Cyanobacterial Toxicity Expression Within An Interconnected Multi-Lake Suburban Lake System In NY

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Abstract

The most ancient phytoplankton on Earth, cyanobacteria cause toxic algal blooms in freshwater environments (J.M. O'Neill et al., 2012). It is widely acknowledged that harmful algal blooms are complicated phenomena that are usually not brought on by a single environmental factor but rather by a number of interrelated ones (Heisler et al., 2008). This study examines the relationship between the freshwater ecosystem in Lake Hemlock at Mountain Lakes Park, located at a higher elevation (~915 ft above sea level), to those within the three lakes council (Lake Waccabuc, Lake Oscaleta, and Lake Rippowam), located at a lower elevation (~650 ft above sea level), in South Salem, NY. This study focuses on analyzing microcystin and saxitoxin concentrations found in each body of water as a function of the generic diversity and relative abundance of the “dirty dozen” of cyanobacteria, and the phycocyanin and chlorophyll levels present. This study found a positive relationship between phycocyanin levels and microcystin concentration, a significant positive relationship between microcystin concentration and cyanobacteria diversity, and a significant relationship between phycocyanin levels and saxitoxin concentration. This study found a significant similarity in the ecology of freshwater systems located in The Three Lakes Council in South Salem, NY and that Lake Hemlock in Mountain Lakes Park has a different ecology from Lake Waccabuc, Lake Oscaleta, and Lake Rippowam.

Introduction

Freshwater systems are exposed to a variety of chemical, biological, and anthropogenic stressors (e.g climate change, nutrient pollution, and habitat modification) (Cihelio Alves Amorim, et al., 2020). Because of these stressors, certain species are experiencing population losses causing extinction rates to rise thus endangering freshwater biodiversity (Cihelio Alves Amorim, et al., 2020). Communities with greater diversity can maintain improved ecological functioning, leading to increases in productivity, temporal stability, and nutrient retention (Eisenhauer et al., 2019). Ten percent of all animal species and about 35% of all vertebrate species worldwide are supported by freshwater habitats (Stendera et al., 2012). The relative role of freshwater ecosystems in global biodiversity and how that role will change due to the increasing pressure from human activity and changing climate is poorly understood in the scientific community (Bridgham et al., 2014, Mitsch et al., 2013). Therefore, maintaining the services provided by freshwater ecosystems depends on our ability to comprehend how biodiversity trends vary in response to biotic and abiotic stresses and to consolidate our knowledge of these patterns (Tickner et al., 2020).

Algae play a significant role in the aquatic food chain (Shalaby, Emad et al., 2011). These animals provide food for upper trophic levels. More algae in the water implies more oxygen is released into the atmosphere and more carbon dioxide is taken up from the atmosphere (Shalaby, Emad et al., 2011). In order to develop and proliferate, algae need warmth, sunlight, and nutrients (Heisler et al., 2008). The type of algae that exists in freshwater systems can also be a beneficial indicator for a freshwater systems trophic status (Systems, APEC Water). Green algae and diatoms suggest a relatively "clean" oligotrophic lake and bloom-forming blue-green algae indicate a more polluted or eutrophic environment, usually due to the problematic cyanobacteria (Heisler et al., 2008).

Since their genetic material is not arranged in a membrane-bound nucleus, cyanobacteria are categorized as bacteria rather than algae (Burford, M. A., Davis et al., 2012). They use the sun as an energy source and possess chlorophyll, in contrast to other bacteria (Mchau et al.,). They are commonly referred to as "blue-green" since the first cyanobacteria to be discovered had a bluish-green color (Environmental Protection, Maine Department). Not every member, though, is this color. Some have olive or dark green hues, while some even have purplish hues. Surface waters are naturally home to cyanobacteria (Environmental Protection, Maine Department). Even though they are typically very low in abundance, cyanobacteria can experience a phenomenon called blooming, under the right circumstances. This happens when algae multiply quickly and the individual algae form clumps that are visible to the naked eye (Burford, M. A., Davis et al., 2012).

Phycocyanin and Chlorophyll level ratios estimate the relative abundance of cyanobacteria and is a low-cost surrogate for tracking hazardous algal blooms and the trophic condition of a body of water. While chlorophyll pigments are present in a wide variety of species, including plants, cyanobacteria, and algae, phycocyanin pigments are mostly found in freshwater cyanobacteria and are connected to the toxicity of cyanobacteria.

Harmful Algal Blooms (HABS)

In lakes and oceans, harmful algal blooms, or HABS, have existed since the 19th century (Sha, Jun, et al., 2021). When harmful algae "bloom" and proliferate quickly, the surrounding aquatic environment is severely impacted (J.M. O'Neil a, et al., 2011). Some of these "blooms" can create poisons that are lethal to fish, animals, and birds, as well as sicken or even kill humans in severe situations (Burford, M. A. Davis et al., 2012). Hepatotoxins and neurotoxins are the two main types of toxins that cyanobacteria produce. Different types of neurotoxins have varying biological effects on the nervous system (Rutkowska et al., 2019). The genus *Anabaena* and *Aphanizomenon* are known to include cyanobacteria-derived neurotoxins, such as anatoxins and saxitoxins. These neurotoxins can produce drowsiness, burning, numbness in the skin, salivation, confused speech, and respiratory paralysis that can be fatal (US EPA., 2013). One major type of hepatotoxin found in HABs is *Microcystin*. Microcystins are the most commonly acknowledged cyanotoxins and are known to have been associated with *Microcystis*, *Anabaena*, and *Oscillatoria*.

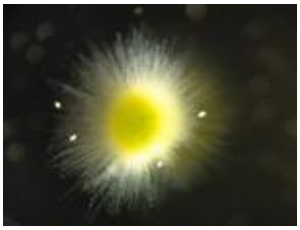
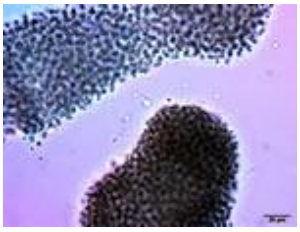
Case studies in varying research locations have shown how some species have evolved due to anthropogenic climate changes, to be larger, more morphologically similar, or more tolerant of drought or extremely high temperatures (such as heat waves, which are maximum temperatures), or short-term droughts, which are lowest precipitation (Kingsolver, Joel G., and Lauren B. Buckley 2017). The timing (phenology) of important biological processes, species distributions, and basal metabolic functioning are all affected by warming (Andrew W. Griffith a b, et al., 2019). Many eutrophic habitats that host recurring HAB's already experience thermal extremes, low dissolved oxygen, and low pH, making these locations potential examples for conditions that will become more common in larger-scale systems as climate change accelerates (Andrew W. Griffith a b, et al., 2019).

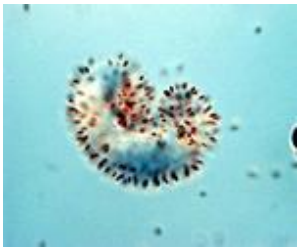
The “Dirty Dozen” Of Cyanobacteria

This study compares cyanobacterial toxicity expression against cyanobacterial population signatures of an undeveloped reference lake within the Mountain Lakes Park against three developed residential lakes within Three Lakes Council in South Salem, NY. The name "The Dirty Dozen” was given by the UNH freshwater ecology lab. The "Dirty Dozen" are collections of cyanobacteria that are frequently seen in New England and produce the highest levels of toxins. Below is a chart showing the “dirty dozen” of cyanobacteria genus, a photo example, and known associated toxins.

Table 1: *The “dirty dozen” of cyanobacteria genus, a photo example, and known associated toxins found in New England.*

	Genus Groups	**Photo Example**	**Associated (known) Toxins**
1	*Anabaena/Anabaenopsis*		anatoxin-a, microcystins
2	*Aphanizomenon*		neosaxitoxin, microcystins
3	*Aphanocapsa/Aphanothece*		microcystins

4	*Coelosphaerium*		microcystins
5	*Gloeocapsa/Chroococcus*		microcystins
6	*Gloeotrichia*		microcystins
7	*Lyngbya/Phormidium*		anatoxin, microcystins
8	*Merismopedia*		microcystins
9	*Microcystis*		microcystins

10	Nostoc		BMAA, microcystins
11	*Oscillatoria/Planktothrix*		microcystins
12	*Woronichinia*		anatoxin, microcystins

Trophic Status Of Freshwater Systems

As a result of runoff from homes and businesses, a freshwater system can get enriched with nutrients (Shalaby, Emad et al., 2011). An algal bloom results from an excess of nutrients being deposited in a freshwater system. When algae grow, they absorb sunlight by blocking it from reaching the water's surface. Algae use photosynthesis to create oxygen, which they then release into the water when they get enough sunshine. When algae are abundant, they will stop producing oxygen and consume it instead. When algae die, bacteria consume algal remains and consume oxygen during the decomposition process. The breakdown eventually results in the water holding less oxygen over time. Toxins are produced and the freshwater system's oxygen levels are reduced further (Andrew W. Griffith et al., 2019). The lack of oxygen and the creation of toxic substances cause the death of aquatic animals (Woodward, Guy, et al., 2010). Large areas of water may turn into hypoxic dead zones, which are places with no oxygen in the

water and cause a mass extinction of aquatic life. If climate change continues to warm freshwater surface temperatures, this could put Lake Waccabuc, which is already eutrophic (Table 2), at a higher risk for hypoxia and potentially decrease the stability of the other two lakes (Lake Oscaleta and Lake Rippowam) that are connected to Lake Waccabuc (Table 2).

Table 2: *CSLAP summary scorecard from Three Lakes Council data report 2022.*

Water quality indicators		Waccabuc		Oscaleta		Rippowam	
		Typical Year	2022	Typical Year	2022	Typical Year	2022
Trophic Status	Phosphorus	Mesotrophic	Eutrophic	Mesotrophic	Mesotrophic	Eutrophic	Mesotrophic
	Chlorophyll	Mesotrophic	Eutrophic	Mesotrophic	Mesotrophic	Eutrophic	Eutrophic
	Secchi	Mesotrophic	Eutrophic	Mesotrophic	Mesotrophic	Mesotrophic	Eutrophic

Generic Diversity and Relative Abundance Of Cyanobacteria

An old but still important question in biology is assessing patterns of biodiversity, or how they differ among taxonomic groups, biomes, and ecosystems (Singer, David, et al., 2020). Assessing patterns in biodiversity gives us a foundation for understanding how ecosystems work and the services that the organisms that live there provide (Singer, David, et al., 2020). Protists appear to comprise a sizable portion of the microbial variety that makes up the majority of life on Earth (Carvalho da Silva and Fernandes et al., 2023). Relative abundance of a species/genus describes how evenly individuals are distributed in a community, whereas genetic diversity refers to the variety of unique inherited characteristics found in a species. For a population to be able to adapt to changing surroundings, genetic variety is essential (Carvalho da Silva and Fernandes et al., 2023).

Study Area

Lakes Waccabuc, Oscaleta, and Rippowam comprise The Three Lakes Council, and are located in the northeast corner of the Town of Lewisboro in New York. The watershed extends into Ridgefield, Connecticut. The lakes have glacial origins, being formed twenty thousand years ago during the Wisconsinan ice period. The three linked lakes are supplied by streams and springs, both intermittent and permanent. In order to manage environmental stewardship for

Lakes Waccabuc, Oscaleta, and Rippowam, the Three Lakes Council was established in 1970 (Andersen, Janet, and Jean Lewis 2020). Native Americans referred to this region as Wepack or Wepuck in early records, and is translated as “Long Pond " (Andersen, Janet, and Jean Lewis 2020). This phrase appears to allude to the grouping of the three lakes rather than any one lake.

According to “Reflections On Our Lake”, the first European inhabitants most likely came in this region before 1730. North Salem and South Salem—later renamed Lewisboro—were formed from the partition of the Town of Salem. Documents started referring to the lakes as North Pond, South Pond, and West Pond based on their respective locations by the 1830s. The Mead family, a wealthy family that occupied the North Pond, developed and marketed “Waccabuc House” as a resort around 1860. The hotel's name was moved to the lake. An early settler called Richard Lawrence changed the name of South Pond to Lake Oscaleta, which is a play on the words "little kiss." The third lake's name, Lake Rippowam, has no known origin. Around 1857, the Mead family constructed the Waccabuc House, which has a view of Lake Waccabuc. In 1895, the Mead family sold the Waccabuc House. In 1896, a fire brought the resort to an end. A boathouse on the lake close to the hotel was the rental boat company for the Lake Waccabuc Boat Corporation. It seemed to be a victim of the Great Depression, yet it carried on operation until 1934. Along the lake, the Mead family constructed boathouses and cottages. They guarded Lake Waccabuc extremely carefully. The land wasn't developed until the Mead family ordered a road survey in 1863 so that plots of land could be sold. On the southern bank of Lake Waccabuc, there was an eight-acre property owned by Robert Hoe III, another member of the Mead family. Lake Waccabuc came to rely heavily on this tract. He constructed a boathouse next to the water. This became the club's beach area when the Meads established the Waccabuc Inn, which would subsequently become the Waccabuc Country Club.

In “Reflections On Our Lakes,” the authors state that in the early 1900s, individuals started constructing cottages on Lake Oscaleta's south side, and the first dwelling on the lake is mentioned in documents dating back to 1882. A boathouse owned by the Rippowam Estate,

which was founded in 1902 by Johathan Bulkley of New York City, is located at the east end of Rippowam. These days, the docks and rights-of-way are maintained by the Lake Waccabuc Association. Additionally, the association hosts social gatherings for its members all year round. According to some geologists, the three lakes were formerly a single, bigger lake due to the glacier's retreat. The three lakes are now hydrologically connected by marshes and tiny streams. The necessity for water to supply a burgeoning New York City led to the creation of the channels between each of the three lakes (Fig. 2).

Long Pond Indians transferred land ownership of the Mountain Lakes Park area to Connecticut landowners in the early 1700s which was then transferred to NY in 1731 as part of the Oblong Lands (Lewisboro Land Trust). This parcen was then purchased by Westchester County as parklands in 1961. There are 1,038 acres in Mountain Lakes Park and has three lakes that are open for fishing, canoeing, and ice skating. The park also has an extensive network of well-marked trails that are open to visitors year-round. Known custody of Mountain Lakes Park is important to this study because it demonstrates that Mountain Lakes Park is a property that was never developed and therefore, shows that Lake Hemlock has different land use than that of Three Lakes Council.

Figure 1. *Lake Hemlock at Mountain Lakes Park is a small, "round", "undeveloped", hilltop, and wooded lake. Lake Waccabuc, Oscaleta, and Rippowam which are part of the Three Lakes Council are larger, "developed", valley bottom, and residential lakes. This map represents each lake that was included in this study.*

As seen in table 3, the lakes in this study vary hydrologically in size, depth, volume, flushing rate, and water residence time. Please note that no available information has been documented on Lake Hemlock's lake but it is important to keep in mind for this study that Lake Hemlock is situated within Mountain Lakes Park in a protected area with no development unlike the lakes within the Three Lakes Council.

Table 3: *Data from The Three Lakes Council 2022 lake survey showing the differences in size, depth, volume, flushing rate, and water residence time (yrs).*

Lake Data	Rippowam	Oscaleta	Waccabuc
Size (acres / ha)	34 /13.7	65 / 26.4	138 / 55.9
Max depth (ft/m)	20 ft / 5.8 m	35 ft / 10.8 m	45 ft / 14.2 m

Mean depth (ft/ m)	13.5 ft / 4.1 m	19.4 ft / 5.9 m	23.3 ft / 7.1 m
Volume (mil gals)	150	412	3696
Hypolimnion volume	<1	61	369
Flushing rate (x/yr)	3.8 – 4.7	2.6 – 3.2	1.1 – 1.4
Water residence time (yrs)	0.2 – 0.3	0.3 – 0.4	0.7 – 0.9

Hypothesis

In a typical year, Lake Waccabuc is usually eutrophic, and if freshwater surface temperatures rise further due to climate change, this might increase the danger of Lake Waccabuc becoming hypoxic and possibly weakening the stability of Lake Waccabuc's two associated lakes, Lake Oscaleta and Lake Rippowam (Table 2). The Citizens Statewide Lake Assessment initiative, or CSLAP, is the main research initiative that supplies the Three Lakes Council with information (Three Lakes Council 2023). The NYS CSLAP program evaluates the quality of lake water around the state. Volunteers sample the lakes' waters and do physical observations. Certified labs get samples of water to analyze (Department of Environmental Conservation 2023). The testing's scope has evolved over time to take into account the DEC's shifting perception of critical variables and the application of visual evaluation in the detection of hazardous algal blooms (Three Lakes Council 2023). Three Lakes Council members then make the annual report available for historical and archiving purposes.

CSLAP provides a detailed analysis of the water quality of the lakes but the results take a few months to get back. In order to determine if an algal bloom is too harmful for recreational use, immediate analysis of the toxicity of a lake will be useful if HABS are expected to occur more often as the climate continues to change. Allowing for different water quality monitoring techniques will also give lake stewards a chance to make faster more effective mitigation

because it will allow for faster analysis between the connections among freshwater ecosystems and if one freshwater system may be influencing another. CSLAP also does not analyze the generic diversity and relative abundance of cyanobacteria within a freshwater system. Using a different monitoring technique like an algal net will give lake stewards an insight on what toxic populations are in a freshwater system.

