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Management Strategies for Harmful Algal Blooms In Worcester, MA

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Management Strategies for Harmful Algal Blooms In Worcester, MA

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Abstract

Many lakes and ponds have experienced growth of Harmful Algal Blooms (HABs), which are colonies of cyanobacteria that can produce toxins that can be a threat to public's health. This project includes an assessment of monitoring, prevention, and treatment methods, along with development of suitable advisory thresholds that can support the control of HABs in the City of Worcester, MA. Research included archival research, data analysis, and interviews with researchers and scientists. Results were compared to understand which methods for prevention, treatment and advisory thresholds should be used for the city of Worcester to protect the public's health. Some results were the recommendation to raise the cell density threshold and testing for other cyanotoxins.

Executive Summary

Throughout the United States, Harmful Algal Blooms (HABs) have started to appear more frequently in lakes and ponds and have become a concern for many cities and towns. These blooms are formed by cyanobacteria and can grow at a very rapid pace, resulting in large algae-contaminated regions within the lakes. When they develop into large masses, they absorb a lot of the oxygen from the water, which can reduce the amount of oxygen getting to the other surrounding organisms, potentially killing them. When the cyanobacteria cells die or get ruptured, they can release cyanotoxins into the water. These cyanotoxins can cause health problems with animals and humans ranging from very mild effects all the way to death. When these blooms form in the city’s water bodies, the city is forced to shut down recreation in the lake to protect the public's health. Closing the water body is very frustrating for the residents as they rely on these water bodies for recreation and to relax.

The City of Worcester, MA has also experienced cyanobacteria blooms in a number of its lakes and ponds, and has been working to prevent and manage them more effectively. The City’s goal is to be able to keep the city's water bodies open as much as possible while at the same time keeping the public safe. The city has focused a lot of time on researching the causes of their blooms and has found some very good information that they will be able to use to hopefully reduce the frequency of bloom occurrences. The city currently performs frequent water tests to monitor the growth of these blooms and will treat the water if possible to try and prevent the blooms from occurring. If the bloom is already too big to treat, the water body will be closed until the bloom has gone away.

For this project, we are working with the Department of Public Works and Parks to help them research monitoring, prevention, and treatment techniques that they can use to improve their current harmful algal bloom management strategy. The goal of our IQP was to provide the Worcester DPW&P with research and recommendations on their current management plan to prevent premature and unnecessary closure of the local water bodies during the summer and fall seasons. We accomplished this goal by pursuing the following objectives:

1) Understand the current procedures that the Worcester DPW&P use for monitoring their water bodies.

2) Acquire information from research scientists and universities to better understand cyanotoxins and testing procedures for these toxins.

3) Compare and contrast researchers' preventive methods to current Worcester DPW&P preventive methods.

4) Provide recommendations and alterations to the current monitoring techniques and testing procedures.

The approach to meet these objectives included archival research, data analysis, and interviews with researchers and scientists. We conducted archival research to get a better understanding of cyanobacteria and cyanotoxins. We then analyzed the data of water tests that have been conducted on water bodies in Worcester to better understand the cyanobacteria that they are experiencing and also the cyanotoxins that are being produced. Finally we conducted interviews with researchers and scientists that focus a lot of their work on harmful algal blooms. This helped us to see the methods used in other parts of the country as well as the techniques that have been the most effective in combating harmful algal blooms.

Once we collected all of our information from conducting archival research, data analysis, and interviews with researchers and scientists, we started to analyse it by breaking it down into separate sections. We broke the information down into monitoring, prevention and treatment so that we could analyze each section individually. We found common trends and found which methods within each section that would work the best for the issues occurring in Worcester as well as the resources available for harmful algal bloom management.

The common trends that we found within our research and interviews was that frequent water tests are common practice to monitor the nutrient levels in water bodies as well as the amount of cyanobacteria present. The cell density threshold for cyanobacteria used by most of our interviewees was in the 70,000 - 100,000 cells/mL range and this was also the range that we found in our research. Treatment of blooms is always a last resort and is something that is not practiced very often due to the potential negative effects on the surrounding ecosystems. Everyone agreed that prevention was the most important part of their management strategy.

Their main goal was to prevent excess nutrients from reaching the water bodies. This can be done in a variety of ways.

After finding these trends and analysing Worcester’s current management strategy, we were able to provide a few recommendations to help them improve their already proactive management strategy. We recommended adding testing for saxitoxins and anatoxins, recommending looking into raising Worcester’s cyanobacteria threshold to 100,000 cells/mL, and to continue their effective prevention methods. We hope that these recommendations will help the city of Worcester improve their harmful algal bloom management strategy to keep recreation open as long as possible for residents while also keeping everyone safe.

Acknowledgements

We would like to thank Professor Mathisen for advising us throughout our project. We appreciated all the hard work you put into making sure that our project was still manageable considering the circumstances of working remotely and through zoom. We felt that we gained so much knowledge and great work experience while constructing this paper over the last seven weeks that we can continue to learn from this project and improve ourselves moving forward with our school and careers.

We would also like to thank Jacquelyn Burmeister of the Worcester DPW&P for spending her time analyzing and giving us feedback week in, week out on our progress, findings and results over the last seven weeks. She helped us find contacts to interview for our project which were vital in finalizing our recommendations to Worcester DPW&P.

Thanks to all of the interviewees that were a part of our interview process, joining our zoom calls with a positive attitude and giving us great, thorough answers to our questions.

Lastly, we would like to thank WPI for giving us this unbelievable opportunity to work with the Worcester DPW&P and truly try to make a positive impact on the Worcester community, especially the lakes and ponds throughout the City.

This experience was one that we know we will never forget. We learned valuable lessons and skills working together with a small group that will carry on with us in school and forever.

