# Cyanotoxin and Cyanobacterial density variation Among Small Freshwater Lakes within the Hudson Highlands of the New York/Connecticut Border

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## Abstract

Cyanobacterial populations which photosynthesize and multiply rapidly, potentially releasing toxins known as cyanotoxins, are known as Harmful Algal Blooms (HABs). Phycocyanin is a photosynthetic pigment within cyanobacteria. Phycocyanin abundance can be quantified in freshwater lakes using a handheld fluorometer which reports readings as Raw Fluorescent Units (RFUs). Microcystin is a hepatotoxin commonly present in HABs. Microcystin abundance was quantified using Enzyme-Linked Immunosorbent Assays (ELISA). This study found no universal relationship between microcystin concentrations and phycocyanin concentrations across five local lakes located in northern Fairfield and southeastern Putnam Counties. While two study sites (Putnam Lake and Squantz Pond) did produce strong positive relationships between phycocyanin and microcystin concentrations, no site exceeded public health guideline for concentrations of microcystin throughout the duration of this study.


## Introduction

Cyanobacteria, commonly known as blue-green algae, are photosynthetic microorganisms found across the world, in oceans, freshwater systems, bare rock, and soil (Catherine et al., 2013). Cyanobacteria are ancient microscopic organisms, responsible for the oxygenation of the planet over 3.5 billion years ago (Schopf., 2002). Most cyanobacteria species occur in low, concentrations however, when environmental conditions are favorable, cells can multiply rapidly to large concentrations, forming harmful algal blooms (HABs). HABs typically occur in summer through early fall and can occur multiple times per year in the same bodies of water.

Harmful Algal Blooms (HABs) are a prominent public safety concern in many bodies of water across the world (Moore et el., 2008). The release of cyanotoxins such as hepatotoxins and neurotoxins can be harmful to exposed humans, pets, fish, birds, and other wildlife (Breinlinger et al., 2021; Hillborn & Beasley., 2015).

What is the Harm of increased HAB expression?

Cyanobacteria can be dangerous because they possess genes that can produce toxic metabolites, potentially causing bodies of water and waterways, to become toxic. Cyanobacterial toxins naturally exist intracellularly (in the cytoplasm) and are retained within the cells of cyanobacteria... these variants of cyanotoxins are found intracellularly approximately 95% of the time during the growth stage of the bloom. When weather conditions are right, these toxins can be released into the water body by lysed cells following the breakdown of a cyanobacterial bloom (McElhiney & Lawton, 2005). For those species, when the cell dies or the cell membrane ruptures the toxins are released into the water and can remain present for up to three weeks (United States EPA, 2014; Kosiba et al., 2018). The two major toxins produced by cyanobacteria are, hepatotoxins and neurotoxins. Neurotoxins are a group of compounds that have a biological effect on the nervous system with different modes of action, dependent upon the type of neurotoxin (Rutkowska et al., 2019). Neurotoxins from cyanobacteria such as, Anatoxins and Saxitoxins, are known to be found in *Anabaena/Dilochospermum and Aphanizomenon* genera. These neurotoxins can cause tingling, burning, numbness in the skin, drowsiness, incoherent speech, salivation, and respiratory paralysis leading to death (US EPA., 2013).

Hepatotoxins are toxic chemicals that damage the liver and even lead to hepatocyte necrosis and hemorrhaging, potentially resulting in death (Sangolkar et al., 2006). 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, Oscillatoria, and Nostoc* genera. Microcystins are produced and retained in cyanobacterial cells during the growth and stationary phases of blooms and often these blooms appear in eutrophic freshwater ecosystems. The long-term exposure to low concentrations of microcystin can also

cause long term liver damage and in some cases have even been implicated in tumor promotion (Ito et al., 1997). Microcystin pose a high risk to drinking water supplies, fisheries, and aquatic recreational areas as they are the most abundant and frequently occurring cyanotoxins (Duan et al., 2019). As knowledge about the harmful effects of cyanobacteria increases, it is becoming clear that, with an increasing trend in the frequency of harmful algal bloom occurrence, so must there be an increase in efforts to monitor these blooms throughout the blooming season. This is critical to determining the safety of drinking water and recreational water supplies.

As the number of waterways in the United States supporting frequent and intense HABs increases, humans are increasingly exposed to cyanotoxins through the consumption of contaminated drinking water or recreational activities such as swimming (McElhiney & Lawton, 2005). Since the 1950's harmful algal bloom expression has increased worldwide in biomass, frequency, and duration (Planas & Paquet, 2015).

While rare, some human exposures have resulted in dire consequences. In 2002, several healthy teenage boys swam in a pond containing high concentrations of blue-green algae in Dane County, WI. All became ill with mild to severe symptoms of nausea and diarrhea. As for one teenager, who swallowed the water, died of heart failure approximately 48 hours after the exposure (Weirich & Miller, 2014). Another instance where the detrimental effects of HABs has been documented in Kansas. In 2011, 38 water bodies were confirmed to have the presence of a harmful algal bloom. With that said, 34 reports of human and animal illnesses were known to be associated with exposure to the neurotoxins found in cyanobacteria. In one of these lakes alone, five human illnesses, two dog illnesses and five dog deaths were all associated to the exposure of cyanobacteria (Trevino-Garrison et al., 2015).

Given the direct human harm caused by cyanotoxins, drinking water supplies are particularly vulnerable to the negative effects of HABs (US EPA, 2013). It is estimated that the lakes and reservoirs which serve as sources of drinking water for 30-48 million Americans are periodically contaminated by algal toxins (Chapra et al., 2017). Many bodies of water that supply drinking water for communities come from artificial water bodies such as these. Kosiba et al (2018), found that artificial ponds were more susceptible to harmful cyanobacterial blooms, and for a more extended period, than the natural oxbow lakes. "In addition, several cyanobacterial genera produce off-flavor compounds, such as geosmin and methylisoborneol, which may also contaminate municipal drinking water systems." (Kasinak et al., 2015, Juttner and Watson., 2007).

Known and Potential Drivers of HAB Expression

HABs are a form of eutrophication (nutrient enrichment) in lakes, streams, rivers, swamps, wetlands, and even coastal waters, known to be promoted by excessive nutrient loadings (O'Neil et al., 2012). As temperatures increase annually, so too does the rate of photosynthetic activity and, consequently, the expression of HABs (Clean Water Action, 2020; Hudnell., 2010). The abundance and occurrences of harmful algal blooms is suspected that the increase is due to climate change (Thomson-Laing et al., 2020). As the occurrence of blooms increases, so too do the threats of human health, as well as wildlife.

Complicating this trend is the fact that certain artificial lakes do not accurately represent the characteristics of a natural body of water. Some of these bodies of water may be too shallow, or have higher retention time, than other bodies of water do (Hudnell et al., 2010; Kosiba et al., 2018; Princetonhydro, 2015). Retention times for bodies of water, is a measure of how long water will remain present in that body of water. An increase in the flowrate of water suppresses

the increase of cyanobacteria, due to the suppression of sunlight absorption, but when flow rate is low, cyanobacteria can remain still and have more time to absorb heat, sunlight, and photosynthesize before cyanobacteria are washed out of a water body (Li et al., 2013). This is because when flow rate is low, this means water is moving at a slower rate or sitting still and in turn, low flowrate increases sunlight absorption. The longer the retention time, the more time for cyanobacteria to absorb heat and sunlight therefore, increasing the photosynthetic rate and growth in cyanobacteria.

Study Sites

HAB conditions are commonly expressed throughout the Hudson highlands of the New York/Connecticut Border, a region with high residential populations and human-lake interactions. Many recreational areas within local bodies of water in this region may host cyanobacteria blooms and become toxic before they are visibly present which can cause unknown exposures to cyanotoxins and threaten their recreational use. This study is sought to determine if the timing and peak of cyanobacterial bloom along with their toxicity levels are correlated to each other and through the use of this data further understand the relationship between phycocyanin and microcystin along with the similarities and differences between the expression of HABs within regional freshwater lakes.

Since this study sought to better understand these occurrences, five lakes similar to one another in location and size were selected for examination. Based on relative proximities and physio-geographic similarities and differences such as surrounding landforms, suburban influences, elevation as well as drainage features, five bodies of water all within a 15-mile radius of one another were selected. These bodies of water were determined to be suitable for comparison due to being within the same local region, experiencing the same weather,

temperatures, and similar suburban influences. These bodies of water are located in northwestern Fairfield County, CT, and southeastern Putnam County, NY.

Across these lakes a comparison of the seasonal trends in timing and peaks of cyanobacterial bloom development along with their toxicity levels was drawn in effort to try and find patterns, as well investigating any correlation between biovolume and toxicity that may exist.

