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Assessing Environmental Factors that Influence Cyanobacterial Blooms in Skinn Lake

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Introduction

Toxic cyanobacterial harmful algal blooms are becoming increasingly observed in the global lake regions (Bullerjahn et al. 2016, and references therein). Specifically, the microbiomes of these blooms, which is the microbial community associated with the algae, are being examined to determine their composition and function in the establishment of the blooms. The main component of many blooms that are studied in the Ohio lakes is a genus of the cyanobacteria known as *Planktothrix.* This genus of cyanobacteria creates toxins and is part of “a global ecological problem that directly threatens human health and crop safety” (Zhang, et al., 2020). *Planktothrix* is the dominant organism in these studied blooms and thrives in low light environments, which gives them an advantage over competing organisms as others do not thrive in similar conditions (Kyle et al., 2015). Within these *Planktothrix* blooms, there is also a co-occurrence of strains that are related but genetically distinct (Sønstebø and Rohrlack, 2011).

The *Planktothrix* strains present in most blooms are closely related by virtue of the rRNA sequences (Sønstebø and Rohrlack, 2011). Indeed, two closely related species inhabit Ohio lakes- *Planktothrix rubescens* and *Planktothrix agardhii.* When compared visually, *P. rubescens* creates a bloom with a reddish-brown tint due to the presence of the red accessory photopigments phycoerythrin while *P. agardhii* is green, so each absorbs different wavelengths of visible light for photosynthesis. Phycoerythrin allows for efficient light absorption in deeper waters (Oberhaus et al. 2007). Despite the visible differences in the species, when analyzed at the molecular level, DNA sequencing of both of these strains shows a very similar genome composition (Humbert and Le Berre, 2001).

Concerning abiotic factors of an ecosystem, these two *Planktothrix* species exist in different environmental specifications. For example, *Planktothrix rubences* tend to be found in parts of an aquatic ecosystem that have low light and low temperatures, while *Planktothrix agardhii* is found in places with higher light and high temperatures (Oberhaus et al., 2007). When the water column of bloom is examined, *Planktothrix agardhii* is located in the shallow sections of the water column while *Planktothrix rubescens* is located deeper in the column (Oberhaus et al., 2007). These species are co-dominant until winter when *Planktothrix agardhii* dies off and *Planktothrix rubences* becomes the dominant strain as it can survive in the colder temperatures (Oberhaus et al., 2007). This scenario shows the differences in adaptability in these two strains regardless of their similar genotypes.

These genetic similarities despite physical differences point to the occurrence of genetic recombination between these strains (Humbert and Le Berre, 2001). It is important to understand the relationship between strains like these because we hypothesize that these two strains may harbor different microbiomes. In blooms where there are multiple types of cyanobacteria, it has been found that the "non-cyanobacterial prokaryotic community" has affected the bacterial dominance and bloom, overall (Zhang et al., 2021). The microbial community associated with a bloom should be known in order to understand how these microbes may assist in bloom formation, persistence, and decline.

The specific environment being examined in this research is Skinn Lake in Norwalk, Ohio. This lake began as a pit where gravel was quarried for road construction and was subsequently landscaped and converted into a privately owned recreational lake. Recently, this lake has been experiencing red blooms during the winter and the toxicity of these blooms is a matter of concern. This bloom has been occurring annually and the current source of the bloom is unknown. It is believed that the microbiome of this bloom may have answers as to what is causing the toxin producing *Planktothrix* to bloom.

For these reasons, it is important to understand what makes up the microbiome of the bloom and to understand what other microbes allow for the growth of a bloom. The ultimate purpose of this research is to find if there is a link between microbiome and growth of *Planktothrix*. First, we need to identify all the members of the microbial community. If samples from lakes with *Planktothrix* blooms are examined, we hypothesize that the microbes found within these samples will change based on the season and bloom presence.

Methods

Skinn Lake is a former gravel quarry located in Norwalk, OH at 41.220783, -82.632246. It has a surface area of approximately 9 hectares, an approximate depth of 2 meters at the dock area and a maximum depth of 12.5 meters.