The dependent (response) variables for this study are (1) Cyanotoxin Profiles– microcystin (hepatotoxin) concentrations and saxitoxin (neurotoxin) concentrations. The independent variables of the study are (2) phycocyanin: chlorophyll levels/ratios. (4) generic diversity/relative abundance of cyanobacteria (4) surface area: volume (or depth) (5) elevation/landscape position. These variables will be related to toxicity within each body of water.

Materials/Methods

Microcystin and Saxitoxin Concentrations

Samples were collected during peak hours of sunlight (12PM-3PM) all within similar atmospheric temperature ranges (80°F/ 26°C and 95°F/ 35°C). This time frame for sampling was chosen because photosynthetic activity within cyanobacteria is at its peak when exposed to sunlight (H. Kenneth Hudnell, et al., 2009). When sunlight warms the water, it facilitates the movement of small organisms like cyanobacteria and speeds up the time it takes for algae to float to the surface.

Due to the potential influence of rainfall on data collection and wind moving cyanobacterial populations to different areas on a body of water, collecting samples was only conducted in dry weather conditions when wind speeds are no higher than 10 mph. Samples were collected every other Sunday from July 10th, 2022 to September 18th, 2022. Every other week the order in which body of water would be sampled from first, rotated. For example, one week Lake Waccabuc would be the first to be sampled, then Lake Oscaleta, Lake Rippowam,

and Lake Hemlock. The next collection date, this order would be randomized. This process reduces variability and the influence of bias within each sample because collecting water samples at the same time of day at the same bodies of water would only be representative of that specific time of day and not be representative of what the water was actually like during peak hours of sunlight (12PM-3PM). All samples were taken from privately owned docks and this was done with the intention of sampling from presumably the “worst” part of each lake where algae scum tends to accumulate (Fig. 1). The goal of this study was to test from areas in the lakes where humans were most likely to be in the water because hepatotoxins can cause liver failure with long-term exposure and this would be the area in the lakes with the greatest toxin amounts (Rutkowska et al., 2019). Samples that were collected from Lake Hemlock were taken from the beach that runs alongside Lake Hemlock (Fig. 2).

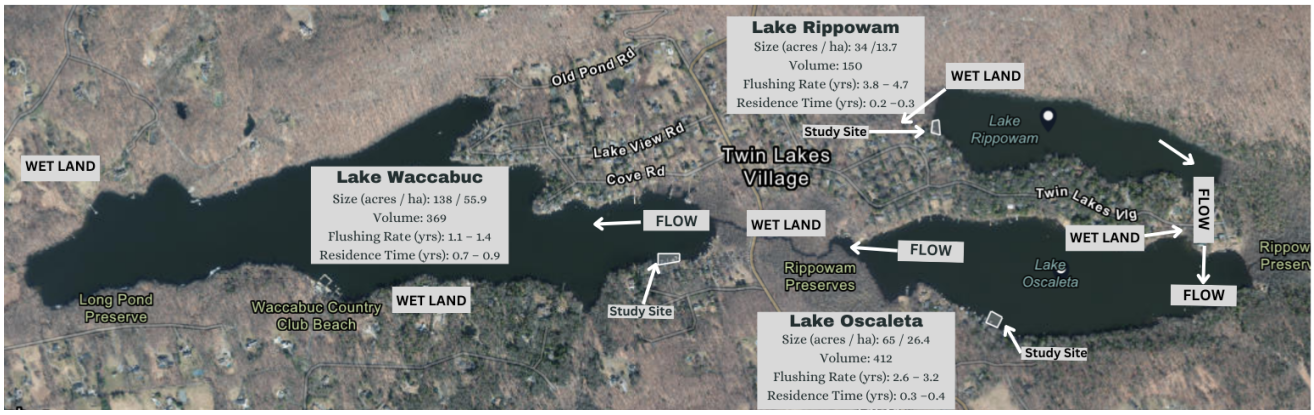


Fig. 2: *Map representing the study site locations, size, volume, flushing rate (yrs), residence time (yrs) and direction of flow of each lake within The Three Lakes Council. Each sample was taken from a privately-owned dock.*

Fig. 3: *Map representing the approximate study site location at Lake Hemlock within Mountain Lakes Park. Each sample was taken from the public beach that runs alongside Lake Hemlock.*

All samples used to determine microcystin and saxitoxin concentrations were stored in the freezer to avoid photodegradation of the algal pigments (Smol, John P., et al. 2001). The ELISA test (Enzyme-Linked Immunosorbent Assay) was used to detect and quantify cyanotoxins (specifically microcystins and saxitoxin) in each of the water samples. During toxin lab analysis, frozen raw water samples were removed from the -20˚C freezer and run through a freeze/thaw cycle twice more for a total of three freeze/thaw cycles. The purpose of the freeze/thaw cycle is to lyse the cyanobacterial cells so potential cyanotoxins can be released. Once the freeze/thaw cycle was complete, the samples were filtered in preparation for the toxin test.

For each individual sample, a clean syringe and a new glass fiber filter membrane was used to prevent contamination among water samples. The lysed (underwent three freeze/thaw cycles) sample was shaken thoroughly. Using a sterile disposable syringe, the sample was drawn up into a plastic syringe up to the 8mL line. A new syringe filter was placed on the tip of the syringe. Before transferring the sample into a clean glass vial, 6 mL of the filtrate was

discarded into a separate beaker. This was done to minimize any debris from getting into the filtrate samples. The remaining 2 mL of the filtrate was plunged into a clean glass vial. These steps were repeated for every water sample that was being tested.

Once all the samples were filtered and ready to be ejected into the microtiter plate, the ELISA kit was removed from the refrigerator and the standards and control were incubated to room temperature (20–22 °C and 68–72 °F) before using them. Multiple pipettes and pipette tips were used by Fisher Scientific along with a prepared Tris wash buffered saline concentrate (Tween 20 and pH 8.0) and a standard timer. The number of microtiter plate strips required were removed. The wash buffer (5x) concentrate was diluted at a ratio of 1:5 with deionized water.

When all materials were prepared, 50µL of the standards, control, and samples were added to wells. The standards and control were used for every test. For every two wells in the same column, one standard, the control, and one sample were added. Once 50 µL of the standards, control, and samples were added to their designated wells, 50 µL of the antibody solution was added to each well using a 100 µL 8 multichannel pipette. When the antibody solution was added to all the wells, the wells were covered with a piece of parafilm, and the well plate was mixed around for 30 seconds in a circular motion. After 30 seconds, the parafilm covered wells incubated for 90 minutes at room temperature. Once the 90-minute incubation was complete, the parafilm was removed and the contents of the wells were discarded into the sink. Then a 300µL 8 multichannel pipette was used to add 250 µL of wash buffer to every row of wells and then the wash buffer was discarded into the sink. This wash cycle was repeated three times.

After washing, the wells were blotted onto paper towels to make sure all contents, debris and dust were removed from the wells. Next, 100 µL of the enzyme conjugate solution was added to the individual wells using the multichannel pipette. Again, the wells were covered with parafilm and stirred around in a circular motion for 30 seconds and after the 30 seconds the wells were left to incubate for 30 minutes. After the 30-minute incubation, the wash step was

repeated three times. After the wash cycle was complete, 100 µL of the substrate color solution was added to the individual wells with the multichannel pipette, covered, mixed, and then left to incubate for 26 minutes. After 26 minutes, 50 µL of the stop solution was added to the wells and the microtiter plate was immediately placed in a microplate ELISA photometer. The absorption was read at 450 nm.

In order to make sure the ELISA test run was successful, multiple parameters were checked to see if the data fit a certain criterion. If the data's R-value for the 4-parameter standard curve fit was greater than 0.990, the run was accepted. If the absorbance for Standard 0 was greater than 0.80, the run was accepted. If the covariation (CV) for the paired absorbency of the control and standards were less than 10% the run was accepted. If the control concentration was between 0.6-0.9 ppb (parts per billion) the run was accepted. Finally, if the CV for the paired absorbency of samples was less than or equal to 15%, the run was successful. If the following data from the ELISA run followed all the following parameters, the toxin test for that run was a success and the concentration data for toxicity was in fact, accurate.

Phycocyanin and Chlorophyll Concentrations

At each lake site, a clean sampling vial was submerged underwater. Once submerged, the top of the sampling vial was slowly opened to let the water inside. The cap was tightly replaced onto the jar and then removed from the water. Each sampling jar was labeled with the name of each body of water and the date the sample was collected and specified whether they were freshwater samples or net collected samples. Sampling vials with raw water samples were brought back to the lab where they were processed through the Cyanoflur for immediate analysis. All samples in the Cyanofluor ran through a filtrate to correct any interference from dissolved organic materials (DOM). A plastic syringe was used to extract the samples from their vial, which were then injected into a glass cuvette. The sides of the cuvette were cleaned with a KIM Wipe following each rinse to get rid of any moisture and/or fingerprints. Raw water sample

material that was leftover in the vial after the Cyanofluor reading was frozen immediately and would stay frozen until toxin lab analysis. PC:CHL (phycocyanin: chlorophyll) ratios were displayed in the first batch of data. The raw fluorescence values (RFUs) for both phycocyanin and chlorophyll were displayed in the following data set. The FTR Blank was the last set of data displayed as CBLK & PBLK. For every sample that was gathered, this process was repeated. When data analysis was completed, samples in the glass cuvette were discarded.

Generic Diversity and Relative Abundance

To determine cyanobacterial diversity and abundance at each lake a plankton net was used to collect a sample and the focus for this sample was to skim the surface of the water. The plankton net was thrown out as far as it could go. Once it was thrown out, the net would skim the surface of the water and collect algae material in a small jar that was attached to the end of the net. All field observations were immediately logged in a journal. When sample collection was complete, the jars from each site were brought back to the lab for microscopy analysis.

Samples collected from the plankton net were left to sit for about 30 minutes to allow any debris to settle. When samples were ready for microscopy analysis, a clean syringe was used to draw up a drop of water where it was then transferred to a clean slide and a coverslip was added to secure the sample on the slide. Standard microscope procedures were used during this process. When cyanobacteria genera were identified visually, one lens cap was removed from the microscope and replaced with an AmScope Camera. The camera was connected to a computer that displayed the image. An image was captured of each cyanobacteria genus that was observed. Species were identified using iNaturalist's list of the Dirty Dozen of Cyanobacteria. To quantify the generic diversity of cyanobacterial populations within each lake a generic point count system was used to determine the total number of each genus of cyanobacteria that were observed within each study lake (e.g genus: Anabaena- Lake Waccabuc total *Anabaena* observed in the study=4). To quantify the relative abundance of

cyanobacterial populations within each lake a generic point count system was used to determine the total number of each genus of cyanobacteria that was observed over the study period (e.g genus: Anabaena- observed 13 times throughout the study).

Results

Phycocyanin Concentrations

Phycocyanin serves as an indicator proxy for HABs and quantifies the biomass of cyanobacteria in freshwater systems (Mchau et al.,). An analysis of phycocyanin levels was done to determine if there was a relationship between phycocyanin levels in each lake.

As seen in figure 4 and 6, at the beginning of the study, all four lakes had relatively low phycocyanin levels at around 1,000-4,000 RFU. Lake Rippowam gradually rose and became the lake with the greatest phycocyanin levels (RFU) on 7/24/2022 then any other body of water. But then dropped to about the same levels as Lake Oscaleta and Lake Hemlock on 8/7/2022. Lake Hemlock is the only one of the four lakes that had consistently low phycocyanin levels (RFU) throughout this study and remained between 1,000-1,500 RFUs. In this study, Lake Waccabuc had the highest phycocyanin levels (RFU) during the entire study period with a significant spike from 8/7/2022 to 9/4/2022 that reached about 18,000 RFU's. That is about 18 x the amount of phycocyanin that Lake Hemlock had the entire study period.

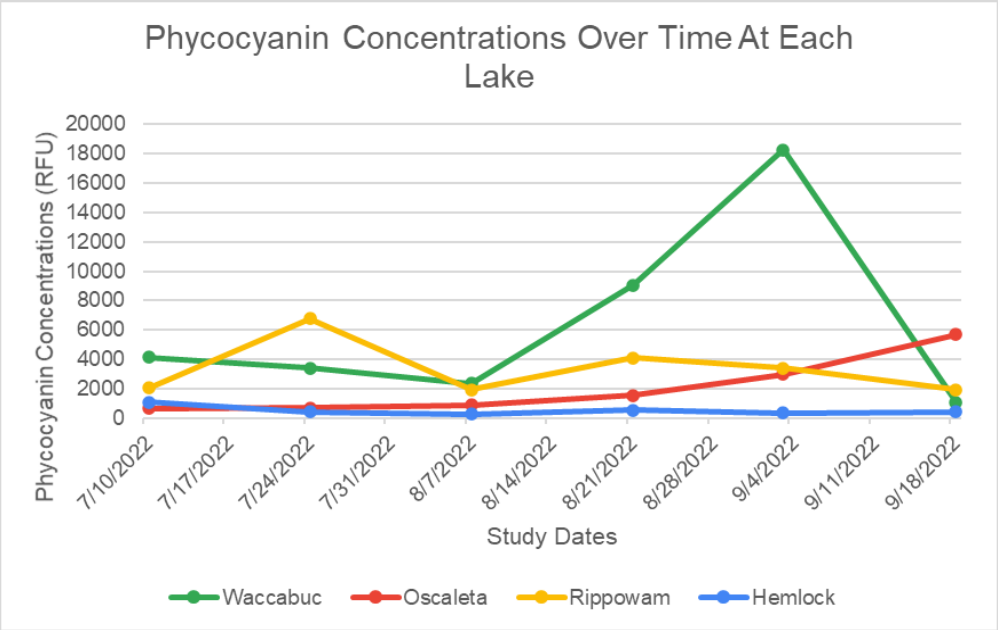


Fig. 4: *Line graph representing the phycocyanin levels over the entire study period at each lake site. Lake Waccabuc had the highest phycocyanin levels (RFU) during the entire study period with a significant spike from 8/7/2022 to 9/4/2022. Lake Hemlock is the only one of the four lakes that had consistently low phycocyanin levels (RFU).*

Then, the phycocyanin levels within the three lakes councils (Lake Waccabuc, Lake Rippowam, and Lake Oscaleta) were averaged and compared with those only at Lake Hemlock. Again, Lake Hemlock remained consistently and significantly lower than the three lakes council's average (Fig. 5 + 7). The three lakes council average showed variation in the phycocyanin levels throughout this study but rose significantly from 8/7/2022 (2,000 RFUs) -9/4/2022 (8,000 RFUs) (Fig. 5 + 7). This was greatly influenced by Lake Waccabuc's coinciding spike that occurred on 9/4/2022 (Fig. 4 + 6).

