-alanine (BMAA) [15]. Combining the aforementioned metabolites as well as those in Table 1, fifteen toxins and bioactive compounds other than microcystin are potentially produced by the cyanobacteria predominant in this study.

The PCA explained 50.85% of the total variability, with nitrate being the main variable associated with principal component 1 (PC1) and orthophosphate associated with principal component 2 (PC2) (Figure 6). However, no clear relationship was observed between the physicochemical gradients and the grouping of sampled blooms related to bloom type, waterbody type, or predominant microalgal bloom (Figure 6A). The MCA (28.7% of total variability; Figure 7) indicated that cyanobacteria with similar ecophysiological traits bloomed in the same environments. N-fixing cyanobacteria (*Aphanizomenon* and *Dolichospermum* spp.) were most strongly associated with water column distribution in ponds and lakes, while Oscillatoriales cyanobacteria such as *Lyngbya* spp. and *Oscillatoria* spp. were primarily associated with benthic mats in creeks. Some environmental gradients were detected when physicochemical variables were fitted to the MCA sample scores. However, the *envfit* test indicated that only orthophosphate ($R^2 = 0.32$; $p < 0.001$), pH ($R^2 = 0.27$; $p = 0.03$), and chlorophyll-α ($R^2 = 0.13$; $p = 0.02$) were significant. Of particular interest for this study was the positive association of orthophosphate with both *Microcystis* spp. blooms and MC production (Figure 7A).
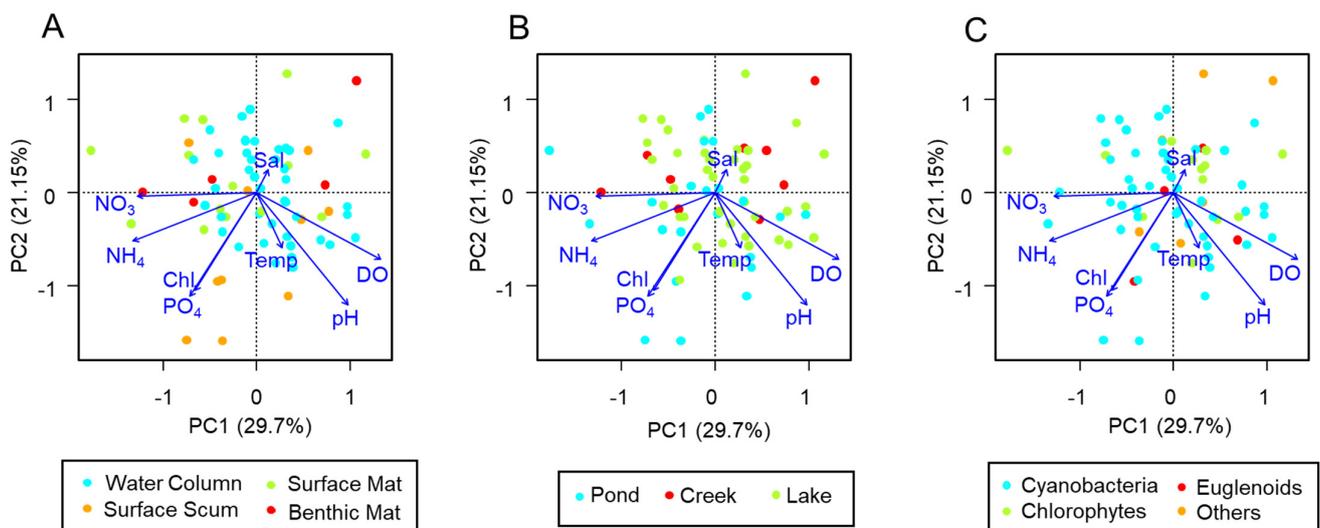

Figure 6. Principal component analysis showing the relationship of physicochemical variables on the distribution of sampled blooms (*n* = 72) according to bloom type (**A**), waterbody type (**B**) and predominant microalgae group (**C**).