Image 1- Skinn Lake during the winter

Image 2- Skinn Lake during the spring

Skinn Lake was sampled at the surface with a handheld 1 L bottle from the main dock and the lakeshore on the northeast, northwest, and western sides (see Table 1 for coordinates). Aliquots from each sampling (180 - 300 mL) were passed through a 0.2 um Sterivex cartridge filter (MilliporeSigma) to yield filtered water samples for analysis of dissolved nutrients. The filter cartridges were drained and immediately frozen on dry ice to preserve biomass for DNA isolation and community profiling by 16S sequencing. Whole water was also frozen upon sampling for analysis of total microcystin toxin and total nutrients. Nutrient analysis was performed by Justin Chaffin at The Ohio State University Stone Laboratory on a SEAL AA3 multichannel nutrient autoanalyzer.

Image 3- Collection of samples

Table 1- Collection location data

Skinn Lake	Dock	NorthEast Shore	Dock	West Shore	Dock	Dock
Date	2/24/21	3/12/21	3/26/21	4/7/21	4/7/21	5/27/21
Site	41.220783	41.2206	41.220783	41.222254	41.220869	41.222254
	-82.632246	-82.632029	-82.632246	-82.633647	-82.632222	-82.633647
	Dock	Sediment	West Shore	Dock	NW shore	
Date	6/10/21	6/10/21	6/10/21	11/12/21	11/12/21	
Site	41.222254	41.220706	41.221944	41.222254	41.221968	
	-82.633647	-81.632227	-82.635244	-82.633647	-82.63314	

DNA was extracted from the filtered water samples. Previously collected samples were taken from storage freezers and extracted from the archived filters. DNA extraction was performed using the Qiagen PowerWater DNA Isolation kit, per the manufacturer's instructions. Wash buffer was added to these samples, the samples were vortexed, and then the top layer of the sample was removed and placed in a new test tube. This process was used to purify the DNA from cells and remove proteins, lipids, and RNA. Then through additional cycles of washing, vortexing, and extracting; a final, concentrated DNA sample was taken from each original Skinn lake filter and assayed by UV absorption for the DNA content in each of the samples. Samples containing more than 2 mg pure DNA were then sent to Discovery Life Sciences (Huntsville, Alabama) for 16S rRNA gene sequencing.

When the sequence data from the outside company were returned, the 16S sequences of the bloom biomass were compared using the Microbial Genomic Module within the CLC Genomic Workbench software platform (Qiagen). The data set combined from all samples included 741,400 16S reads from 19,154 microbial OTUs. *Planktothrix* 16S sequences were identified to determine the genetic diversity of the cyanobacterial community within Skinn Lake. The microbiome of Skinn Lake was also examined to determine if the same microbes are in each bloom period or if each bloom period has a completely different set of microbes. The genomic data that was returned was sequenced through the statistical software R to define the microbial environment and diversity of each sample.

Results

There are many different types of toxic cyanobacteria, but the specific species, *Planktothrix rubescens*, was the primary component of the bloom found in Skinn lake. This species is known for the dark, red blooms that it produces (Images 1 and 2). The bloom that occurred in Skinn Lake occurred in the winter months, which is why the microbiomes differ vastly from the summer (non-bloom) months to the winter (bloom) months.

The data collected from lake samples describes the nutrient and toxin data that was present in the lake during collection. Notably, there were pronounced seasonal differences in nutrients. Specifically, levels of nitrate and nitrite were higher in the winter than in the spring with average values of 2.426 umol/L and 0.6805 umol/L, respectively (Table 2). Ammonium levels were higher in the spring than in the winter with average values of 3.637 umol/L and 1.629 umol/L, respectively (Table 2). Dissolved reactive phosphorus (DRP) was very low in winter samples and below detection in the spring (Table 2), indicating a phosphorus-limited system typical of freshwater lakes (Schindler, 1977). Total phosphorus (TP) was measured at higher levels in the bloom vs. outside of the bloom when sampled at the same time (Table 2).