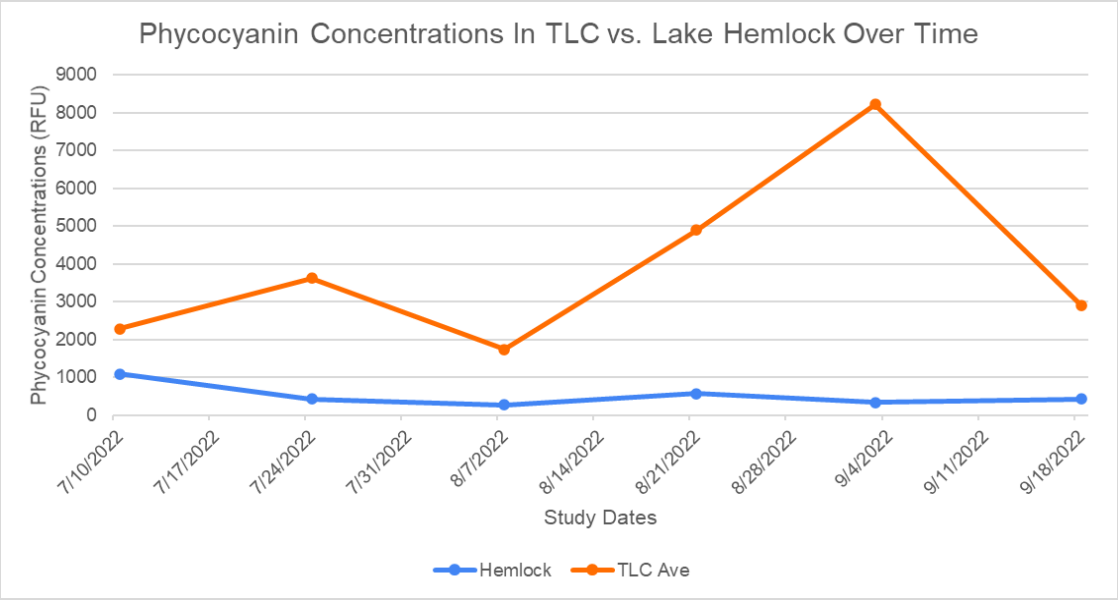


Fig. 5: *Line graph representing phycocyanin levels (RFU) in three lakes council lakes (average of the three lakes phycocyanin levels (RFU) versus Lake Hemlock at Mountain Lakes Park over the duration of the study. The three lakes council average phycocyanin levels (RFU) were significantly higher than Lake Hemlock's levels.*

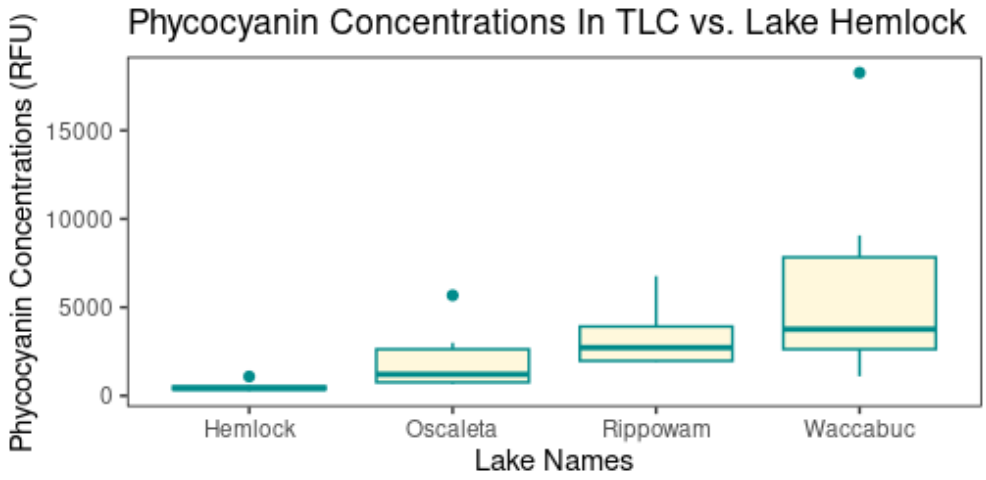


Fig. 6: *Boxplot representing phycocyanin levels (RFU) in three lakes council versus Lake Hemlock at Mountain Lakes Park over the duration of the study. All of the lakes in the three lakes council had higher amounts of phycocyanin when compared to Lake Hemlock. Lake Waccabuc showed the greatest variation in phycocyanin levels when compared to the other three lakes.*

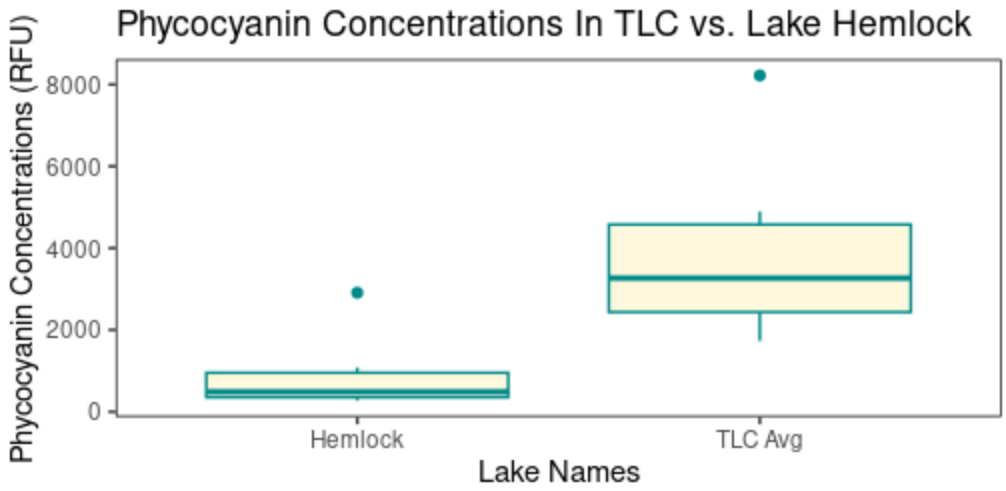


Fig. 7: *Phycocyanin concentrations of the Three Lakes Council averaged against the phycocyanin concentrations within Lake Hemlock. The Three Lakes Council lakes averaged together have a higher phycocyanin concentration throughout the duration of the study when compared to Lake Hemlock.*

Microcystin Concentrations

According to a study conducted by (Preece, Eleen P. et al., 2016), microcystins are short, monocyclic peptides made up of peptide bonds joining seven different amino acids. The peculiar Adda amino acid, which is exclusive to MCs, is frequently linked to the molecule's toxicity.

An analysis of microcystin concentrations were analyzed at each lake during the study period to determine if there was a relationship between microcystin concentrations between each lake. As seen in figure 8 and 10, when comparing microcystin concentrations (µg/l) at each lake site over the duration of the study, all four lakes at the beginning of the study (7/24/2022) had relatively low microcystin concentrations with a slight increase on 8/7/2022. From 8/7/2022 to 8/21/2022 Lake Rippowam and Lake Oscaleta almost doubled their microcystin concentrations. From 8/21/2022 onwards, Lake Rippowam slowly declines in microcystin concentrations and by the end of the study 9/18/2022 falls to almost the same amount as it started the study with (between 0.3 µg/l - 0.6 µg/l). Both Lake Waccabuc and Lake Hemlock had consistently low microcystin concentrations. This is surprising because Lake Waccabuc had the

greatest phycocyanin concentrations (RFU) when compared to the other lakes so it would be assumed that Lake Waccabuc would have greater concentrations of microcystin– but Lake Waccabuc actually has around the same microcystin concentrations as Lake Hemlock does during this study period.

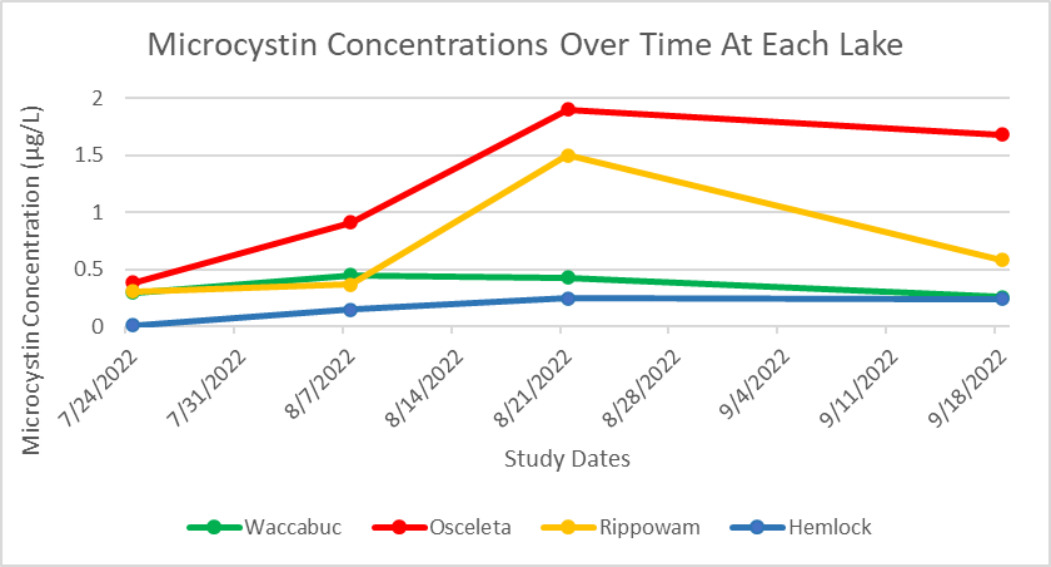


Fig. 8: *Line graph representing microcystin concentrations at each lake for the duration of the study. Lake Oscaleta had the highest microcystin concentrations from 8/7/2022 till the end of the study. Lake Waccabuc and Lake Hemlock had consistently low microcystin concentrations throughout the study period.*

The microcystin concentrations (µg/l) at each of the lakes within three lakes councils (Lake Waccabuc, Lake Oscaleta, and Lake Rippowam) were again averaged and compared against the microcystin concentrations at Lake Hemlock. As seen in figure 9 and 11, Lake Hemlock's microcystin concentrations stayed relatively low throughout the duration of the study (between 0-0.3 µg/l). The Three Lakes Council average microcystin concentration almost doubles between 7/24/2022 and 8/7/2022. From 8/7/2022 to 8/21/2022 the three lakes council average doubles again reaching a total of 3.7 µg/l. This is 4x higher than Lake Hemlock's microcystin concentrations throughout the duration of the study.

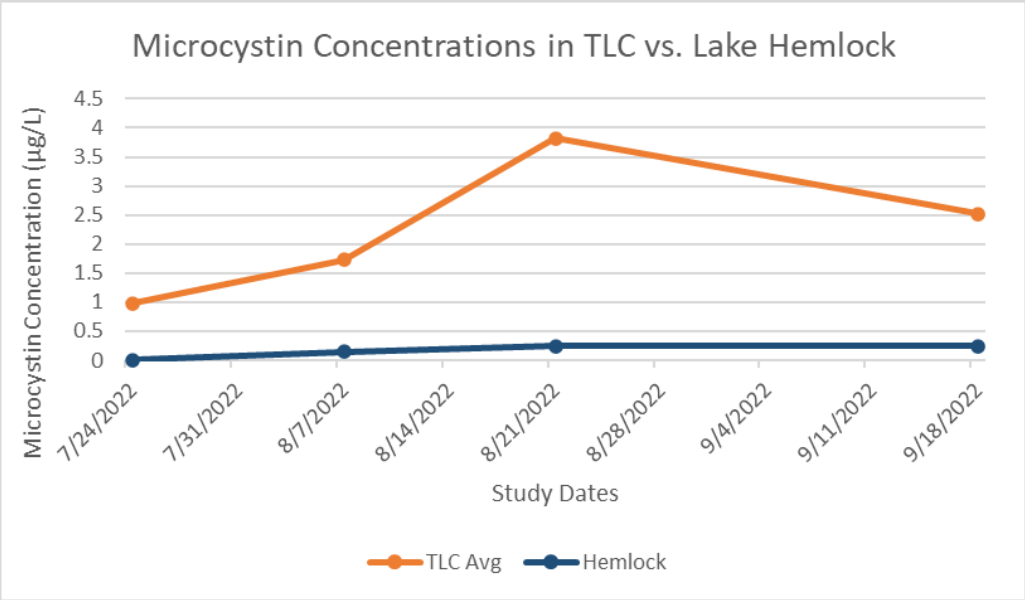


Fig. 9: *Line graph representing the microcystin concentrations in the three lakes council averaged compared to the microcystin concentrations in Lake Hemlock during the duration of the study. The three lakes council average microcystin concentration was higher than Lake Hemlocks during the study period.*

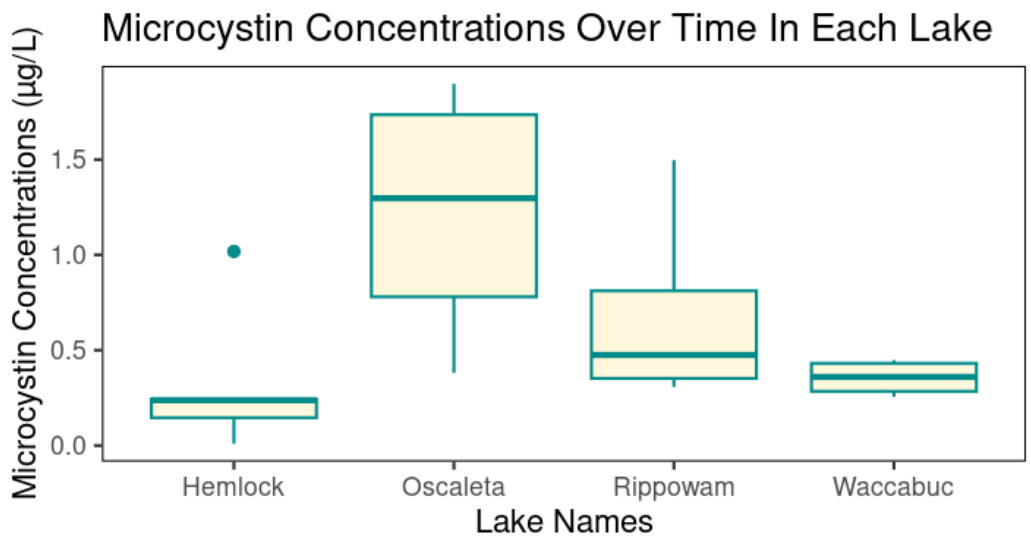


Fig. 10: Boxplot representing microcystin concentrations in each lake site over the duration of the study. *There is little variation in Lake Hemlock's microcystin levels and it remained low during the entire study. Lake Oscaleta's microcystin levels remained high during the entire study but there were variations in the levels within each study date. Lake Rippowam had about the same as Lake Waccabuc's microcystin concentration but showed greater variation throughout the study than Lake Waccabuc did.*

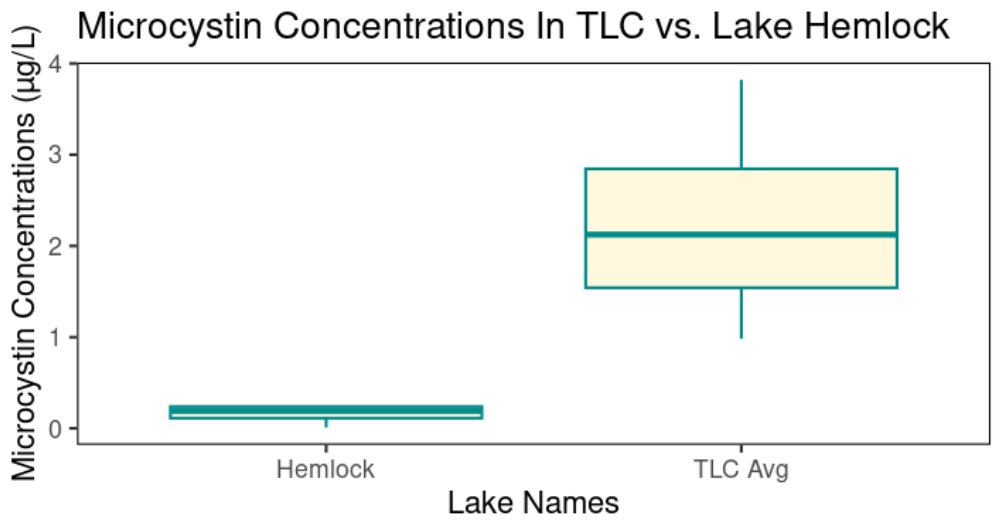


Fig. 11: *Boxplot representing the microcystin concentrations in the Three Lakes council average against Lake Hemlock. The Three Lakes council was about 2.5 x as high as Lake Hemlock and had significantly more variation of the duration of the study period then Lake Hemlock.*

Microcystin Concentrations and Phycocyanin Levels

An analysis of phycocyanin levels (RFU) and microcystin concentrations (µg/L) from the study were compared to determine if there is a universal relationship between phycocyanin levels and toxicity concentrations. As seen in Figure 12, there is a positive logarithmic relationship between phycocyanin levels and microcystin concentrations (y = 0.2118ln(x) - 0.9519) with a R^2 value of 0.1551.

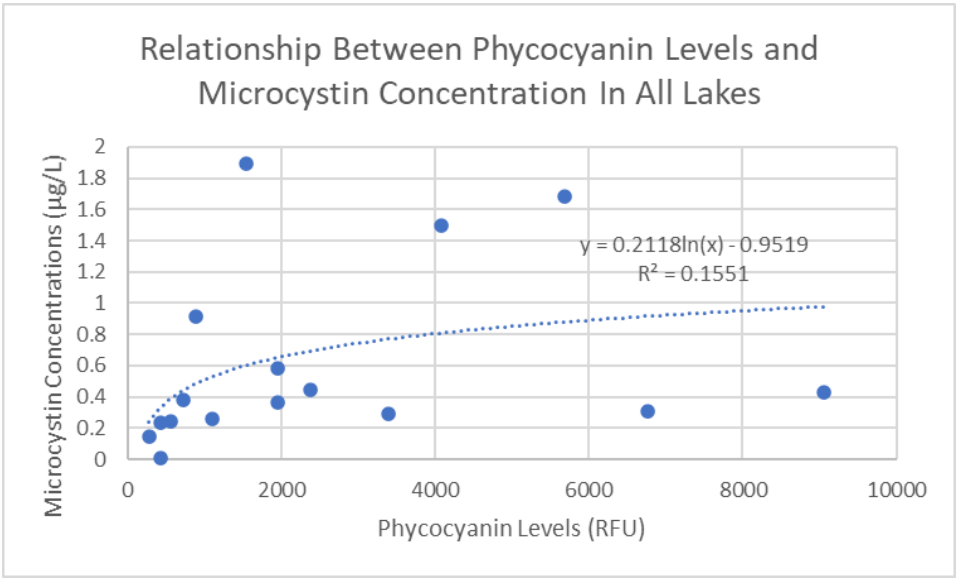


Fig. 12: *Scatter plot representing the logarithmic relationship between phycocyanin levels (RFU) and microcystin concentrations (µg/L) at all lakes over the duration of the study. This analysis revealed that there is a positive logarithmic relationship between phycocyanin levels and microcystin concentrations (y = 0.2118ln(x) - 0.9519) with a R² value of 0.1551.*

Figure 13 represents the logarithmic relationship between phycocyanin levels (RFU) and microcystin concentrations (µg/L) at each lake. The lakes within the Three Lakes Council had a positive logarithmic relationship between phycocyanin levels and microcystin concentrations. Lake Oscaleta had the most significant positive relationship between phycocyanin levels and microcystin concentrations (y = 0.544ln(x) - 2.7716) with an R² value = 0.5237. Lake Rippowam had the second strongest relationship y = 0.0647ln(x) - 0.1615 and R² value of 0.3526, and Lake Hemlock y = 0.1123ln(x) - 0.2178 with an R² value = 0.0154. Lake Waccabuc had the least significant relationship between phycocyanin and microcystin concentrations y = 0.011x0.3614 and an R² value of 0.1096.

