

Article

Relating Cyanotoxin Occurrence to Environmental Cues to Inform Algal Toxin Monitoring and Management in Pennsylvania Lakes

Olive B. Stern ¹, Kristin E. D. Strock ^{1,*} and Beth C. Norman ^{2,3}

¹ Department of Environmental Science, Dickinson College, Carlisle, PA 17013, USA; sterno@dpi.edu

² Lacawac Sanctuary Field Station and Environmental Education Center, Lake Ariel, PA 18436, USA; beth.norman@lacawac.org

³ Department of Biology, Swarthmore College, Swarthmore, PA 19081, USA

* Correspondence: strockk@dickinson.edu

Abstract

Harmful cyanobacteria blooms (CyanoHABs) have become a common problem in freshwater ecosystems. Monitoring at the federal and state level in response to this threat has increased over the last decade across the United States. In 2018, Pennsylvania environmental agencies began collecting CyanoHAB data to inform state regulatory and management efforts. 998 discrete samples from 138 waterbodies were collected over four years. These data were used to determine if indicators often used in CyanoHAB monitoring co-occurred with toxic conditions. Cyanotoxins were detected in more than half of the samples in Pennsylvania's monitoring program with anatoxin, saxitoxins, and microcystins detected in similar proportions. It is important to note that the monitoring program is not designed to be representative of all Pennsylvania lakes but instead is targeted toward sites with greater exposure (recreational beaches) or sites with suspected blooms. Our findings suggest that cyanobacteria growth and cyanotoxin production may be driven by independent variables, as only microcystins concentrations were positively correlated with cyanobacteria counts. While increased cyanobacteria counts were related to all environmental variables considered in this study (warmer water temperatures, increased dissolved oxygen concentrations, and reduced water clarity), the relationships between cyanotoxins and environmental variables were mixed. Our analyses highlight the complexities of bloom occurrence and severity, and the challenges of incorporating these factors into monitoring and management strategies. Long-term monitoring approaches like the one analyzed here can inform management strategies that rely on environmental cues and cyanobacteria enumeration to evaluate risk of cyanotoxin presence.

Keywords: harmful cyanobacteria blooms; cyanotoxins; lake monitoring; lake management; Pennsylvania



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1. Introduction

Harmful blooms of cyanobacteria (CyanoHABs) are increasing in intensity and frequency in lakes across the globe [1]. Blooms cause ecological damage and pose risks to human health [2] as CyanoHABs can produce 2010 known secondary metabolites [3], including neuro- and hepato-toxins [4]. There are four main cyanotoxin classes that are commonly considered in management practices: microcystins, cylindrospermopsin, anatoxin-a,

and saxitoxins [5]. Federal, state, and local monitoring efforts in the United States are expanding as CyanoHABs become more prevalent; however, without a universal agreement on what constitutes a CyanoHAB [6], monitoring and management strategies vary across agencies and states [7]. Monitoring, and even more so managing CyanoHABs is complicated by the interactions between external environmental factors, which are changing with ongoing climate change, and biological traits of bloom-forming cyanobacteria, which range broadly across species [8].

Increased delivery of nutrients to lakes from the surrounding watershed facilitates phytoplankton growth and eutrophication. CyanoHABs are commonly associated with eutrophic systems; however, they have been documented in oligotrophic systems as well [9]. Oligotrophic blooms have produced cyanotoxins on comparable levels to eutrophic blooms despite lower algal biomass [10]. Cyanobacteria can cluster in colonies that create distinct surface scums, shading out other phytoplankton [11]. These scums are often used as visual indicators of toxic bloom conditions to initiate monitoring. Scums are an aesthetic concern for recreation and can lead to anoxic zones, which can exacerbate bloom conditions via nutrient release in anoxic sediments [12]. However, CyanoHABs can exist without visible surface scums [13] and scums and reduced water clarity are not inherently associated with more toxic conditions [6].

Cyanobacteria have numerous adaptations, such as higher temperature tolerance, buoyancy, nitrogen-fixation, phosphorus storage, and akinete production [11]. These adaptations enable cyanobacteria to dominate other phytoplankton groups under adverse conditions expected to increase with climate change [11]. Warm temperatures may promote cyanobacteria growth because of high thermal optima compared to other phytoplankton [14], or because they are well adapted to compete in heavily stratified environments [15,16].

Cyanotoxin occurrence is challenging to predict because of the biological complexity of cyanotoxin production, which is energetically expensive to the cyanobacteria cell [4]. Cyanotoxin production varies across blooms as communities are comprised of a mixture of clones with varying toxin contents, including nontoxigenic ones [17]. Both toxic and nontoxigenic cyanobacteria strains are morphologically indistinguishable [17]. Therefore, toxin concentration is not necessarily related to cyanobacteria cell counts [18] or chlorophyll a concentration [10]. Various environmental variables have been demonstrated to affect toxin production including nutrient availability, light, and temperature thresholds [19,20]. Cyanotoxins are stored intracellularly and are released upon cell lysis [21] or exported out of the cell [22]. Cell lysing may be triggered by factors independent of cell growth [2]. In summary, the factors contributing to increased cyanobacteria growth may differ from those leading to elevated cyanotoxin concentrations in the open water [23].

Incorporating complex factors regulating bloom occurrence and severity into monitoring and management strategies is challenging. Additionally, testing cyanotoxins is resource intensive, making routine sampling unrealistic for many regulatory agencies. Therefore, many rely on visual indicators, such as scum presence, water clarity [24], and measures of cyanobacteria cell abundance as proxies for cyanotoxin threat [25]. The Commonwealth of Pennsylvania, USA is currently developing a robust CyanoHABs management strategy and state-wide monitoring program led by an inter-agency CyanoHAB task force established in 2018. The Pennsylvania Department of Environmental Protection (PA DEP) and the Pennsylvania Department of Health integrate field and laboratory data describing environmental conditions, cyanobacteria abundance, and cyanotoxin concentration collected from lakes across the state into a public, interactive CyanoHAB dashboard [26,27]. We used these data to determine if indicators often used to initiate CyanoHAB monitoring, such as reduced water clarity and high cyanobacteria counts, co-occur with cyanotoxin formation.

These objectives are crucial to strengthening management efforts and limiting community cyanotoxin exposure.

2. Materials and Methods

This study utilized CyanoHAB data contained in the PA HABs Task Force database [27] from the beginning of data collection in April of 2018 to September of 2022. The dataset contained 998 discrete samples collected at 138 waterbodies across Pennsylvania (Figure 1). Samples were collected and analyzed by PA DEP and their partners and utilized PA DEP Environmental Laboratory Standards [28]. Data were entered using Survey123 software in ArcGIS online by trained staff, overseen by PA DEP's Bureau of Clean Water and the PA Department of Natural Resources [26].