*Table 1: This table presents the physical characteristics for each body of water which was sampled from. These factors could influence the biodiversity of cyanobacterial populations among different bodies of water and influence the growth and abundance of HABs, along with their associated cyanotoxins.*

| Physical Characteristics | Ball Pond | Lake Carmel | Margerie Reservoir | Putnam Lake | Squantz Pond |
|---|---|---|---|---|---|
| Elevation (ft) | 775 | 617 | 627 | 493 | 424 |
| Maximum Depth (ft) | 51 | 13 | 28 | 18 | 47 |
| Mean Depth (ft) | 24 | 8 | Unknown | 11 | 22.9 |
| Surface Area (acres) | 83 | 192 | 245 | 226 | 288 |
| Watershed Acreage | 246 | 8414 | 2,942 | 1717 | 3,635 |

As seen in Table 1, Putnam Lake is a shallow artificial lake in Putnam County, New York that is 226 acres and has a maximum depth of 18 feet (mean depth 15ft). Its retention time is approximately 0.7 years (8 months) and is known to be a eutrophic body of water. Putnam Lake has a dam at the southern end of the lake and supplies a watershed of 1717 acres. It supplies water to the Putnam Lake watershed and has frequent occurrences of harmful algal blooms with a large density of cyanobacteria in these blooms. These blooms have minimized recreational activities on Putnam Lake during summers and have become a major nuisance. The blooms in Putnam Lake are noticeably increasing per year and it is almost certain (aside from nutrient loading) that this is due to climate change (CSLAP Report Putnam Lake., 2018).

Constructed at the same time by the same land-development company as Putnam Lake is Lake Carmel (The Putnam Lake Community of Patterson). Another artificial body of water located in Putnam County, New York. Lake Carmel is a 192-acre body of water and has a maximum depth of 13 feet (mean depth, 8 feet). Though is similar in dimensions to Putnam Lake, Lake Carmel has a retention time of 0.1 years (1.2 months) due to its watershed being over four times greater at, 8,414 acres. Despite this increased flowrate, Lake Carmel is also a eutrophic lake and has high susceptibility to harmful algal blooms (CSLAP Report Lake Carmel.,2018).

Nearest to Putnam Lake, however, is the smallest waterbody in the study, Ball Pond. As seen in Table 1, Ball Pond is a 82.44-acre, natural spring fed lake in New Fairfield, Connecticut with a 246-acre watershed. Despite its small area and watershed, Ball Pond is quite deep with a maximum depth of 51 feet (mean depth 24 feet). Although it is within proximity to Putnam Lake, algal blooms are a rare occurrence in Ball Pond. Both bodies of water are local watersheds, which support surrounding communities as water supplies.

Margerie Reservoir is a 245-acre artificial reservoir also located in New Fairfield and Danbury and its watershed is 2,942 acres. Built for the sole purpose of strong and delivering drinking water to the city of Danbury, Connecticut, the water levels in this lake can fluctuate in response to municipal water demands. The dam for Margerie is also located at the south end and the shape of the reservoir is remarkably similar to Putnam Lake. No statistic could provide data on its mean depth, but the lake has a maximum depth of 28 feet (Danbury Hazard Mitigation Plan, 2016). Margerie Reservoir is known for having cyanobacterial blooms occur at least once a year though it is just one of three drinking water supplies to the City of Danbury.

The final lake studied is, Squantz Pond. Squantz Pond is a natural spring fed body of water located in New Fairfield, CT. As seen in Table 1, Squantz Pond today has a maximum depth of 45 feet (mean depth 23 feet) and takes up 266 acres with its watershed taking up 3,635 acres. While Squantz Pond itself is a naturally occurring lake, when Candlewood Lake was constructed in 1938, for the purpose of power generation, the filling of Candlewood ended up raising (and controlling) the water levels in Squantz Pond. As a result, of its hydrologic connection to Candlewood lake, water levels in this water body are artificially manipulated in response to power production needs. Over the past several years, Squantz Pond has had harmful algal bloom occurrences which have caused for the state park's beach to be closed to the public.

Measurements were taken for water temperatures, wind direction/speed, air temperature, toxicity, and levels of cyanobacteria at these five freshwater bodies. To quantify the levels of cyanobacteria (biovolume) Cyanobacteria specific pigments were used to quantify cyanobacterial abundance (Kasinak et al., 2015) using a handheld fluorometer. To compare toxicity to biovolume, a Microcystin ELISA toxin assay kit and the benchtop fluorometer were used to determine if microcystin levels increase with phycocyanin fluorescence (RFUs). Measurements other environmental factors were documented such as water temperature, air temperature, and wind speed to see if there is a correlation between these environmental influences and the increase of cyanobacteria and its levels of toxicity. In addition, this also helped to determine whether there is a correlation in the timing and rate of change between harmful algal blooms on different bodies of water.

The goal of this study was to see if there is a relationship between phycocyanin and microcystin concentrations in order to more rapidly predict the range of toxicity within a body of water dependent upon how much phycocyanin is present. In addition, sampling from five local

bodies of water within similar regions would be able to provide a better ecological understanding on the behavior of phycocyanin and microcystin to see how certain environmental factors can influence the behavior of HABs.

## Materials & Methods

### Phycocyanin Concentrations

The phycobilin pigments cyanobacteria contain, include phycocyanin, which is an accessory pigment to chlorophyll that gives many cyanobacterial taxa their distinctive blue-green color (Kasinak et al., 2015). Phycocyanin concentrations can be detected using a fluorometer which is a handheld device that can detect and quantify photosynthetic pigments.

The levels of cyanobacteria were determined with a CyanoFluor Handheld HAB Indicator by Turner Designs, Inc. This device is a field-portable fluorometer which can quickly estimate the abundance of cyanobacteria through measuring levels of phycocyanin. Studies that used the CyanoFluor to conduct their research were, Choo et al. 2019, Kasinak et al., 2014.

Samples that were collected from each body of water were taken at the same sampling site every week in order to reduce sampling error.

Samples for each body of water were collected once a week for 12 consecutive weeks, beginning August 20$^{th}$, 2020 and ending on November 26$^{th}$, 2020. Sample collecting would begin every Wednesday or Thursday (depending on the weather) between 12:00pm and 1:00pm and end between 3:00pm and 4:00pm. This was done so samples would be collected during peak hours of sunlight all within similar temperature ranges. Collecting samples would only be suitable in dry weather conditions when wind speeds are no higher than 10 mph, due to the

influence rainfall could have on data collection and wind moving cyanobacterial populations to different areas on a body of water since photosynthetic activity within cyanobacteria is at its peak when exposed to sunlight (Hudnell, 2010).

Every week the order in which body of water would be sampled from first, rotated. For example: in the first week of sampling, samples were collected in order from Squantz Pond, Ball Pond, Lake Carmel, Putnam Lake, and finish sample collection on Margerie Reservoir. Then the next week, collection started with Ball Pond, following the same order in which the lakes were sampled previous week, and completing sample collections at Squantz Pond. The method of rotating the order in which samples were collected at each body of water was done so the collection of samples would be done at different times within the three-hour sample collection time frame.

Before going into the field to collect samples, a small pouch was packed with materials; a thermometer to measure water temperature, five sampling vials to collect water at each site, and a Kestrel 4500 Pocket Weather Tracker by Forestry Supplies, Inc., to measure air temperature and wind speed/direction. When approaching the site, wind speed and air temperature were measured using the Kestrel 4500. To make sure the Kestrel was pointed in the direction in which the wind is blowing, the propeller on the Kestrel was referred to which measures wind speed precisely. Then water temperature was measured using a stick thermometer.

When measuring water temperature, the thermometer was submerged at least six inches below the surface of the water and was held there for 10 seconds to get an accurate water temperature reading. Once the water temperature data was collected, the sampling jar was completely submerged underwater before opening the top, once submerged the top was opened to let the water fill the jar and the cap was tightly sealed before bringing the sampling jar back to

the surface. All the field data was immediately logged in a journal. These steps were repeated at each sampling site.

After sample collection was complete, the jars were brought back to the lab within half an hour after collecting the final water sample to test the samples using the CyanoFluor. Each sampling jar was labeled with the name of each body of water and the date. When extracting these samples from the jar for the fluorometer a plastic syringe was used to take up the sample.

All samples in the Cyanofluor were ran using a filtrate to correct for the interference from dissolved organic materials (DOM). Samples were collected using a plastic syringe and then ejected into the glass cuvette. After running the filtrate, the sample was discarded from the cuvette. Then filter capsules were used to place at the end of the 60cc syringe with the sample. The glass cuvette was rinsed three times with the filtrate. Once rinsing was complete the rinsed cuvette was filled ¾ full with the filtrate. After every rinse a KIM Wipe was used to wipe the sides of the cuvette to remove away any moisture and/or fingerprints. The first set of data shown was the ratio; PC:CHL (phycocyanin : chlorophyll). The next set of data showed the raw fluorescence values (RFUs) for both phycocyanin and chlorophyll. The final set of data shown was the FTR Blank values which was displayed as PBLK & CBLK. After data was collected, the sample was discarded from the cuvette and rinsed thoroughly before running another sample through the fluorometer. All data was collected from the CyanoFluor for each sample reading. This was repeated for each sample that had been collected.

Microcystin Concentrations

Separately, concentrations of the hepatotoxin microcystin which is commonly produced by HABs were also measured in water bodies. Multiple studies have also used an ADDA ELISA Kit as well as liquid chromatography to test for cyanotoxins found within waterbodies (Zhang &

Zhang, 2014; Stauffer et al., 2019). Therefore, once each sample was tested in the fluorometer, the remaining water sample in the individual jars were transferred to a smaller vial, using a syringe. The samples were stored in a -20˚F freezer to preserve the potential microcytin. This is where samples were stored which would be tested for levels of toxicity using a Eurofins Abraxis Microcystin-ADDA SAES ELISA. The ELISA test (Enzyme-Linked Immunosorbent Assay) is used to detect and quantify cyanotoxins (specifically microcystins) in water samples.