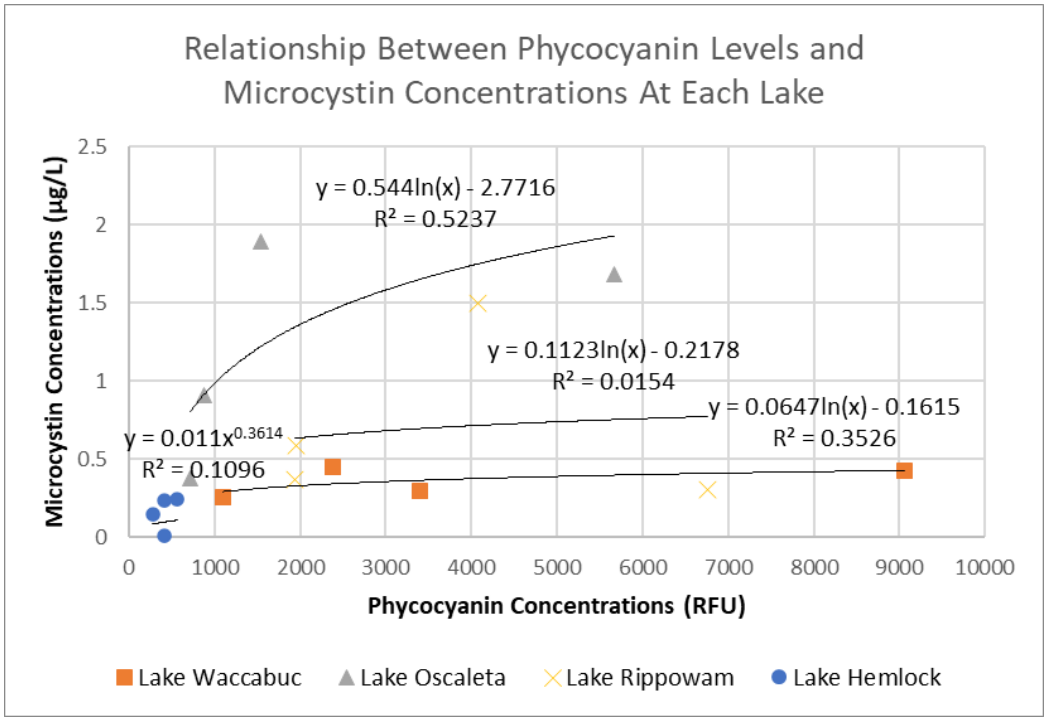


Fig. 13: *Scatter plot representing the logarithmic relationship between phycocyanin levels (RFU) and microcystin concentration (µg/L) at each lake. Lake Oscaleta had a positive relationship between phycocyanin levels and microcystin concentrations (y = 0.544ln(x) - 2.7716) with an R² value = 0.5237. Lake Hemlock had the least significant relationship between phycocyanin levels and microcystin concentrations y = 0.1123ln(x) - 0.2178 with an R² value = 0.0154.*

Microcystin Concentrations and Generic Abundance of Cyanobacteria

A generic point-count system was used to assess the abundance of “the dirty dozen” of cyanobacteria that were present in all of the lakes during the duration of the study. A number was given to each lake to represent how many of the “Dirty Dozen” of cyanobacteria species were present in each lake over the study period. These values were compared against microcystin concentrations (µg/L) at each lake to analyze if there was a relationship between cyanobacteria abundance and microcystin concentration. As seen in Figure 15, when analyzing all of the lakes over the study period, there is no linear relationship between microcystin concentrations (µg/L) and cyanobacteria abundance.

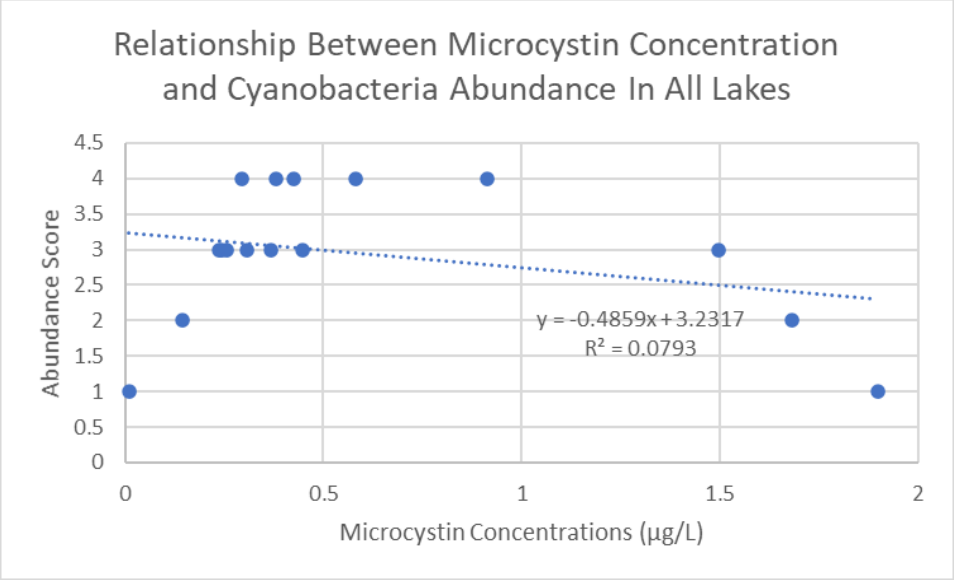


Fig. 14: *Scatter plot representing the linear relationship between microcystin concentrations (µg/L) and cyanobacteria abundance in all lakes over the duration of the study. This analysis revealed that there is no linear relationship between microcystin concentrations and cyanobacteria abundance (y = -0.4859x + 3.2317 / R² = 0.0793).*

When analyzing the microcystin concentrations (µg/L) and cyanobacteria abundance in each lake over the study period, only Lake Hemlock (y = 8.7406x + 0.8581 / R² = 0.9909) and Lake Oscaleta (y = 8.7406x + 0.8581 / R² = 0.9909) had a significant positive relationship between microcystin concentration (µg/L) and cyanobacteria abundance (Fig. 15). While Lake

Waccabuc (R^2 = 0.0024)and Lake Rippowam (R^2 = 0.0155) had a positive but not significant relationship between microcystin concentration (µg/L) and cyanobacteria abundance (Fig. 15).

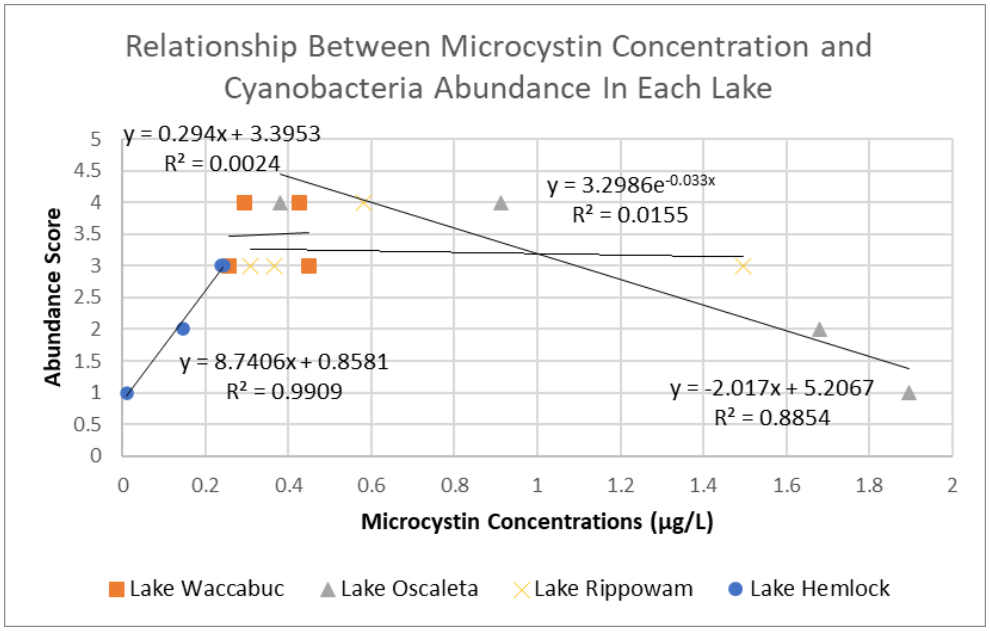


Fig. 15: *Scatter plot representing the linear relationship between microcystin concentration (µg/L) and cyanobacteria abundance in each lake. This analysis revealed that only Lake Hemlock (y = 8.7406x + 0.8581 / R^2 = 0.9909) and Lake Oscaleta (y = 8.7406x + 0.8581 / R^2 = 0.9909) had a strong positive relationship between microcystin concentration (µg/L) and cyanobacteria abundance.*

Microcystin Concentrations and Generic Diversity of Cyanobacteria

To analyze if there was a relationship between microcystin concentrations (µg/L) and generic diversity of the “Dirty Dozen” of cyanobacteria in each lake over the duration of the study, each lakes separate microcystin concentrations from the study period were averaged to determine one number for each lake to represent as its microcystin concentration average. These averages were compared against the total amount of cyanobacteria species that were observed in each lake. As seen in Figure 16, there is a significant positive linear relationship between microcystin concentration (µg/L) and cyanobacteria diversity in all of the lakes during the duration of the study (y = 3.1613x + 4.8358 / R^2 = 0.735).

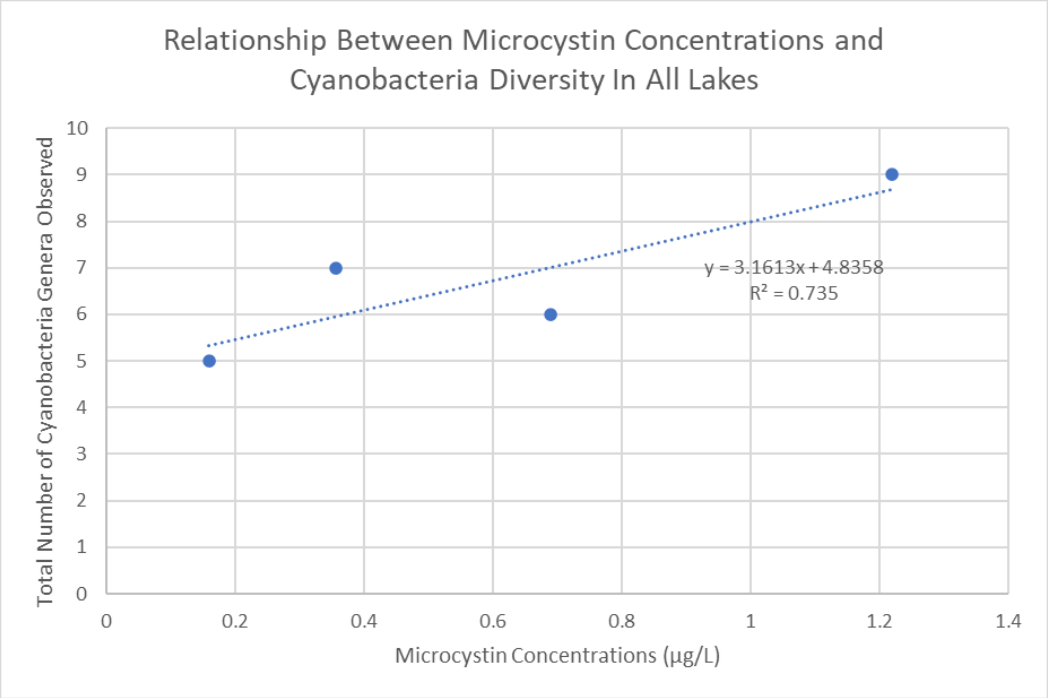


Fig. 16: *Scatter plot representing the linear relationship between microcystin concentration (µg/L) and cyanobacteria diversity in all of the lakes during the duration of the study. This analysis revealed that there is a significant positive linear relationship between microcystin concentration (µg/L) and cyanobacteria diversity in all of the lakes during the duration of the study.*

As seen in Figure 17, when analyzing the linear relationship between microcystin concentration (µg/L) and cyanobacteria diversity in each individual lake during the duration of the study there is a significant positive linear relationship between them. Lake Oscaleta had the most significant positive relationship between microcystin concentration (µg/L) and cyanobacteria diversity (y = 7.782x - 6.564). Lake Waccabuc (y = 6.644x - 6.288) and Lake Rippowam (y = 5.3113x - 4.6225) had similar positive relationships between microcystin concentration (µg/L) and cyanobacteria diversity but were less significant relationships than Lake Oscaleta. Lake Hemlock (y = 4.8408x - 4.6815) had a positive relationship between microcystin concentration (µg/L) and cyanobacteria diversity but was the least significant relationship when compared to the other three lakes.

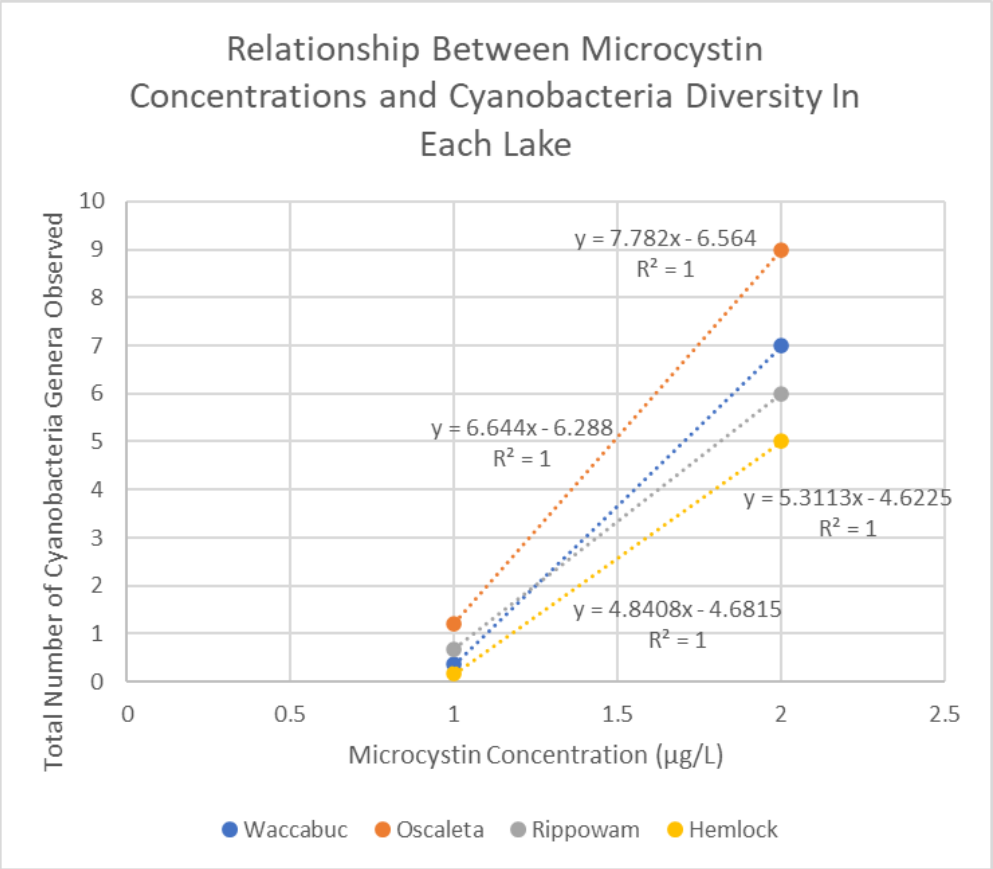


Fig. 17: *Scatter plot representing the linear relationship between microcystin concentration (µg/L) and cyanobacteria diversity in each lake during the duration of the study. This analysis revealed that when observing each individual lake's microcystin concentration (µg/L) against the cyanobacteria present in each lake, that there is a strong positive relationship between them.*

Saxitoxin Concentrations

According to Anderson et al., (1990), saxitoxin is commonly classified as a marine neurotoxin that is produced by *dinoflagellates* and causes paralytic shellfish poisoning in both humans and animals. However, it was discovered in 1995 (Negri et al., 1995) that the freshwater cyanobacterium *Anabaena circinalis* also produced this toxin. Since then, other taxa of widely dispersed freshwater cyanobacteria have been identified as saxitoxin producers.

Potential known producers of saxitoxin include: *Dolichospermum*, *Cuspidothrix*, *Phormidium*, and *Planktolyngbya* (Podduturi, Raju, et al., 2021).

The ecophysiological adaptability of saxitoxin allows it to develop blooms in a wide range of settings, from relatively pristine conditions to contaminated, and from tropical to temperate climates (Ramos, Tanise Klein, et al., 2021). When saxitoxins are released into water, the water becomes a large, potentially significant source of dissolved toxins that could surpass the approximate 3 µg/L WHO guideline threshold (Humpage, Andrew 2017).