The intensity of all algal blooms (as indicated by chlorophyll-α) had a significant relationship with both total phosphorous (TP) ($R^2 = 0.41$, $p < 0.001$) and total nitrogen (TN) ($R^2 = 0.75$, $p < 0.001$) concentrations. However, specific nitrogen species, ammonium (NH_4) and nitrate (NO_3), and total dissolved inorganic nitrogen (DIN) were not significant predictors of bloom intensity (Table S2). As DIN is the labile form of nitrogen, TN was not likely a cause of elevated bloom intensity but rather the result of enormous concentrations of nitrogen-dense algal cells (particularly those of cyanobacteria). Regardless of the dominant microalgal taxa, bloom intensity significantly increased with dissolved inorganic phosphorous (DIP) concentration ($R^2 = 0.26$, $p < 0.001$), demonstrating that labile phosphorous is likely a driving factor of bloom formation and proliferation (Figure 7B).
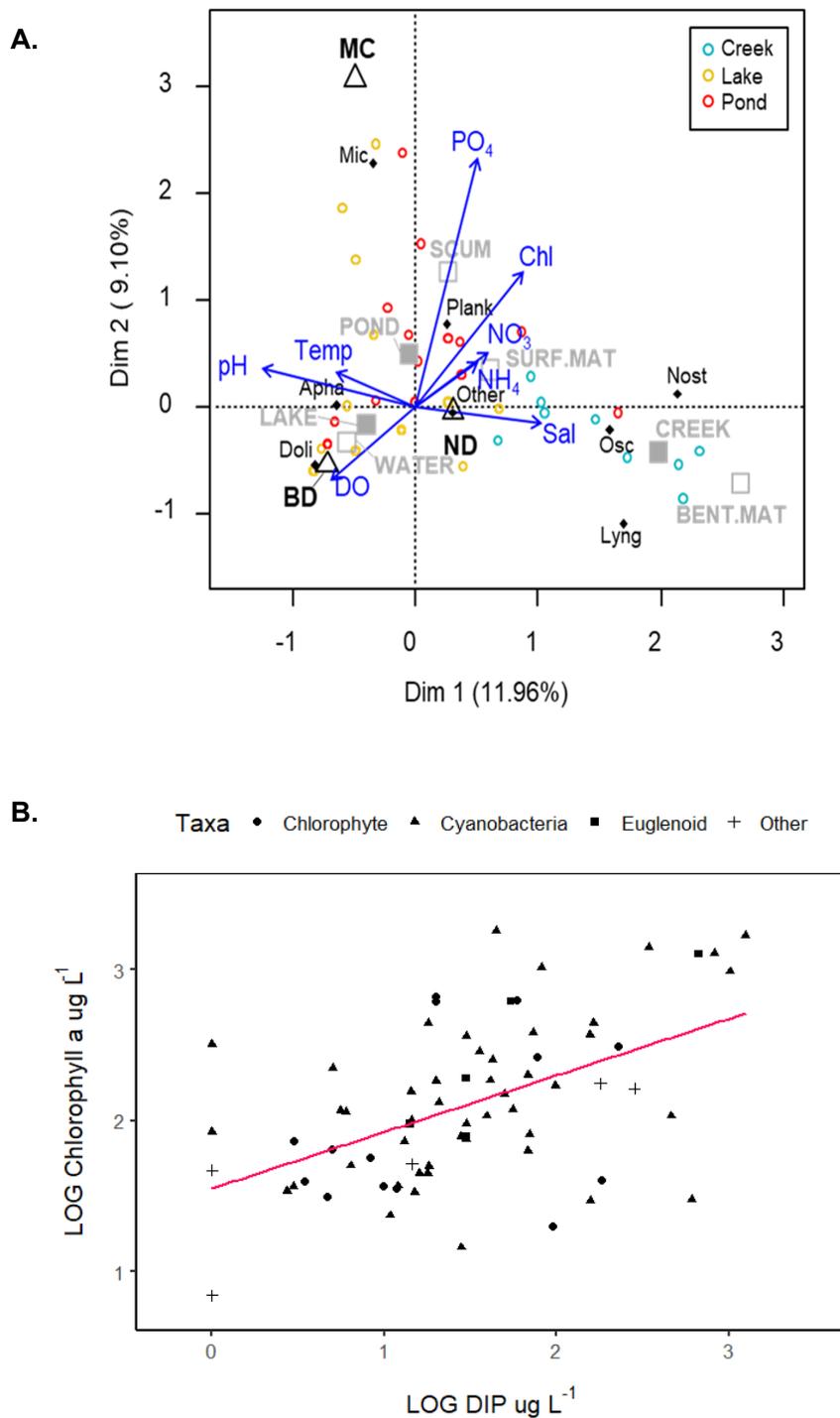
Figure 7. (**A**) Multiple Correspondence Analysis (MCA; n = 72) showing the distribution of the main potentially toxic cyanobacteria related to waterbody type (lake, water, creek), bloom type (water = water-column distributed, surf.mat = surface mats, bent.mat = benthic mats, scum = surface scum) and microcystin detection (ND = non determined, BD = below detection, MC = > 5 ppb). Apha = *Aphanizomenon flos-aquae*, Doli = *Dolichospermum* spp., Lyng = *Lyngbya* spp., Mic = *Microcystis* spp., Nost = benthic Nostocales, Osc = benthic Oscillatoriales, Plank = *Planktothrix* spp., PO_4 = orthophosphate, NO_3 = nitrate, Chl = Chlorophyll-α, Temp = Temperature, Sal = Salinity. (**B**) Linear regression of bloom intensity (as defined by chlorophyll-α) and dissolved inorganic phosphorous (DIP) concentrations (n = 72, R^2 = 0.26, $p < 0.001$).

4. Discussion

The results of this study demonstrate that stormwater ponds and stormwater receiving waterbodies host frequent and intense algal blooms (chlorophyll-α concentration ranging 40–1290 µg L^{-1}) mostly dominated by cyanobacteria although other taxonomic groups occasionally formed blooms (i.e., chlorophytes, euglena, chrysophytes, dinoflagellates, and diatoms). The predominance of cyanobacteria blooms observed in the sampled systems, indicates that the environmental conditions therein are often favorable to this group.

A total of twenty-five cyanobacteria genera were identified, seventeen of which were potentially toxin-producing taxa (Figure 5). Of the twenty-five total identified cyanobacteria only fifteen were found to be the dominant bloom taxa and all fifteen are documented toxin-producing genera (Table 1). Among cyanobacteria genera, *Dolichospermum* spp. blooms were the most frequent. Several *Dolichospermum* and *Microcystis* species were detected demonstrating that stormwater-receiving waterbodies provide an ideal environment for a diversity of potentially toxic taxa. MC-producing blooms were dominated by *Microcystis* and/or *Dolichospermum* spp. however, the production of toxins other than microcystins (e.g., anatoxin, cylindrospermopsin, guanitoxin, and saxitoxin) by *Dolichospermum* species have also been documented in the literature (Table 1). *Dolichospermum* species notably synthesizes cyanotoxins of various chemical structures and functional types i.e., neurotoxins, hepatotoxins, cytotoxins, and dermatotoxins [41]. Though only microcystin presence was evaluated by this study, the large diversity of *Dolichospermum* species identified across blooms indicates that other toxins and secondary metabolites were potentially present.

Microcystins (MC) are the most routinely tested for cyanotoxins [42,43] but they are not the only toxins regularly produced by cyanoHABs [44]. Cylindrospermopsin (CYN) has been detected at "above guideline values" globally, demonstrating large knowledge gaps and lacking mitigation efforts [45]. Nodularin molecules are water soluble and have long residence times due to their cyclic structure [46]. Though they are primarily produced by benthic cyanobacteria, nodularins are often released into the water column. As nodularins originate from benthic taxa the identification of a bloom or dense filamentous algal mats may go unnoticed and therefore cyanotoxin presence untested. The potent neurotoxins anatoxin (ATX) and saxitoxin (STX) are widespread in cyanoHABs but critically understudied compared to MC [47]. Immunochromatographic tests, such as the Abraxis test strips used by this study, and enzyme-linked immunosorbent assays (ELISA) produce rapid results but are limited to a chosen target cyanotoxin and do not yield exact concentrations. Along with the bias towards MC, one of the greatest challenges in cyanotoxin detection is the resource availability and time-consuming nature of the most sensitive and precise analyses, i.e., molecular methods, HPLC, and mass spectrometry [48,49].

In addition to the potential bias of toxin selective testing, the dichotomy of taxa [50–53] and production of toxins [52–55] may vary over the course of a bloom. Ideally, blooms should be examined daily or on alternate days throughout their duration to track community assemblage and toxicity, but the constraints of time and manpower make this unrealistic. As such, if potentially toxic cyanobacteria represent the dominant taxa in a bloom there is a possibility that toxins were or will be produced within the lifespan of the bloom.