When samples were ready to be ran for the ELISA toxin assay these procedures were followed. The frozen water samples were removed from the -20˚C freezer and were ran them through a freeze/thaw cycle twice more since the samples had already been frozen once. The Eurofins Abraxis Microcystin-ADDA SAES ELISA test kit came with 96 wells. Several extra columns of wells were provided by Dr. Edwin Wong (Western Connecticut State University). To reduce contamination of samples and mixing samples, it was determined it was best to divide the toxin test of all samples into three separate testing days. Through splitting the water samples into three separate groups and then running the toxin test on each group of samples on a different day. For the first day of toxin testing, 10 Samples were ran for toxicity levels the first day of toxin testing, 20 samples the next testing day, and the last 20 samples being tested on the final day. Due to overfilling the vials containing the water samples from the week of August 20$^{th}$, 2020, and freezing them, the vials had broken, and therefore the water samples from August 20$^{th}$ could not be tested for toxicity.

The first day of toxin tests, the water samples that were tested were collected from, August 26$^{th}$ & September 3$^{rd}$, 2020. For the freeze/thaw cycle all samples were stored in a -20˚F freezer until the scheduled day of testing. The purpose of the freeze/thaw cycle is to lyse the cyanobacterial cells so potential cyanotoxins can be released. Since samples had be frozen

initially, the freeze/thaw cycle had to be repeated only two more times. When samples were removed from the freezer they were then thawed in an incubator or water bath. Once the samples were completely thawed out, the samples were then placed in a -80˚F freezer so the samples could quickly freeze. After the samples were completely frozen, they were thawed out again and this was repeated once more.

Once the freeze/thaw cycle was complete samples were brought into the lab where each sample had to be 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 of water samples. The lysed sample was shaken thoroughly and then using a sterile disposably syringe, we drew the sample up into the 8 mL graduation line in the plastic syringe. A new syringe filter was then placed on the tip of the syringe.

Before transferring the sample into a clean glass vial, 5- 6 mL of the initial filtrate was discarded into the vial in which the water sample initially came from. This was done incase these samples needed to be retested, and to minimize debris getting into our filtrate samples which we used for the ELISA test. 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 on the designated testing day.

Once all the samples were filtered and ready to be ejected into the microtiter plate, the Eurofins Abraxis Microcystin-ADDA SAES ELISA kit was removed from the refrigerator and the standards and control were incubated to an ambient temperature before using them. Multiple pipettes and pipette tips were used by Fisher Scientific along with wash buffer solution and a timer. The number of microtiter plate strips required was removed from the resealable pouch and

the remaining strips were stored in the pouch. The wash buffer (5x) concentrate was diluted at a ratio of 1:5 with deionized or distilled water.

Once all materials were prepared, 50µL of the standards, control, and samples were added to the wells. The standards and control were used for every test. For every two wells in the same column, you added one standard, the control, one sample. Once 50 µL of the standards, control, and samples was added to their designated wells, 50 µL of the antibody solution was added to each well using a 100 µL 8 multichannel pipette.

After adding the antibody solution 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 the 30 seconds the strips incubated for 90 minutes at room temperature. Once the 90-minute incubation was complete, the parafilm was removed and the 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 criterium. If the data's R-value for the 4-parameter standard curve fit was greater than 0.990, the run was accepted. If the absorbency 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. Also, 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. These steps were repeated two more times on separate days.

On the second day of toxin tests, twenty samples were ran, five samples from each week September 9$^{th}$, September 17$^{th}$, September 24$^{th}$, and October 1$^{st}$. For the third day of toxin testing, samples from October 8$^{th}$, October 15$^{th}$, October 22$^{nd}$, and November 6$^{th}$ were tested. The week between October 22$^{nd}$ and November 6$^{th}$ we did not collect water samples, due to weather conditions which would affect our results and did not follow our parameters for proper weather conditions. Once all the phycocyanin and toxin data was thoroughly checked and collected, data analysis was conducted.

## Results

An analysis of all the phycocyanin and microcystin measurements from the sampling season was compared to see if there was a universal relationship between all phycocyanin and toxicity concentrations across all sample sites. As seen in Figure 1 below, there appeared to be a potential positive trend between phycocyanin and microcystin concentrations. ($R^2$ = 0.01375).

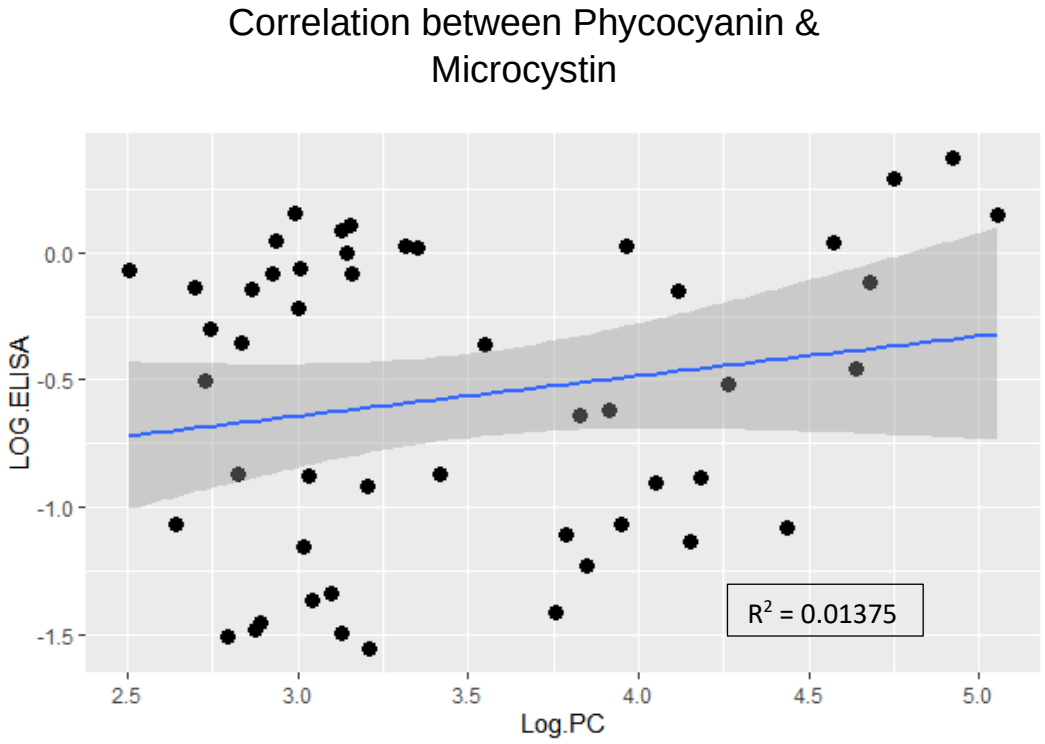


*Figure 1 All Phycocyanin & Microcystin concentrations from the sampling season were compared in a scatter plot to determine if there was a universal relationship between the two.*

However, after running a simple linear regression, it was found that there was no significant correlation between phycocyanin and microcystin (F= 1.683, P-value: 0.2007).

With no obvious universal pattern in the pooled data, the analysis was expanded to see if the cyanobacterial populations among each individual body of water possess a relationship between their phycocyanin and microcystin concentrations. The data in Figure 2 below presents

the correlation between the phycocyanin and microcystin of the cyanobacterial populations among each separate body of water.

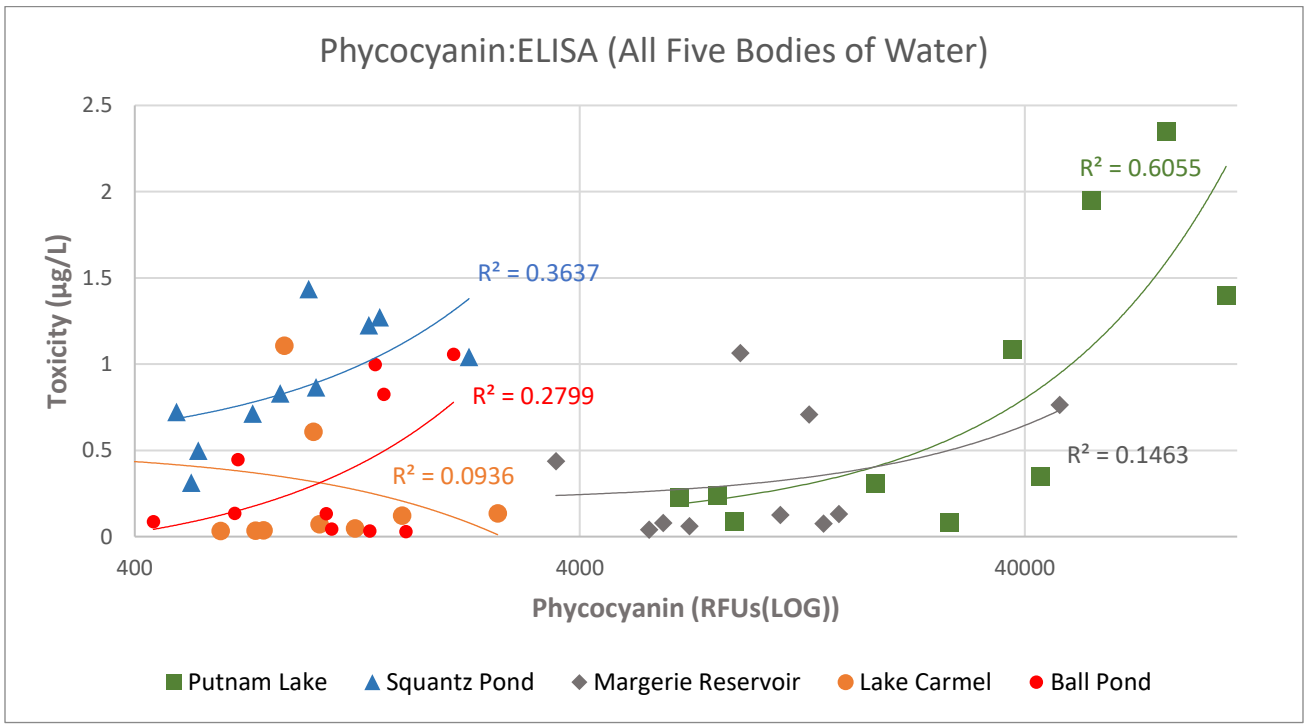


*Figure 2 A scatter plot was created to determine the linear relationship between phycocyanin and microcystin among each individual body of water. This was created to determine if certain bodies of water and their cyanobacterial populations may have a closer relationship between phycocyanin and microcystin.*