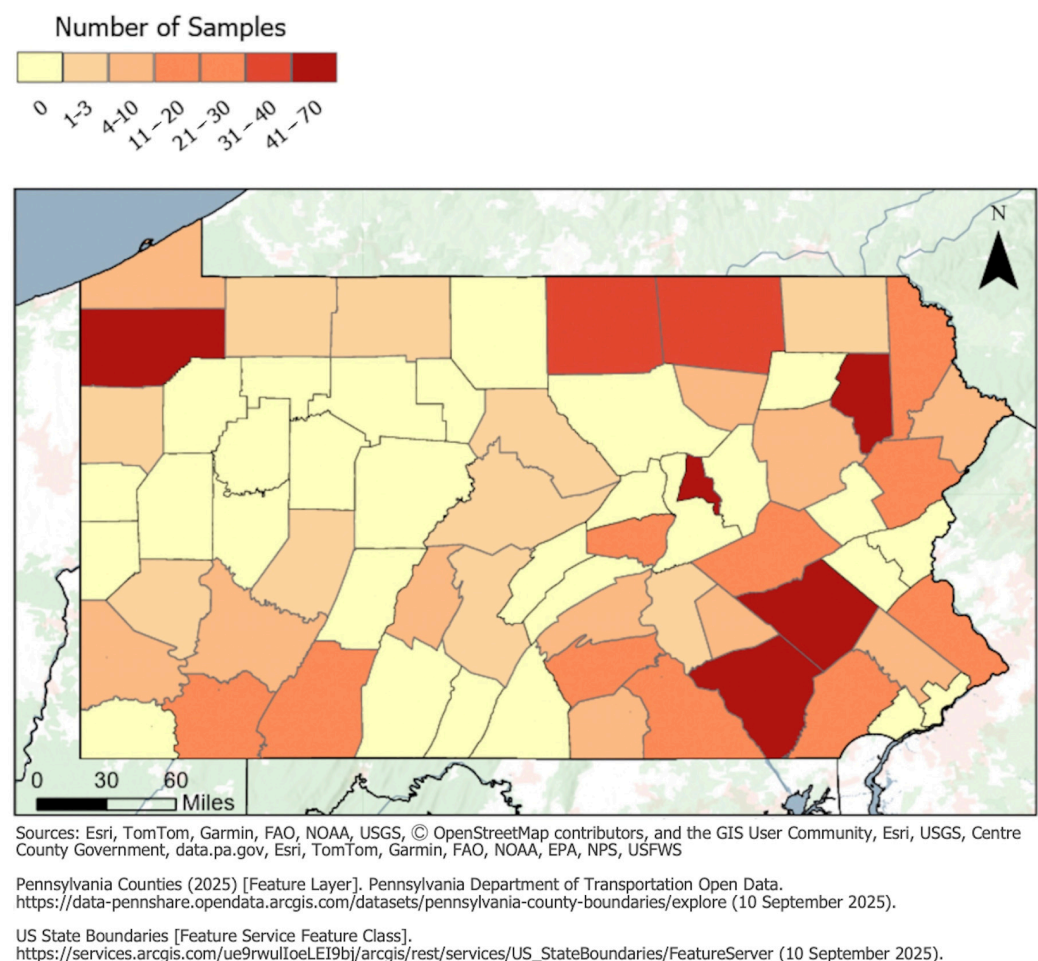


Figure 1. The number of distinct samples in each Pennsylvania county over the four-year sampling period included in this study, 2018–2022 [29,30].

Samples were collected using one of four collection methods, determined by site and bloom conditions: samples collected at depth, from a scum, a surface water grab, and from surface water along a transect [28]. Transect sampling was used when a beach or boat launch was the primary focus, scum sampling was used when there was visible accumulation, surface sampling was done when there was a uniform, widespread bloom or as part of routine monitoring, and depth sampling was generally in the photic zone when there was a visible bloom below the surface. Out of the samples in both subsets, 51% were surface samples, 30% were transect samples, 15% were depth samples, and 3% were scum samples.

The PA DEP protocol uses a priority assessment for cyanotoxin analysis. As a result, not all samples collected through this monitoring program were analyzed for cyanotoxins. Samples were prioritized for analysis based on initial cyanobacteria counts that characterize algal communities in the sample, historical knowledge of biology in waterbodies throughout the state, likelihood for human exposure (e.g., sites with swimming beaches), and visual cues or suspicion of a CyanoHAB. If a sample did not meet specific criteria indicating a likelihood of detecting cyanotoxins or a considerable human health concern, then the sample was not tested for cyanotoxins.

When analyzed for cyanotoxins, samples could have been tested for four cyanotoxins: total anatoxin-a, total microcystins/nodularins, cylindrospermopsin, and saxitoxins. Depending on algal communities identified in the sample, samples could have been analyzed for one or more of these cyanotoxins. Cylindrospermopsin values were excluded from our analyses due to infrequent detection (only detected in 1.1% of total samples). “Cyanotoxins” in this analysis refer to anatoxin-a, microcystins/nodularins, and saxitoxins.

Cyanotoxin analyses were completed by the PA DEP Bureau of Laboratories (starting in 2021) or GreenWater CyanoLab (from 2018 to 2021). PA DEP quantified microcystin/nodularin concentration according to the EPA 546 method [31], which measures a single combined concentration and does not measure microcystins and nodularins separately. Anatoxin-a and saxitoxin concentrations were measured using enzyme-linked immunosorbent assays (ELISA) (Abraxis, Gold Standard Diagnostics, Horsham, PA, USA): ANA (Cat. #520060), STX (Cat. #52255B). GreenWater CyanoLab quantified microcystins/nodularins according to the EPA 546 method [31], saxitoxin concentration using ELISA (Abraxis, Gold Standard Diagnostics, Horsham, PA, USA): STX (Cat. #52255B), and anatoxin-a concentration using liquid chromatography and tandem mass spectrometry (LC-MS/MS). We refer to microcystins/nodularins and anatoxin-a as microcystins and anatoxin, respectively, for simplicity. Cyanotoxin analyses that resulted in values below the detection limits for each respective methodology and laboratory were considered “non-detected” samples while samples with concentrations at or above detection limits were considered “detected.” The development of this monitoring program happened over several years with both GreenWater CyanoLab and PA DEP Bureau of Laboratories contributing to cyanotoxin measures. As a result, detection limits have shifted over time. We distinguished “detected” from “non-detected” based on the detection limit reported in the database at the time of analysis. We also relied on quality assurance and quality control completed by the PA DEP [28].

Cyanobacteria enumeration was completed by the PA DEP Bureau of Laboratories in Harrisburg and was measured in natural units (N.U./mL) [27]. A natural counting unit is defined by each natural grouping of algae as either an individual filament, colony, or unicell, each of which is counted as one unit [32]. Cyanobacteria enumerations represent total cyanobacteria counts which include both toxic and non-toxic genera. A cyanobacteria count of at least 300 N.U./mL triggers a further look at algal community assemblages [28]. For each sample, cyanotoxins were selected for analysis depending on what species of cyanobacteria were present and the current understanding of their potential to produce cyanotoxins, which included referencing historical knowledge of biology in waterbodies across the state. Samples with a cyanobacteria count of zero (i.e., the sample was analyzed but no cyanobacteria were present) were included in our data, while samples with no recorded cyanobacteria count (i.e., the sample was not analyzed) were excluded.

The PA DEP protocol allows for the optional collection of a range of environmental data in the field; therefore, samples in the dataset are associated with a variable combination of parameters. Environmental data that were included in the database were collected as discrete field measurements at the same depth and time as the water collected for cyanotoxin

analysis [28]. The optional nature of environmental data collection limits the availability of specific water quality field measurements associated with CyanoHAB monitoring, resulting in considerable variability across analytes and over time. Environmental data included in this analysis were selected based on frequency of collection and association with cyanotoxin or cyanobacteria count data, and include dissolved oxygen (DO) (membrane electrode), water clarity (Secchi disk depth), and water temperature (field-thermometric). The term “environmental data” in this analysis refers only to these three variables. Nutrient data had infrequent representation across the dataset (only present in 7% of total samples) and were excluded from any analyses despite their importance to phytoplankton growth.

To account for the variability in available data among samples, the data were analyzed as the following subsets (Figure 2): samples with cyanotoxin data, samples with cyanotoxin and environmental data, samples with cyanobacteria counts and cyanotoxin data, and samples with cyanobacteria counts and environmental data. Samples were included as having “cyanotoxin data” if at least one of the three cyanotoxins contained in the database were measured (anatoxin, microcystins, or saxitoxins).