Table 2- Nutrient values from samples

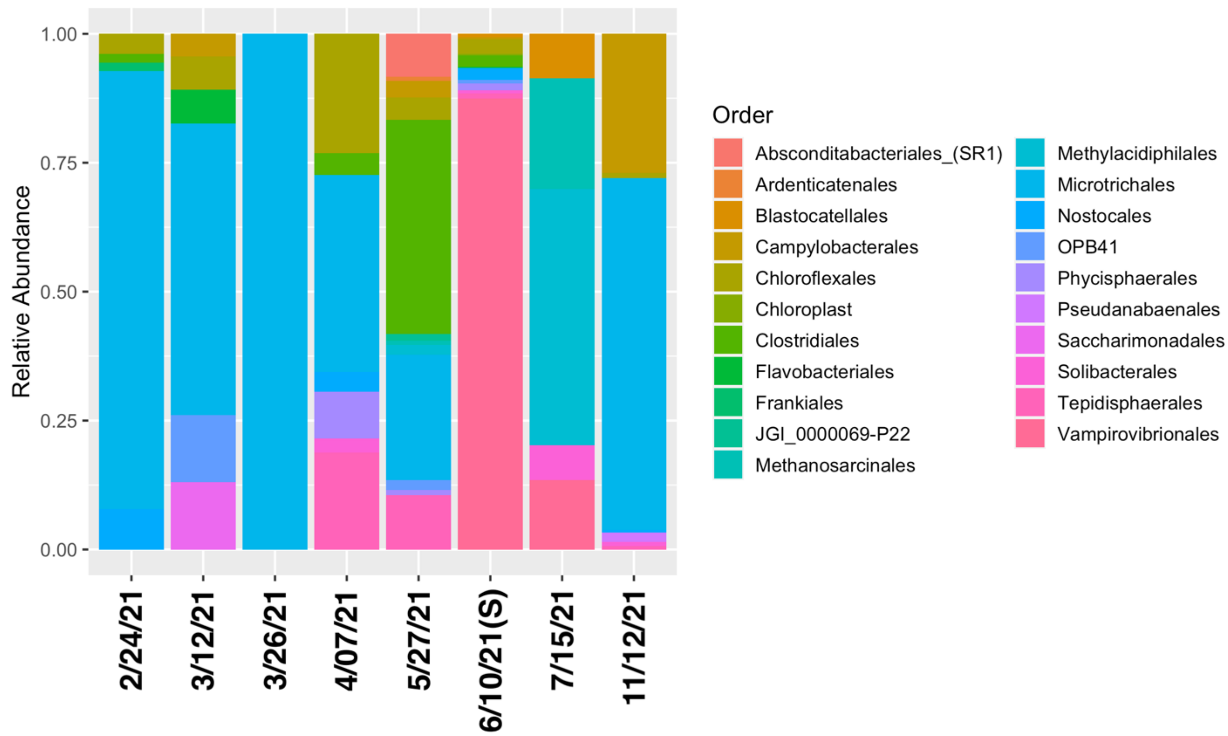
Date	Nitrate+Nitrite (umol/L)	Ammonium (umol/L)	Nitrite (umol/L)	DRP (umol/L)	Silicate (umol/L)
2/24/21	2.381	1.249	0.372	0.018	60.411
3/12/21	2.471	2.008	0.285	0.002	62.467
3/26/21	0	0	0.027	0	71.942
4/7/21	1.283	6.757	0.098	0	70.794
4/7/21	0.078	1.018	0.057	0	66.826
5/27/21	0	3.136	0	0	61.227
7/15/21	0.126	0	0	0	42.44
Date	Nitrate (umol/L)	TP (umol/L)	TKN (umol/L)	TN (umol/L)	TN:TP (molar)
2/24/21	2.009	3.092	62.97	65.351	21.136
3/12/21	2.186	39.64	4438.9	4441.371	112.043
3/26/21	0	1.133	54.104	54.104	47.753
4/7/21	1.185	21.468	894.77	896.053	41.739
4/7/21	0.021	1.029	45.988	46.066	44.768
5/27/21	0				
7/15/21	0.131	0.331	28.416	28.542	86.230

These nutrient values explain in part how the environment of Skinn Lake impacts the bloom, in addition to how the bloom changes the ecosystem it is in. This shows that the blooms preferentially use ammonium over nitrate due to the decreasing levels during the bloom season. Elevated nitrate levels increasing throughout the bloom months support data that *Planktothrix* preferentially assimilates ammonium, and those losses of nitrate due to denitrification is likely suppressed in colder waters as has been documented in other systems (Small et al., 2016).