Initially Putnam Lake seems to have a strong relationship between its phycocyanin and microcystin readings ($y = 2E\text{-}05x + 0.0668$, $R^2 = 0.6055$, P-value = 0.01035). Likewise, Squantz Pond ($y = 0.0004x + 0.488$, $R^2 = 0.3637$, P-value = 0.01702) also has a significant positive relationship. On the other hand, while both Margerie ($y = 1E\text{-}05x + 0.199$, $R^2 = 0.1463$, P-value = 0.3235) and Ball Pond ($y = 0.0004x – 0.1557$, $R^2 = 0.2779$, P-value = 0.5743) both also potentially carried positive correlations neither were considered significant Furthermore of the five lakes, only Lake Carmel presented no significant relationship, ($y = -0.0002x + 0.5117$, $R^2 = 0.0936$, P-value = 0.6097).

With patterns in lake-specific relationships between phycocyanin and microcystin established, the between lake-variation of each parameter was analyzed. Statistics of the data presented in Figure 3 below illustrates, in terms of the independent variable of this study, Putnam Lake and Margerie Reservoir both produced high levels of phycocyanin. Putnam Lake had concentrations of phycocyanin as high as 113,318 RFUs. Margerie Reservoir had concentrations of phycocyanin as high as 47,932 RFUs. Whereas Ball Pond Lake Carmel & Squantz Pond produced low concentrations of phycocyanin. Ball Pond also had low concentrations of phycocyanin with concentrations no greater than 2,081 RFUs. Lake Carmel had concentrations no greater than 2615 RFUs. Squantz Pond produced consistently low concentrations of phycocyanin no greater 2255 RFUs.

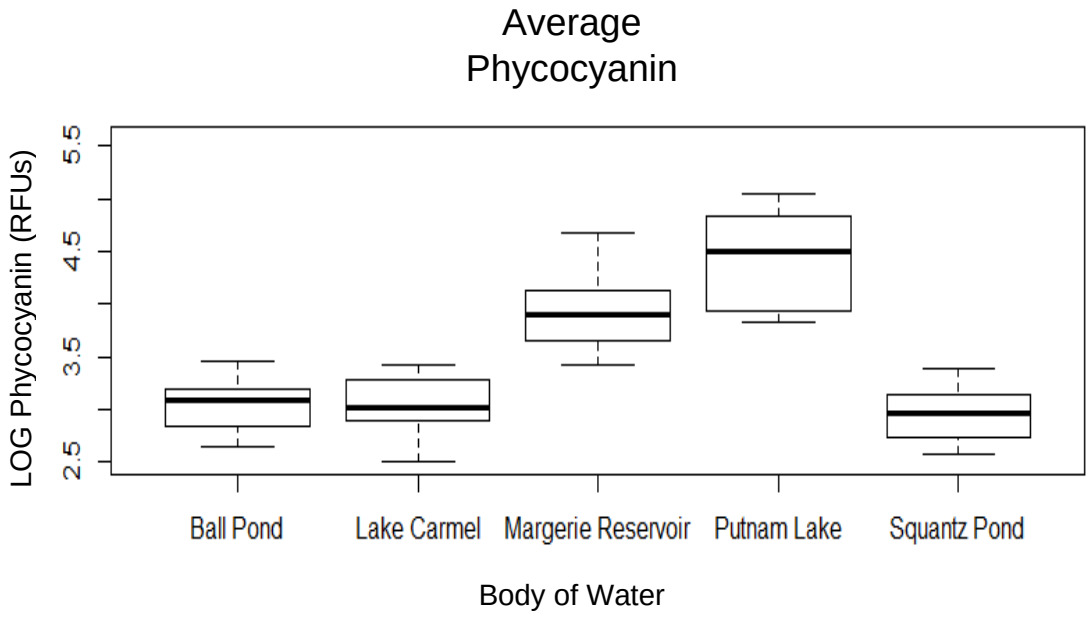


*Figure 3 A box & whisker plot representing phycocyanin concentrations among each body of water. Margerie Reservoir & Putnam Lake had exceedingly higher levels of phycocyanin compared to Ball Pond, Lake Carmel & Squantz Pond. (F-statistic = 49.05 P-value:<2e-16 ***).*

*Table 2: This table presents an ANOVA which compares the differences in phycocyanin concentrations among the five waterbodies.*

| Body of Water | Diff | Lower | Upper | Adjusted P-value |
|---|---|---|---|---|
| Lake Carmel-Ball Pond | -0.003052949 | -0.3780257 | 0.3719198 | 0.9999999 |
| Margerie Reservoir-Ball Pond | 0.870772097 | 0.4957994 | 1.2457448 | 0.0000002 |
| Putnam Lake-Ball Pond | 1.383598419 | 1.0086257 | 1.7585712 | 0.0000000 |
| Squantz Pond-Ball Pond | -0.078021084 | -0.4529938 | 0.2969517 | 0.9764966 |
| Margerie Reservoir-Lake Carmel | 0.873825046 | 0.4988523 | 1.2487978 | 0.0000002 |
| Putnam Lake-Lake Carmel | 1.386651367 | 1.0116786 | 1.7616241 | 0.0000000 |
| Squantz Pond-Lake Carmel | -0.074968136 | -0.4499409 | 0.3000046 | 0.9797069 |
| Putnam Lake-Margerie Reservoir | 0.512826322 | 0.1378536 | 0.8877991 | 0.0027017 |
| Squantz Pond-Margerie Reservoir | -0.948793181 | -1.3237659 | -0.5738204 | 0.0000000 |

Putnam Lake and Margerie Reservoir contained concentrations of phycocyanin no less than 3535 RFUs. Ball Pond, Lake Carmel, & Squantz Pond contained levels of phycocyanin concentrations no greater than 2615 RFUs. After running an ANOVA, there was a difference among these water bodies (P<2e-16***). As seen in Figure 3 among these bodies of water, Ball Pond, Lake Carmel, and Squantz Pond were all similar whereas Putnam Lake was different from each body of water as well as Margerie Reservoir. Although Putnam Lake and Margerie Reservoir had the highest levels of phycocyanin, as seen in Table 2 they also were different from one another (P-value = 0.0027017). The bodies of water similar in phycocyanin concentrations were combined and a t-test was ran to see if there is a significant difference between Putnam Lake and Margerie Reservoir's average phycocyanin concentrations compared to Ball Pond, Lake Carmel, & Squantz Pond's phycocyanin concentrations.

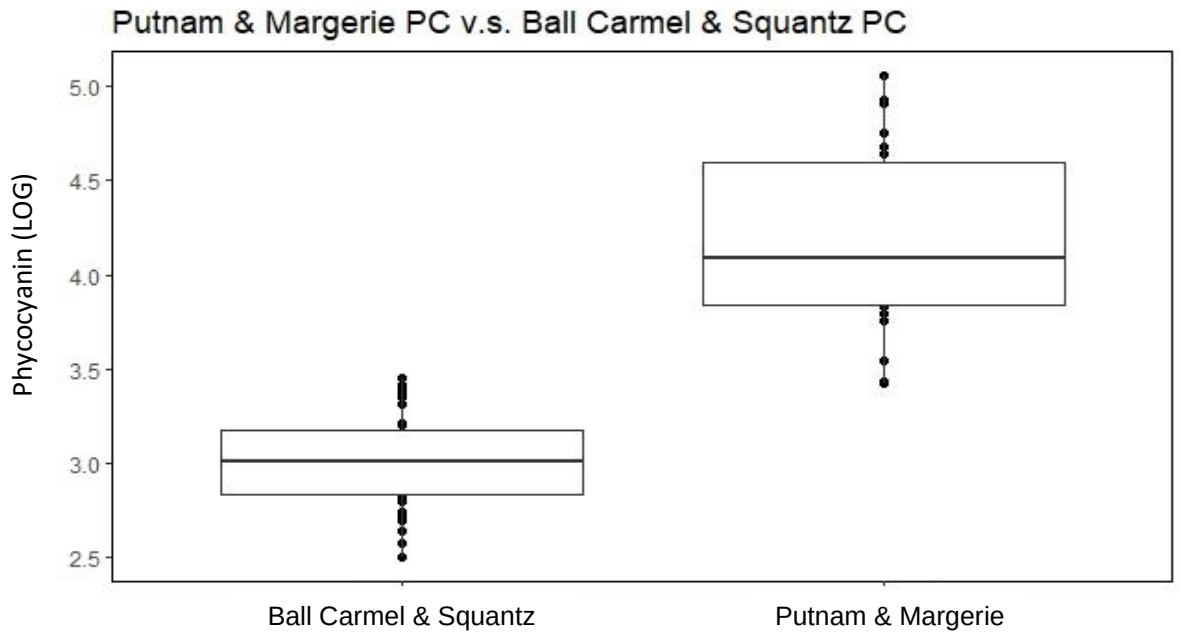


*Figure 4 This t-tests presents the significant difference between Putnam Lake & Margerie Reservoir's grouping of phycocyanin compared to Ball Pond, Lake Carmel, & Squantz Pond's grouped phycocyanin concentrations. (P-value = 3.066e-12). These groupings are significantly different from one another in terms of their phycocyanin ranges.*

This log scaled t-test was conducted to see how the two bodies of water, containing the highest fluorescent units of phycocyanin (Margerie Reservoir & Putnam Lake), compared to the three bodies of water with the lowest phycocyanin data (Ball Pond, Lake Carmel, Squantz Pond). There was a significant difference between these two groups and their average phycocyanin concentrations (t = -10.888; p-value = 3.066e-12). The average phycocyanin readings in the Ball Carmel & Squantz combined group was 3.014975 RFUs (Log scaled) whereas Putnam & Margerie had an average of 4.169184 RFUs (Log scaled).