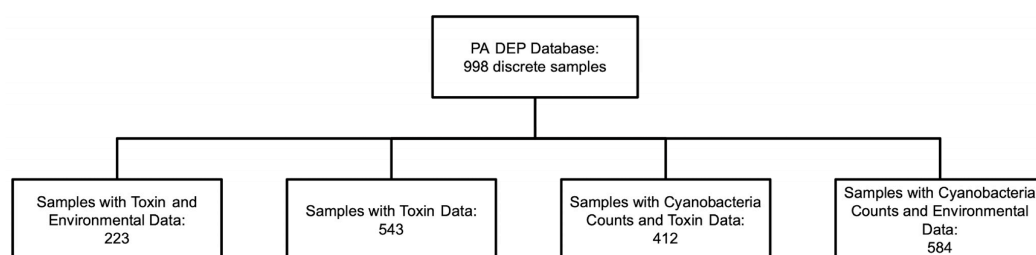


Figure 2. The number of samples in each data subset considered in this analysis. Samples with cyanotoxin data were included if they were analyzed for anatoxin, microcystins and/or saxitoxins. Cyanobacteria count data were measured as natural units per mL. Environmental data included dissolved oxygen (DO), water clarity, and water temperature.

The data collection effort was not designed to serve as a representative probability-based statistical survey of all Pennsylvania lakes. Instead, there is an unbalanced sampling design with varying numbers of observations per lake and irregular sampling intervals across years. Repeated observations from individual lakes may introduce non-independence. With limited explanatory variables to pair with toxin data and an unbalanced design, we are using an exploratory analysis as the first assessment of these data in the hopes of informing future data collection efforts.

Data were cleaned using R software version 4.4.0 [33] and tools in the tidyverse package [34]. Excel pivot tables were used to select detected and non-detected samples that included cyanobacterial counts or environmental data. Cyanobacteria samples above and below 300 N.U./mL were also separated along with the corresponding environmental data. SigmaPlot version 13.0 (2019) was used for statistical analyses and data visualization. As data were largely non-normal with unequal variances (determined by the Shapiro–Wilk test $p < 0.05$), non-parametric tests were applied. Spearman’s rank correlation coefficient was used to determine the relationship between environmental conditions and cyanotoxin concentration, excluding non-detected samples. Mann–Whitney U tests were used to determine significance between environmental variables among “detected” and “non-detected” cyanotoxin samples and cyanobacteria counts grouped as ≥ 300 N.U./mL or < 300 N.U./mL, corresponding to the PA DEP thresholds for cyanotoxin analysis [28].

3. Results

3.1. Cyanotoxin Detection

Samples analyzed for cyanotoxins (anatoxin, microcystins, and saxitoxins) were split into groups by those with concentrations above the detection limit (“detected”) and those that were below the detection limit (“non-detected”) (Figure 3). Over half (61%) of the 543 samples analyzed for cyanotoxins between 2018 and 2022 had at least one cyanotoxin detected. Out of all samples with at least one detected cyanotoxin, microcystins were detected most frequently (57% of samples), followed by saxitoxins (53% of samples) and anatoxin (50% of samples). These percentages exceed 100, as a single sample could have tested positive for more than one toxin. Anatoxin and saxitoxins were detected concurrently in 19% of samples, microcystins and saxitoxins in 9% of samples, and anatoxin and microcystins in 8% of samples. All three cyanotoxins were detected in 10% of samples. Sample sizes for comparing detected and non-detected groups were skewed slightly, with more non-detected results than detected results across all three cyanotoxins. Detected samples for each individual cyanotoxin made up between 40 and 44% of cyanotoxin analyses.

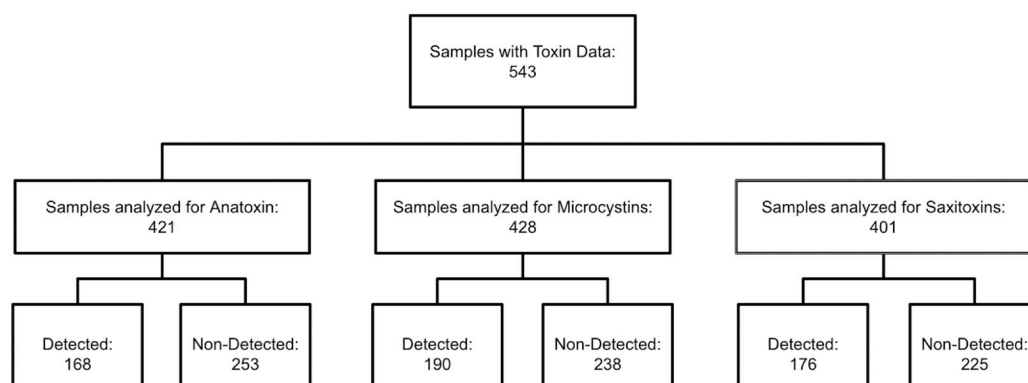


Figure 3. The number of detected and non-detected samples for each cyanotoxin included in the cyanotoxin data subset (left to right: anatoxin, microcystins, saxitoxins). Samples were split into groups by those with concentrations above the detection limit (“detected”) and those that were below the detection limit (“non-detected”).

Detected cyanotoxin concentrations can fall under two management-relevant categories for lakes with recreational primary contact in Pennsylvania: “advisory” or “avoid contact.” An “advisory” category includes cyanotoxin concentrations ranging from the detection limit to the “avoid primary contact” threshold concentration. Microcystin concentrations exceeded the “avoid primary contact threshold” (>8 ng/mL) in 11% of samples analyzed; however, not all these lakes may be managed for primary contact. None of the anatoxin ($n = 421$) or saxitoxin ($n = 401$) samples exceeded the Pennsylvania avoid-contact thresholds.

3.2. Cyanobacteria Counts and Cyanotoxin Detection

A smaller subset of data contained both cyanobacteria counts and cyanotoxin analyses ($n = 412$, Figure 4) as compared to samples with only cyanotoxin analyses ($n = 543$, Figure 3). There was a relatively even distribution of samples across the three cyanotoxins considered in these analyses: anatoxin ($n = 311$), microcystins ($n = 326$), and saxitoxins ($n = 292$). Sample sizes for comparing detected and non-detected groups within each cyanotoxin subset were skewed slightly, with more non-detected results than detected results across all three cyanotoxins. Detected samples for each individual cyanotoxin made up between 41 and 48% of samples.

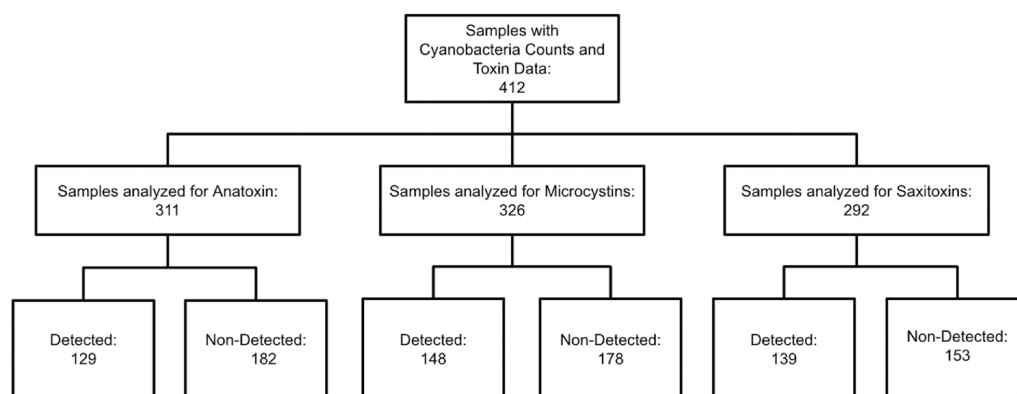


Figure 4. The number of samples containing both cyanobacteria count data and cyanotoxin analyses. Samples were included if they had both cyanobacteria counts and analysis for at least one cyanotoxin. Samples sizes are listed for each cyanotoxin (middle row, left to right: anatoxin, microcystins, and saxitoxins) and the number of samples where toxin concentrations were above the detection limit (“detected”) and below the detection limit (“non-detected”) (bottom row).