In this study, saxitoxin concentrations (µg/L) were analyzed at each of the lakes in the Three Lakes Council and Lake Hemlock on one day 9/18/2022. Figure 18 represents the saxitoxin concentrations in each lake on 9/18/2022. On this day, Lake Rippowam had the highest saxitoxin concentrations when compared to Lake Oscaleta and Lake Waccabuc but Lake Rippowams levels were not significantly higher than the other two lakes. Lake Hemlock's saxitoxin concentration on this day was 0.06 (µg/L) more than Lake Rippowam, which is about 3 times as much saxitoxin as Lake Rippowam.

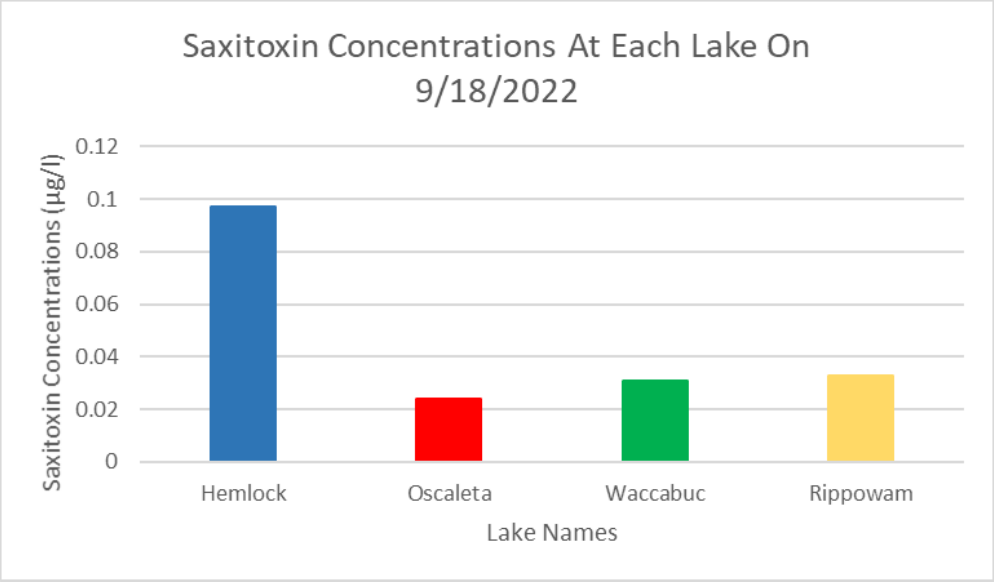


Fig. 18: *Bar graph representing the saxitoxin concentrations (µg/L) in each lake on 9/18/2022. On this day, Lake Rippowam had the highest saxitoxin concentrations when compared to Lake Oscaleta and Lake Waccabuc. Lake Hemlock had about 3 times the amount of saxitoxin than Lake Rippowam did.*

As seen in Figure 19, when analyzing the Three Lakes Council average saxitoxin concentrations against Lake Hemlock's saxitoxin concentration on 9/18/2022 Lake Hemlock had 0.09 (µg/L) more saxitoxin in its water than the Three Lakes council average. These results could suggest biological variation between Lake Hemlock and the lakes within the Three Lakes Council.

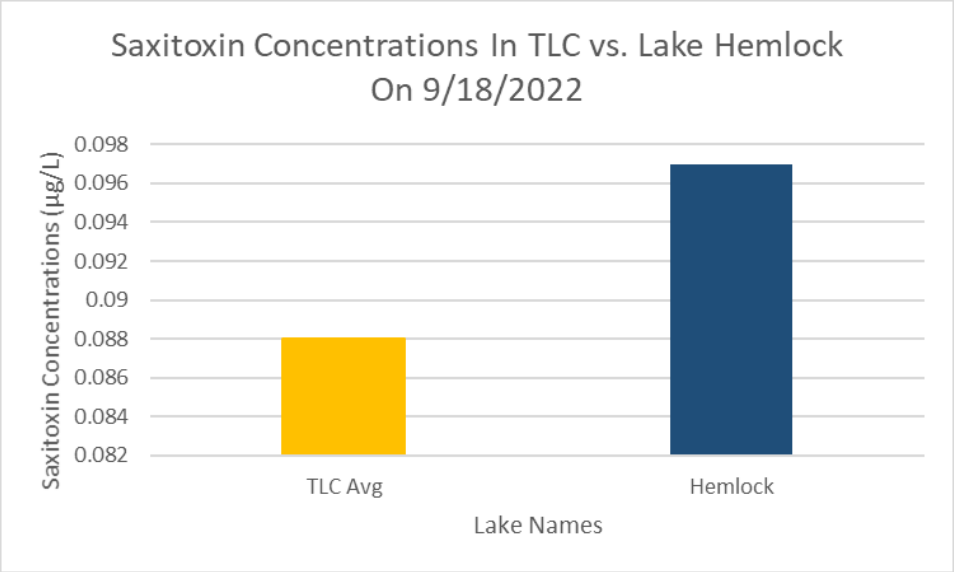


Fig. 19: *Bar graph representing the saxitoxin concentrations (µg/L) in the Three Lakes Council average against Lake Hemlock. Lake Hemlock had 0.09 (µg/L) more saxitoxin than the Three Lakes Council averaged together on 9/18/2022.*

Saxitoxin Concentrations and Phycocyanin Levels

An analysis of phycocyanin levels (RFU) and saxitoxin concentrations (µg/L) from 9/18/2022 were compared to determine if there is a universal relationship between phycocyanin levels and toxicity concentrations. As seen in Figure 20, there is a significant power relationship between phycocyanin levels (RFU) and saxitoxin concentrations (µg/L) in all of the lakes ($y = 9.7104x-1.557$ / $R^2 = 0.7154$).

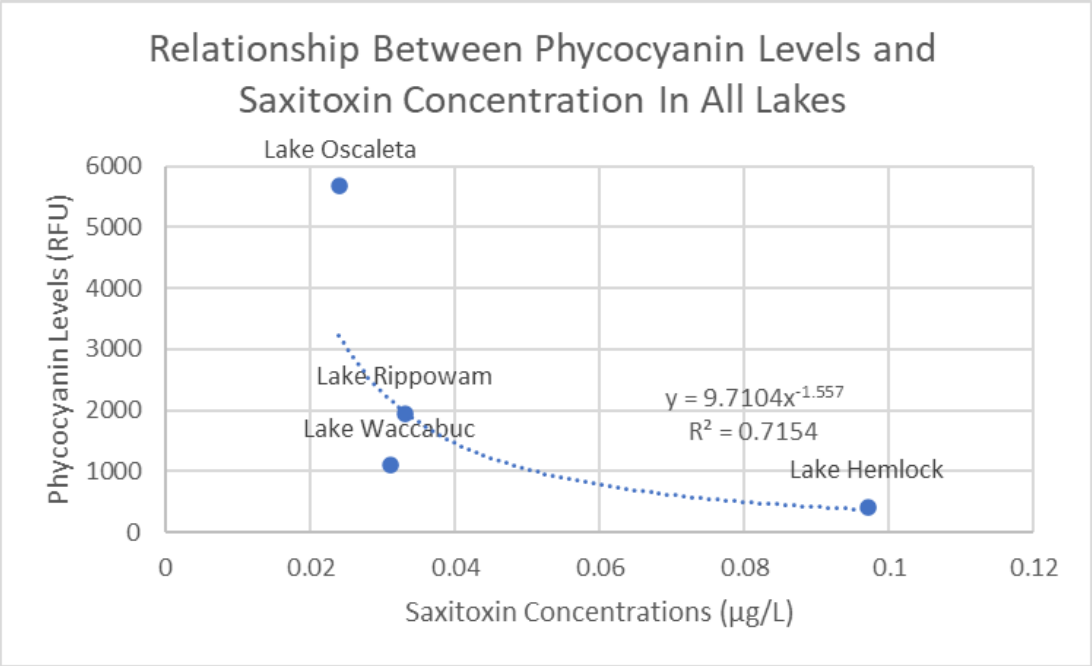


Fig. 20: *Scatter plot representing the power relationship between phycocyanin levels (RFU) and saxitoxin concentrations (µg/L) in all lakes on 9/18/2022. This analysis revealed that there is a significant power relationship between phycocyanin levels (RFU) and saxitoxin concentrations (µg/L).*

As seen in Figure 21, when analyzing the linear relationship between phycocyanin levels (RFU) and saxitoxin concentration (µg/L) in each individual lake on 9/18/2022, there is a significant positive linear relationship between them, with all R^2 values = 1. Lake Oscaleta had the most significant positive linear relationship between phycocyanin levels (RFU) and saxitoxin concentration (µg/L) (y = 5676.3x - 5676.3 / R^2 = 1). Lake Rippowam (y = 1950.2x - 1950.1 / R^2 = 1) and Lake Waccabuc had the second and third most significant linear relationship between phycocyanin levels (RFU) and saxitoxin concentration (µg/L), and Lake Hemlock (y = 417.04x - 416.95 / R^2 = 1) had the least significant linear relationship between phycocyanin levels (RFU) and saxitoxin concentration (µg/L) when compared to the other three lakes.

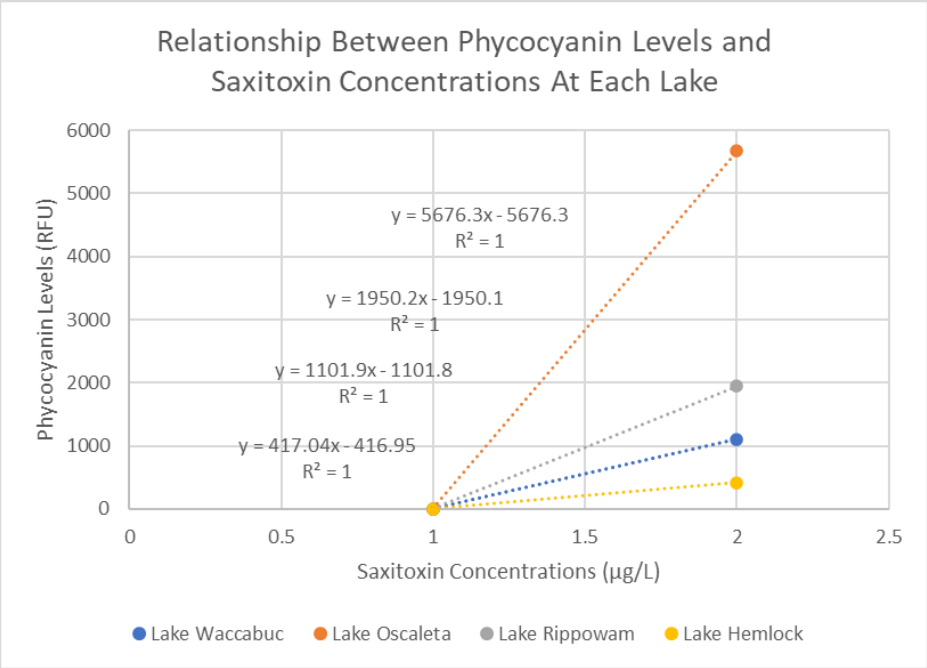


Fig. 21: *Scatter plot representing the linear relationship between phycocyanin levels (RFU) and saxitoxin concentrations (µg/L) in each lake on 9/18/2022. This analysis revealed that there is a significant positive linear relationship between phycocyanin levels (RFU) and saxitoxin concentrations (µg/L) in each lake.*

Saxitoxin Concentrations and Generic Abundance of Cyanobacteria

A generic point-count system was used to assess the abundance of “the dirty dozen” of cyanobacteria that were present in all of the lakes on 9/18/2022. A number was given to each lake to represent how many of the “dirty dozen” of cyanobacteria species were present on this day. These values were compared against the saxitoxin concentrations (µg/L) at each lake on 9/18/2022 to analyze if there was a relationship between cyanobacteria abundance and saxitoxin concentration. As seen in Figure 22, there is no linear relationship between saxitoxin concentrations (µg/L) and cyanobacteria abundance in all lakes on 9/18/2022 ($y = 2.5871x + 2.8803$ / $R^2 = 0.0116$).

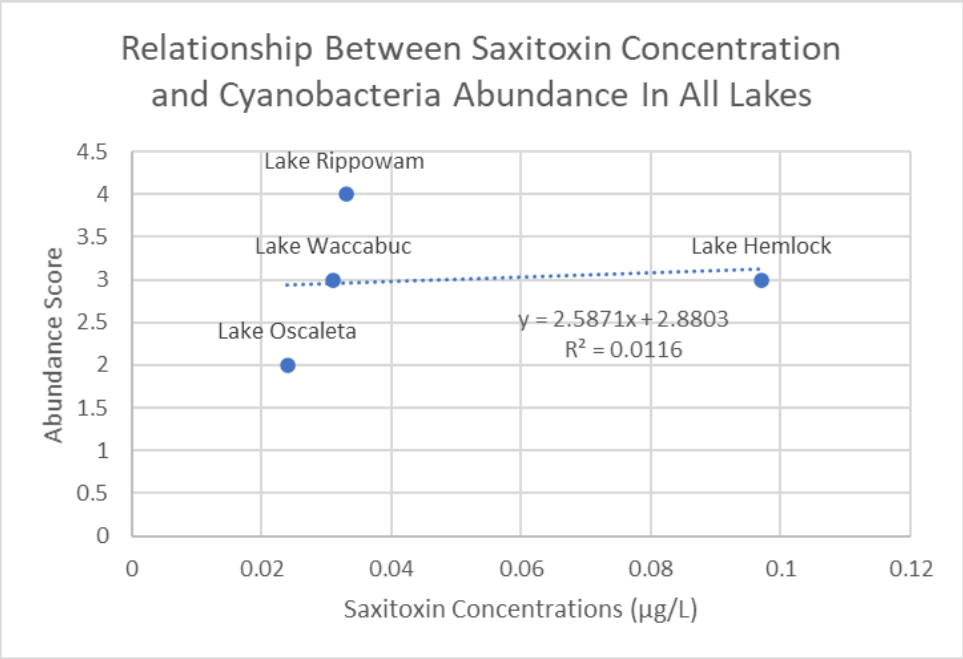


Fig. 22: *Scatter plot analyzing the linear relationship between saxitoxin concentrations (µg/L) and cyanobacteria abundance in all lakes on 9/18/2022. This analysis revealed that there is no relationship between saxitoxin concentrations (µg/L) and cyanobacteria abundance in all lakes on 9/18/2022 (y = 2.5871x + 2.8803 / R² = 0.0116).*

As seen in Figure 23, there is a significant positive relationship between saxitoxin concentrations (µg/L) and cyanobacteria abundance when analyzing each individual lake on 9/18/2022. Lake Rippowam had the most significant positive linear relationship between saxitoxin concentrations (µg/L) and cyanobacteria abundance (y = 3.967x - 3.934). Lake Hemlock (y = 2.969x - 2.938) and Lake Waccabuc (y = 2.903x - 2.806) had almost identical positive linear relationships between saxitoxin concentrations (µg/L) and cyanobacteria abundance. Lake Oscaleta had the least significant positive linear relationship between saxitoxin concentrations (µg/L) and cyanobacteria abundance (y = 1.976x - 1.952).

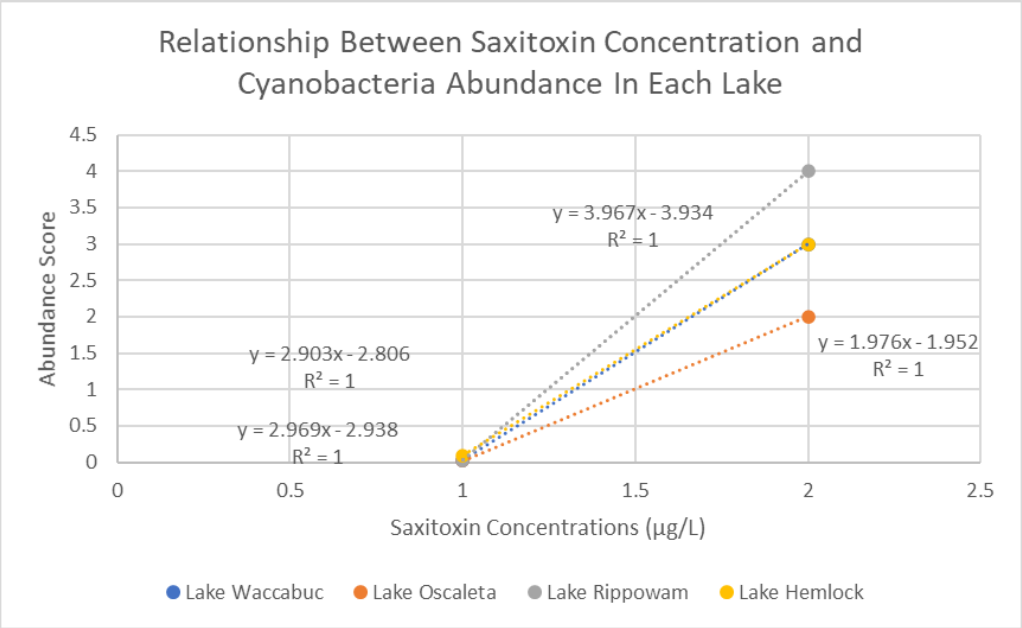


Fig. 23: *Scatter plot representing the linear relationship between saxitoxin concentrations (µg/L) and cyanobacteria abundance in each lake on 9/18/2022. This analysis revealed that there is a significant positive linear relationship between them.*

Saxitoxin Concentrations and Generic Diversity of Cyanobacteria

To analyze if there was a relationship between saxitoxin concentrations (µg/L) and generic diversity of the "dirty dozen" of cyanobacteria in all lakes on 9/18/2022, each lakes saxitoxin concentrations was compared against the total amount of cyanobacteria species that were observed in each lake over the entire study period. As seen in Figure 24, there is no logarithmic relationship between saxitoxin concentrations (µg/L) and generic diversity of the "dirty dozen" of cyanobacteria in all lakes on 9/18/2022 ($y = -0.349\ln(x) + 5.6203$ / $R^2 = 0.016$).