Several study sites experienced predictable and reoccurring blooms due to established cyanobacteria populations. Dormant akinetes (cyanobacteria resting cells) in the sediments are stimulated by warming temperatures and the associated release of phosphorous from sediments [56,57]. Our findings suggest warmer temperatures foster cyanobacteria blooms. Though bloom taxa did not significantly differ by temperature, (Kruskal-Wallis, $p = 0.36$), seventy percent of cyanobacteria blooms occurred at temperatures 26 °C or above, whereas forty-four percent of chlorophyte blooms, the second most common taxa, occurred at temperatures 26 °C or lower (Table S3, Figure S1).

Eutrophication is one of, if not the most, significant factors driving algal bloom formation and the growth of harmful cyanobacteria [2,4,6,28,58–62]. Following cyanobacteria bloom initiation, biogeochemical cycling promotes the succession from genera to genera,

creating a positive feedback loop perpetuating cyanoHABs [56,63]. As observed in other studies, dissolved inorganic phosphorous (DIP) concentration significantly increased the intensity of blooms and was the primary factor associated with toxin production [3,5,50,64]. Blooms sampled in this study occurred almost exclusively in highly eutrophic waterbodies, particularly stormwater ponds.

Stormwater ponds are specifically designed to retain stormwater and reduce suspended solids. As their construction does not require the retention of a given percentage of nutrients, they retain nutrient-rich stormwater runoff, becoming eutrophic themselves. Sediments in eutrophic waterbodies function as a reservoir for phosphorous [57]. Phosphorous is liberated from sediments in low-oxygen environments characteristic of shallow ponds in warmer months [65]. The eutrophic, warm, and stagnant pond water acts as an incubator for toxic cyanobacteria [7,28,66,67]. As the creation of these ponds is a dominant standard practice for stormwater management, land development for residential and commercial use is another factor increasing the pervasiveness of harmful algae blooms in urban and suburban areas [9,68].

Coastal zones are densely populated and continue to develop rapidly in the southeast US. Development and land use changes negatively impact coastal environments via increased impervious surface area, construction, and erosion, as well as nutrient pollution, increased pathogens in water, saltwater intrusion, food web disruptions, and flooding [69,70]. Increasing impervious surface area is closely tied to the eutrophication of receiving waters [71], and few coastal zones are unimpacted by the destruction of natural habitats [72]. In addition to environmentally detrimental land use changes, climate change dynamics are predicted to increase the frequency of cyanoHABs.

Increasing temperatures have been shown to change plankton community dynamics and enact shifts toward cyanobacteria-dominated systems [73,74]. Higher temperatures [63,75,76] and elevated carbon dioxide concentrations [77] have been demonstrated to increase the growth rates and toxin production of harmful cyanobacteria. Temperature and carbon dioxide increases, coupled with extreme storms and flooding (facilitating transportation of vegetative cells and akinetes to other waterbodies), are likely to intensify the occurrence and distribution of cyanoHABs in coastal regions.

Shallow water tables in low-elevation coastal zones and flooding during heavy rains and hurricanes are a regular occurrence in the southeast US [78,79]. Overflow drainage from eutrophic stormwater ponds that enters tidal creeks can facilitate the potential spread of harmful cyanobacteria to brackish systems. Many potentially toxic cyanobacteria genera including *Anabaena*, *Anabaenopsis*, *Aphanizomenon*, *Microcystis*, *Oscillatoria*, *Phormidium* [42], *Cylindrospermopsis* [80], *Dolichospermum* [80,81], and *Nodularia* [82], have significant salt tolerance and can proliferate in estuarine waters [83–85]. *Microcystis* cells have been found in salinities ranging from 5 to 35 ppt [42,86] and microcystins have been found in conductivities as high as 41 mS cm^{-1} (equivalent to ~25 ppt), [20]. Thus, eutrophication of stormwater ponds, development of cyanoHABs in them, and export to coastal receiving waters may have far-reaching effects.

Cyanobacteria akinetes may persist in sediments in otherwise unfavorable conditions enabling long-term residence in brackish and marine systems [87]. Once encysted cells are established in sediment, nutrient pollution entering estuaries could spur the onset of localized blooms [88]. Cyanotoxins entering estuarine environments can accumulate in filter-feeding shellfish [89–91], biomagnify in food webs [92], and cause human illness via shellfish consumption.

Additionally concerning is the threat of cyanotoxin contamination of public drinking waters. This hazard is increasing globally [22,93–96]. The Cape Fear River, which provides most of the public water to the Wilmington, NC, area, has hosted toxic cyanobacteria blooms [97]. The Cape Fear is also the outfall for Greenfield Lake, a recreational city park where regular blooms of potentially toxic cyanobacteria occur (44 sampled blooms in this study, including two microcystin-producing blooms). CyanoHABs at Greenfield Lake occur annually and have persisted lake-wide for as long as 6 weeks, resulting in

its closure to boating throughout the summer of 2019. The lake is, in essence, a large stormwater pond as it drains highly polluted runoff from a 1033-ha watershed and has been designated an NC 303d impaired waterbody since 2014 [98]. NC 303d status is defined as exceeding a 10% occurrence of water quality criteria set as numeric levels and/or narrative statements established by the State of North Carolina [99]. Due to its history of regular microalgae blooms, Greenfield Lake failed to meet water quality criteria for chlorophyll-α (<40 μg L^{-1}). The introduction of harmful cyanobacteria and cyanotoxins to the Cape Fear River Estuary from Greenfield Lake is imminent without dedicated efforts to improve the lake's water quality.

5. Conclusions

The findings herein demonstrated that potentially toxic cyanobacteria are the predominant group across diverse freshwater systems in the Wilmington, NC area. The dominant cyanobacteria taxa identified represent genera capable of producing several cyanotoxins including, ATX, CYN, MC, and STX, among others.

The blooms sampled by this study were all within residential, commercial, or recreational areas easily accessible to people. The diverse array of potentially toxic cyanobacteria identified is both a public and environmental health concern. Stormwater ponds and small lakes in housing developments are often marketed to residents as aesthetic or recreational features and lack water quality monitoring and pollution mitigation efforts. Microcystin-producing blooms in two heavily utilized Wilmington city parks (Long Leaf Park and Greenfield Lake) were documented by this study, and similar events are likely in urban lakes and ponds elsewhere.

Efforts to educate planners, developers, and residents in urban and suburban areas where stormwater ponds are pervasive are essential to mitigate the pollution-driven harmful bloom formation, proliferation, and negative impacts. The onset of a changing climate is irreversible, but land-use practices and development strategies (including nutrient reduction at the source, infiltration into greenspace, constructed wetlands to enhance denitrification, etc.) can and must be improved to stop the spread of cyanoHABs.