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Acronym List

AFA- Aphanizomenon flos-aquae

ELIZA- Enzyme-linked Immunosorbent Assay

qPCR- Real-time Polymerase Chain Reaction

LC-MS/MS- Liquid Chromatography Mass Spectrometry

HAB- Harmful Algal Bloom

ILWA- Indian Lake Watershed Association

MassDEP - Massachusetts Department of Environmental Protection

MassDPH - Massachusetts Department of Public Health

MichiganEGLE- Michigan Department of Environment, Great Lakes and Energy

NJDEP - New Jersey Department of Environmental Protection

NJDEP DSREH - NJDEP Division of Science, Research and Environmental Health

PCR - Polymerase Chain Reaction

UDWQ- Utah Department of Water Quality

VANR- Vermont Agency of Natural Resources

WHO - World Health Organization

Worcester DPW&P - Worcester Department of Public Works & Parks

WDPH -Worcester Division of Public Health

OEPA- Ohio Environmental Protection Agency

ODPH- Ohio Department of Public Health

ODNR- Ohio Department of Natural Resources

ELISA- Enzyme-Linked Immunosorbent Assay

CYL = cylindrospermopsin

MC = microcystin

NOD = nodularin

ATX = anatoxin-a and homoanatoxin

SAX = saxitoxin and decarbamoylsaxitoxin

NEO = neo saxitoxins

BMAA = β-N-methylamino-L-alanine

Chapter 1: Introduction

Blue-green algae, also known as cyanobacteria, grows in water bodies with warm environmental conditions with excess sunlight and high nutrient levels of phosphorus and nitrogen (Anderson 106). The cyanobacteria that thrive in these nutrient-rich environments can lead to a condition known as a harmful algal bloom(HABs). HABs are algae-based colonies that can grow into massive blooms that release toxins into bodies of water that pose a health risk to recreational users and its surrounding natural habitat.

Over the past decade, Harmful Algal Blooms have become more prominent in the northeastern part of the United States. Primarily, the state of Massachusetts has dealt with HABs, but the state has no strict procedures or regulations for dealing with the HABs. The state currently uses advisory thresholds as a guide on when to advise municipalities to close down bodies of water for safety. This means that cities are not obligated to test their water bodies or shut them down if the threat is too high.

In Worcester, Massachusetts, these algal blooms have temporarily closed down several lakes and ponds in the area during the summer season where water bodies' recreational use is at its peak(Mass.gov). Indian Lake is one of the most popular lakes in the city and has become more susceptible to HABs over the years. The lake provides many recreational options for the citizens of Worcester, receiving a lot of traffic from families, swimmers, boaters, fishermen and dog walkers. Since Indian Lake is such a high traffic area, HABs pose a serious threat to this lake and the community users of this water body because anyone who comes in contact with the HABs can lead to health problems for example rashes, skin irritation, headaches, and on a more serious circumstance, liver failure or respiratory failure. The Worcester Department of Public Works and Parks (Worcester DPW&P) requests assistance developing guidelines to help protect the public health and recreation of the surrounding community.

Furthermore, to contain these HABs, the Worcester DPW&P is responsible for testing and identifying if the water has been affected by HABs. If the water has been affected, they need to decide whether or not the area should be closed to begin treatment. They are dedicated to providing the city of Worcester with accessible and safe public areas for recreation as well as more prominent areas for local businesses(worcesterma.gov). To accomplish this they have become proactive in trying to protect public health especially with the growing HAB threat in their community.

The goal of our IQP is to provide the Worcester DPW&P with research and recommendations on their current management plan to prevent premature and unnecessary closure of the local water bodies during the summer and fall seasons. The main concern is to determine if the current Worcester management methods are adequate or if they should be altered in order to keep the water bodies in Worcester open as long as possible without putting the public health at risk. We accomplished this goal by pursuing the following objectives:

1) Understand the current procedures that the Worcester DPW&P use for monitoring their water bodies.

2) Acquire information from research scientists and universities to better understand cyanotoxins and testing procedures for these toxins.

3) Compare and contrast researchers' preventive methods to current Worcester DPW&P preventive methods.

4) Provide recommendations and alterations to the current monitoring techniques and testing procedures.

Chapter 2: Background

This background chapter presents an in-depth review of HABs, issues associated with HABs, and the current advisory thresholds in place. Specifically, we will discuss the threat that HABs pose in the Worcester area. The stakeholders involved believe HABs are a problem and need to be addressed to protect the public's health. We have analyzed literature about our topic and examined two case studies that involve regulations for managing HABs.

2.1 History of Harmful Algal Blooms (HABs)

The HABs issue is no new threat to local communities' water sources, but people have not had a complete understanding of the causes and threats that HABs have on the public's health. Meaning there are still many things to learn about HABs and it's unpredictable nature. As the situation has worsened over the most recent years and became more prominent especially in