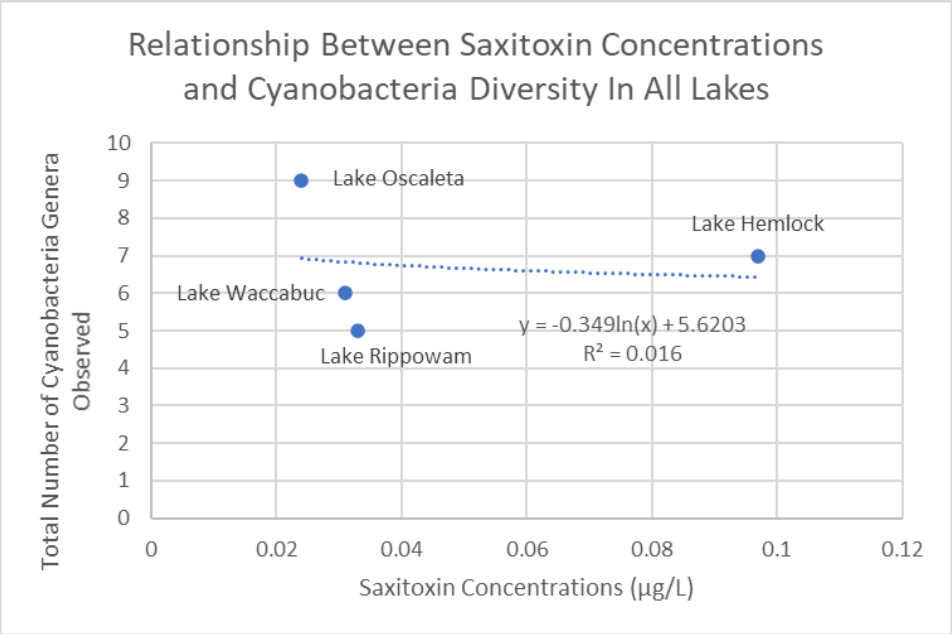


Fig. 24: *Scatter plot representing the logarithmic relationship between saxitoxin concentrations (µg/L) and generic diversity of the "dirty dozen" of cyanobacteria in all lakes on 9/18/2022. This analysis revealed that there is no relationship between saxitoxin concentrations (µg/L) and generic diversity of the "dirty dozen" of cyanobacteria in all lakes on 9/18/2022.*

As seen in Figure 25, when analyzing the linear relationship between saxitoxin concentration (µg/L) and cyanobacteria diversity in each individual lake on 9/18/2022 there is a significant positive linear relationship between them, with all R^2 values = 1. Lake Oscaleta had the most significant positive linear relationship between saxitoxin concentration (µg/L) and cyanobacteria diversity ($y = 6.903x - 6.806$ / $R^2 = 1$). Lake Hemlock ($y = 4.967x - 4.934$ / $R^2 = 1$), Lake Waccabuc ($y = 8.976x - 8.952$ / $R^2 = 1$), and Lake Rippowam ($y = 5.969x - 5.938$ / $R^2 = 1$) all had similar positive linear relationships but Lake Hemlock was the most significant relationship when compared to the other two lakes.

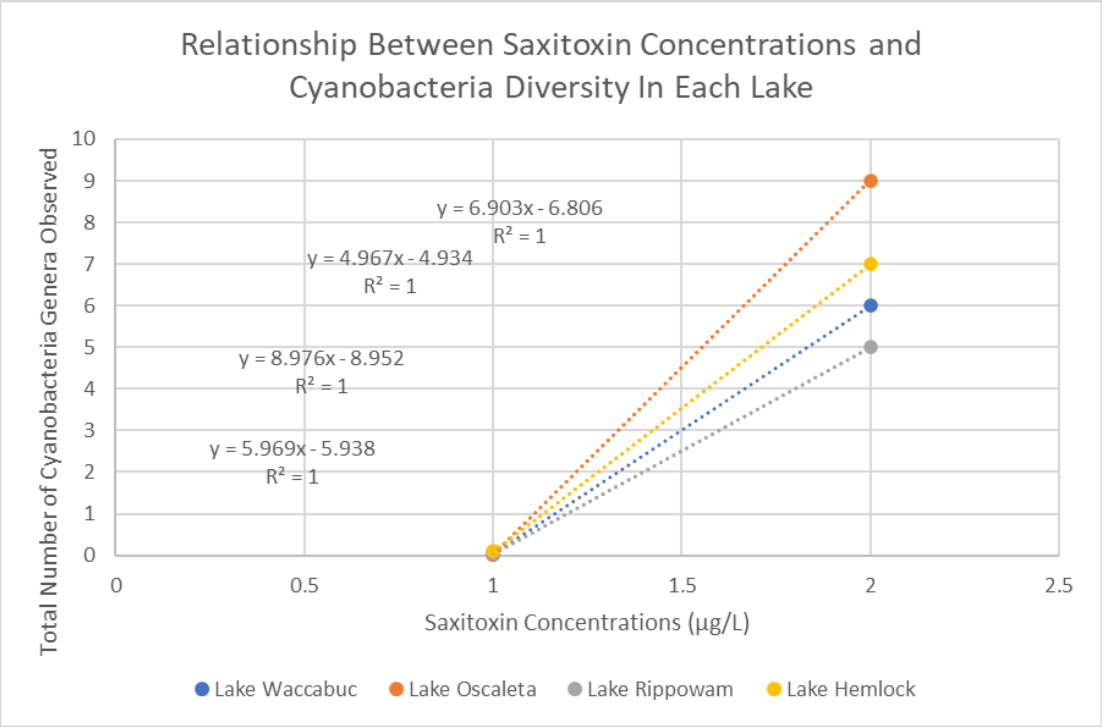


Fig. 25: *Scatter plot representing the linear relationship between saxitoxin concentrations (µg/L) and generic diversity of the "dirty dozen" of cyanobacteria when observing each individual lake on 9/18/2022. This analysis revealed that there is a significant positive relationship between saxitoxin concentrations (µg/L) and generic diversity of the "dirty dozen" of cyanobacteria when observing each individual lake on 9/18/2022.*

Chlorophyll Concentrations

An indicator of the quantity of phytoplankton in a freshwater system can be determined by the concentration of chlorophyll in the body of water. Climate variables like winds and water surface temperatures have an impact on phytoplankton numbers (Carlson, Robert et al., 1996). An analysis of the chlorophyll levels at each study site during the duration of the study was conducted to see if there was a relationship among chlorophyll levels in each lake. As seen in figure 28 and 30, at the beginning of the study, Lake Hemlock, Lake Rippowam, and Lake Waccabuc had higher levels of chlorophyll between 5000-6000 RFUs and Lake Oscaleta had much lower chlorophyll levels at around 2750 RFUs. All four lakes' chlorophyll levels dropped on 7/24/2022 and began to rise again on 8/7/2022. Lake Oscaleta continued to slowly rise in chlorophyll levels from 8/7/2022 till the end of the study. Lake Hemlock's chlorophyll levels remained lower than the other three lakes from 7/24/2022 till the end of the study. Lake

Waccabuc showed variation every 2 weeks throughout the entire study with the highest chlorophyll levels being on 9/4/2022, also coinciding with the lake's peak phycocyanin levels (Fig. 4 + 6). Lake Rippowam had similar chlorophyll levels with Lake Waccabuc from 7/10/2022 through 9/18/2022. Lake Rippowam had contained the highest chlorophyll levels on 9/4/2022 and continued to have the highest chlorophyll levels till the end of the study (9/18/2022) (Fig. 28 + 30).

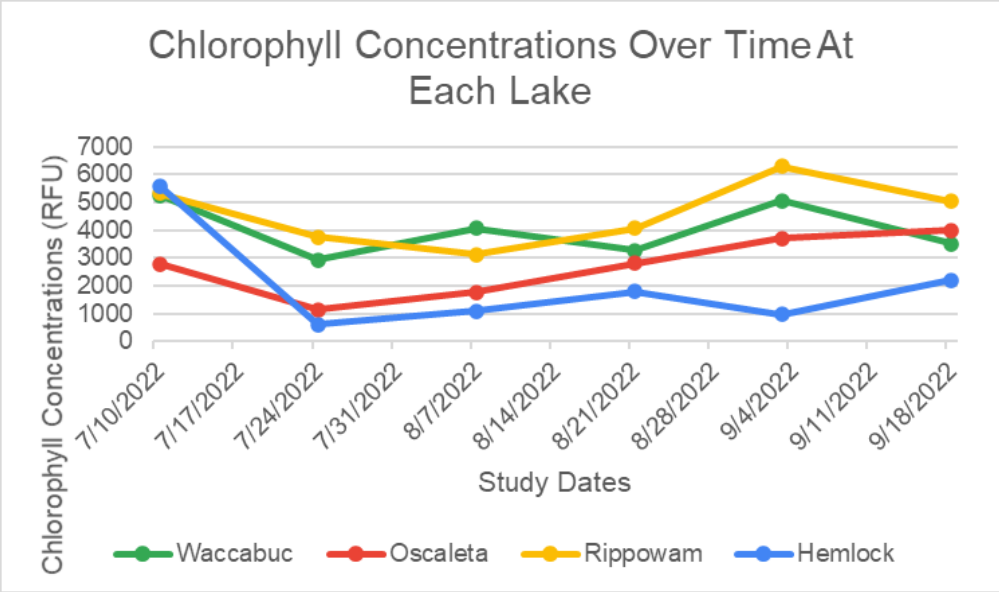


Fig. 26: *Line graph representing the chlorophyll levels (RFU) in the three lakes council versus the chlorophyll levels (RFU) in Lake Hemlock at Mountain Lakes Park over the duration of the study. Lake Hemlock consistently had less chlorophyll levels (RFU) when compared to the three lakes council. All four lakes showed a spike in chlorophyll levels from 8/21/2022 to 9/4/2022.*

The chlorophyll levels (RFUs) at each of the lakes within three lakes councils (Lake Waccabuc, Lake Oscaleta, and Lake Rippowam) were averaged and compared against the chlorophyll levels at Lake Hemlock. As seen in Figure 27 and 29, the Three Lakes Council average chlorophyll levels over the duration of the study were significantly higher than the chlorophyll levels found in Lake Hemlock. Both the three lakes council and Lake Hemlock dropped chlorophyll levels on 7/24/2022 but Lake Hemlock's levels continued to stay under 2,000 RFUs till the end of the study. Again, the three lakes council showed the greatest spike in chlorophyll levels on 9/4/2022.

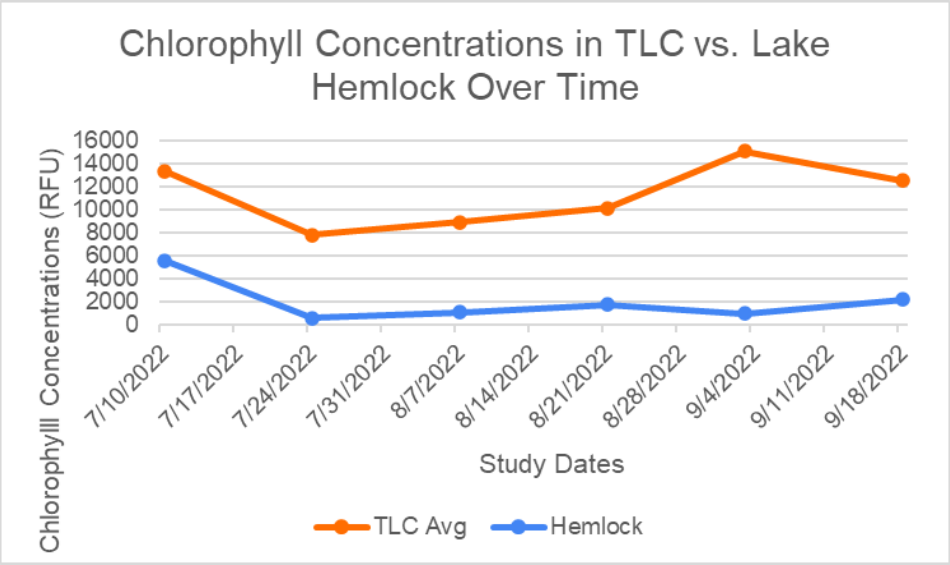


Fig. 27: *Line graph representing chlorophyll levels (RFU) in the three lakes council average versus Lake Hemlock at Mountain Lakes Park over time. Lake Hemlock's chlorophyll levels (RFU) remained consistently low throughout the study. The three lakes council average was about twice as high as Lake Hemlocks chlorophyll levels (RFU) throughout the study.*

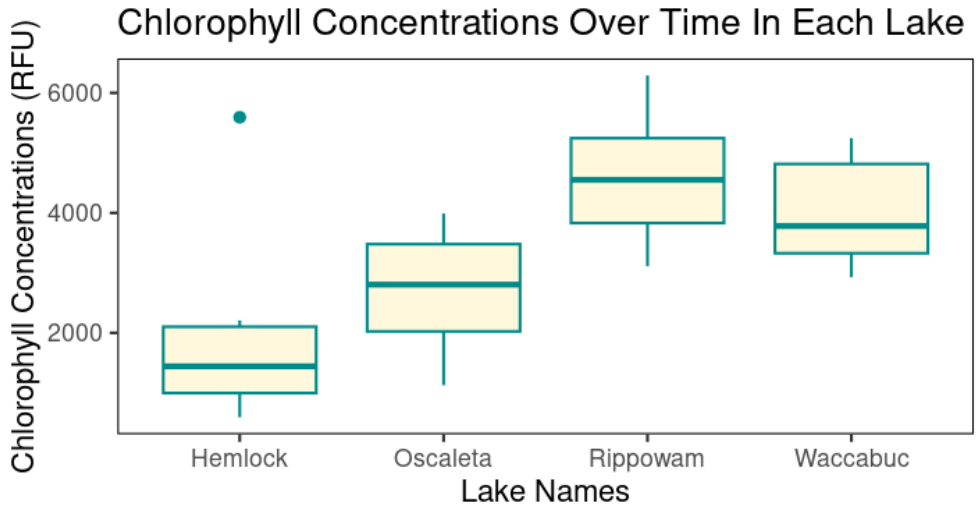


Fig. 28: *Boxplot representing the chlorophyll levels (RFU) in each lake over the duration of the study. Lake Rippowam had the highest levels of chlorophyll but was not significantly higher than Lake Waccabuc when compared to the other three lakes. Lake Hemlock had the greatest variation in chlorophyll levels (RFU) most likely due to mishaps in the first week of data collection.*

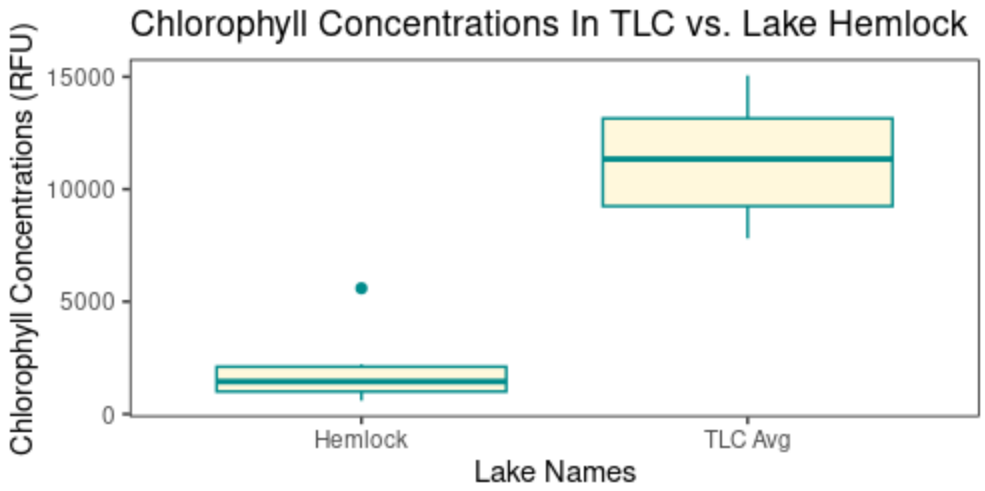


Fig. 29: *Boxplot representing the chlorophyll levels (RFU) in the three lakes council average versus Lake Hemlock at Mountain Lakes Park. The three lakes council average had significantly higher levels of chlorophyll (RFU) then Lake Hemlock, despite Lake Hemlock showing greater variation.*

Relative Abundance and Generic Diversity

A point-count system was used to quantify the relative abundance of The Dirty Dozen that were identified during this study. As seen in figure 30, *Anabaena (22%)*, *Microcystis (19%)* *Phormidium (16%),* and *Oscillatoria (15%)* were the most identified genus of cyanobacteria. *Aphanizomenon (7%)* and *Merismopedia (9%)* were the second most identified genus. *Arthropods*, *Gloeocaspa*, *Coelosphaerium*, and *Woronichinia* were all less than 10% of the total genus identified in this study.