Results from this data also show how the specific microbiome of the bloom shifts from bloom to non-bloom months. As shown in Figure 1, the order of the microbes in the lake during times of collection varies greatly from periods of bloom to periods of no bloom. The diversity of the lake microbiome increases as time increases, with the most diverse months being May and June (Figure 1). It can also be shown that a sample taken from the shore of the lake had minimal

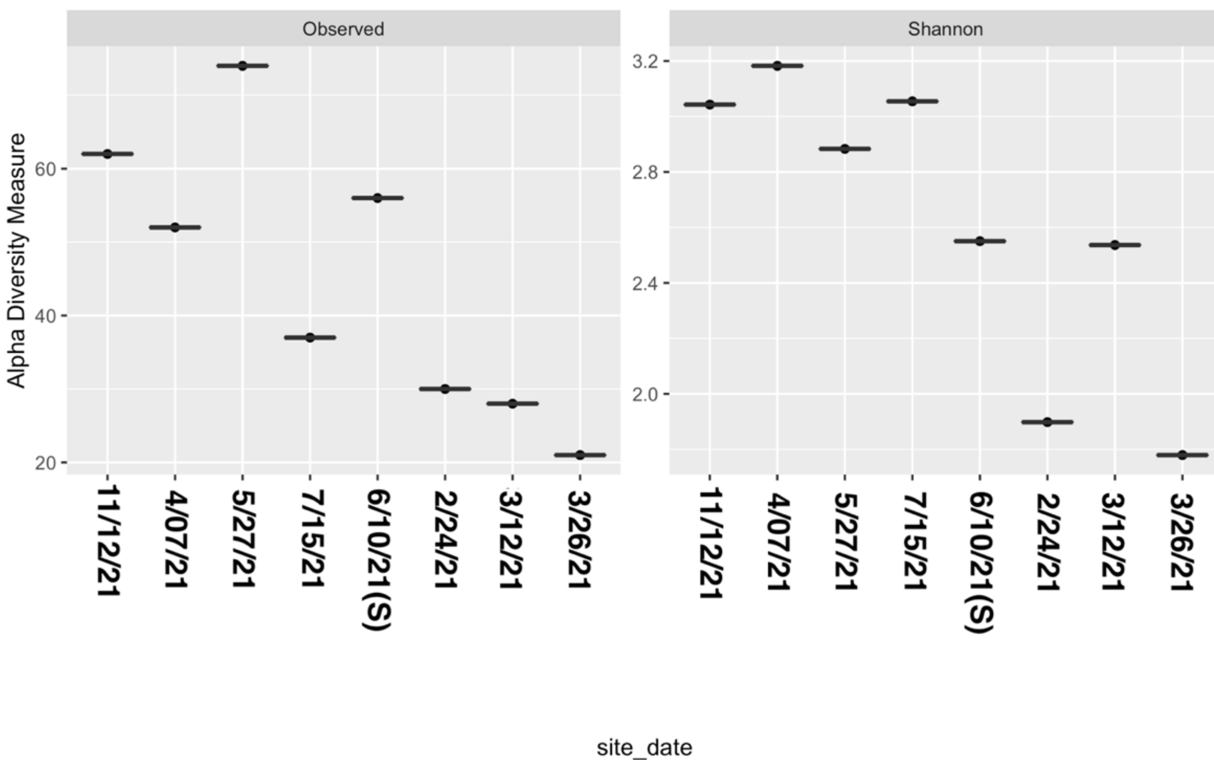
Planktothrix present, but a majority of the population is another type of cyanobacteria (Figure 1). The order *Nostocales (*includes *Planktothrix)* is found in all samples taken throughout the year, with the exception of July (Figure 1). It is important to note that during bloom months, the bacterial population was dominated by *Nostocales (Planktothrix)* (Figure 1).