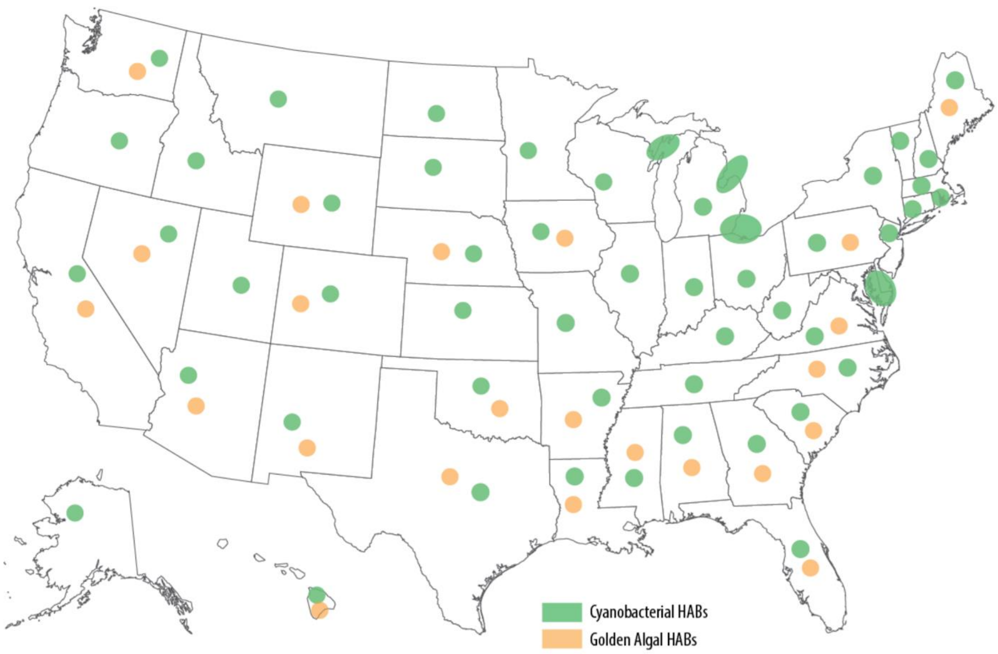

Figure 2.1: Map Depicts Widespread of HABs in The U.S (made by the National Office of Harmful Algal Blooms at Woods Hole Oceanographic Institution)

the United States. Some of the worst HABs outbreaks attacked major lakes and coasts of the U.S causing detrimental damage to the environment, local economy and communities. The widespread occurrence of HABs can be seen in Figure 2.1. The map depicts locations of two types of HABs that appeared across the country between the years 2006 and 2015 (Congressional Research Service, 2019). This rising issue caused people to begin looking into how they can prevent and treat these HABs and minimize the effects they cause to the surrounding areas where the HAB is located. To figure out what causes these HABs, they looked at the base of HAB and that is the small colony of algae it originates as.

Algae is a naturally occurring component of an aquatic ecosystem in lakes, ponds, and rivers. Although algae can be harmless in small amounts and is even a vital asset to marine and fresh-water ecosystems, it can pose a serious threat under certain conditions. For example, higher temperatures and increased light as well as excess nutrients can cause the algal community to "bloom". Also, nutrient run-off from roads, agriculture, and waste is theorized as another cause for increased nutrients into a water body. Increases of nutrients into the water is a known driver in the rapid growth of algae in water. Once a harmful algal bloom (HAB) has developed, it tends to be easy to spot because it appears as a green scum substance on top of the water, as seen in Figure 2.2, and usually has a very pungent smell. These scums can easily develop into immense masses which can absorb a great amount of oxygen resulting in fish die-offs and restrict growth of aquatic plants. In addition, certain species of cyanobacteria, such as Blue-Green Algal, when decaying, produce toxins, known as cyanotoxins that are harmful to humans and aquatic life (Mass.gov, 2019).

Cyanotoxins can have hepatic (liver), respiratory, neurologic, dermatologic and other health effects. These health effects can range from very mild to fatal. The cyanotoxins that are produced by cyanobacteria are *Microcystins*, C*ylindrospermopsin*, *Anatoxins*, and *Saxitoxins*. (Congressional Research Service, 2019).

Once researchers and scientists finally got concrete causes and characteristics to look for to identify these HABs, they started to develop tests for water monitoring to check for different aspects in the water which may lead to a potential bloom. Also, testing for cyanotoxins was developed which allowed the pinpointing of which toxins were present in the water. The capability to test the water gave people an edge to hopefully prevent a bloom from ever reaching its full form.

Figure 2.2: Example of HAB Appearance (taken by The New York Times)

2.1.1 Issues Associated with Harmful Algal Blooms

When HABs affect a local water body, it creates environmental, social, and economic issues that pose a serious threat to the community. From an environmental position, HABs affect the ecosystems associated with that waterbody because the toxins released can kill fish and other aquatic life. Land animals that use that water body as a source of water can get exposed to the toxins through drinking and coming in contact with it (Ibrahim, 2014). The algal blooms can also cause large dense mats on the surface of the water that can prevent fishing and swimming. These mats can also odor problems and can deplete the oxygen in the water, negatively affecting the other plants and wildlife around it (EPA.gov).

On a societal level, HABs affect the community in a negative way because they can prevent residents from using water bodies for recreation. Many people in the Worcester area rely on these sources of water for recreation through swimming, fishing, boating, and among other activities. When HABs infiltrate these areas, it causes them to be unsafe, so the city of Worcester must close the recreational areas limiting the citizen’s options for recreation in the summer months. The closing of these water bodies also greatly affects the residents that live on the lake. The residents pay a lot of money to live on these beautiful water bodies, but because of HABs, they cannot even enjoy the water. Not only does it affect the residents that live on the water body, it affects the other residents that live in the city as well. Most residents that cannot afford to go away on vacation or cannot find the time to get out of the city, tend to utilize these public recreation water sources to relax and get away from their work life through swimming, boating, fishing, walking, running, etc. But, if the water body is closed due to HABs it takes away a lot of those recreation options from them. This is why if Worcester can do its best to mitigate and prevent these blooms, then it can reduce the frustration and negative effects they can cause to the communities that utilize these water sources.

The economic effects of HABs are another issue that seriously affects the communities' local businesses and the local government. Economists can break the economic effects into four different categories, public health, fisheries, recreation and prevention/management (Hoagland, 2002). If water sources are closed, then all these categories can be affected. For example, if there is a bloom, then the city must increase spending on management and prevention in order to mitigate the risks that the HABs will cause. Therefore, the closure of these water bodies creates a domino effect. If the water sources get closed, fishing activities will cease and bait shops will lose business concluding in financial struggles for numerous community members.

The testing required for HABs also causes a great financial burden on the city of Worcester. After talking with our sponsor Jacquelyn, she told us that each test costs around $500 to perform. Over the course of the summer, the city is forced to spend many thousands of dollars just to test the water for these blooms. If a test indicates that a bloom is going to occur soon, the city will then treat the water in hopes of preventing the bloom, but this costs around $25,000-$30,000 every time an aluminum sulfate treatment is used and around $3,000 for each copper sulfate treatment used.