Microcystins had the most consistent, positive relationship with cyanobacteria counts among the three cyanotoxin types (Figure 5). Microcystin concentration was positively correlated with increasing cyanobacteria counts (Figure 5F), and samples where microcystins were detected occurred with higher cyanobacteria counts than non-detected samples (Figure 5C). The median difference in cyanobacteria counts between microcystin detected and non-detected groups was 2648 N.U./mL. Saxitoxins and anatoxin did not have a significant relationship with cyanobacteria counts in either analysis (Figure 5A,B,D,E). While detected microcystin samples had significantly higher cyanobacteria counts than non-detected samples, both detected and non-detected samples overlapped throughout the range of cyanobacteria counts (Figure 5A–C). Detected and non-detected samples generally occur at similar frequencies across the range of cyanobacteria counts (0 to 100,000 N.U./mL), with peak numbers of samples for both detected cyanotoxins and non-detected cyanotoxins occurring at the same cyanobacteria density (1000–5000 N.U./mL; Figure 5A–C).

3.3. Cyanotoxin Detection and Environmental Data

This analysis had the smallest subset of data, with 223 samples containing both cyanotoxin analyses and environmental data (water temperature, dissolved oxygen, and/or Secchi disk depth) (Figure 6). Sample sizes were similar when comparing cyanotoxin detection with water temperature ($n = 221$) and dissolved oxygen ($n = 222$); however, the number of cyanotoxin analyses with Secchi disk depth data were less than half this size ($n = 95$). Sample sizes for comparing detected and non-detected groups within each cyanotoxin subset were skewed, with more non-detected results than detected results across all three cyanotoxins (bottom row, Figure 6). For most environmental variables and cyanotoxins, the number of non-detected samples were 1.4 to 1.8 times higher than the respective number of detected samples. The greatest difference in the number of detected and non-detected samples was for microcystin concentrations that also had Secchi disk depth data. In that case, the number of non-detected microcystin samples was 3.3 times higher than the number of detected samples. A single sample, with one set of environmental data, could have been analyzed for all three cyanotoxins and as a result would be included across the cyanotoxins as either detected or non-detected for each specific cyanotoxin.

Samples where saxitoxins were detected had warmer water temperatures than samples without detectable saxitoxins (Figure 7A), with a median difference between detected and non-detected groups of 1.5 °C. However, the relationship between saxitoxins and water temperature was not consistent across analyses. Saxitoxin concentration and water

temperature were not correlated (Figure 7B). This inconsistency across detected/non-detected group analyses and cyanotoxin concentration analyses was similar for anatoxin. There was no difference between median water temperature of detected and non-detected anatoxin groups; however, anatoxin concentration and water temperature were positively correlated (Figure 7C). There was no significant difference in water temperature across microcystin detected and non-detected samples (Figure 7A), and no correlation between microcystin concentration and water temperature (Figure 7D).

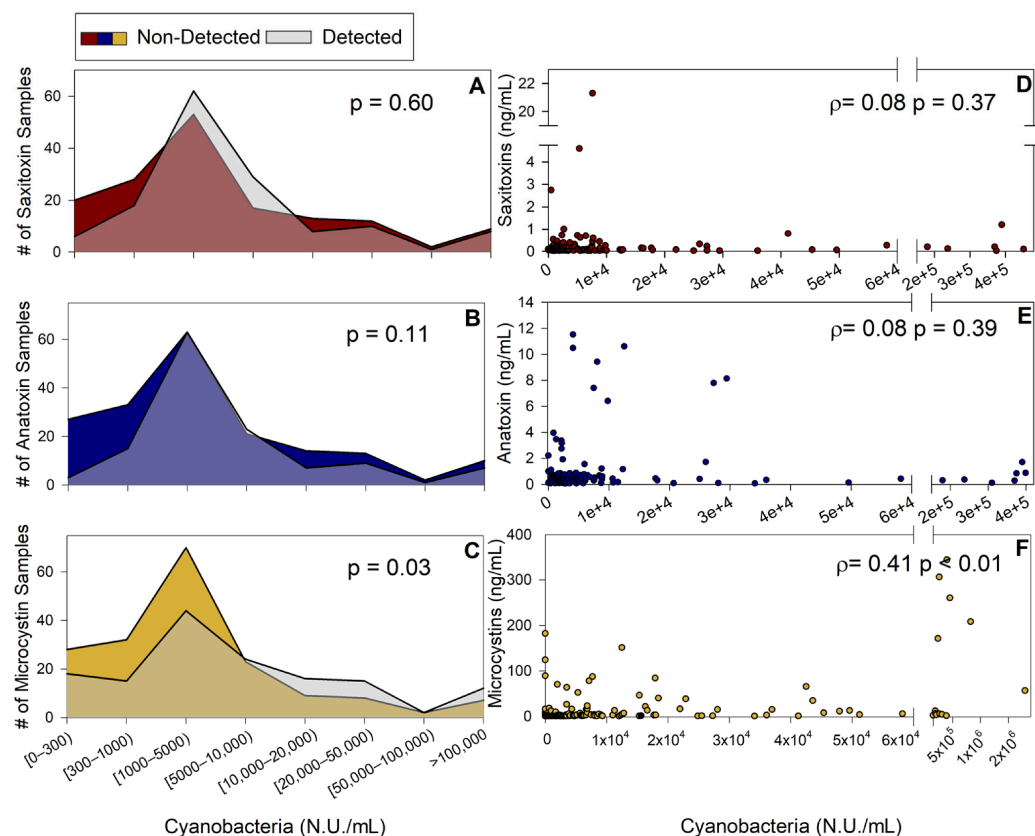


Figure 5. Panels (A–C) represent the distribution of samples with detected cyanotoxins (light gray) and non-detected samples (saxitoxins (red), anatoxin (blue), and microcystins (yellow)) along a continuum of cyanobacteria counts (N.U./mL). p values are of a Mann–Whitney U test comparing cyanobacteria counts between detected and non-detected groups for each cyanotoxin (saxitoxins (A), anatoxin (B), and microcystins (C)). Panels (D–F) represent the relationship between cyanotoxin concentration and cyanobacteria count (N.U./mL) with the rho and p -value of a Spearman’s correlation for each cyanotoxin (saxitoxins (D), anatoxin (E), and microcystins (F)).

There were no differences in DO concentrations between detected and non-detected cyanotoxins (Figure 8A), or significant relationships between cyanotoxin concentrations and DO concentration (Figure 8B–D). There was no evidence of increased cyanotoxin presence co-occurring with increased DO, which can occur with high levels of algal growth in photic conditions. Similarly, there was no evidence of increased cyanotoxin presence co-occurring with high levels of decomposition, respiration, and anoxia. Instead, there was a large amount of variability in DO concentration across cyanotoxin conditions.