References

1. Anderson, D.M.; Burkholder, J.M.; Cochlan, W.P.; Glibert, P.M.; Gobler, C.J.; Heil, C.A.; Kudela, R.M.; Parsons, M.L.; Rensel, J.E.J.; Townsend, D.W.; et al. Harmful Algal Blooms and Eutrophication: Examining Linkages from Selected Coastal Regions of the United States. *Harmful Algae* **2008**, *8*, 39–53. [CrossRef] [PubMed]
2. Heisler, J.; Glibert, P.M.; Burkholder, J.M.; Anderson, D.M.; Cochlan, W.; Dennison, W.C.; Dortch, Q.; Gobler, C.J.; Heil, C.A.; Humphries, E.; et al. Eutrophication and Harmful Algal Blooms: A Scientific Consensus. *Harmful Algae* **2008**, *8*, 3–13. [CrossRef]
3. Beaver, J.R.; Tausz, C.E.; Scotese, K.C.; Pollard, A.I.; Mitchell, R.M. Environmental Factors Influencing the Quantitative Distribution of Microcystin and Common Potentially Toxigenic Cyanobacteria in U.S. Lakes and Reservoirs. *Harmful Algae* **2018**, *78*, 118–128. [CrossRef] [PubMed]
4. Glibert, P.M.; Burkholder, J.M. Causes of Harmful Algal Blooms. In *Harmful Algal Blooms*; Wiley: Hoboken, NJ, USA, 2018; pp. 1–38.
5. Davis, T.W.; Berry, D.L.; Boyer, G.L.; Gobler, C.J. The Effects of Temperature and Nutrients on the Growth and Dynamics of Toxic and Non-Toxic Strains of Microcystis during Cyanobacteria Blooms. *Harmful Algae* **2009**, *8*, 715–725. [CrossRef]
6. Beaulieu, M.; Pick, F.; Gregory-Eaves, I. Nutrients and Water Temperature Are Significant Predictors of Cyanobacterial Biomass in a 1147 Lakes Data Set. *Limnol. Oceanogr.* **2013**, *58*, 1736–1746. [CrossRef]
7. Walls, J.T.; Wyatt, K.H.; Doll, J.C.; Rubenstein, E.M.; Rober, A.R. Hot and Toxic: Temperature Regulates Microcystin Release from Cyanobacteria. *Sci. Total Environ.* **2018**, *610–611*, 786–795. [CrossRef] [PubMed]
8. Ho, J.C.; Michalak, A.M. Exploring Temperature and Precipitation Impacts on Harmful Algal Blooms across Continental U.S. Lakes. *Limnol. Oceanogr.* **2019**, *65*, 992–1009. [CrossRef]
9. Cotti-Rausch, B.; Majidzadeh, H.; DeVoe, R. Stormwater Ponds in Coastal South Carolina. In *2019 State of Knowledge Full Report*; S.C. Sea Grant Consortium: Charleston, SC, USA, 2019.
10. Drescher, S.; Messersmith, M.; Davis, B.; Sanger, D. *State of Knowledge Report: Stormwater Ponds in the Coastal Zone*; S.C. DHEC's Office of Ocean and Coastal Resource Management: Charleston, SC, USA, 2007.
11. Lewitus, A.J.; Brock, L.M.; Burke, M.K.; DeMattio, K.A.; Wilde, S.B. Lagoonal Stormwater Detention Ponds as Promoters of Harmful Algal Blooms and Eutrophication along the South Carolina Coast. *Harmful Algae* **2008**, *8*, 60–65. [CrossRef]
12. Siegel, A.; Cotti-Rausch, B.; Greenfield, D.I.; Pinckney, J.L. Nutrient Controls of Planktonic Cyanobacteria Biomass in Coastal Stormwater Detention Ponds. *Mar. Ecol. Prog. Ser.* **2011**, *434*, 15–27. [CrossRef]
13. Brooks, B.W.; Lazorchak, J.M.; Howard, M.D.A.; Johnson, M.-V.V.; Morton, S.L.; Perkins, D.A.K.; Reavie, E.D.; Scott, G.I.; Smith, S.A.; Steevens, J.A. Are Harmful Algal Blooms Becoming the Greatest Inland Water Quality Threat to Public Health and Aquatic Ecosystems? *Environ. Toxicol. Chem.* **2016**, *35*, 6–13. [CrossRef]
14. Zurawell, R.W.; Chen, H.; Burke, J.M.; Prepas, E.E. Hepatotoxic Cyanobacteria: A Review of the Biological Importance of Microcystins in Freshwater Environments. *J. Toxicol. Environ. Health Part B Crit. Rev.* **2005**, *8*, 1–37. [CrossRef]
15. Rastogi, R.P.; Madamwar, D.; Incharoensakdi, A. Bloom Dynamics of Cyanobacteria and Their Toxins: Environmental Health Impacts and Mitigation Strategies. *Front. Microbiol.* **2015**, *6*, 1254. [CrossRef] [PubMed]
16. Nowruzi, B.; Porzani, S.J. Toxic Compounds Produced by Cyanobacteria Belonging to Several Species of the Order Nostocales: A Review. *J. Appl. Toxicol.* **2021**, *41*, 510–548. [CrossRef] [PubMed]
17. Loftin, K.A.; Graham, J.L.; Hilborn, E.D.; Lehmann, S.C.; Meyer, M.T.; Dietze, J.E.; Griffith, C.B. Cyanotoxins in Inland Lakes of the United States: Occurrence and Potential Recreational Health Risks in the EPA National Lakes Assessment 2007. *Harmful Algae* **2016**, *56*, 77–90. [CrossRef] [PubMed]
18. Cheung, M.Y.; Liang, S.; Lee, J. Toxin-Producing Cyanobacteria in Freshwater: A Review of the Problems, Impact on Drinking Water Safety, and Efforts for Protecting Public Health. *J. Microbiol.* **2013**, *51*, 1–10. [CrossRef]
19. Bullerjahn, G.S.; McKay, R.M.; Davis, T.W.; Baker, D.B.; Boyer, G.L.; D'Anglada, L.V.; Doucette, G.J.; Ho, J.C.; Irwin, E.G.; Kling, C.L.; et al. Global Solutions to Regional Problems: Collecting Global Expertise to Address the Problem of Harmful Cyanobacterial Blooms. A Lake Erie Case Study. *Harmful Algae* **2016**, *54*, 223–238. [CrossRef]
20. Metcalf, J.S.; Banack, S.A.; Wessel, R.A.; Lester, M.; Pim, J.G.; Cassani, J.R.; Cox, P.A. Toxin Analysis of Freshwater Cyanobacterial and Marine Harmful Algal Blooms on the West Coast of Florida and Implications for Estuarine Environments. *Neurotox. Res.* **2021**, *39*, 27–35. [CrossRef]
21. Schaefer, A.M.; Yrastorza, L.; Stockley, N.; Harvey, K.; Harris, N.; Grady, R.; Sullivan, J.; McFarland, M.; Reif, J.S. Exposure to Microcystin among Coastal Residents during a Cyanobacteria Bloom in Florida. *Harmful Algae* **2020**, *92*, 101769. [CrossRef]
22. Melaram, R.; Lopez-Dueñas, B. Detection and Occurrence of Microcystins and Nodularins in Lake Manatee and Lake Washington-Two Floridian Drinking Water Systems. *Front. Water* **2022**, *4*, 899572. [CrossRef]
23. Heil, C.A.; Muni-Morgan, A.L. Florida's Harmful Algal Bloom (HAB) Problem: Escalating Risks to Human, Environmental and Economic Health With Climate Change. *Front. Ecol. Evol.* **2021**, *9*, 646080. [CrossRef]
24. Olokotum, M.; Mitroi, V.; Troussellier, M.; Semyalo, R.; Bernard, C.; Montuelle, B.; Okello, W.; Quiblier, C.; Humbert, J.F. A Review of the Socioecological Causes and Consequences of Cyanobacterial Blooms in Lake Victoria. *Harmful Algae* **2020**, *96*, 101829. [CrossRef]
25. Chernova, E.; Sidelev, S.; Russkikh, I.; Korneva, L.; Solovyova, V.; Mineeva, N.; Stepanova, I.; Zhakovskaya, Z. Spatial Distribution of Cyanotoxins and Ratios of Microcystin to Biomass Indicators in the Reservoirs of the Volga, Kama and Don Rivers, the European Part of Russia. *Limnologica* **2020**, *84*, 125819. [CrossRef]