Not only do HABs cause all of these issues to the community, they also pose an issue to the people in charge of monitoring them. When Worcester conducts testing, the tests indicate if the living cyanobacteria cells are accumulating toxins within. These blooms only release toxins into the water when the cells die, or if the cells are ruptured (EPA.gov). This gives them a gage of how much toxin will get released once the cells die off. This means that when management employees close down a water body for a high cyanobacteria cell count, the water could still be completely safe because no toxins have been released into the water. This has caused a lot of uncertainty with these employees because they want to protect the residents of the community but they also want to leave the water bodies open as long as possible for community recreational use.

2.2 Common Monitoring Techniques for HABs

There are several monitoring techniques that are used throughout the world in testing water bodies for HABs. The frequent monitoring of water bodies in areas where environments are at a greater risk of HABs occurring are essential in trying to control the size of the bloom. Accurate testing for HABs allows for quick prevention methods to surface keeping recreational waters safe and open to the public. The common monitoring techniques used are cell count testing, ELISA and LC-MS/MS for toxin testing, transparency testing, satellite imaging, qPCR testing, Chlorophyll testing and Nutrient (Phosphorus and Nitrogen) testing;

Cell Count Testing- This is a widely used technique, especially in Worcester. This technique requires gathering small water samples that are then sent to a lab to be analyzed into the count of cyanobacteria cells included in a water body per mL of water. The higher the cell count of cyanobacteria means there is a greater risk for cyanotoxins in the body of water. Advisory cell density thresholds are in place to advise water body closure based on whether or not cell count exceeds threshold levels.

ELISA (Enzyme-Linked Immunosorbent Assay) Method- When it comes to cyanotoxins, ELISA is a testing method that determines the presence of a specific cyanotoxin. Antibodies of the cyanotoxin are generated and then inserted into a water sample, the results are based on the response of the toxin, if it binds to the antibodies, presence is confirmed (Biobest). For HAB testing the ELISA method has the validation to test for the most common cyanotoxins like *Microcystin-LR*, *Anatoxin-a*, *Saxitoxins* and *Cylindrospermopsin* (Enviroscience).

LC-MS/MS (Liquid Chromatography Triple Quadrupole Mass Spectrometry Method- The LC-MS/MS testing method is used for various testings. In terms of cyanobacteria, it is validated to measure a lot more known cyanobacteria species congeners, if not all, compared to ELISA. The method of sample ionization paired with mass spectrometry breaks down mixtures into its individual components and then each individual component's structural identity and molecular breakdown is evaluated and quantified.

Transparency Testing(Secchi Disk)- The Secchi Disk testing tests for the overall transparency of the water body. A black and white 8-inch diameter disk is lowered into the water body until it cannot be seen. How far the disk drops before the disk is not visible shows how transparent the water is. When water is very clouded and anything but clear, that points towards a growth of algal bloom. As transparency of the water bodies goes down could mean that HABs start to develop (Boyd 2004), it is an indirect way of testing for cyanobacteria and further testing needs to be done to obtain accurate results.

Satellite Imaging- Satellite imaging is a monitoring technique actively used by states like Maine and California as well as in China. The satellite detects and measures the phytoplankton density which directly correlates to cyanobacteria concentrations in water bodies (USGS 2020).

qPCR (Polymerase Chain Reaction) Testing - qPCR testing is a method that has been developed and applied within the past decade. PCR testing is a polymerase chain reaction in which the DNA of miniscule specimens are analyzed, specifically to, "identify and estimate toxic phytoplankton species in marine natural samples" (Penna, A) DNA of a specific cyanotoxin producing gene will be present in these tests and the quantity of these genes will determine whether ELISA testing will follow.

Chlorophyll Testing- Cyanobacteria contain chlorophyll which means that testing on chlorophyll levels would help predict future HABs. Specifically cyanobacteria possess a specific chlorophyll called blue-green chlorophyll which is then evaluated in testing. According to NYSDEC developed 4 levels of bloom status and the "confirmed bloom" status includes that blue green chlorophyll levels are greater than 25 ug/L (NYDEC).

Nutrient Testing - Nutrient testing is used to test for nitrogen and total phosphorus levels. Cyanobacteria feed off of nutrient rich water bodies and grow under these circumstances. One way nutrient testing is displayed is with the trophic state index which ranks on a scale of 1-100 how nutrient rich a water body is and how susceptible it is to algae blooms. The greater the presence of nutrients, the higher the number (Carlsen 1998). Measuring these nutrient levels are key in helping predict and detect an environment perfect for HABs to appear. Monitoring nutrients will help catch the growth of HABs.

2.2.1 Common Prevention Methods

In addition to monitoring, to manage and avoid HABs requires methods to prevent cyanobacteria from growing in the first place. The most common prevention methods for battling

HABs are chemical management measures including alum, barley straw and copper sulfate, as well as biological management such as vegetation buffers(US EPA);

Alum- Aluminum Sulfate is a bloom prevention technique that produces heavy particle clumps heavier and denser than water called floc. The floc drops to the bottom of water bodies and creates a matt over the bottom floor of the water body which prevents the releasing of phosphorus from sediments on the bottom of the body. Limiting nutrient release into the water will limit HABs (NALMS 2004).

Barley Straw- Barley Straw is a prevention technique used to decompose itself and limit cyanobacteria cell proliferation by producing Dissolved Organic Carbon (DOC) and other oxidizing agents. It takes around two to eight weeks for barley straw to begin decomposition and this method should only be used twice a year in the summer if necessary(Newman, 2012).

Copper Sulfate- Copper Sulfate is an algicide that kills off algae, especially HABs. If too much of the copper sulfate is released into water bodies it can deplete oxygen and suffocate aquatic life so dosage levels are extremely important (Princeton Hydro).

Vegetation Buffers- Vegetation buffers are a very eco friendly prevention technique. Vegetation buffers are permanent vegetation crops that are placed on land around a water body in an attempt to intercept and absorb all excess nutrients and waste water runoff. The idea of the vegetation buffers is to limit the amount of nutrients that flow into water bodies. This technique is very important in very densely populated areas or inner city areas where there is more waste and fertilizer runoff (MDNR 2007).