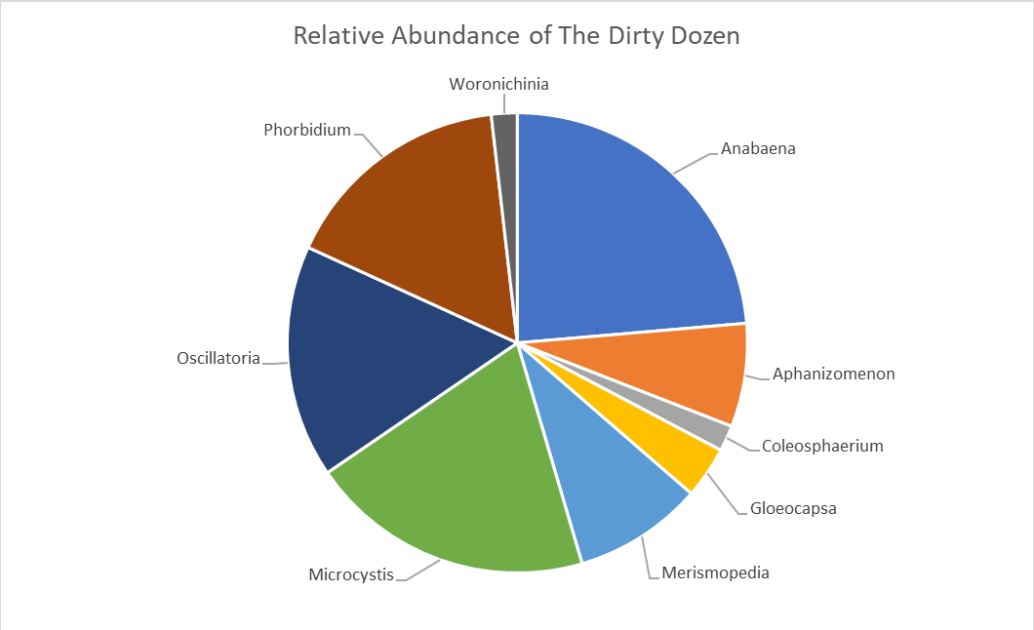


Fig. 30: *Pie chart representing the relative abundance of the "Dirty Dozen" that were identified within all lakes during the duration of the study. Anabaena, Oscillatoria, Phorbidium, and Microcystis were identified the most frequently throughout this study.*

As seen in Figure 31, *Anabaena (13)*, *Microcystis (11)*, *Phorbidium (9)*, and *Oscillatoria (9)* were identified most frequently throughout this study. *Merismopedia (5)* and *Aphanizomenon (4)* were the second most identified genus. *Arthropods* were identified three times throughout the study period. *Gloeocapsa (2)*, *Coelosphaerium (1)*, and *Woronichinia (1)* were identified the least throughout the study period.

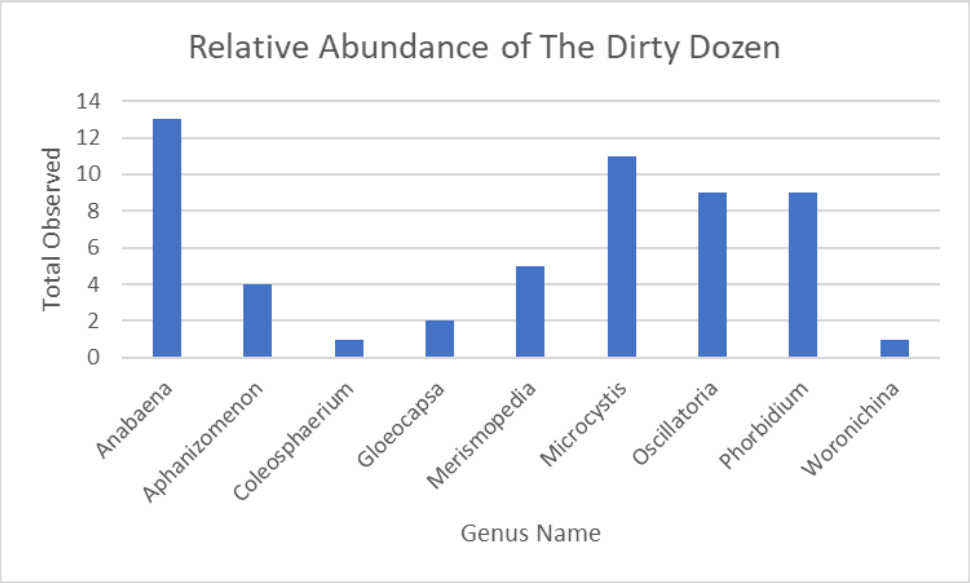


Fig. 31: *Boxplot representing the relative abundance of The Dirty Dozen throughout the duration of the study. Anabaena, Microcystis, Phorbidium, and Oscillatoria were observed the most times.*

The generic diversity of The Dirty Dozen was then observed at each lake to determine if there was a relationship between genus observed and toxicity concentrations in each lake. As seen in figure 32, Lake Waccabuc and Lake Hemlock contained the most *Anabaena* that was identified throughout the study. Lake Oscaleta was the only one of the lakes that had the genus *Coelosphaerium* and *Gloeocapsa*. Lake Waccabuc and Lake Rippowam contained the highest amount of *Microcystis*, almost twice the amount that Lake Hemlock had. Lake Waccabuc also had the highest amount of the genus *Oscillatoria* and *Phormidium* and was the only of the four lakes to have *Woronichinia*. Phycocyanin and microcystin levels were higher in Lake Waccabuc so identifying these species could be used as a proxy for toxicity levels in Lake Waccabuc.

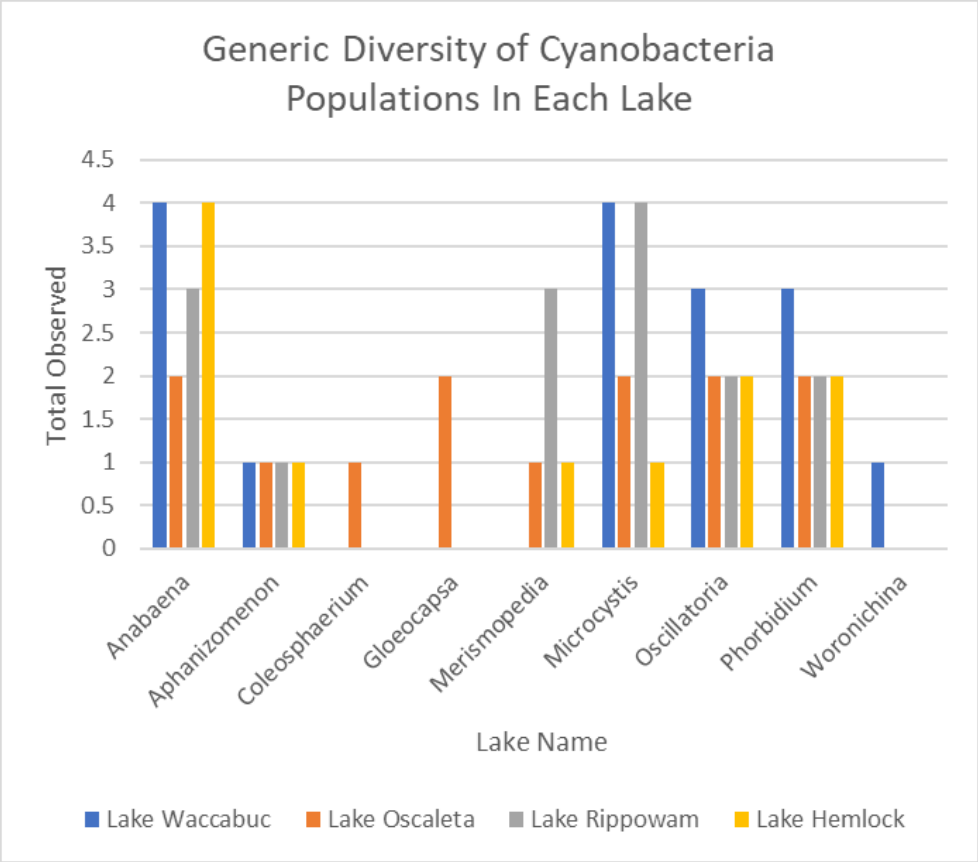


Fig. 32: *Bar graph representing the relative abundance of The Dirty Dozen within each lake during the duration of the study. Lake Waccabuc and Lake Hemlock contained the most Anabaena. Lake Waccabuc and Lake Rippowam contained the most amount of Microcystis.*

Discussion

This study found a positive relationship between phycocyanin levels and microcystin concentrations in all lakes (Fig. 12). Lake Oscaleta had the most significant positive relationship between phycocyanin levels (RFU) and microcystin concentration when compared to the other three study lakes (Fig. 13). “Peak” Toxicity levels seemed to occur around 2,000-3,000 RFU and then levels out for the duration of the study (Fig. 12). This could imply that toxicity levels within these lakes reach a certain RFU value and then remain around that value. These results conclude that phycocyanin levels could be used as a proxy for determining microcystin concentration within a body of water but only until a certain RFU value.

Phycocyanin levels in Lake Rippowam were the highest towards the beginning of the study (Fig. 4), but Lake Waccabuc was highest overall when compared to the other four study lakes (Fig. 4). On 9/4/2022, Lake Waccabuc, Lake Oscaleta, and Lake Rippowam spiked in phycocyanin levels (Fig. 4). Lake Oscaleta had the highest microcystin concentrations overall when compared to the other four study lakes (Fig. 8). It spiked on 8/21/2022 along with Lake Rippowam, but Lake Rippowam gradually lowered till the end of the study and Lake Oscaleta remained high (Fig. 4). Lake Waccabuc and Lake Rippowam had the highest chlorophyll levels throughout the duration of the study period (Fig. 26). Lake Waccabuc, Lake Oscaleta, and Lake Rippowam's chlorophyll levels spiked on 9/4/2022 but not Lake Hemlock (Fig. 26). Lake Oscaleta's chlorophyll levels at the beginning of the study were low and gradually raised throughout the duration of the study (Fig. 26). Lake Hemlock contained the highest saxitoxin concentration when compared to the other four study lakes (Fig. 18). All of these variables above confirm that Lake Hemlock contains a different biology than Lake Waccabuc, Lake Oscaleta, and Lake Rippowam and that these three lakes within the Three Lakes Council share a similar biology with each other.

There was no linear relationship between cyanobacteria abundance and microcystin concentrations in all of the lakes during this study period (Fig. 14). Lake Hemlock and Lake Rippowam were the only two lakes that had a positive relationship between cyanobacteria abundance and microcystin concentration (Fig. 14). These results conclude that there is no significant relationship between how abundant cyanobacteria populations are and microcystin concentrations in any of the study lakes. But, there was a positive and significant linear relationship between cyanobacteria diversity and microcystin concentrations at all of the study lakes (Fig. 16). Lake Oscaleta had the most significant relationship between cyanobacteria diversity and microcystin concentrations when compared to the other three study sites (Fig. 15). Lake Waccabuc and Lake Rippowam had similar positive relationships between cyanobacteria diversity and microcystin concentrations, and Lake Hemlock had the least significant

relationship between cyanobacteria diversity and microcystin concentrations, when compared to the three lakes within the Three Lakes Council (Fig. 17). These results conclude that the more diverse cyanobacteria populations are in a freshwater system, the more toxic they are.

When analyzing the relationship between phycocyanin levels and the saxitoxin concentration from 9/18/2022, there was a significant positive relationship in all of the study lakes (Fig. 20). Lake Oscaleta had the most significant relationship between phycocyanin levels and the saxitoxin concentration (Fig. 21). Lake Rippowam and Lake Waccabuc had similar relationships between phycocyanin levels and the saxitoxin concentration and Lake Hemlock had the least significant relationship between phycocyanin levels and saxitoxin concentration (Fig. 21). Saxitoxin samples were only collected on one study day (9/18/2022). The small sample size for saxitoxin concentrations can be misrepresenting this information and therefore these results from this study conclude that phycocyanin levels do not really give an accurate representation of the toxicity in a freshwater system despite having a significant power relationship in this study.

When analyzing the relationship between cyanobacteria abundance and saxitoxin concentrations there was no linear relationship in all of the study lakes (Fig. 22). Lake Rippowam had the most significant relationship between cyanobacteria abundance and saxitoxin concentrations. Lake Hemlock and Lake Waccabuc had similar relationships between cyanobacteria abundance and saxitoxin concentrations, and Lake Oscaleta had the least significant relationship (Fig. 23). These results conclude that cyanobacteria abundance could potentially result in higher toxicity (saxitoxin concentrations), but due to the small sample of saxitoxin concentrations in this study, it is unclear if this relationship is legitimate.

This study found there was no logarithmic relationship between cyanobacteria diversity and saxitoxin concentrations in all of the study lakes (Fig. 24). Lake Oscaleta had the most significant relationship between cyanobacteria population diversity and saxitoxin concentrations and Lake Hemlock, Lake Waccabuc, and Lake Rippowam all had similar relationships between

cyanobacteria population diversity and saxitoxin concentrations (Fig. 25). These results cannot conclude that cyanobacteria population diversity influences saxitoxin toxicity in freshwater systems. If more saxitoxin concentrations are collected, these relationships with saxitoxin could change and look more similar to the results from the relationships with microcystin.

Alternatively, the variations in concentration ranges between the various freshwaters could be due to genuine biological variations, like bloom episodes, or the analytical techniques used (Jørgensen, Niels O., et al. 2022). There were some issues with data collection during this study that could have influenced the results. Some of the freshwater samples that were over-filled and put in the freezer cracked, resulting in some of the samples to be lost during transportation between labs. Microcystin samples from 7/10/2022 had to be completely scratched due to overfilling. Samples from 9/3/2022 were most of the time not included in this study analysis because there weren't four samples to compare with the other variables. These mishaps with data collection and analysis could have potentially resulted in skewed results. Saxitoxin samples were only collected on 9/18/2022 because the saxitoxin kit arrived very late. If it arrived on time and this study included a full analysis of saxitoxin concentrations during the study period, there might have been similar positive relationships between saxitoxin concentrations and phycocyanin levels, cyanobacteria abundance and cyanobacteria diversity like the microcystin concentration relationships showed.

All samples collected during this study only went through one freeze/thaw cycle before performing the ELISA tests. One study by Śmietańska, Rastawicki W., and Rokosz N. Jagielski concludes that there is no significant difference between antibodies that were frozen once or went through a 30-day freeze thaw cycle. But this does not completely guarantee that multiple freeze/thaw cycles could not influence the abundance or diversity of cyanobacteria populations.

It is also important to note that all samples in this study were collected from privately owned docks on each of the three lakes within the Three Lakes Council and from a beach on Lake Hemlock. This was intentional for this study because these samples would be

representative of presumably the “worst” part of each lake, and also where people and dogs are usually located on the lakes. Sampling from this area on the lake would represent the toxicity found in these freshwater systems where people spend the most time and where their exposure to these toxins would be the greatest.

It is also important to note the different stages of development on each of the study lakes. Lake Rippowam is the smallest of the three lakes in the Three Lakes Council. Lake Rippowam flows into Lake Oscaleta– the second smallest lake in the Three Lakes Council. Lake Oscaleta flows into Lake Waccabuc–the biggest lake in the Three Lakes Council. Lake Waccabuc has a country club on it and was the first of the three lakes to be developed on. Lake Waccabuc has a much longer residence time than the other two lakes– almost 3 times as long as Lake Rippowam’s water resides in its system (Table 2). Lake Rippowam also turns over 3-4 times a year while Lake Waccabuc turns over just once.

The differing hydrologic conditions between each of the lakes in this study could also be creating favorable or unfavorable environments for “The Dirty Dozen” of cyanobacteria. During microscopy observations the cyanobacteria genus *Anabaena*, *Microcystis*, *Oscillatoria*, and *Phorbidium* were identified in all four study lakes (Fig. 32). This is an interesting result to find that Lake Hemlock also contains these cyanobacteria genus that are known to produce microcystin toxins (Table 1) and could possibly be an explanation for why Lake Hemlock had a high saxitoxin concentration on 9/18/2022 (Fig. 18 + 19).

The three lakes within The Three Lakes Council all contained the genus of cyanobacteria *Aphanizomenon,* which is known to produce dense blooms that produce toxins such as: *Anatoxin-a*, *Cylindrospermopsin*, *Microcystins*, and *Saxitoxins* (Lyon-Colbert, Amber, et al., 2017). The genus *Merismopedia* was identified in Lake Oscaleta, Lake Rippowam, and Lake Hemlock and *Arthropods* were only identified in Lake Waccabuc and Lake Oscaleta (Fig. 32). The genus *Woronchinia* that is known to produce *Anatoxins* (Table 1) was identified in only Lake Waccabuc (Fig. 32). The genus *Gelocaspa* and *Coelosphaerium* of cyanobacteria were only

identified in Lake Waccabuc and Lake Oscaleta (Fig. 32). Identifying different genus of cyanobacteria in each of the four study lakes led to a comprehensive understanding of the taxonomy of phytoplankton in each of the study lakes and could explain why there were differing toxin concentrations in each of the lakes.