In order to deduce if there were any discernable temporal patterns in these data, that may be associated with bloom development patterns, Figure 5 below was produced. This figure presents phycocyanin concentrations from each body of water over time.

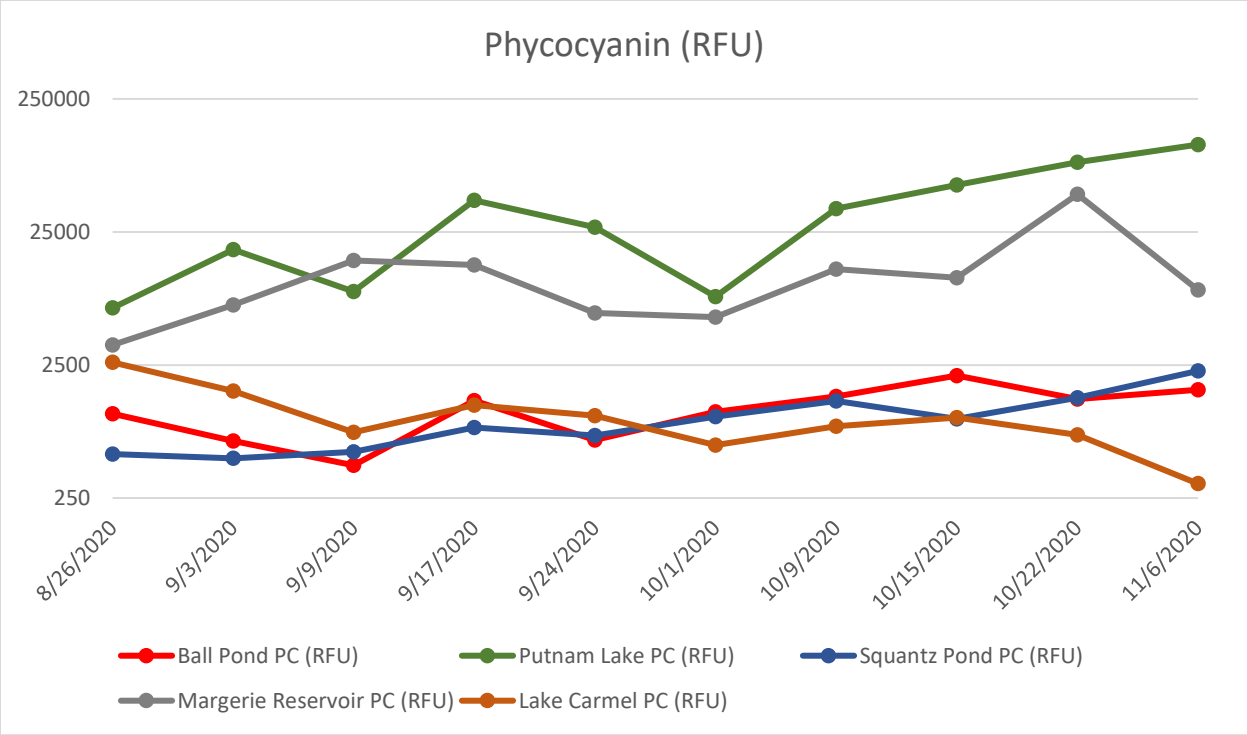


*Figure 5 Over time, Putnam Lake & Margerie Reservoir remained persistently higher than the other three bodies of water which possessed low concentrations of phycocyanin. The three bodies of water which persistently remained low in phycocyanin levels, were linear to one another. Based on this line graph we can see how these bodies of water significantly differed in terms of phycocyanin.*

From this graph it was observed that the two bodies of water with the highest levels of phycocyanin do not intersect with the three bodies of water containing the lowest levels of phycocyanin. Putnam Lake and Margerie Reservoir are linear to one another whereas Ball Pond, Lake Carmel, & Squantz Pond are grouped together. This demonstrates the differences between these two sets of lakes persisted throughout the entirety of the sampling season.

Next an ANOVA was conducted on the dependent variable of the study to see if the sample bodies of water differed in their average concentrations of microcystin. As figure 6 presents below, Putnam Lake had an average microcystin concentration of 0.8071 µg/L, Squantz Pond's average of microcystin was 0.8909 µg/L, Margerie Reservoir = 0.3477 µg/L, Ball Pond = 0.3776 µg/L, & Lake Carmel's microcystin concentration was 0.304 µg/L.

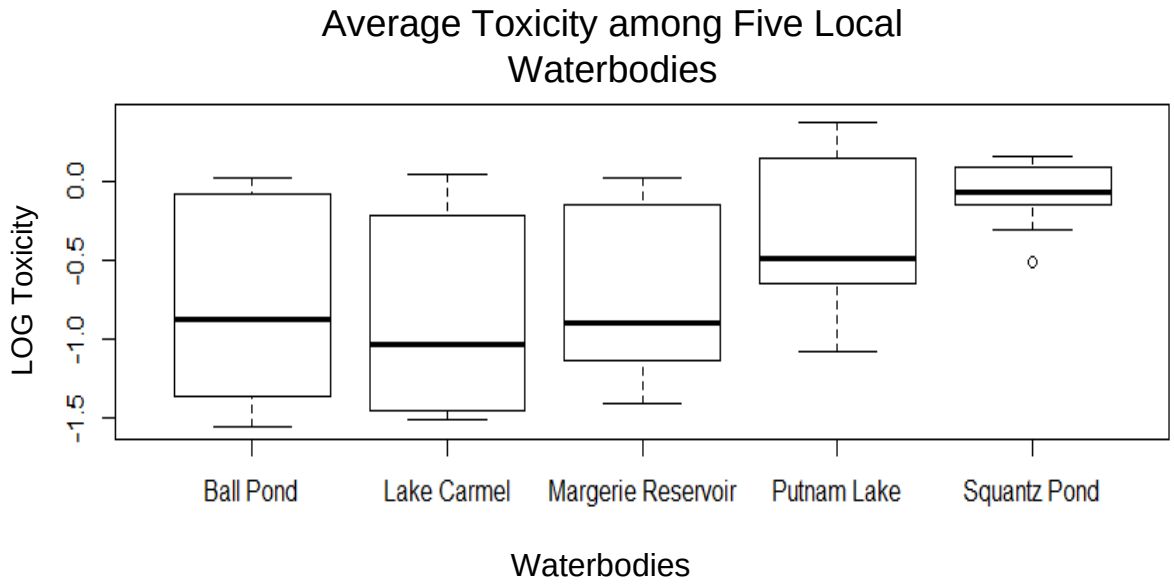


*Figure 6. This box & whisker plot presents the individual ranges and averages of microcystin levels among each individual body of water. Squantz Pond had a small but high-level range of microcystin compared to the other four bodies of water. Putnam Lake possessed the highest concentration reading of microcystin but had a wider range of readings compared to Squantz Pond. (F-statistic = 4.13; P-value: 0.00619 **)*

*Table 3: This table presents the similarities and differences in microcystin concentrations among the five waterbodies*

**Tukey multiple comparisons of means**

**95% family-wise confidence level**

| Body of Water | Diff | Lower | Upper | Adjusted P-valu |
|---|---|---|---|---|
| Lake Carmel-Ball Pond | -0.1322664 | -0.79433783 | 0.5298050 | 0.9790842 |
| Margerie Reservoir-Ball Pond | 0.0382246 | -0.62384679 | 0.7002960 | 0.9998309 |
| Putnam Lake-Ball Pond | 0.4106533 | -0.25141814 | 1.0727247 | 0.4076363 |
| Squantz Pond-Ball Pond | 0.6770072 | 0.01493582 | 1.3390786 | 0.0427982 |
| Margerie Reservoir-Lake Carmel | 0.1704910 | -0.49158036 | 0.8325624 | 0.9479420 |
| Putnam Lake-Lake Carmel | 0.5429197 | -0.11915170 | 1.2049911 | 0.1543629 |
| Squantz Pond-Lake Carmel | 0.8092737 | 0.14720226 | 1.4713450 | 0.0096295 |
| Putnam Lake-Margerie Reservoir | 0.3724287 | -0.28964274 | 1.0345000 | 0.5061848 |
| Squantz Pond-Margerie Reservoir | 0.6387826 | -0.02328878 | 1.3008540 | 0.0633466 |
| Squantz Pond-Putnam Lake | 0.2663540 | -0.39571743 | 0.9284254 | 0.7828417 |