26. Li, J.; Hansson, L.A.; Persson, K.M. Nutrient Control to Prevent the Occurrence of Cyanobacterial Blooms in a Eutrophic Lake in Southern Sweden, Used for Drinking Water Supply. *Water* **2018**, *10*, 919. [CrossRef]

27. Qin, B.; Zhu, G.; Gao, G.; Zhang, Y.; Li, W.; Paerl, H.W.; Carmichael, W.W. A Drinking Water Crisis in Lake Taihu, China: Linkage to Climatic Variability and Lake Management. *Environ. Manag.* **2010**, *45*, 105–112. [CrossRef]

28. Facey, J.A.; Michie, L.E.; King, J.J.; Hitchcock, J.N.; Apte, S.C.; Mitrovic, S.M. Severe Cyanobacterial Blooms in an Australian Lake; Causes and Factors Controlling Succession Patterns. *Harmful Algae* **2022**, *117*, 102284. [CrossRef]

29. Moreira, M.G.A.L.; Hinegk, L.; Salvadore, A.; Zolezzi, G.; Hölker, F.; Domecq, S.R.A.M.; Bocci, M.; Carrer, S.; De Nat, L.; Escribá, J.; et al. Eutrophication, Research and Management History of the Shallow Ypacaraí Lake (Paraguay). *Sustainability* **2018**, *10*, 2426. [CrossRef]

30. Gurbuz, F.; Uzunmehmetoğlu, O.Y.; Diler, Ö.; Metcalf, J.S.; Codd, G.A. Occurrence of Microcystins in Water, Bloom, Sediment and Fish from a Public Water Supply. *Sci. Total Environ.* **2016**, *562*, 860–868. [CrossRef] [PubMed]

31. Tavakoli, Y.; Mohammadipanah, F.; Te, S.H.; You, L.; Gin, K.Y.-H. Biodiversity, Phylogeny and Toxin Production Profile of Cyanobacterial Strains Isolated from Lake Latyan in Iran. *Harmful Algae* **2021**, *106*, 102054. [CrossRef]

32. Catherine, Q.; Susanna, W.; Isidora, E.S.; Mark, H.; Aurélie, V.; Jean-François, H. A Review of Current Knowledge on Toxic Benthic Freshwater Cyanobacteria—Ecology, Toxin Production and Risk Management. *Water Res.* **2013**, *47*, 5464–5479. [CrossRef]

33. Hudon, C.; De Sève, M.; Cattaneo, A. Increasing Occurrence of the Benthic Filamentous Cyanobacterium Lyngbya Wollei: A Symptom of Freshwater Ecosystem Degradation. *Freshw. Sci.* **2014**, *33*, 606–618. [CrossRef]

34. McAllister, T.G.; Wood, S.A.; Hawes, I. The Rise of Toxic Benthic Phormidium Proliferations: A Review of Their Taxonomy, Distribution, Toxin Content and Factors Regulating Prevalence and Increased Severity. *Harmful Algae* **2016**, *55*, 282–294. [CrossRef] [PubMed]

35. Toxic Algae Found in Wilmington Pond after Death of Dogs. *WWAY News*. 27 August 2019. Available online: https://www.wwaytv3.com/toxic-algae-found-in-wilmington-pond-after-death-of-dogs/ (accessed on 1 June 2023).

36. Welschmeyer, N.A. Fluorometric Analysis of Chlorophyll a in the Presence of Chlorophyll b and Pheopigments. *Limnol. Oceanogr.* **1994**, *39*, 1985–1992. [CrossRef]

37. APHA. *4500-P J. Persulfate Method for Simultaneous Determination of Total Nitrogen and Total Phosphorous*; Eaton, A.D., Clesceri, L.S., Rice, E.W., Greenberg, A.E., Eds.; American Public Health Association, American Water Works Association, Water Environmental Federation: Washington, DC, USA, 2005.

38. Zimmermann, C.F.; Keefe, C.W. *Method 365.5 Determination of Orthophosphate in Estuarine and Coastal Waters by Automated Colorimetric Analysis*; U.S. Environmental Protection Agency: Washington, DC, USA, 1997; pp. 1–9.

39. Nienaber, M.A.; Steinitz-Kannan, M. *A Guide to Cyanobacteria*; The University Press of Kentucky: Lexington, KY, USA, 2018; ISBN 9780813175614.