2.3 Case Study on New Jersey Department of Environmental Protection (NJDEP)

This section is an evaluation of the New Jersey Department of Environmental Protection (NJDEP) strategies towards managing, detecting, and alerting surrounding communities about HABs in their water bodies. The NJDEP is proactive in researching and managing HABs in New Jersey. Researching the NJDEP management strategies will give us a better idea on how effective Worcester's techniques, thresholds and strategies are and how they can be improved.

2.3.1 NJDEP Harmful Algal Bloom Response Strategy

The NJDEP performs multiple cyanobacteria testing methods beginning with a visual assessment of the water bodies to determine whether or not there is a visual green matt of algae on the surface of water bodies. If the assessment of the water body confirms a potential HAB, further testing precedes. Beginning with phycocyanin pigment testing, handheld field fluorometer tests or samples for laboratory screening are taken. The screening process identifies the various taxa that are found in the sample. Additional samples are taken to test using ELISA method specifically for toxin analysis of *Microcystin*, *Cylindrospermopsin* and *Anatoxin-a*. NJDEP also uses Chlorophyll 'a' and cell count tests to compare to the WHO thresholds for chlorophyll 'a' and cyanobacteria cell count to decide whether or not a closure of the water body is necessary.

NJDEP's Harmful Algal Bloom Response Strategy analyzes response, closure and notification strategies for harmful algal blooms in lakes, rivers and streams with human use. New Jersey formed an HAB Workgroup specializing in the management and prevention of HABs in lakes, ponds and streams.

The NJDEP Division of Science, Research and Environmental Health developed a specific threshold for three cyanobacterial toxins. The primary cyanotoxins released and tested for are *Microcystin-LR*, *Cylindrospermopsin* and *Anatoxin-a* group. The threshold for recreational exposure to individual cyanotoxins for *Microcystins* are 3 μg/L, *Cylindrospermopsin* is 8 μg/L and *Anatoxin* is 27 μg/L (NJDEP 2018). New Jersey DEP recommends that if the cyanobacteria cell count is greater than or equal to 20,000 cells/ml in active human use areas, then Danger advisory signs will be posted. New Jersey goes by the World Health Organization (WHO) cell count guidelines. The initial threshold for the beginning of management procedures is when the toxin levels reach 20,000-100,000 cells/ml (NJDEP 2018).

In response to HABs in recreational waters, community members report these blooms via the DEP hotline and the HAB reporting form based on their visual assessment of the body for water. After further visual assessment, agencies then perform field screenings to confirm HABs presence in these bodies of water. First, testing is held using a field fluorometer to test for cyanobacteria taxa and if this water sample tests positive for it, further testing is done for toxin and cell counts. Specific toxin strips are used to determine if certain toxins are in abundance in these bodies. Lastly, in testing, chlorophyll is tested for amount per liter because it is found in both cyanobacteria and blue-green algae. Greater than 10 μg/L presents a moderate risk (NJDEP 2018).

After all of their testing, advisories are given out which is based on the NJ Action Level and Health Advisory Guidelines for Recreational Exposure. These are signs that are posted by recreational waters based on suspected HABs.The advisories have wording such as "Warning:

Avoid contact or Ingestion" if there is a bloom on the rise or if the cell counts or toxin counts have reached or surpassed the advisory threshold, signs of, "Danger: No contact or ingestion" will be posted. Monitoring is constant throughout the time the recreational waters are closed. Water bodies reopen once it is deemed the waters are safe again to ensure that the people in the communities using these waters stay healthy and safe.

2.3.2 Case Study on Ohio Environmental Protection Agency, Department of Public Health and Department of Natural Resources (OEPA, ODPH, ODNR)

The OEPA, ODPH and ODNR monitors, prevents and responds to HABs in water bodies in Ohio. Ohio is a major state in the US that pours a lot of time and resources into battling HABs in areas like their Ohio River and Lake Erie. As a state constantly dealing with HABs and having to use management strategies to combat them, their tactics can be analyzed and dissected to help Worcester with their battle with HABs on Indian Lake.

2.3.3 OEPA, ODPH, ODNR Monitoring, Prevention and Response Strategies

The purpose of OEPA, ODOH and ODNR response strategy is to, "provide a unified statewide approach to addressing harmful algal blooms (HABs) in Ohio recreational waters and to protect people from cyanotoxins produced by cyanobacteria (Butler 2016)." Ohio water bodies experience a cyanotoxin presence consisting of *Microcystins, Saxitoxin, Cylindrospermopsin* and *Anatoxin-a*. Detecting these toxins require first detecting when algal blooms are forming. The National Oceanic and Atmospheric Administration (NOAA) and NASA generate remotely sensed imagery that capture HABs as deep as one to two feet below the surface especially used for Lake Erie and Ohio River (Butler 2016).

After initial visual assessment, satellite imaging results or algal bloom reports, sample collection is conducted. Phytoplankton samples are collected to determine if cyanobacteria are present amongst other phytoplankton species. Molecular HAB testing is conducted using the qPCR method which helps identify the presence of toxin-producing genes in cyanobacteria. Lastly, ELISA method is implemented to collect cyanotoxin samples and test for specific cyanotoxins like *Microcystin*.

Following testing, data and results are compared to cyanotoxin thresholds in recreational waters. Below is a table from the State of Ohio 2016 Harmful Algal Bloom Response Strategy for Recreational Waters displaying the specific toxin thresholds and response strategy aligned with each threshold.