Samples with detected anatoxin had shallower Secchi disk depths (lower water transparency) than non-detected samples (Figure 9A). Similarly, samples with detected saxitoxins had lower Secchi disk depths than non-detected samples (Figure 9A). The median difference in Secchi disk depth between sample groups was 0.4 m and 0.2 m for anatoxin and saxitoxins, respectively. However, there were no correlations between cyanotoxin concen-

tration and Secchi disk depth for any of the three cyanotoxins (Figure 9B–D). Microcystins detected and non-detected samples did not have a significant difference in Secchi disk depth (Figure 9A), and microcystin concentration was not significantly correlated with Secchi disk depth (Figure 9C).

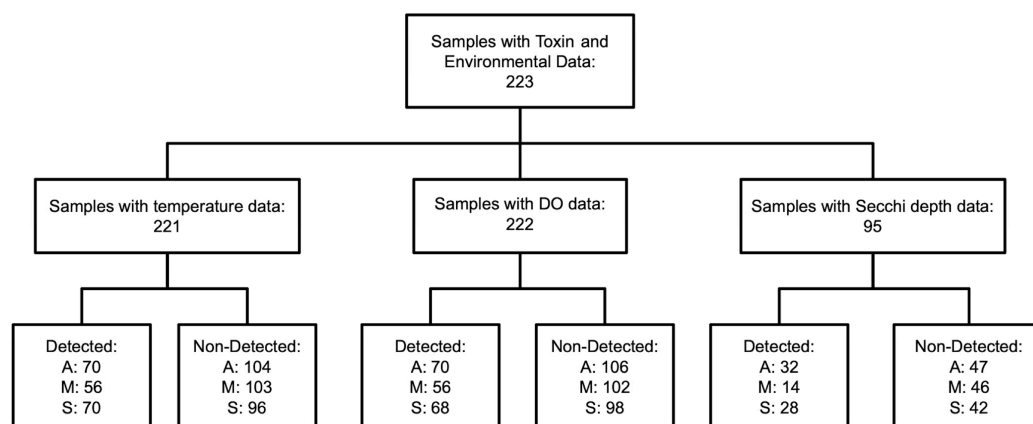


Figure 6. The total number of samples with cyanotoxin analyses and associated environmental data, and the number of samples associated with specific environmental variables (middle row from left to right: water temperature, dissolved oxygen (DO), and Secchi disk depth). The number of samples where cyanotoxin concentrations were “detected” (above detection limit) and “non-detected” (below detection limit) in association with at least one environmental variable for each cyanotoxin are in the bottom row (listed for each cyanotoxin as “A” (anatoxin), “M” (microcystins), and “S” (saxitoxins)). Samples were included if they contained analysis for at least one cyanotoxin and at least one environmental parameter. Note that samples are included in multiple categories if they had more than one type of associated parameter. Similarly, samples analyzed for more than one cyanotoxin may have been counted as detected for one cyanotoxin and non-detected for another.

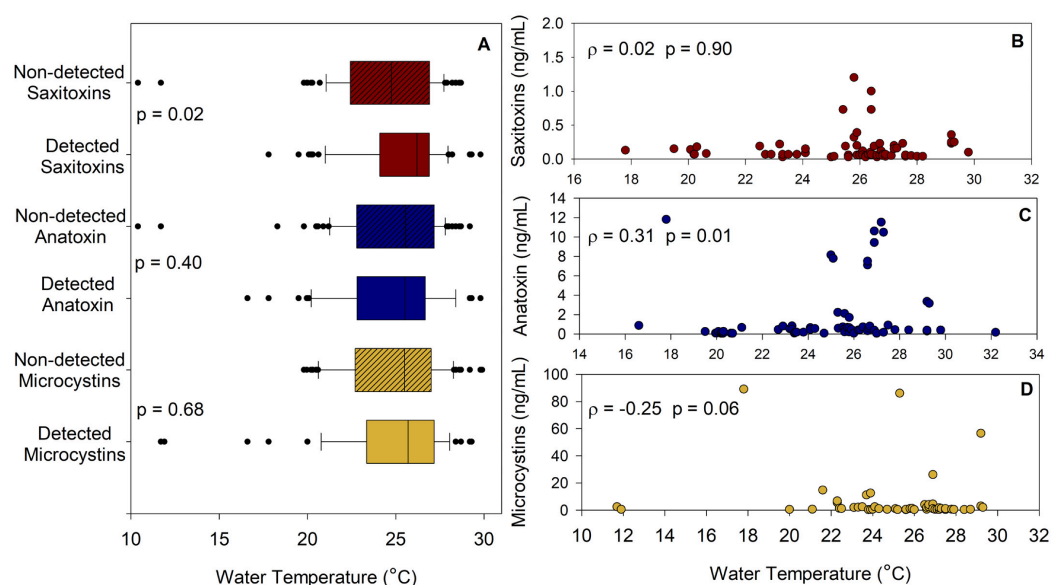


Figure 7. (A) Distribution of samples with detected cyanotoxins (solid boxes) and non-detected samples (cross-hatched boxes) along a continuum of water temperature (°C). Associated statistics represent results of a Mann–Whitney U test comparing water temperature between detected and non-detected groups for each cyanotoxin. (B–D) Relationship between cyanotoxin concentration and water temperature. Associated statistics represent both the rho and p-value of a Spearman’s correlation.

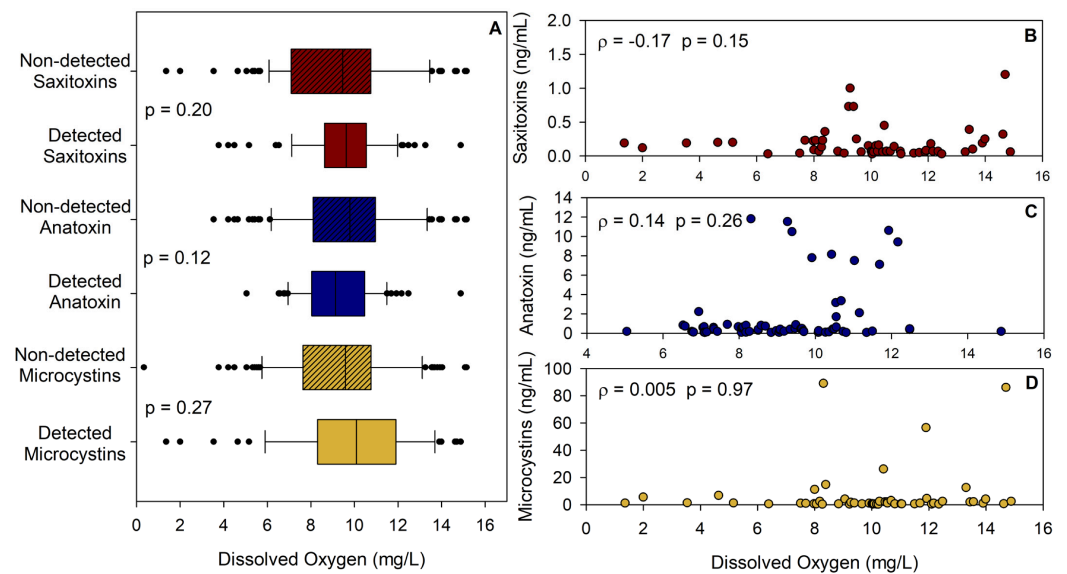


Figure 8. (A) Distribution of samples with detected cyanotoxins (solid-colored boxes) and non-detected samples (cross-hatched boxes) along a continuum of dissolved oxygen concentration (mg/L). Associated statistics represent results of a Mann–Whitney U test comparing dissolved oxygen concentration among detected and non-detected groups for each cyanotoxin. (B–D) Relationship between cyanotoxin concentration and dissolved oxygen concentration. Associated statistics represent both the rho and p -value of a Spearman’s correlation.