40. Rattner, B.A.; Wazniak, C.E.; Lankton, J.S.; McGowan, P.C.; Drovetski, S.V.; Egerton, T.A. Review of Harmful Algal Bloom Effects on Birds with Implications for Avian Wildlife in the Chesapeake Bay Region. *Harmful Algae* **2022**, *120*, 102319. [CrossRef]

41. Li, X.; Dreher, T.W.; Li, R. An Overview of Diversity, Occurrence, Genetics and Toxin Production of Bloom-Forming Dolichospermum (Anabaena) Species. *Harmful Algae* **2016**, *54*, 54–68. [CrossRef] [PubMed]

42. Preece, E.P.; Hardy, F.J.; Moore, B.C.; Bryan, M. A Review of Microcystin Detections in Estuarine and Marine Waters: Environmental Implications and Human Health Risk. *Harmful Algae* **2017**, *61*, 31–45. [CrossRef]

43. Díez-Quijada, L.; Prieto, A.I.; Guzmán-Guillén, R.; Jos, A.; Cameán, A.M. Occurrence and Toxicity of Microcystin Congeners Other than MC-LR and MC-RR: A Review. *Food Chem. Toxicol.* **2019**, *125*, 106–132. [CrossRef]

44. Janssen, E.M.L. Cyanobacterial Peptides beyond Microcystins—A Review on Co-Occurrence, Toxicity, and Challenges for Risk Assessment. *Water Res.* **2019**, *151*, 488–499. [CrossRef]

45. Scarlett, K.R.; Kim, S.; Lovin, L.M.; Chatterjee, S.; Scott, J.T.; Brooks, B.W. Global Scanning of Cylindrospermopsin: Critical Review and Analysis of Aquatic Occurrence, Bioaccumulation, Toxicity and Health Hazards. *Sci. Total Environ.* **2020**, *738*, 139807. [CrossRef]

46. Chen, G.; Wang, L.; Wang, M.; Hu, T. Comprehensive Insights into the Occurrence and Toxicological Issues of Nodularins. *Mar. Pollut. Bull.* **2021**, *162*, 111884. [CrossRef]

47. Christensen, V.G.; Khan, E. Freshwater Neurotoxins and Concerns for Human, Animal, and Ecosystem Health: A Review of Anatoxin-a and Saxitoxin. *Sci. Total Environ.* **2020**, *736*, 139515. [CrossRef]

48. Kulabhusan, P.K.; Campbell, K. Recent Trends in the Detection of Freshwater Cyanotoxins with a Critical Note on Their Occurrence in Asia. *Trends Environ. Anal. Chem.* **2021**, *32*, e00150. [CrossRef]

49. Massey, I.Y.; Wu, P.; Wei, J.; Luo, J.; Ding, P.; Wei, H.; Yang, F. A Mini-Review on Detection Methods of Microcystins. *Toxins* **2020**, *12*, 641. [CrossRef]

50. Tanvir, R.U.; Hu, Z.; Zhang, Y.; Lu, J. Cyanobacterial Community Succession and Associated Cyanotoxin Production in Hypereutrophic and Eutrophic Freshwaters. *Environ. Pollut.* **2021**, *290*, 118056. [CrossRef]

51. Kim, S.G.; Rhee, S.K.; Ahn, C.Y.; Ko, S.R.; Choi, G.G.; Bae, J.W.; Park, Y.H.; Oh, H.M. Determination of Cyanobacterial Diversity during Algal Blooms in Daechung Reservoir, Korea, on the Basis of CpcBA Intergenic Spacer Region Analysis. *Appl. Environ. Microbiol.* **2006**, *72*, 3252–3258. [CrossRef] [PubMed]

52. Sabart, M.; Crenn, K.; Perrière, F.; Abila, A.; Leremboure, M.; Colombet, J.; Jousse, C.; Latour, D. Co-Occurrence of Microcystin and Anatoxin-a in the Freshwater Lake Aydat (France): Analytical and Molecular Approaches during a Three-Year Survey. *Harmful Algae* **2015**, *48*, 12–20. [CrossRef]

53. Ruiz, M.; Galanti, L.; Ruibal, A.L.; Rodriguez, M.I.; Wunderlin, D.A.; Amé, M.V. First Report of Microcystins and Anatoxin-a Co-Occurrence in San Roque Reservoir (Córdoba, Argentina). *Water. Air. Soil Pollut.* **2013**, *224*, 1593. [CrossRef]

54. Harland, F.; Wood, S.A.; Broady, P.; Williamson, W.; Gaw, S. Changes in Saxitoxin-Production through Growth Phases in the Metaphytic *Cyanobacterium Scytonema* Cf. *Crispum. Toxicon.* **2015**, *103*, 74–79. [CrossRef] [PubMed]

55. Chaffin, J.D.; Westrick, J.A.; Furr, E.; Birbeck, J.A.; Reitz, L.A.; Stanislawczyk, K.; Li, W.; Weber, P.K.; Bridgeman, T.B.; Davis, T.W.; et al. Quantification of Microcystin Production and Biodegradation Rates in the Western Basin of Lake Erie. *Limnol. Oceanogr.* **2022**, *67*, 1470–1483. [CrossRef]

56. Cottingham, K.L.; Ewing, H.A.; Greer, M.L.; Carey, C.C.; Weathers, K.C. Cyanobacteria as Biological Drivers of Lake Nitrogen and Phosphorus Cycling. *Ecosphere* **2015**, *6*, 1–19. [CrossRef]

57. Mallin, M.A.; Cahoon, L.B. The Hidden Impacts of Phosphorus Pollution to Streams and Rivers. *Bioscience* **2020**, *70*, 315–329. [CrossRef]

58. Anderson, D.M.; Fensin, E.; Gobler, C.J.; Hoeglund, A.E.; Hubbard, K.A.; Kulis, D.M.; Landsberg, J.H.; Lefebvre, K.A.; Provoost, P.; Richlen, M.L.; et al. Marine Harmful Algal Blooms (HABs) in the United States: History, Current Status and Future Trends. *Harmful Algae* **2021**, *102*, 101975. [CrossRef]

59. Glibert, P.M.; Allen, J.I.; Bouwman, A.F.; Brown, C.W.; Flynn, K.J.; Lewitus, A.J.; Madden, C.J. Modeling of HABs and Eutrophication: Status, Advances, Challenges. *J. Mar. Syst.* **2010**, *83*, 262–275. [CrossRef]

60. O'Neil, J.M.; Davis, T.W.; Burford, M.A.; Gobler, C.J. The Rise of Harmful Cyanobacteria Blooms: The Potential Roles of Eutrophication and Climate Change. *Harmful Algae* **2012**, *14*, 313–334. [CrossRef]

61. Reed, M.L.; DiTullio, G.R.; Kacenas, S.E.; Greenfield, D.I. Effects of Nitrogen and Dissolved Organic Carbon on Microplankton Abundances in Four Coastal South Carolina (USA) Systems. *Aquat. Microb. Ecol.* **2015**, *76*, 1–14. [CrossRef]