Table 2.1 Cyanotoxin Threshold table issued for State of Ohio (Table from https://epa.ohio.gov/portals/35/hab/HABResponseStrategy.pdf)

Threshold (µg/L)	Microcystin	Cylindrospermopsin	Anatoxin-a	Saxitoxin
Information Sign	**<6**	**<5**	**<80**	**<0.8**
Recreational Public Health Advisory	**6**	**5**	**80**	**0.8**
Elevated Recreactional Public Health Advisory	**20**	**20**	**300**	**3**

Table 2.2 Cyanotoxin Threshold table issued for State of NJ

Threshold (µg/L)	Microcystin	Cylindrospermopsin	Anatoxin-a
Danger/ High Risk - No Contact and Ingestion	**≥4**	**≥9**	**≥28**

Table 2.3 Cyanotoxin Threshold table issued for State of MA

Threshold (µg/L)	Microcystin
Advisory Warning	**≥8**

Informational Signs still advise to "have fun in the water" but inform those who use these water bodies to be aware of waters that look like spilled paint, with scum mats covering the surface. Recreational Public Health Advisory is a sign with a bolded, all capital letters WARNING sign informing people that an algal bloom and toxins are present in the water and swimming is not recommended for selective groups of people including children, pregnant or nursing women, pets and those specific medical conditions. Lastly, the Elevated Recreational

Public Health Advisory is a DANGER sign that states very clearly to avoid all contact with the water and HABs have reached an "Unsafe" level. Additional public awareness methods Ohio uses are website postings on OEPA, ODNR and ODPH pages (Butler 2016). In comparison to other states like Massachusetts and New Jersey, Ohio has the most tested toxins with thresholds. Massachusetts only has *Microcystin* and New Jersey only implements *Microcystin*, *Anatoxin-a* and *Cylindrospermopsin*.

2.4 Harmful Algal Blooms in the Worcester Area

In Worcester alone, there are over twenty lakes and ponds that provide the community with various recreational activities and boost the city's economy by creating businesses to support citizens working in the recreation industry. Due to the effects of the industrial development in the area along with urbanization, maintenance and preservation of the appropriate water quality to accommodate people's needs is increasingly challenging (worcesterma.gov 2020).

HABs are one of the immediate threats to these water sources due to the shallowness of most ponds in combination with the high nutrient content of the water, promoting their growth. The City of Worcester focuses its efforts towards coping with this issue in four of the largest lakes in Worcester. Our research will examine closely how the existing preventive measures affect the case of Indian Lake.

2.4.1 Impacts of Harmful Algal Blooms on Indian Lake

Indian Lake, shown in Figure 2.3 below, was originally a small natural lake which was later impounded to provide water to the Blackstone Canal during the 1800s, which expanded it to over 200 acres. Finally when Route 190 was built the Lake was shrunk to its current 190 acres with its deepest point being 17 feet on the north-eastern side. The lake is connected to two smaller ponds, Salisbury Pond to the east and Little Indian Lake to the south (ilwa.org). During summer months, Indian Lake provides numerous activities including swimming, watersports, and fishing to the public.

Figure 2.3. A picture of Indian Lake (Taken by Indian Lake Watershed Association Inc.)

Because Indian lake is so close to residential land and the Interstate 90 highway,it has been faced with many issues including low water quality, threatened aquatic life and restricted public use with temporary closure. According to the Water Quality Monitoring Report of the DPW&P for Indian Lake, the lake has been affected by HABs multiple times in the past (worcesterma.gov). One of the most severe appearances of HABs was during the summer of 2014 when algal blooms developed during one night. The following morning the water was covered by the green scum and the lake was closed for the rest of the summer. Later, when the City of Worcester tested the lake, it was found that it had a high density of cyanobacteria cells that could produce toxins. This issue has arisen multiple, most recently in the summer of 2018 with results of fish die-off and complaints of unpleasant smells coming from the lake by the residents (worcesterma.gov 2020).

2.4.2 Harmful Algal Blooms Management on Indian Lake

Since 2016, when Worcester DPW&P started the Lakes and Ponds Program in order to ensure safety and protection of the state's water bodies (worcesterma.gov 2020), Indian Lake has been monitored more closely. The Worcester DPW&P conducts both direct and indirect tests to monitor the cyanobacteria presence in Indian Lake. Over the summer months, the lake is tested weekly, for the following factors that greatly affect the growth of cyanobacteria: temperature, pH, total Nitrogen and Phosphorus levels, dissolved oxygen and general water clarity. The results are inexpensive and fast, offering clear indications of possible algae bloom occurrence and the need for more effective measures.

The next step of Worcester's monitor plan after reviewing the results of the first tests, is to proceed to cyanobacteria cell density testing. Multiple samples collected from the lake are sent for cell density analysis and further testing and treatments are applied accordingly to prevent blooms. The density analysis offers an understanding of which kinds of bacteria live in the water and based on the density level of the harmful cyanobacteria, it is determined if an algal bloom is possible to occur. The disadvantage of this test is that the results are available in 4-7 days due to the few labs that can process these tests as our sponsor,Jacquelyn Burnmeister, mentions. At an early stage, based on the cell density test results and in line with the MassDPH Guidelines on Harmful Algal Blooms (Mass.gov 2020), Worcester DWP&P has to act appropriately and apply treatments to lower the cell count.

The Worcester DPW&P has been managing the HAB by applying preventative treatments to avoid formation and ultimately, unnecessary closing. Indian Lake has been treated with aluminum sulfate and copper sulfate. Aluminum Sulfate (Alum) is applied to the coast of the lake, the swimming area for the residents, to reduce the excess elements of phosphorus in the waters. It is a nontoxic compound which when in touch with the water binds the particles of phosphorus into sediments and drags them to the bottom of the lake, decelerating phosphorus release (nalms.org 2004). This process reduces the nutrient level of the lake and blocks the development of algae, however, rain allows excess nutrients to access the waters again , so it is not certain that the treatment will be effective. Unfortunately, as our sponsor has explained to us in an interview, the treatment is very expensive, thus it is usually applied once per year.

In the meantime, cell density tests are conducted biweekly to maintain the accurate status of the cyanobacteria presence and an insight to the dangers it entails. If the cell density appears to be high again, Worcester DPW&P then proceeds to apply Copper Sulfate. A treatment that contains a compound toxic to cyanobacteria and other aquatic microorganisms which binds to their proteins damaging their cells and killing them (npic.orst.edu 2012). These methods are a short-term solution to lower the cyanobacteria cell count in the lake, and they do not offer permanent results as phosphorus is re-released back into the water column..