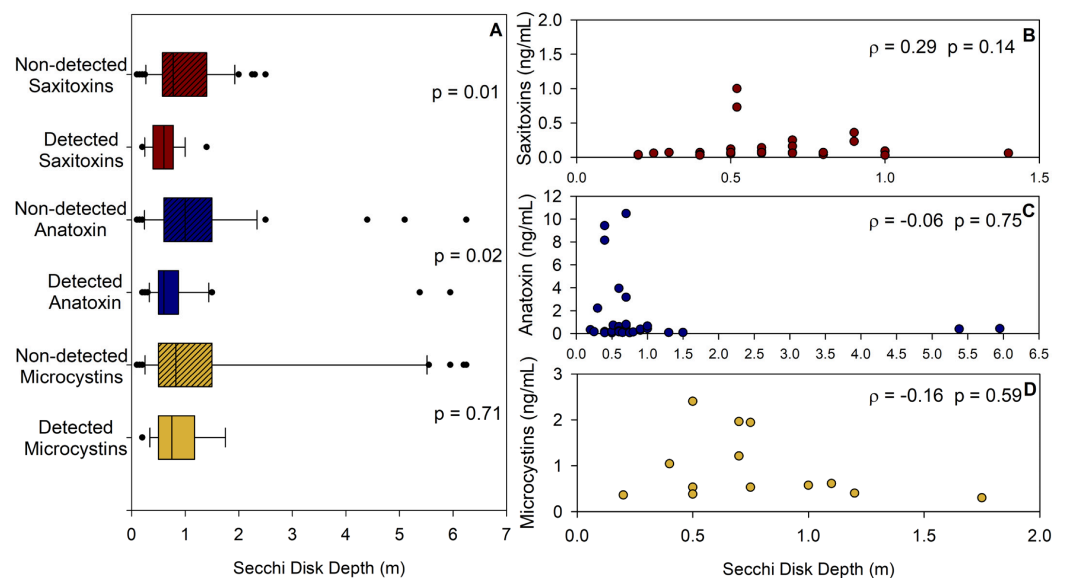


Figure 9. (A) Distribution of samples with detected cyanotoxins (solid boxes) and non-detected samples (cross-hatched boxes) along a continuum of Secchi disk depth (m). Associated statistics represent results of a Mann–Whitney U test comparing Secchi disk depth between detected and non-detected groups for each cyanotoxin. (B–D) Relationship between cyanotoxin concentration and Secchi disk depth. Associated statistics represent both the rho and p -value of a Spearman’s correlation.

3.4. Cyanobacteria Counts and Environmental Data

This subset of data, containing any sample with both cyanobacteria count and environmental data (Figure 10), was the largest out of all subsets in the database ($n = 584$). This is likely because it does not require resource-intensive cyanotoxin analyses. Sample sizes were similar when comparing cyanobacteria counts with water temperature ($n = 304$) and dissolved oxygen ($n = 301$); however, the number of cyanobacteria count samples that also contain Secchi disk depth data was less than half this size ($n = 138$). The number of samples

≥ 300 N.U./mL and <300 N.U./mL was similar across all three environmental variables (Figure 10).

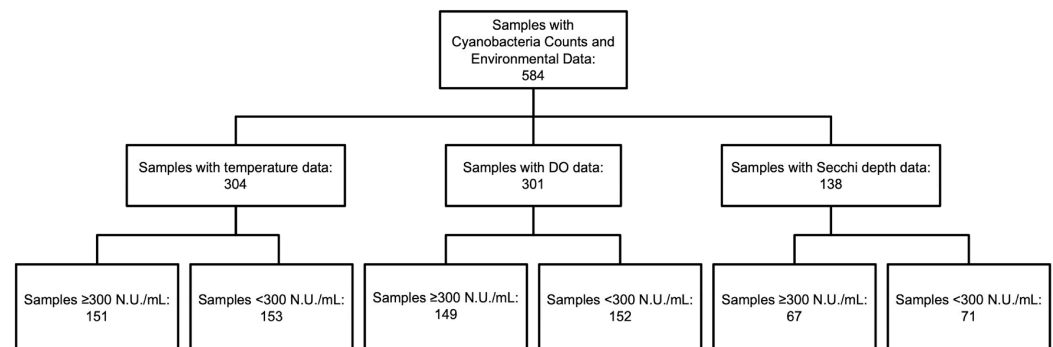


Figure 10. The number of samples with cyanobacteria counts and associated environmental data (middle row from left to right: water temperature ($^{\circ}\text{C}$), dissolved oxygen (DO) (mg/L), and Secchi disk depth (m)). Samples were included if they contained both cyanobacteria counts and at least one environmental parameter. The number of samples was further categorized by counts below the PA DEP advisory threshold of 300 N.U./mL and those above. Note that samples were included in multiple categories if they had more than one type of associated parameter.

Cyanobacteria counts ≥ 300 N.U./mL occurred at warmer water temperatures (median water temperature 1.6°C degrees warmer), higher DO concentrations (median DO 0.7 mg/L higher), and reduced water clarity (median Secchi disk depth 1.0 m shallower) as compared to samples with cyanobacteria counts below 300 N.U./mL (Figure 11A–C). Similarly, cyanobacteria natural unit counts were positively correlated with water temperature (Figure 11D) and DO concentration (Figure 11E) and negatively correlated with Secchi disk depth (Figure 11F).

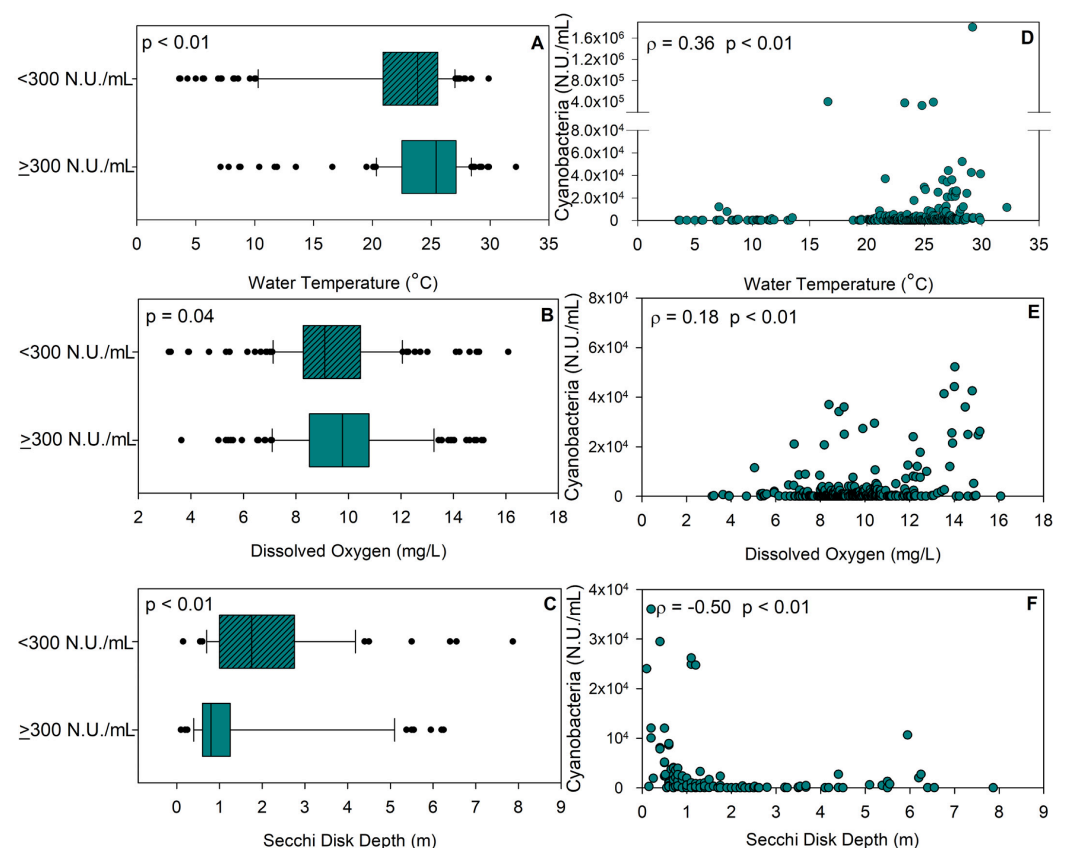


Figure 11. Distribution of samples with cyanobacteria counts ≥ 300 N.U./mL (solid boxes) and

<300 N.U./mL (cross-hatched boxes) along a continuum of (A) water temperature (°C), (B) dissolved oxygen (mg/L), and (C) Secchi disk depth (m). Associated statistics represent results of a Mann–Whitney U test comparing cyanobacteria count categories within each environmental parameter. (D–F) Relationships between cyanobacteria counts and environmental data. Associated statistics represent both the rho and *p*-value of a Spearman’s correlation.