62. Mallin, M.A.; McIver, M.R.; Wambach, E.J.; Robuck, A.R. Algal Blooms, Circulators, Waterfowl, and Eutrophic Greenfield Lake, North Carolina. *Lake Reserv. Manag.* **2016**, *32*, 168–181. [CrossRef]

63. Yan, X.; Xu, X.; Wang, M.; Wang, G.; Wu, S.; Li, Z.; Sun, H.; Shi, A.; Yang, Y. Climate Warming and Cyanobacteria Blooms: Looks at Their Relationships from a New Perspective. *Water Res.* **2017**, *125*, 449–457. [CrossRef]

64. Kramer, B.J.; Jankowiak, J.G.; Nanjappa, D.; Harke, M.J.; Gobler, C.J. Nitrogen and Phosphorus Significantly Alter Growth, Nitrogen Fixation, Anatoxin-a Content, and the Transcriptome of the Bloom-Forming Cyanobacterium, Dolichospermum. *Front. Microbiol.* **2022**, *13*, 955032. [CrossRef]

65. Hupfer, M.; Lewandowski, J. Oxygen Controls the Phosphorus Release from Lake Sediments—A Long-Lasting Paradigm in Limnology. *Int. Rev. Hydrobiol.* **2008**, *93*, 415–432. [CrossRef]

66. González-Madina, L.; Levrini, P.; de Tezanos Pinto, P.; Burwood, M.; Crisci, C.; Cardozo, A.; Lagomarsino, J.J.; Pacheco, J.P.; Fosalba, C.; Méndez, G.; et al. Blooms of Toxic Raphidiopsis Raciborskii in Laguna Del Sauce (Uruguay): Environmental Drivers and Impacts. *Hydrobiologia* **2022**, *849*, 4041–4058. [CrossRef]

67. Gibbs, M.M.; Roygard, J.; Patterson, M.; Brown, L.; Brown, D. Factors Influencing Cyanobacteria Blooms: Review of the Historical Monitoring Data to Assess Management Options for Lake Horowhenua. *N. Z. J. Mar. Freshw. Res.* **2022**, 1–27. [CrossRef]

68. Gagrani, V.; Diemer, J.A.; Karl, J.J.; Allan, C.J. Assessing the Hydrologic and Water Quality Benefits of a Network of Stormwater Control Measures in a SE U.S. Piedmont Watershed. *J. Am. Water Resour. Assoc.* **2014**, *50*, 128–142. [CrossRef]

69. Sanger, D.; Blair, A.; DiDonato, G.; Washburn, T.; Jones, S.; Riekerk, G.; Wirth, E.; Stewart, J.; White, D.; Vandiver, L.; et al. Impacts of Coastal Development on the Ecology of Tidal Creek Ecosystems of the US Southeast Including Consequences to Humans. *Estuaries Coasts* **2015**, *38*, 49–56. [CrossRef]

70. Mallin, M.A.; Burkholder, J.M.; Cahoon, L.B.; Grogan, A.E.; Sanger, D.M.; Smith, E. *An Environmental Assessment of the North and South Carolina Coasts*, 2nd ed.; Elsevier Ltd.: Amsterdam, The Netherlands, 2018; ISBN 9780128050682.

71. Brabec, E.; Schulte, S.; Richards, P.L. Impervious Surfaces and Water Quality: A Review of Current Literature and Its Implications for Watershed Planning Impervious Surfaces and Water Quality: A Review of Current Literature and Its Implications. *J. Plan. Lit.* **2002**, *16*, 499–514. [CrossRef]

72. Williams, B.A.; Watson, J.E.M.; Beyer, H.L.; Klein, C.J.; Montgomery, J.; Runting, R.K.; Roberson, L.A.; Halpern, B.S.; Grantham, H.S.; Kuempel, C.D.; et al. Global Rarity of Intact Coastal Regions. *Conserv. Biol.* **2022**, *36*, e13874. [CrossRef]

73. Carey, C.C.; Ibelings, B.W.; Hoffmann, E.P.; Hamilton, D.P.; Brookes, J.D. Eco-Physiological Adaptations That Favour Freshwater Cyanobacteria in a Changing Climate. *Water Res.* **2012**, *46*, 1394–1407. [CrossRef]

74. Jovanović, J.; Popović, S.; Simić, G.S.; Jovanović, V.; Predojević, D.; Jovanović, D.; Karadžić, V. Freshwater Cyanobacteria in Waters Intended for Human Consumption in Serbia: Two Decades of Changes in Diversity. *Arch. Biol. Sci.* **2022**, *74*, 217–226. [CrossRef]

75. Cordeiro-Araújo, M.K.; Lorenzi, A.S.; Chia, M.A.; Mota, E.C.; do Carmo Bittencourt-Oliveira, M. Insights into the Impact of Increasing Temperature, Light Intensity, and UV-B Exposure on the Circadian Rhythm of Microcystin Production and Release, and the Expression of Mcy Genes in the Cyanobacterium Microcystis Aeruginosa. *J. Appl. Phycol.* **2022**, *34*, 231–242. [CrossRef]

76. Kramer, B.J.; Gobler, C.J. Simulated Heat Waves Promote the Growth but Suppress the N2 Fixation Rates of Dolichospermum Spp. and Cyanobacterial Communities in Temperate Lakes. *Ecol. Indic.* **2023**, *147*, 109983. [CrossRef]

77. Kramer, B.J.; Hem, R.; Gobler, C.J. Elevated CO2 Significantly Increases N2 Fixation, Growth Rates, and Alters Microcystin, Anatoxin, and Saxitoxin Cell Quotas in Strains of the Bloom-Forming Cyanobacteria, Dolichospermum. *Harmful Algae* **2022**, *120*, 102354. [CrossRef]

78. Wahl, T.; Jain, S.; Bender, J.; Meyers, S.D.; Luther, M.E. Increasing Risk of Compound Flooding from Storm Surge and Rainfall for Major US Cities. *Nat. Clim. Chang.* **2015**, *5*, 1093–1097. [CrossRef]

79. Manda, A.K.; Klein, W.A. Adaptation Strategies to Address Rising Water Tables in Coastal Environments Under Future Climate and Sea-Level Rise Scenarios. In *Coastal Zone Management*; Elsevier: Amsterdam, The Netherlands, 2019; pp. 403–409.