In case the bloom has already appeared on the waters or the density of the cyanobacteria is above the set threshold of 70,000 cell/ml of water, the only option is for the lake to be temporarily shut down to secure public health. In this case, Worcester carries out the ELIZA toxin test to check the existence of specific toxins in Indian Lake and inform the citizens to protect their well-being. The lake remains closed until two consecutive cell density counts tests result below the thresholds, which usually occurs later in the fall when the weather cools down.Unfortunately, the proposed guidelines by the MassDPH do not guarantee the prevention of premature closure of the lakes or provide a solution to the problem as up to date, there is a lack of information on the root causes of Harmful Algal Blooms.

2.5 Stakeholders

The management of harmful algal blooms in the Worcester area is the job of several different agencies throughout the city and the state. These municipalities work together to form regulations as well as form prevention and treatment methods.

2.5.1 Worcester Department of Public Works & Parks (Sponsor)

The Worcester DPW&P is in charge of maintaining city infrastructure and facilities to ensure a beneficial quality of life for the city. Their goal is to upkeep the cleanliness and to protect and maintain the resources of the city. Their responsibilities include everything from delivering safe, potable water, to designing and managing the construction of city infrastructure and even protecting the city's natural resources. The DPW&P monitors the water quality of the city's four largest bodies of water, also known as "blue spaces". They work with the Commonwealth, watershed groups, and other local organizations to monitor and deal with any threats to these bodies of water. They also strive to educate the community on ways to help protect their natural resources and also ways to help improve their quality (Mass.gov 2019).

2.5.2 Massachusetts Department of Environmental Protection

The Massachusetts Department of Environmental Protection(MassDEP) is in charge of keeping the state's natural resources clean. These resources include air, land, and water. Their work includes preservation efforts as well as cleaning up hazardous waste sites and spills. The MassDEP's Watershed Planning Program administers the Water Quality Monitoring Program to evaluate the state bodies of water. They ensure the bodies of water are in accordance with the Federal Clean Water Act (314 CMR 4). The program is composed of staff and volunteers who sample the state's waters for data. The data is then used to prepare water quality reports which are then used to regulate the waters. (Mass.gov 2019)

2.5.3 Massachusetts Department of Public Health

The Massachusetts Department of Public Health (MDPH) is in charge of promoting the health and well-being of the state's residents. They accomplish this by ensuring access to high-quality healthcare services and by working to prevent possible health, wellness, and equity concerns. The MassDPH works with the World Health Organization (WHO) to develop guidelines for water testing. The current guidelines advise that if 70,000 cyanobacteria cells/mL are contained in the waters, the toxin level is possible to be high, endangering someone that comes into contact with it. At this threshold, the area must be shut down until it is cleared. (Mass.gov 2019)

2.5.4 Indian Lake Watershed Association (ILWA)

The ILWA is a non profit organization that focuses on, "combating the effects of development within the urban watershed on Indian Lake (ILWA About)." They work with the Worcester DPW&P to prevent HABs from surfacing in Indian Lake. ILWA organizes semi-annual cleanups around Indian Lake to ensure the Lake's surroundings are in tip top shape and won't negatively affect the water body in any way. ILWA partners with the Worcester DPW&P especially in detecting areas of nutrient sources outside of the water body and also conducting sediment and cost analysis on the bottom of Indian Lake and the cost for the Lake to be dredged and cleansed of sediments and nutrient resources from inside the water. Along with the DPW&P, ILWA is an important group involved in finding ways to prevent the next HAB from occurring in Indian Lake.

2.6 Conclusion Summary

HABs are an increasing problem in Worcester and more evident in the seasons of spring and summer and fall due to the warmer climates that allow algae to grow. The growth of HABs have led to temporary closures of many water bodies, including Indian Lake, a central recreational area for the Worcester Community. Aquatic life and businesses of the area are affected by these HABs. The Worcester DPW&P which has responsibility in managing and promoting public health needs to respond to this threat. The MassDEP and MassDPH are in charge of developing guidelines for monitoring HABs in Massachusetts. Research on these two organizations, out of state HAB management departments like NJDEP and OEPA and examination of requirements for regulating HABs will provide valuable insight on how effective Worcester's DPW&P current monitoring and advisory threshold strategies are and how they can be improved.

Chapter 3: Methodology

The goal of our IQP is to provide the Worcester DPW&P with research and recommendations on their current management plan to prevent closing the local water bodies. For this project we plan to see if the current Worcester management methods are suitable or if they should be altered to prevent premature closure of local water bodies and ensure the mitigation of HABs is adequate. We plan to accomplish this goal by pursuing the following objectives:

- Understand the monitoring and prevention methods used by Worcester DPW&P.
- Acquire information from research scientists and other states to understand the possible impacts of cyanotoxins as well as testing techniques.
- Compare and contrast researchers' preventive methods.
- Provide recommendations and alterations to the current advisory thresholds and monitoring techniques.

3.1 Understand the Monitoring and Prevention Methods Used by Worcester DPW&P.

In order to meet the goal of improving the regulations for Worcester, the first step was to understand the current methods. Through research and data analysis we examined the implemented techniques which Worcester DPW&P uses to monitor their water bodies and we learned about the cyanobacteria and cyanotoxins we encountered in data sets from Indian Lake. In addition to that, we interviewed with our sponsor, Jacquelyn Burmeister, about the testing procedures at Indian Lake and the analysis of the resulting data sets. It was important for us to familiarize ourselves with guidelines and management plans used to mitigate HABs. Our team evaluated and analyzed the present monitoring methods compared to the known information for cyanobacteria and cyanotoxins to provide the best recommendations for the Worcester DPW&P.