4. Discussion

Detectable levels of microcystins, anatoxin, and saxitoxins were consistently found in lakes during the period of this study. This suggests that a comprehensive monitoring program for Pennsylvania lakes is important to detect this emerging threat to communities and lake health. It is important to note that this monitoring program was not designed to serve as a representative probability-based statistical survey of all Pennsylvania lakes. Instead, it is targeted to sites with potential for community exposure (swimming beaches) or lakes with suspected blooms. In addition, cyanotoxin testing is dependent on cyanobacteria counts greater than 300 natural units/mL. Many CyanoHAB monitoring programs are reliant on prioritization schemes to meet cost and time restraints within management agencies. Long-term monitoring approaches like the one analyzed here can inform management strategies that rely on environmental cues and cyanobacteria enumeration to evaluate risk of cyanotoxin presence.

Over half of the samples analyzed for cyanotoxins had at least one cyanotoxin detected, and 11% of microcystin concentrations exceeded the “avoid primary contact” threshold as defined by interim PA guidelines for lakes with primary contact [35]. Guidelines are an important component to safeguarding community exposure to cyanotoxins. In the absence of federal guidelines, state recommendations for cyanotoxin guidelines vary across the United States. For example, many varieties of cyanotoxins exist; however, microcystins are the most commonly measured and detected cyanotoxins [36]. As a result, some states only regulate microcystins [37]. While microcystins were detected at slightly higher frequencies as compared to saxitoxins and anatoxin in this database (57% detected across the full database as compared to 53% and 50%, respectively) and a small percentage of concentrations exceeded current advisory guidelines, this analysis found similar detection frequencies for both saxitoxins and anatoxin. As this database is comprised of sites with suspected blooms or the potential for community exposure, frequencies of toxin detection may differ in a more probability-based statistical survey of the state. As guidelines continue to be refined for additional cyanotoxins, these analyses suggest that monitoring of anatoxin and saxitoxins is an important component for effective lake management.

As testing for cyanotoxins is resource intensive, many regulatory agencies rely on visual indicators such as scum presence, water clarity [24], and measures of cyanobacteria cell abundance as proxies for cyanotoxin threat [25]. Pennsylvania’s cyanotoxin monitoring program prioritizes samples for cyanotoxin analysis through the enumeration of cyanobacteria via natural unit counts. Understanding the relationship between a cell enumeration method and toxicity is crucial in these instances. There were some significant relationships between cyanobacteria natural unit counts and cyanotoxin concentration, though they were not uniform across all three cyanotoxins. Microcystin concentration was positively correlated with increasing cyanobacteria counts, and samples where microcystins were detected contained higher cyanobacteria counts than non-detected samples. Saxitoxins and anatoxin presence and concentration may be more challenging to predict using cyanobacteria natural unit counts. As cyanotoxin testing is often initiated by cyanobacteria counts in this monitoring program, the more nuanced relationship between cyanobacteria counts

and the production of saxitoxins and anatoxin might be driving a reduced frequency of detection within the database.

Despite a significant relationship between microcystins and cyanobacteria count, the ability to predict toxicity with count data is constrained by small differences in median natural unit counts between samples with and without detectable cyanotoxins. For example, the distribution of detected and non-detected samples overlap throughout the range of cyanobacteria counts included in this database (ranging from 0 N.U./mL to 100,000 N.U./mL). Even at the highest cyanobacteria counts observed in the database, samples with no detectable cyanotoxins occurred in similar frequencies as samples with detectable cyanotoxin concentrations. The peak number of samples for both detected microcystins and non-detected microcystins occurred at the same cyanobacteria density (1000–5000 N.U./mL). The median difference in cyanobacteria counts between samples that detected microcystins and the non-detected group was 2648 N.U./mL. Analysis of this database would suggest that toxicity is equally likely to occur at high and low cyanobacteria counts, especially for saxitoxins and anatoxin. According to DEP protocol, most samples were only analyzed for cyanotoxins if cyanobacteria counts were ≥ 300 N.U./mL; therefore, results presented here are biased towards samples ≥ 300 N.U./mL. Cyanotoxin analysis of samples regardless of cyanobacteria count may reveal additional insights into cyanotoxin production at low cyanobacteria counts [38].

Whether or not cell enumerations are reliable indicators of cyanotoxin concentration is debated in the literature, with some studies finding positive relationships between cyanotoxins and cyanobacteria cell counts [39,40] and others finding that cell counts were not proportional to toxicity [41,42]. Both Loftin et al. (2016) [41] and Christensen (2019) [42] found that using cell counts may overestimate toxic potential since toxic and non-toxic strains are morphologically indistinguishable [17]. Natural unit counts also have the potential to underestimate cell abundance when algal communities are dominated by colonial species as both colonies and individual cells are counted as one natural unit.

In addition to cyanobacteria counts, other visual and environmental indicators, such as reduced water clarity and warming water temperatures, have been considered to initiate cyanotoxin monitoring. In Pennsylvania lakes, increased cyanobacteria counts had predictable relationships with warmer water temperatures, higher DO concentrations, and reduced water clarity. However, cyanotoxins had fewer and more variable relationships with these environmental conditions. This suggests that the environmental conditions promoting cyanobacteria growth may differ from those that promote toxic conditions. In addition, the three cyanotoxins examined in this study may respond differently to similar environmental conditions. There are nuances among these relationships that are important when utilizing environmental conditions to inform CyanoHAB monitoring and management. An expanded set of predictive environmental variables, including nutrient concentrations, would help to clarify these complex relationships. As sample sizes increase with additional monitoring, analyses that explore more complex, non-linear relationships between cyanotoxin concentrations and environmental variables should be considered.

Higher cyanobacteria counts were positively correlated with increasing water temperature and samples ≥ 300 N.U./mL were found at higher temperatures. This finding is consistent with other results reported in the literature, finding elevated biomass above 25 °C [2,43]. Thomas and Litchman (2016) [44] reported that multiple strains, both toxic and non-toxic, had a growth optimum temperature above 28 °C in an experimental setting. However, this relationship of increased water temperature and increased cyanobacteria biomass is not uniform across studies [45] and the direct influence of temperature is not entirely understood [46].