After running an ANOVA, it was found that there is a difference in microcystin concentrations among these bodies of water (P-value = 0.00619**). The only body of water which was different from other bodies of water was Squantz Pond. Squantz Pond was

significantly different from every water body except Putnam Lake (P-value = 0.7828417). From these averages and observing the graph it appeared Putnam Lake & Squantz Pond are most similar in toxicity whereas Ball Pond, Lake Carmel, & Margerie Reservoir are similar with lower toxicity rates. It is noted, that while the range of Putnam Lake's measurements overlap with both Margerie and Squantz Pond the decision to group Putnam Lake with Squantz Pond and not Margerie Reservoir was based on Putnam Lake's measurements overlapping completely with all of Squantz Pond's measurements in microcystin concentrations, while only partially overlapping with those of Margerie. Putnam Lake and Squantz Pond possessed the highest concentrations of microcystin. Putnam Lake's concentration of microcystin ranged from 0.083 to 2.346 µg/L. Squantz Pond's readings for microcystin ranged from 0.312 to 1.433 µg/L. Ball Pond had a concentration range of 0.028 to 1.056 µg/L. Lake Carmel had a range from 0.031 to 1.107 µg/L and Margerie Reservoir's range of microcystin spread from 0.039 to 1.064 µg/L.

Among these two separate groups of freshwater lakes/ponds, there may be a difference between their average concentrations of microcystin. Figure 7 below represents Putnam lake and Squantz Pond's combined microcystin averages compared to Ball Pond, Lake Carmel, & Margerie Reservoir's combined microcystin averages. To help determine if there is a significant difference, a t-test was conducted on these groupings.

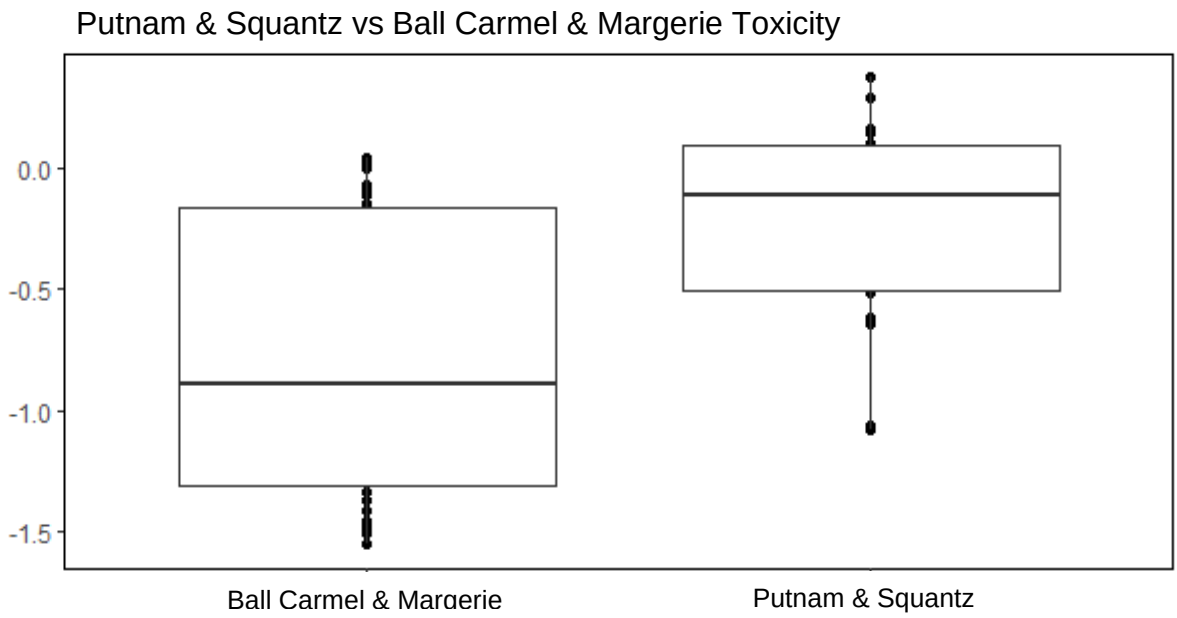


*Figure 7 Ball Pond, Lake Carmel, & Margerie Reservoir possessed low concentrations of microcystin when compared to Putnam Lake & Squantz Pond's microcystin concentrations. Although none of these bodies of water exceeded federal and state health limits, there was a significant difference in terms of toxicity between these two grouping of lakes (P-value = 0.0001512).*

After running a t-test there was in fact a significant difference in average microcystin concenrations between Putnam Lake & Squantz Pond compared to Ball Pond, Lake Carmel, & Squantz Pond (t = -4.1184; P-value=0.0001512).

In order to deduce if there were any discernable temporal patterns in these data, that may be associated with bloom development patterns, Figure 8 below was produced. This figure presents the trends of microcystin concentrations among the five sample freshwater lakes/ponds over time.

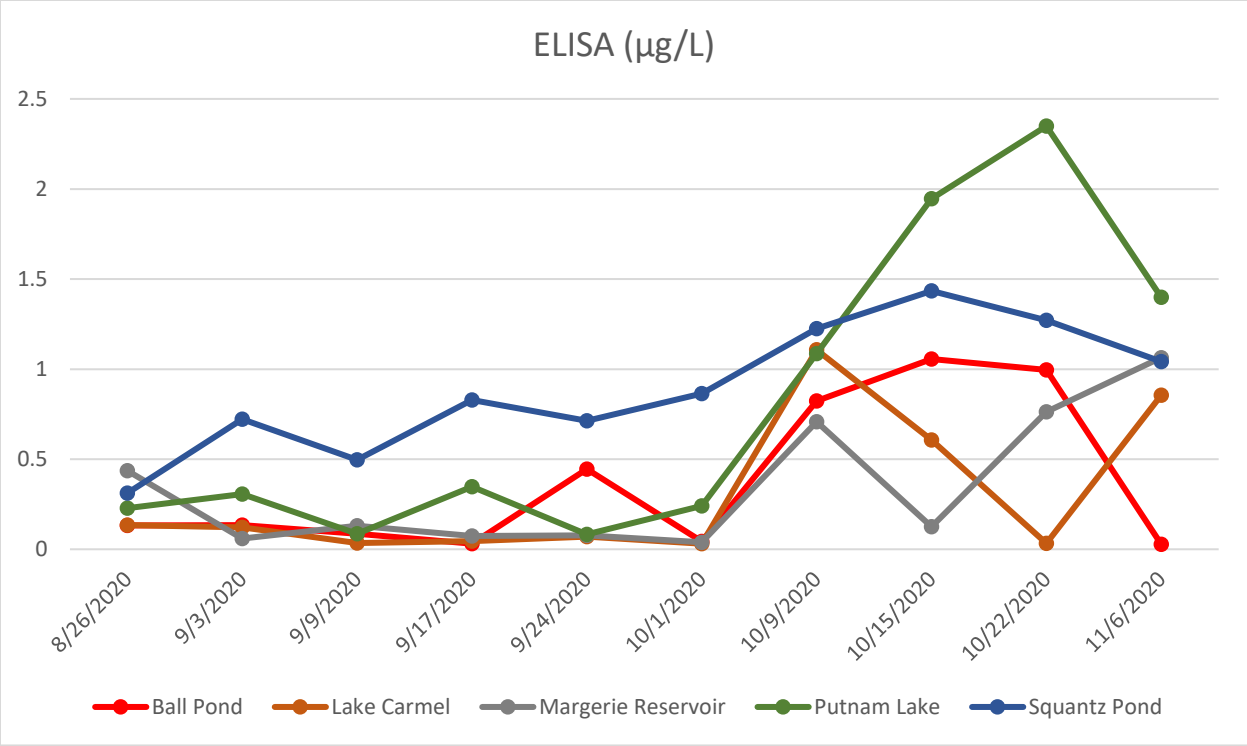


*Figure 8. From this line graph, Squantz Pond remained persistently higher in microcystin compared to all other bodies of water. It was not until later in the sampling season when Putnam Lake significantly increased in microcystin concentrations. Putnam Lake possessed the highest concentrated level of microcystin out of all five waterbodies (2.349 µg/L). This does not exceed the public health threshold for microcystin at 8 µg/L.*

## Averages of all Lake/Ponds

*Table 4: This table presents the averages of all five waterbodies. It is sorted in phycocyanin from high-low as Phycocyanin was the independent variable for this study.*

| Body of Water | ELISA (µg/L) | Phycocyanin (RFU) | Chlorophyll (RFU) | PC:CHL Ratio |
|---|---|---|---|---|
| Putnam Lake | 0.8071 | 40340 | 10326 | 11.01 |
| Margerie Reservoir | 0.3477 | 13340 | 7823 | 3.5 |
| Ball Pond | 0.3776 | 1187 | 4369 | 0.28 |
| Lake Carmel | 0.304 | 1085 | 2776 | 0.42 |
| Squantz Pond | 0.8909 | 1019 | 3351 | 0.32 |

After collecting all the data, averages of phycocyanin, chlorophyll, and microcystin were calculated for each body of water. As highlighted in Table 4Table, Putnam Lake's cyanobacterial population possessed high concentrations of both phycocyanin and microcystin whereas Ball Pond and Lake Carmel had low concentrations of both phycocyanin and microcystin. Margerie Reservoir and Squantz Pond however fluctuated. Margerie Reservoir contained high concentrations of phycocyanin but low levels of microcystin whereas Squantz Pond contained low readings of phycocyanin but the highest levels of microcystin.