Figure 1- Relative Abundance of Bacterial Orders by 16S sequencing of lake samples



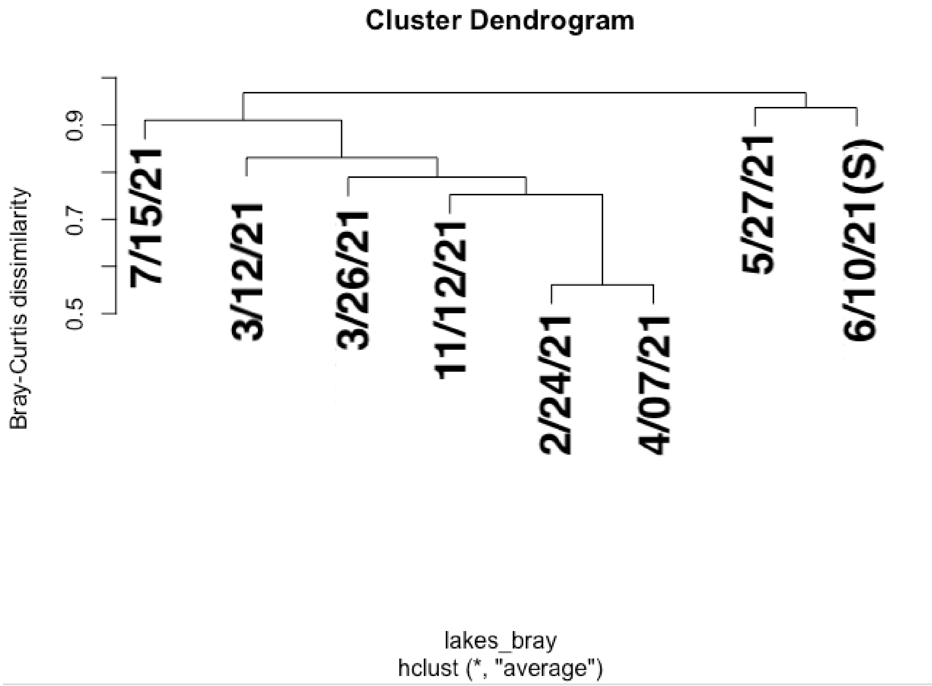
The diversity of Skinn lake can also be better defined through the results of sampling. In terms of alpha diversity, the diversity of a single ecosystem follows a trend: the higher the Shannon index, the higher the diversity of different species in a sample. The Shannon value indicates similarity of species in a community, a value of 0 would represent no diversity or an entire ecosystem made up of only one species. The typical range for these values is 1.5-3.5, with values around 2.5 showing moderate diversity in water samples. In Figure 2, the three lowest Shannon values all fall within the samples taken from the early months (February-March). This shows that diversity decreases when a bloom is present, which can also explain the highest recorded diversity occurring in the months without a bloom (Figure 2). Beta diversity, the measurement of diversity between separate locations over time, shows that values taken when the bloom was present are closely related based on similar diversities (Figure 3). The values taken in the summer vs. the values taken in the winter are not closely related because they do not share similar microbiomes (Figure 3).

Figure 2- Rarified richness of lake samples (Alpha Diversity)



Observed diversity values focus on the number of different species in a sample (richness), while the Shannon diversity values show both richness and evenness (equal distribution) of a sample.

Figure 3- Cluster Dendrogram of lake samples (Beta Diversity)



Bray-Curtis values measure dissimilarity, a value of 0 is completely the same type of species and 1 is completely different types of species.