Lake Hemlock is a lake in a county park that is protected and not developed at all. While there is no data available on Lake Hemlock's residence time or turn-over rate, Lake Hemlock is visually much smaller than Lake Rippowam (the smallest of the Three Lakes Council). Lake Hemlock sits at a much higher elevation (~915 ft above sea level) than Lake Waccabuc, Lake Oscaleta, and Lake Rippowam (~650 ft above sea level) (Fig. 33). These biological, hydrologic, and environmental conditions could be influencing Lake Hemlocks freshwater system and be different conditions than those within the Three Lakes Council.

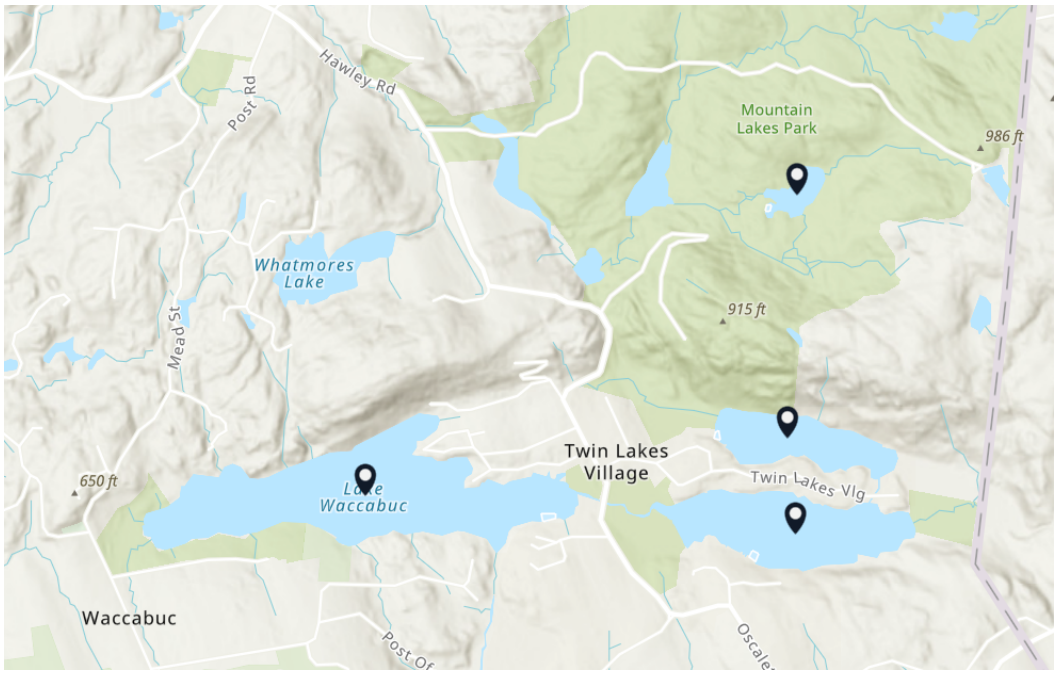


Fig. 33: *Topographic map representing the varying elevations of the lakes within this study. Lake Hemlock is close to 915 ft above sea level and the lakes in the Three Lakes Council are close to 650 ft above sea level.*

Lake Waccabuc's trophic level for a typical year is eutrophic, Lake Oscaleta and Lake Rippowam are usually mesotrophic (Table 2). Lake Waccabuc could be receiving additional

nutrient loading from Waccabuc Country Club or residential homes surrounding the lake and due to the hydrology (turnover, residence time, size and depth) of Lake Waccabuc (Table 3) the nutrients could be sitting in Lake Waccabuc for a longer time than Lake Oscaleta and Lake Rippowam. These nutrients could be making a favorable environment for cyanobacteria to persist. Microcystis concentrations in the water might rise due to the concentration of algae caused by a consistent wind direction (Huang, Jian, et al., 2021) causing cyanobacteria populations in Lake Waccabuc to move into the other two lakes.

In this study, Lake Oscaleta had the greatest diversity of cyanobacteria when compared to the other three study lakes (Fig. 30 + 31). Increased diversity in a community has been shown to sustain better ecological functioning, which boosts temporal stability, production, and nutrient retention (Eisenhauer et al., 2019).

Conclusion

This study found a significant similarity in the ecology of freshwater systems located in The Three Lakes Council in South Salem, NY and that Lake Hemlock in Mountain Lakes Park has a different ecology from Lake Waccabuc, Lake Oscaleta, and Lake Rippowam proving the hypothesis of this study correct. This study found a positive relationship between phycocyanin levels and microcystin concentration (Fig 12), no relationship between microcystin concentration and cyanobacteria abundance (Fig. 15), and a significant positive relationship between microcystin concentration and cyanobacteria diversity (Fig. 16). This study found a significant relationship between phycocyanin levels and saxitoxin concentration (Fig. 20) and potential positive relationships between cyanobacteria abundance and saxitoxin concentration (Fig. 22), and cyanobacteria diversity and saxitoxin concentration (Fig. 24). Suggestions for future studies include testing a wider variety of toxins such as anatoxins and cylindrospermopsin along with collecting more saxitoxin samples. Through running a wider range of toxin tests, it can be

determined which toxins are most abundant in each of the study lakes and if there are other toxins influencing the ecology of these lakes. Future research should include an annual study that can be analyzed over time to determine the behavior of cyanobacteria in these lakes along with changes within the lakes ecosystems. This could be useful information for The Three Lakes Council to conduct in order to monitor the health of the three lakes in the presence of anthropogenic environmental / climatic changes.

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Citations

Andersen, Janet, and Jean Lewis. *Reflections on Our Lakes: Three Lakes Council 1970-2020: Lakes Waccabuc, Oscaleta, and Rippowam*, Three Lakes Council, South Salem, NY, NY, 2020, pp. 1–35.

Anderson, Donald M., et al.,. “Progress in Understanding Harmful Algal Blooms ... - Annual Reviews.” *Annual Review Of Marine Science*, Jan. 2012, www.annualreviews.org/doi/abs/10.1146/annurev-marine-120308-081121.

Andrew W. Griffith, et al., “Harmful Algal Blooms: A Climate Change Co-Stressor in Marine and Freshwater Ecosystems.” *Harmful Algae*, Elsevier, 21 May 2019, www.sciencedirect.com/science/article/pii/S1568988319300344.

Bižić M. Klintzsch; Ionescu D. Hindiyeh ;Günthel M. Muro-Pastor; Eckert W. Urich ;Keppler F. Grossart; “Aquatic and Terrestrial Cyanobacteria Produce Methane.” *Science Advances*, U.S. National Library of Medicine, pubmed.ncbi.nlm.nih.gov/31998836/. Accessed 22 Nov. 2023.

Bridgham, Scott D., et al., “The Carbon Balance of North American wetlands.” *Wetlands*, vol. 26, no. 4, 2006, pp. 889–916, https://doi.org/10.1672/0277-5212(2006)26[889:tcbona]2.0.co;2.

Burford, M. A., Davis, T. W., Gobler, C. J., & O’Neil, J. M. (2012). The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. Harmful Algae, 14, 313– 334.

Buschke, Falko. “Neutral theory exposes the challenge of bending the curve of biodiversity loss for the post-2020 Global Biodiversity Framework.” *Bending the Curve of Global Freshwater Biodiversity Loss: An Emergency Recovery Plan*, 2021, https://doi.org/10.22541/au.161605601.11680229/v1.

Carlson, Robert, and Johnathon Simpsom. “A Coordinator’s Guide to Volunteer Monitoring (Digital Download).” *North American Lake Management Society (NALMS)*, 5 Sept. 2023, www.nalms.org/product/a-coordinators-guide-to-volunteer-monitoring/.

Carvalho da Silva Vanessa, and Noemi Fernandes. “Protist taxonomic and functional diversity in aquatic ecosystems of the Brazilian Atlantic Forest.” *PeerJ*, vol. 11, 2023, https://doi.org/10.7717/peerj.15762.

Cihelio Alves Amorim, et al., “Ecological Impacts of Freshwater Algal Blooms on Water Quality, Plankton Biodiversity, Structure, and Ecosystem Functioning.” *Science of The Total Environment*, Elsevier, 17 Nov. 2020, www.sciencedirect.com/science/article/abs/pii/S0048969720371369.

Cirés, Samuel, et al., “Temperature Influences the Production and Transport of Saxitoxin and the Expression of SXT Genes in the Cyanobacterium Aphanizomenon Gracile.” *MDPI*, Multidisciplinary Digital Publishing Institute, 13 Oct. 2017, doi.org/10.3390/toxins9100322.

Department of Environmental Conservation. “Citizens Statewide Lake Assessment Program (CSLAP).” *Department of Environmental Conservation*, dec.ny.gov/environmental-protection/water/water-quality/cslap. Accessed 15 Dec. 2023.

Eisenhauer, Nico, et al., “A Multitrophic Perspective on Biodiversity-Ecosystem Functioning Research.” *Advances in Ecological Research*, U.S. National Library of Medicine, 2019, www.ncbi.nlm.nih.gov/pmc/articles/PMC6944504/.

Environmental Protection, Maine Department. “Cyanobacteria (Blue-Green Algae).” *Cyanobacteria, Aka Blue-Green Algae, Maine Department of Environmental Protection*, www.maine.gov/dep/water/lakes/cyanobacteria.html#:~:text=Cyanobacteria%2C%20formerly%20known%20as%20blue,%2Dgreen%20or%20brownish%2Dgreen. Accessed 15 Dec. 2023.

Etienne, Dominique, et al., SUNY Purchase College, Purchase, NY, 2023, pp. 1–30, *Climate Change: Why We HABS To Talk About It*.

H. Kenneth Hudnell et al., “Freshwater Harmful Algal Bloom (FHAB) Suppression with Solar Powered Circulation (SPC).” *Harmful Algae*, Elsevier, 31 Oct. 2009, www.sciencedirect.com/science/article/abs/pii/S1568988309001218.

Heisler J. Gilbert; P. Burkholder; Anderson D. Cochlan; W. Dennison Gobler C. Dortch;Heil C. Humphries; Lewitus A. Magnien ;Marshall H. Sellner ;Stockwell D. Stoecker;Suddleson M. “Eutrophication and Harmful Algal Blooms: A Scientific Consensus.” *Harmful Algae*, U.S. National Library of Medicine, pubmed.ncbi.nlm.nih.gov/28781587/. Accessed 22 Nov. 2023.

Huang, Jian, et al., “Effects of hydrological and climatic variables on cyanobacterial blooms in four large shallow lakes fed by the Yangtze River.” *Environmental Science and Ecotechnology*, vol. 5, 2021, p. 100069, https://doi.org/10.1016/j.ese.2020.100069.

Humpage, Andrew. “World Health Organization.” *World Health Organization (WHO)*, Mar. 2017, apps.who.int/iris/bitstream/handle/10665/338069/WHO-HEP-ECH-WSH-2020.8-eng.pdf?sequence=1.

Ingrid, Martin, and Welker Chorus. “Toxic Cyanobacteria in Water: A Guide to Their Public Health Conseque.” *Encyclopedia of Microbiology*, Taylor Francis Books, 8 Mar. 2021, www.taylorfrancis.com/books/oa-edit/10.1201/9781003081449/toxic-cyanobacteria-water-ingrid-chorus-martin-welker.

J.M. O'Neil , et al., "The Rise of Harmful Cyanobacteria Blooms: The Potential Roles of Eutrophication and Climate Change." *Harmful Algae*, Elsevier, 29 Oct. 2011, www.sciencedirect.com/science/article/abs/pii/S1568988311001557.

Jørgensen, Niels O., et al., "Fate of saxitoxins in Lake Water: Preliminary testing of degradation by microbes and sunlight." *Water*, vol. 14, no. 21, 2022, p. 3556, https://doi.org/10.3390/w14213556.

Kingsolver, Joel G., and Lauren B. Buckley. "Quantifying thermal extremes and biological variation to predict evolutionary responses to changing climate." *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 372, no. 1723, 2017, p. 20160147, https://doi.org/10.1098/rstb.2016.0147.

Kirwan, M. L., and L. K. Blum. *Enhanced Decomposition Offsets Enhanced Productivity and Soil Carbon Accumulation in Coastal Wetlands Responding to Climate Change*, 2011, https://doi.org/10.5194/bgd-8-707-2011.

Lewisboro Land Trust. "Mountain Lakes Park." *Lewisboro Land Trust*, lewisborolandtrust.org/mountain-lakes-park#:~:text=Mountain%20Lakes%20Park%20was%20 purchased,of%20the%20 Long%20 Pound%20Indians. Accessed 15 Dec. 2023.

Lyon-Colbert, Amber, et al., "A systematic literature review for evidence of aphanizomenon Flos-Aquae toxigenicity in recreational waters and toxicity of dietary supplements: 2000–2017." *Toxins*, vol. 10, no. 7, 2018, p. 254, https://doi.org/10.3390/toxins10070254.

Mathys, Werner. "Analysis of Microcystins in Freshwater Samples Using High Performance Liquid Chromatography and an Enzyme-Linked Immunosorbent Assay."

International Journal of Hygiene and Environmental Health, Urban & Fischer, 21 June 2005, www.sciencedirect.com/science/article/abs/pii/S143846390570328X.

Mchau, Geofrey J., et al.,. “Phycocyanin as a proxy for algal blooms in surface waters: Case study of Ukerewe Island, Tanzania.” *Water Practice and Technology*, vol. 14, no. 1, 2019, pp. 229–239, https://doi.org/10.2166/wpt.2019.005.

Negri, Andrew P, et al. “Sheep mortality associated with paralytic shellfish poisons from the cyanobacterium anabaena circinalis.” *Toxicon*, vol. 33, no. 10, 1995, pp. 1321–1329, https://doi.org/10.1016/0041-0101(95)00068-w.

Podduturi, Raju, et al. “Monitoring of saxitoxin production in lakes in Denmark by molecular, chromatographic and microscopic approaches.” *Harmful Algae*, vol. 101, 2021, p. 101966, https://doi.org/10.1016/j.hal.2020.101966.

Preece, Eleen P. “A Review of Microcystin Detections in Estuarine and Marine Waters: Environmental Implications and Human Health Risk.” *Harmful Algae*, Elsevier, 29 Nov. 2016, www.sciencedirect.com/science/article/pii/S1568988316302141.

Rutkowska, M., Płotka-Wasylka, J., Majchrzak, T., Wojnowski, W., Mazur-Marzec, H., & Namieśnik, J. (2019). Recent trends in determination of neurotoxins in aquatic environmental samples. TrAC Trends in Analytical Chemistry, 112, 112–122. https://doi.org/10.1016/j.trac.2019.01.001

Sangolkar, Lalita N., et al. “Methods for determining microcystins (peptide hepatotoxins) and microcystin-producing cyanobacteria.” *Water Research*, vol. 40, no. 19, 2006, pp. 3485–3496, https://doi.org/10.1016/j.watres.2006.08.010.

Sha, Jun, et al. “Harmful algal blooms and their eco-environmental indication.” *Chemosphere*, vol. 274, 2021, p. 129912, https://doi.org/10.1016/j.chemosphere.2021.129912.

Shalaby, Emad. “Algae as promising organisms for environment and health.” *Plant Signaling & Behavior*, vol. 6, no. 9, 1 Sept. 2011, pp. 1338–1350, https://doi.org/10.4161/psb.6.9.16779.

Singer, David, et al. “Protist Taxonomic and Functional Diversity in Soil, Freshwater and Marine Ecosystems.” *Environment International*, Pergamon, 19 Nov. 2020, www.sciencedirect.com/science/article/pii/S0160412020322170.

Śmietańska, Rastawicki W., and Rokosz N. Jagielski. “[Effect of Multiple Freeze-Thaw Cycles on Detection of IGA, IGG and IGM Antibodies to Selected Bacterial Antigens].” *Medycyna Doswiadczalna i Mikrobiologia*, U.S. National Library of Medicine, pubmed.ncbi.nlm.nih.gov/22808733/. Accessed 21 Dec. 2023.

Smol, John P., et al. “Tracking environmental change using lake sediments.” *Developments in Paleoenvironmental Research*, 2001, https://doi.org/10.1007/0-306-47668-1.

Stendera, Sonja, et al. “Drivers and stressors of freshwater biodiversity patterns across different ecosystems and scales: A Review.” *Hydrobiologia*, vol. 696, no. 1, 2012, pp. 1–28, https://doi.org/10.1007/s10750-012-1183-0.

Systems, APEC Water. “Water Quality Information - Can Algae Have Beneficial Effects on Water Supplies?” *APEC Water Systems*, www.freedrinkingwater.com/water_quality/quality2/j-3-08-algae-beneficial-effects-on-water-supplies.htm. Accessed 15 Dec. 2023.

Three Lakes Council. “Water Quality.” *Three Lakes Council*, 13 Mar. 2023, threelakescouncil.org/reference/water-quality/.

US EPA, O. (2013, June 3). Harmful Algal Blooms [Collections and Lists]. Retrieved May 6, 2020, from US EPA website: https://www.epa.gov/nutrientpollution/harmful-algalbloom

Woodward, Guy, et al. “Climate change and freshwater ecosystems: Impacts across multiple levels of organization.” *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 365, no. 1549, 2010, pp. 2093–2106, https://doi.org/10.1098/rstb.2010.0055.