Putnam Lake and Margerie Reservoirs were the only waterbodies out of the five with a PC:CHL ratio greater than 1 (Putnam Lake = 11.01; Margerie Reservoir = 3.5). This indicates that these two bodies of water possessed a cyanobacteria-dominated phytoplankton community while the other lakes experienced green algae-dominated phytoplankton communities.

## Discussion

Among the five local bodies of water that were sampled within northern Fairfield County and Putnam County no universal correlation was presented between phycocyanin and microcystin. A result similar to what was concluded in a study conducted by McQuaid et al., 2011, a study taking place in the Missiquoi Bay in Quebec, Canada. Although that study only took place on a single body of water, it lasted two years and was chosen on the basis of historical taste & odor events along with frequent occurrences of cyanobacterial blooms (McQuaid et al., 2011). This led to the presumption supported by Kasinak et al., 2015, that although there may be a correlation between phycocyanin and cyanobacterial volume between years within a single lake, there is potentially no correlation between phycocyanin and cell density. Cyanobacterial

populations with low cell density may produce lower concentrations of photosynthetic pigments compared to cyanobacterial communities with high cell density. In addition, phycocyanin production also depends upon cyanobacterial growth stages as well as morphology, size, and relative abundance to other phytoplankton taxa (Kasinak et al., 2015). Microscopy of the water samples taken from each waterbody would help better understand the taxonomy of phytoplankton and the differences in the populations that make up these different bodies of water. This would also further provide an explanation for the differing concentrations in phycocyanin among the five bodies of water.

This is pattern seems to reinforce laboratory studies using photosynthetic pigments to predict toxin concentrations have also widely varied in finding a correlation between the two (Thomson-Laing et al., 2020; McQuaid et al., 2011; Francy et al., 2020). Although there was no universal correlation between phycocyanin fluorescence and microcystin, it is presumed from the data and numerous statistical tests that different bodies of water and their cyanobacterial populations can be classified and differentiated from one another. Even if there was a correlation between phycocyanin and microcystin, such results would not be of use as a rapid test to estimate toxicity unless a multiple year study was conducted on multiple bodies of water.

Results from this study,however demonstrate two lakes within which a statistically significant positive relationship between phycocyanin and microcystin appears to exist. Putnam Lake and Squantz Pond both contained cyanobacterial population that produced the highest amounts of microcystin and had a positive relationship between microcystin levels and phycocyanin.

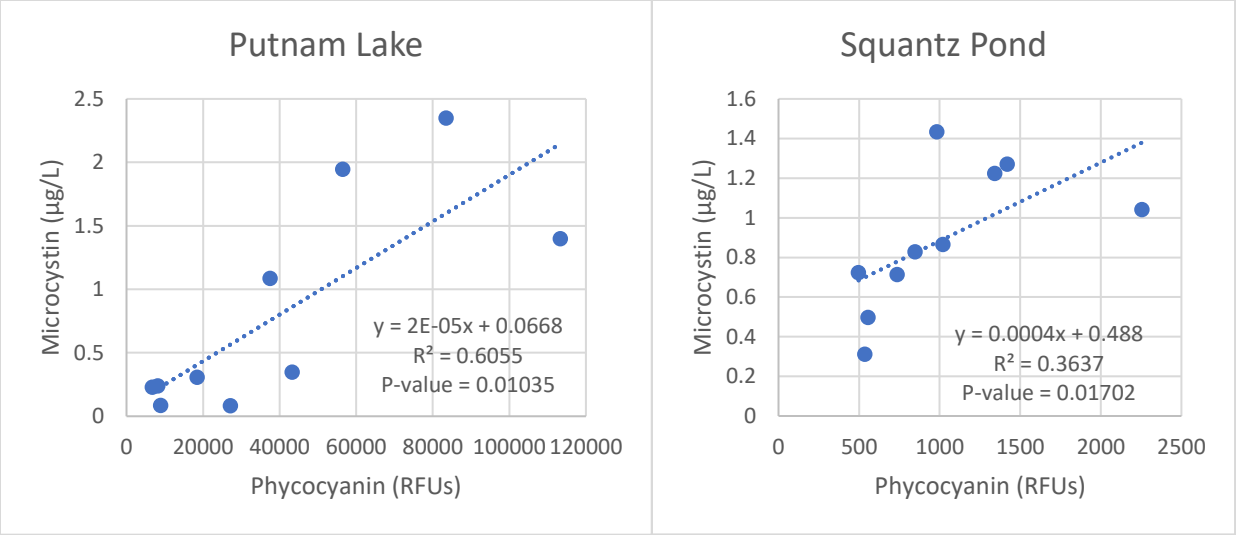


*Figure 9: These figures present the positive relationship of Putnam Lake and Squantz Pond's phycocyanin and microcystin concentrations.*

Putnam Lake also maintained a cyanobacterial population which the second highest average amount of microcystin compared to the other bodies of water (0.8071 µg/L) as well as the highest average amount of phycocyanin (40,340 RFUs). Again, this is not too surprising as Putnam Lake's average phycocyanin:chlorophyll (PC:CHL) ratio of over 11 fits the characterization of a lake experiencing harmful algal blooms during this sampling season.

Despite Putnam Lake's high overall cyanobacterial community. Within Squantz Pond is one of the greatest concerns in terms of potential harm to public health. Squantz Pond contained the highest average amount of microcystin (0.8909 µg/L) among all five bodies of water but the lowest average amount of phycocyanin fluorescence (1019 RFUs) and with a PC:CHL ratio that was below 1 suggesting the toxicity present was being produced despite the lake not having a cyanobacteria dominated phytoplankton community. Ball Pond and Lake Carmel both had low amounts of microcystin, averaging at 0.3776 & 0.304 µg/L as well as low average readings of phycocyanin within their cyanobacterial populations (1187 & 1085 RFUs) which were sampled

from. Margerie Reservoir however had a high range of concentrations for phycocyanin whereas the concentration levels of microcystin for Margerie Reservoir's cyanobacterial population had an average of 0.3477 µg/L. During the sampling period Putnam Lake and Margerie Reservoir had a range of phycocyanin readings from 3535 RFUs to 113,318 RFUs. Ball Pond, Lake Carmel, and Squantz Pond had a range from 321 RFUs to 2615 RFUs.

Potential Biological Drivers of Variation

Cyanobacterial populations within Putnam Lake and Squantz Pond possessed the highest concentrations for microcystin out of all five bodies of water and both presented positive relationships between microcystin and their phycocyanin concentrations. Although Squantz Pond had the lowest average reading for phycocyanin out of all five sampled bodies of water, Squantz Pond produced the highest average concentration of microcystin (0.8909 µg/L). In addition, although Putnam Lake and Margerie Reservoir produced the highest amounts of phycocyanin, that does not necessarily mean they are likely to be the most toxic populations. This is supported by Squantz Pond's data which contained the lowest concentration of phycocyanin (1019 RFUs) out of all five sampled bodies of water yet Squantz had the highest average concentration for microcystin. Hisbergues et al., 2003 conducted research using PCR to identify microcystin-producing genotypes of different cyanobacterial genera. Using similar methods from Hisbergues et al., 2003, could potentially support an argument that Squantz Pond may support a population of cyanobacteria which possess a correlation between genotypes and genera of cyanobacteria that can produce microcystin. Hisbergues et al., 2003 found that *Nodularia* may not possess the gene capable of producing microcystin.

Squantz Pond potentially supports the most toxic cyanobacterial population for microcystin of all five bodies of water due to its low phycocyanin and high microcystin readings.

In addition, Margerie Reservoir may support the least toxic cyanobacterial population out of all five bodies of water. This is due to the matter that Margerie Reservoir produced the second highest average of phycocyanin (13340 RFUs) yet the second lowest average concentration for microcystin.

Toxin Variation

The United States Environmental Protection Agency, establishes that the recommended magnitude for microcystin is 8 µg/L. From Table 8 no single body of water within this study ever reached this threshold for microcystin. With that said, microcystin is not the only cyanotoxin considered to be a threat to public drinking and recreational water supplies. Although this study only tested for microcystin, it is important to consider different types of cyanotoxins may vary dependent upon the water body.

It is important to recall that the only cyanotoxins which were tested for was microcystin. Other bodies of water within this study may have supported cyanobacterial populations which produce more anatoxins or saxitoxins over microcystin. Neurotoxic alkaloid toxins such as anatoxin and saxitoxin can be produced mainly by strains of the genera *Dolichospermum, Oscillatoria, Aphanizomenon,* and *Cylindrospermopsis*. (Welker et al., 2004). The sampled bodies of water that did not produce much microcystin may produce a higher concentration of anatoxins and saxitoxins, which are some of the most acknowledged cyanotoxins, next to microcystin (Quiblier et al., 2013). With that said it is possible Putnam Lake supports cyanobacterial communities that are largely made-up of genera such as, Anabaena, Aphanizomenon, & Oscillatoria, which can produce anatoxins. It is not established that Putnam Lake does not support toxic algal blooms, since the ELISA test for this study did not test for any other cyanotoxin that could potentially reside in Putnam Lake. According to a report from the

World Health Organization., 2004, the most common cyanobacterial genera which can produce microcystin are; *Microcystis*, Anabaena, Aphanizomenon, & Planktothrix. With microscopy and conducting more ELISA tests for a wider range of cyanotoxins, we might better be able to find a correlation between phycocyanin and cyanotoxin concentrations among local bodies of water and their supported cyanobacterial populations as certain genera may produce higher concentrations of toxicity than others. As well as different types of cyanotoxins.