Since the relationship between temperature and cyanobacteria is complex [40,45,47], it is useful from a management perspective to know when higher water temperature is related to increased cyanobacteria presence and if that consistently leads to increased production of cyanotoxins. Unlike previously published literature where water temperature positively increased both cyanobacteria growth and cyanotoxin concentration [20,48], this relationship is more nuanced here. Warmer water temperatures did not always lead to increased cyanotoxins. For instance, saxitoxin detection occurred in warmer water (with no correlation between concentration and temperature) while anatoxin concentration was positively correlated with water temperature (but there were no consistent differences in median water temperature between detected and non-detected groups). The most studied cyanotoxins, microcystins, had no observable relationship to water temperature in this database. In a lab study, Walls (2018) [20] saw reduced microcystins up to 19 °C and increased toxicity above 19 °C, suggesting that water temperature could be used to forecast microcystin bloom severity. It is important to note that toxicity varied widely across water temperature once the higher threshold was reached [20]. The majority of samples in the PA DEP CyanoHABs database were collected when water temperatures exceeded the threshold identified by Walls (2018) [20]. Some studies suggest reduced cyanotoxin production may be a tradeoff for increased reproduction in warmer environments [49]. Walls (2018) [20] also reported an increase in microcystin concentration over a range of 5 °C while biomass declined. These results are similar, where there was a small difference in water temperature (1.5 °C) between detected and non-detected saxitoxins samples. In a field setting, however, these changes may be more difficult to track as water temperature can fluctuate between 4 °C and 7 °C over the course of the day, particularly in shallow lakes [50].

Different environmental factors can regulate cyanotoxin production for specific co-existing toxic strains, but there is variability in which environmental factors are at play across sites [51]. The role of cyanotoxins as secondary metabolites is also not fully elucidated, but it is understood that relationships to environmental parameters can vary across metabolites [52]. Considering other factors in relation to temperature may strengthen our understanding of these relationships. Compared to water temperature, relationships between cyanobacteria, cyanotoxins, and DO were more variable. As with water temperature, higher DO conditions coincided with higher cyanobacteria counts, and increasing cyanobacteria counts were positively correlated to increasing DO. However, both detected and non-detected cyanotoxin samples were associated with well-oxygenated conditions. Relating DO to CyanoHABs and cyanotoxin production can be circular, as CyanoHABs are photosynthesizing during the day while also contributing to high levels of decomposition and respiration that lower DO levels in aphotic conditions. The positive relationship between DO and cyanobacteria counts in the PA DEP database may be related to sample collection, as it often took place in surface waters during daylight hours, biasing towards well-oxygenated conditions. Neilan et al. (2013) [52] hypothesized a link between cyanotoxin production and photosynthesis due to the regulation of cyanotoxin genes and cyanotoxin production by light across cyanobacteria; however, this mechanism did not result in detectable relationships between cyanotoxin concentration and DO across these PA lakes.

Visual indicators have been integrated into many national and community-engaged programs for assessing CyanoHAB occurrence and threat of cyanotoxin exposure [24,53]. Visual indicators can be the sole CyanoHAB assessment tool for smaller inland lake communities that lack the resources for quantitative monitoring. With many regulatory agencies and management protocols relying on cyanobacteria quantification or visual indicators to determine health risk, it is crucial to understand how effective they are as predictors of cyanotoxin detection and concentrations.

As compared to both water temperature and DO, there were more significant relationships between Secchi disk depth and cyanotoxin presence despite a lower sample size in our dataset. Shallow Secchi disk depths co-occurred with anatoxin and saxitoxin detection and, therefore, may be useful in determining cyanotoxin presence for these cyanotoxins. The median difference in Secchi disk depth between detected and non-detected groups was 0.4 m and 0.2 m for both anatoxin and saxitoxins, respectively. There is little published work investigating the accuracy and precision of Secchi disk depth [54], and error values were not reported in the PA DEP Water Quality Monitoring Protocols for Surface Waters [28]. This makes it challenging to understand the application of this finding in a field setting. The state sampling methodology uses a Secchi disk marked at 0.50 m increments, and an additional measuring tape to measure to the nearest tenth place if the Secchi disk depth reading falls between marked increments [28]. The final Secchi disk depth is reported as an average of these values [28], which may misconstrue minute shifts in Secchi disk depth further. These analyses suggest using a Secchi disk depth to assess risk for cyanotoxin exposure, particularly for anatoxin and saxitoxins, could be a helpful tool for lake managers. Secchi disk depth measures are an accessible and easily quantifiable measure of water clarity.

Relating water clarity to cyanotoxin presence is complicated as cyanotoxins have been detected across a range of cyanobacteria counts/algal biomass, which are directly linked to water clarity measures. For example, toxic blooms have been documented in the absence of visible indicators [10,13,55]. Vareli et al. (2015) [10] found an oligotrophic system could have comparable levels of microcystins to a eutrophic one, but with lower algal biomass. More attention is being drawn to toxic bloom events in oligotrophic systems, which are often occurring regardless of water clarity conditions and may be driven by separate factors from those in eutrophic systems [56], such as infrequent nutrient inputs unlocked from shore sites when lake levels rise [57].

5. Conclusions

As CyanoHABs become an increasingly common concern for lake managers and a point of alarm for lake users, Pennsylvania is working to better understand CyanoHAB occurrence across the state and what tools will be useful when making public safety decisions. Cyanotoxins were detected in more than half of the samples in Pennsylvania's monitoring program with anatoxin, saxitoxins, and microcystins detected in similar proportions. Microcystins were the only cyanotoxin with measured concentrations exceeding Pennsylvania's avoid-contact guidelines. Our findings suggest that cyanotoxin production may be driven by different variables than cyanobacteria growth as only microcystin concentrations were positively related to cyanobacteria counts. As cyanotoxin testing in this monitoring program was often initiated only when cyanobacteria counts exceeded 300 natural units/mL, this relationship may be biased to sites with higher algal biomass and suspected bloom conditions.

While increased cyanobacteria counts were related to all environmental variables considered in this study (warmer water temperature, increased DO concentrations, and reduced water clarity), the relationship between cyanotoxins and environmental variables was mixed. There was some evidence to suggest saxitoxins and anatoxin were related to increased water temperature and reduced water clarity, but there was variability across analyses. This variability supports the growing acknowledgement that CyanoHAB management may be most effective when understanding blooms on a site-by-site basis [51]. Long-term, large-scale datasets are valuable assets to make sense of variable patterns in cyanotoxin production that could inform future decisions in CyanoHAB management [58]. We may improve our understanding of CyanoHABs and co-occurring environmental condi-

tions if we can relate toxic samples to a broader range of ancillary data. While the PA HABs database contains a wider range of ancillary data (e.g., pH, specific conductance) than what was considered here, they were collected infrequently. This led to the exclusion of most ancillary data from this study due to a lack of reporting in conjunction with cyanotoxin values. Of particular interest would be nutrient data, due to the demonstrated relationship among nutrient inputs, cyanobacteria growth and cyanotoxin production [40]. Future analyses should include expanded independent variables (including nutrient concentrations) and consider multivariate approaches to achieve a more in-depth understanding of environmental predictors of cyanotoxin occurrence.

Our analyses highlight the challenges to predicting cyanotoxin occurrence due to the biological complexity inherent in cyanotoxin production. While challenging, incorporating complex factors regulating bloom occurrence and severity into monitoring and management strategies is important as regulatory agencies tackle this emerging problem.

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Data Availability Statement: A complete dataset for all CyanoHABs data collected as a part of Pennsylvania's recreation monitoring program can be accessed through the Pennsylvania Harmful Algal Blooms (HABs) Dashboard, which is maintained by the Pennsylvania Department of Health and is accessible at the following link: <https://www.pa.gov/agencies/health/programs/environmental-health/habs-dashboard> (accessed on 30 September 2022).

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Abbreviations

The following abbreviations are used in this manuscript:

CyanoHABs	Harmful Cyanobacteria Blooms
PA DEP	Pennsylvania Department of Environmental Protection
DO	Dissolved Oxygen

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