Discussion

Based on the results of the processed samples, the contents of an algae bloom have been shown to affect the surrounding environment. This is portrayed through the nutrient levels of ammonia and nitrate compared during bloom vs. non-bloom months. Ammonia is used as a preferred N source over nitrate by the bloom community, and withing the blooms there may be other bacteria that use ammonia as both an energy source, yielding nitrate as an end product of ammonia oxidation (Small et al., 2016). This is represented in the data with low ammonia levels in the winter because it is being consumed by the cyanobacteria, and high nitrate levels in the winter because other bacteria may be producing nitrate. Alternatively, nitrate can be consumed as an electron acceptor by denitrifying bacteria (Salk et al., 201), and it may be that denitrification is suppressed during the cold winter months.

Another place where cyanobacterial effects on an ecosystem can be seen is diversity. The diversity of the aquatic ecosystem decreases as the cyanobacteria increases, meaning that these bacteria can be observed as taking control of the environment during times of abundance. It is important to note the harm that cyanobacteria, specifically *Planktothrix*, can have on an ecosystem.

If nothing is done to prevent the further growth of these blooms, the environment can suffer a devastating impact. Cyanobacteria, such as *Planktothrix*, release toxic metabolites into the environment that can travel up the food chain, from the fish that live in the water to the people that eat the fish. As the *Planktothrix* grows in bloom months, it can release the liver toxin, microcystin, and the neurotoxins anatoxins and saxitoxins. These toxins can affect zooplankton, fish, pets, livestock, and humans, so both human health and aquatic food webs can be altered.

Apart from influencing the death of surrounding organisms, these cyanobacterial blooms have influenced the expansion of multidrug resistant microbes that harbor antibiotic resistance genes (ARGs) within the bloom microbiome (Zhang, et al., 2020). A connection between the growth of ARGs and algae blooms related to cyanobacteria has been noted and studied. The results of these studies show that "human activities such as the discharge of domestic sewage and the fertilization of soil by the addition of human and animal feces with subsequent leaching cause freshwater ecosystems to become an important repository for ARGs" (Zhang, et al., 2020). ARGs are able to grow and thrive in these bacterial environments due to the selection of resistant genes that may be found in a bloom (Zhang, et al., 2020). This study also shows that direct harm can be caused by these ARGs from contact or ingestion of products that come from the environment (Zhang, et al., 2020). If nothing is done to prevent the continuous growth of cyanobacterial blooms, not only will human health be threatened now, but it will become increasingly threatened in the future by the spread of ARGs.

To improve the status of the bloom that occurs in Skinn Lake, the source of pollution should be defined. This information would give those in charge of the lake an idea of what nutrients are causing the bloom to grow and create ways to stop the pollution from entering the lake. Another recommendation for Skinn Lake is prohibiting winter contact with the lake. Because of the toxins that are present, animals such as pets and other wildlife could die upon consuming lake water. Humans should also be prohibited from coming into contact during bloom season in order to prevent negative health consequences.

Further research should be done in Skinn Lake to locate possible sites of pollution and how this pollution is feeding the bloom, changing its microbial diversity. More collection sites can be set up in the lake to analyze nutrient layout, possibly directing toward the pollution source. This could be used as a standard for future blooms relating to *Planktothrix rubescens* and other cyanobacterial blooms in lakes such as Skinn Lake.

Conclusion

This study reported on nutrient levels and diversity in Skinn Lake and demonstrated that the chemistry and microbiology of the lake is highly variable during bloom periods vs. non-bloom periods. The goal of this research was to take a first step to determine the microbiomes of a *Planktothrix* bloom in order to better understand the bloom, in general. A bloom cannot be defined unless its environment is defined, so it is important to know what is in a bloom, what it is made of, and the surrounding microbes. These factors lead to a better understanding of why blooms occur and what makes them grow or die off.

Recognizing how to better control a bloom or prevent it from reoccurring can help create safer environments and ecosystems. Identifying the microbes surrounding a bloom and how they affect the bloom and its toxicity can be important to assess if water is safe for consumption and recreation. The consequences that a *Planktothrix* bloom can have on a community are potentially very serious, which is why it is necessary to create a better understanding of these blooms in order to better control and treat the causes. Ultimately, this work will help us understand *P. rubescens* blooms as they occur in other temperate lakes around the world so that effective management decisions can be made to mitigate them.

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