80. Houliez, E.; Briand, E.; Malo, F.; Rovillon, G.A.; Hervé, F.; Robert, E.; Marchand, L.; Zykwinska, A.; Caruana, A.M.N. Physiological Changes Induced by Sodium Chloride Stress in *Aphanizomenon gracile*, *Cylindrospermopsis raciborskii* and *Dolichospermum* sp. *Harmful Algae* **2021**, *103*, 102028. [CrossRef]

81. Duval, C.; Thomazeau, S.; Drelin, Y.; Yéprémian, C.; Bouvy, M.; Couloux, A.; Troussellier, M.; Rousseau, F.; Bernard, C. Phylogeny and Salt-Tolerance of Freshwater Nostocales Strains: Contribution to Their Systematics and Evolution. *Harmful Algae* **2018**, *73*, 58–71. [CrossRef] [PubMed]

82. Mazur-Marzec, H.; Żeglińska, L.; Pliński, M. The Effect of Salinity on the Growth, Toxin Production, and Morphology of Nodularia Spumigena Isolated from the Gulf of Gdańsk, Southern Baltic Sea. *J. Appl. Phycol.* **2005**, *17*, 171–179. [CrossRef]

83. Tonk, L.; Bosch, K.; Visser, P.M.; Huisman, J. Salt Tolerance of the Harmful Cyanobacterium Microcystis Aeruginosa. *Aquat. Microb. Ecol.* **2007**, *46*, 117–123. [CrossRef]

84. Bormans, M.; Amzil, Z.; Mineaud, E.; Brient, L.; Savar, V.; Robert, E.; Lance, E. Demonstrated Transfer of Cyanobacteria and Cyanotoxins along a Freshwater-Marine Continuum in France. *Harmful Algae* **2019**, *87*, 101639. [CrossRef] [PubMed]

85. Wurtsbaugh, W.A.; Paerl, H.W.; Dodds, W.K. Nutrients, Eutrophication and Harmful Algal Blooms along the Freshwater to Marine Continuum. *WIREs Water* **2019**, *6*, e1373. [CrossRef]

86. Lapointe, B.E.; Herren, L.W.; Paule, A.L. Septic Systems Contribute to Nutrient Pollution and Harmful Algal Blooms in the St. Lucie Estuary, Southeast Florida, USA. *Harmful Algae* **2017**, *70*, 1–22. [CrossRef]

87. Wood, S.M.; Kremp, A.; Savela, H.; Akter, S.; Vartti, V.-P.; Saarni, S.; Suikkanen, S. Cyanobacterial Akinete Distribution, Viability, and Cyanotoxin Records in Sediment Archives From the Northern Baltic Sea. *Front. Microbiol.* **2021**, *12*, 681881. [CrossRef]

88. Burkholder, J.M.; Dickey, D.A.; Kinder, C.A.; Reed, R.E.; Mallin, M.A.; McIver, M.R.; Cahoon, L.B.; Melia, G.; Brownie, C.; Smith, J.; et al. Comprehensive Trend Analysis of Nutrients and Related Variables in a Large Eutrophic Estuary: A Decadal Study of Anthropogenic and Climatic Influences. *Limnol. Oceanogr.* **2006**, *51*, 463–487. [CrossRef]

89. Preece, E.P.; Moore, B.C.; Hardy, F.J. Transfer of Microcystin from Freshwater Lakes to Puget Sound, WA and Toxin Accumulation in Marine Mussels (Mytilus Trossulus). *Ecotoxicol. Environ. Saf.* **2015**, *122*, 98–105. [CrossRef]

90. Gibble, C.M.; Peacock, M.B.; Kudela, R.M. Evidence of Freshwater Algal Toxins in Marine Shellfish: Implications for Human and Aquatic Health. *Harmful Algae* **2016**, *59*, 59–66. [CrossRef]

91. Bukaveckas, P.A.; Franklin, R.; Tassone, S.; Trache, B.; Egerton, T. Cyanobacteria and Cyanotoxins at the River-Estuarine Transition. *Harmful Algae* **2018**, *76*, 11–21. [CrossRef] [PubMed]

92. Miller, M.A.; Kudela, R.M.; Mekebri, A.; Crane, D.; Oates, S.C.; Tinker, M.T.; Staedler, M.; Miller, W.A.; Toy-Choutka, S.; Dominik, C.; et al. Evidence for a Novel Marine Harmful Algal Bloom: Cyanotoxin (Microcystin) Transfer from Land to Sea Otters. *PLoS ONE* **2010**, *5*, e12576. [CrossRef]

93. Caly, L.F.; Rodríguez, D.C.; Peñuela, G.A. Monitoring of Cyanobacteria and Cyanotoxins in a Colombian Tropical Reservoir. *Environ. Sci. Pollut. Res.* **2022**, *29*, 52775–52787. [CrossRef] [PubMed]

94. Grentell, J.; Adhikary, R.K.; Lal, A. Cyanobacteria, Water Quality and Public Health Implications: A Systematic Scoping Review. *Australas. J. Water Resour.* **2022**, *27*, 1–13. [CrossRef]

95. Tito, J.C.R.; Luna, L.M.G.; Noppe, W.N.; Hubert, I.A. First Report on Microcystin-LR Occurrence in Water Reservoirs of Eastern Cuba, and Environmental Trigger Factors. *Toxins* **2022**, *14*, 209. [CrossRef]

96. Aguilera, A.; Almanza, V.; Haakonsson, S.; Palacio, H.; Benitez Rodas, G.A.; Barros, M.U.G.; Capelo-Neto, J.; Urrutia, R.; Aubriot, L.; Bonilla, S. Cyanobacterial Bloom Monitoring and Assessment in Latin America. *Harmful Algae* **2023**, *125*, 102429. [CrossRef]

97. Isaacs, J.D.; Strangman, W.K.; Barbera, A.E.; Mallin, M.A.; McIver, M.R.; Wright, J.L.C. Microcystins and Two New Micropeptin Cyanopeptides Produced by Unprecedented Microcystis Aeruginosa Blooms in North Carolina's Cape Fear River. *Harmful Algae* **2014**, *31*, 82–86. [CrossRef] [PubMed]

98. Iraola, N.D.; Mallin, M.A.; Cahoon, L.B.; Gamble, D.W.; Zamora, P.B. Nutrient Dynamics in a Eutrophic Blackwater Urban Lake. *Lake Reserv. Manag.* **2022**, *38*, 28–46. [CrossRef]

99. North Carolina Water Resources Division. *2024 303(d) Listing and Delisting Methodology*; North Carolina Department of Environmental Quality: Raleigh, NC, USA, 2023.