Potential Physical Drivers of Variation

Certain bodies of water may also contain cyanobacterial populations which can produce higher concentrations of phycocyanin dependent upon environmental factors, such as how long a body of water is within direct sunlight, how fast water is flowing, precipitation events or retention time (Kaebernick & Neilan et al., 2001) (Romo et al., 2013) (W.H.O., 2014). With that said Margerie & Squantz are the only waterbodies out of all five which are manipulated year-round. Yearly, Candlewood Lake (which connects to Squantz Pond) has a drawdown during the winter months (December 1st – mid April). Where the water table is lowered and drained into the Housatonic River. As Candlewood's water table is drawn down, Squantz Pond's water table is lowered as well (Candlewood Lake Authority). Margerie Reservoir is a resource for drinking water which is one of three drinking water reservoirs utilized by the City of Danbury. According to the 2020 Water Quality Report by the City of Danbury, "Our main reservoirs are the West Lake, Margerie, and East Lake located in Danbury… Customers of the Danbury Water System use approximately 7 million gallons of water each day. This water is treated and produced at our West Lake and our Margerie Water Treatment Plants." These artificial bodies of water which are routinely being lowered and raised can have an effect on cyanobacterial populations. This is supported by a study done by Kosiba et al., (2018). The conclusion of this study stated that the

studied artificial ponds within this study were more exposed to harmful cyanobacterial blooms, and for a longer period, than the natural oxbow lakes which had been studied. “The general problem can be expressed in this way: increasing artificiality of the aquatic environment (transformation, destruction, creation of new waterbodies) + eutrophication + global warming = increased proliferation of toxic cyanobacterial blooms + homogenization of plankton species structure” (Kosiba et al., 2018).

Potential Nutrient Variation

One of the major factors which influence the growth of HABs is nutrient loading (CTDEEP, 2020). Nutrient loading is typically caused by fertilization of local lawns, road runoff, groundwater pollution, atmospheric deposition, and point sources. Nitrogen and phosphorus are the two main elements which influence the growth of cyanobacterial blooms. Mainly, nitrate and phosphate enrichment by anthropogenic activities such as household wastes, drainage, and municipal wastes influence cyanobacterial growth and their bloom formation (Jahan et al., 2010). An increase in nutrient loading may cause blooms to rapidly grow as cells multiply, increasing the chances of these blooms to become toxic (Paerl et al., 2001).

Nutrient loading may vary among the sampled bodies of water and influenced the growth and abundance of cyanobacteria and the toxins associated with them. In addition, algal species or species groups have a number of physiological adaptations that allow them to exploit certain nutrients differently. These local bodies of water could potentially vary in biodiversity, causing nutrient loading to rapidly increase cyanobacterial populations and toxicity concentrations in certain bodies of water over others. With that said, differing species groups show preferences for specific nutrient regimes, this includes nutrient ratio or form (Heisler et al., 2008).

Another factor that potentially influences nutrient loading and how long nutrients will remain in a body of water is, retention time/water residency. Retention time is the amount of time in which a droplet of water spends in a stationary phase within a body of water and can influence the growth of cyanobacteria (Romo et al., 2013). Longer retention time can increase and promote photosynthesis and the rate at which blooms photosynthesize and cause nutrients such as phosphorus to deposit into the sediment which increases the growth of cyanobacteria (Jahan et al., 2010). In addition to such, longer retention time allows for nutrients to reside for longer periods of time within a body of water. Romo et al., 2013 found that longer residence time increased total cyanobacteria biomass. It was also found that as residency time increases, so do does the time in which a body of water may remain toxic from a cyanobacterial bloom (Cromar & Fallowfield, 1997).

In addition, there could potentially be environmental conditions favorable within Putnam Lake and Margeire Reservoir that cause cyanobacterial populations to produce higher concentrations of phycocyanin as opposed to Ball Pond, Lake Carmel, and Squantz Pond which produced low concentrations of phycocyanin. To answer this question, microscopy would need to be conducted to attempt to find a correlation between high concentrations of phycocyanin and the physical characteristics of cyanobacteria. The same could also be said for the bodies of water which possessed more toxic cyanobacterial populations.

Potential Hydrologic Variation

Causes of retention time vary, a large factor which may influence retention time and in turn influence the growth of HABs is climate change. Climate change influences a variety of ecological processes such as, ice-cover, wind regimes, solar radiation, precipitation, and weather patterns. Some studies have made predictions about the effects of climate change, increasing,

retention time, nutrient loading, loss of aquatic biodiversity, and a higher presence of phytoplankton and cyanobacteria (Romo et al., 2013; Jeppesen, Søndergaard & Jensen, 2003; Wagner & Adrian, 2009).

Climate change has been known to cause higher rates of precipitation in certain regions, and droughts in others. Specifically in Northeastern America, climate change is predicted to cause events such as extreme precipitation, flooding, and heat waves. Increased rainfall increases the growth of HABs due to increases in road runoff, groundwater draining into local lakes/ponds, and atmospheric deposition. Whereas droughts encourage longer retention time, stable water columns/surfaces which increases water temperature and photosynthetic rates due to direct sunlight and the surface of the water remaining still. Although climate change plays an influential role in the ecological activity which takes place within small freshwater lakes/ponds, physical characteristics of a body of water as well as its surroundings has an influence upon how cyanobacterial populations will behave (USEPA, 2014). Physical characteristics of a body of water can also influence the types of cyanobacterial populations and their diversity which arise within these local bodies of water.

Although research and experiments were designed to see if there was a correlation between phycocyanin and toxicity concentrations. Physical characteristics of a body of water and its surrounding environment may have an influence on the biodiversity of cyanobacterial communities within the local bodies of water which were sampled from.

Putnam Lake and Squantz Pond had the highest average concentrations for microcystin. These two bodies of water also reside at the lowest elevations among the five bodies of water which were sampled from. This observation can be backed up by Singh et al., 2014, where it was found that there was a correlation in cyanobacterial diversity among four high altitude lakes in

India. Multiple studies have found that cyanobacterial populations/species may be more prevalent dependent upon hydrological, ecological, and abiotic factors within local aquatic ecosystems (Kimambo et al., 2019).

Physical factors which are commonly examined within these ecosystems are water temperature, transparency, and alkalinity (Jahan et al., 2010). Water temperatures were measured during this study but had little to no correlation with the abundance of phycocyanin or microcystin. It is evident that HABs are caused by multiple factors, both genetic, physical, and environmental factors. Predicting the actual cause for the onset of HABs is difficult to narrow down and with that said blooming conditions act synergistically and it seems a combination of factors play a role in the influence of HABs. Cyanobacterial blooms are regulated by an interplay of geographically and ecologically diverse environmental variables (Jahan et al., 2010; Paerl et al., 2001). More variables need to be tested an examined to better understand the ecological function and behaviors of cyanobacterial blooms.

Future Research

More bodies of water must be sampled from to see if there are similar environmental factors among bodies of water that negatively or positively influence a bloom. With that said microscopy must be conducted, to determine which species/genera are most abundant and frequently seen among these individual bodies of water. This can help determine the habitat selection and preference for certain genera or species of cyanobacteria.

Next would be to run a wider range of toxin tests. Instead of testing for just one cyanotoxin such as microcystin, ELISA tests should be conducted for multiple cyanotoxins such as, anatoxin, saxitoxin, microcystin, and cylindrospermosin. Perhaps through running a wider

range of toxin tests it can be determined which toxins are most prevalent in individual bodies of water and what environmental factors or genetic factors could be influencing these toxic blooms.

A multiple year study should be conducted in order to determine if behaviors of cyanobacteria among individual bodies of water behave differently year by year or if behaviors remain relatively similar to previous years cyanobacterial behaviors.

## Conclusion

Putnam Lake and Margerie Reservoir have significantly higher concentrations of cyanobacteria than any of the other fresh waterbodies which were sampled from whereas Putnam Lake and Squantz Pond maintain cyanobacterial populations which are significantly more toxic than the other waterbodies. Furthermore, they both possess strong relationships between the phycocyanin and microcystin concentrations. Putnam Lake possessed both high concentrations of microcystin and phycocyanin. In addition, these bodies of water may possess other cyanotoxins within the cyanobacterial populations which they support. Ball Pond and Lake Carmel had both low concentrations of microcystin as well as low densities of cyanobacteria.

Of greatest note, however this study demonstrates that within both lakes with high microcystin levels (exceeding average concentrations of 0.8 µg/L) toxin concentration did positively relate to the concentration of cyanobacteria present. A relationship that exists (in the case of Squantz Pond), even when the phytoplankton community is predominantly composed of cyanobacteria. Of the two artificially manipulated reservoirs in this study, there is no obvious consistent directional relationship, Squantz Pond seems to maintain a cyanobacterial population that can produce high concentration levels of microcystin whereas Margerie Reservoir supports a

cyanobacterial population which does not produce high levels of microcystin. These bodies of water vary among one another in concentrations of phycocyanin and microcystin. This needs further investigation into local environmental influencers of cyanobacterial blooms, and the cyanotoxins associated with them.

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