3.1.1 Analysis of Indian Lake Data

We analyzed the data sets, our sponsor Jacquelyn Burmeister, collected during cell density and cyanotoxin testing procedures at Indian Lake. The cell density analysis shows the amount and the species of the cyanobacteria present in the water. To ensure that testing is valid, samples are extracted from specific locations within the lake where there is evidence of expected or existing blooms. The indications usually include unpleasant odors, water color alteration or green surface scum if the bloom has occurred (Herzfelder, Pritchard and Romney 2005). In addition to the cell density and toxin tests, we also examined more data from the indirect testing done in the lake. While evaluating these factors which promote the growth of HAB, we got an insight of how the Worcester DPW&P examines the water and decides how they approach the potential problem. Researching and analyzing all the available data and information of the testing procedures done on the lake and as our sponsor described it, gave us the ability to correlate the test analysis data to the condition of the lake when the samples were taken. We expected to receive high counts of cells if indications of cyanobacteria existed and vise versa. Examining the results allowed us to notice some trends related to the weather conditions and how the species included in the cell count analysis possibly interact with each other.

3.1.2 Sponsor Interview

In addition, we interviewed Jacquelyn Burmeister, focusing on the various testing processes Worcester DPW&P conducts and what significance cyanobacteria cells and cyanotoxin thresholds from MDPH have on their management strategy. Proposing effective measures to ensure the longer and better safety of the public health in regards to algal blooms requires extensive analysis of the advisories' impact on the management approaches (Refer to Appendix B for a complete list of questions).

3.2 Acquire Information from Researchers, Scientists and other States to Understand the Possible Impacts of Cyanotoxins as well as Testing Techniques.

To provide accurate and useful information to Worcester DPW&P, we had to get a better understanding of different genus of cyanobacteria, the cyanotoxins they produce and research more available prevention methods. The approach included interviews with researchers and scientists who are involved with cyanobacteria research.

3.2.1 Researchers and Scientists Interviews

We reached out to various researchers and scientists that specialize in HABs prevention, treatment and development to interview them about the research they have conducted and to gather information on specific cyanotoxins and cyanobacteria we found in Indian Lakes's data sets. We were able to clarify some confusion ourselves, and the Worcester DPW&P had regarding the impact and the developments of the species. We were then able to implement this knowledge we gained to help the Worcester DPW&P develop revised management approaches for HABs to be more efficient and accurate.

Furthermore, we conducted semi structured interviews with people working in Departments of Environmental Protection (DEP) with interest in cyanobacteria. The goal of conducting the interviews was to become more knowledgeable of the strategies currently used for monitoring HABs and what advisory levels are in place. Gathering information from scientists helped us understand the current methods better and the reasons why they were being used. The researchers were also able to expose us to different testing methods that we were able to present to the Worcester DPW&P as alternative options. Overall conducting research and interviews with researchers and DEP employees gave us valuable information provided a basis for determining alterations in current techniques or recommendations for new proactive monitoring techniques and advisory thresholds.

3.2.2 Interview Details

All our interviews lasted an hour each with recurring questions like; What methods/tests do you use to monitor your cyanobacteria in your water bodies? Do you conduct toxin testing? If so, what toxins do you test for? What was the most frequently recurring cyanobacteria species in your cell density counts data? What is your cell density advisory threshold that you abide by? (Refer to Appendix for complete list of questions)

Most questions circulate around what were their monitoring testing procedures, what were their treatment methods, what toxins they test for and what their advisory thresholds are. Depending on who we interview, we went more into depth asking questions relating to cyanobacteria that Worcester deals with such as *Limnothrix* and *Aphanizomenon flos-aquae*. We picked our interviewees brains asking what toxins they would test for if they were in our position dealing with those specific cyanobacteria species.

Based on all of our interviews, we gained valuable information that we further researched, analyzed and helped compare and contrast the information gathered from researchers and scientists.

3.3 Compare and Contrast Researchers' Preventive Methods

To determine the most suitable methods to protect Indian Lake from HABs, we had to analyze and examine the information about cyanobacteria and the various management methods we were introduced to during the interviews and archival research. We achieved this goal by organizing and evaluating the data we gathered. After objective two was completed and all interviews were conducted, we moved on to organizing all the information that we have learned.

3.3.1 Data Organization

We organized all of the data we acquired in folders by interviewee name in which we included a brief background research of the people we talked to, the questions we asked, the notes taken during the interviews and the recorded audio and video. During the interviews some of us were in charge of taking notes to have easy access to the information we discussed. In order to make sure all the notes were accurate and all the information present, we heard the recordings again and formally summarized them. Furthermore, some of the respondents shared with us data such as tables, maps and tests results to support their answers so we examined these data as well and added more observations and how we wanted to be included in our report.

3.3.2 Interview Analysis

We decided to make slides with the key takeaways of each interview to better understand the differences. This method also helped us highlight the new techniques and who implements them. Data regarding cyanobacteria genuses and cyanotoxins was summarized into tables and visuals in order for us to easily detect the similarities. A lot of the preventive methods and the monitoring testings were similar for all of our interviewees. We categorized the information we gathered in sections like "cyanotoxin tests, treatments, cyanobacteria testing, prevention methods" and filled all the responses we got into a spreadsheet to be able to compare the data (Refer to Table 3.1. for comparison table). There were times that some of the opinions did not line up, in that case we conducted our own research, consulted with our sponsor and decided what sources were more reliable. This issue was created due to the lack of information related to cyanobacteria.

Table 3.1. *Comparing gathered information from interviewees in terms of prevention, testing and treatment.*

	Prevention Plan	**Cyanobacteria Testing**	**Toxin Testing**	**Treatment**
Worcester DPW&P	Waste Water Reduction Plans	Water Chemistry & Cell Density	ELISA for Microcystin/ Nodularin	Alum & Copper Sulfate
Utah Department of Water Quality	Waste Water Reduction Plans	Water Chemistry & Cell Density	ELISA & LC-MS for Microcystin, Anatoxin-a, Future tests for Saxitoxin	Alum to specific marinas
Vermont Watershed Management Division	Waste Water Reduction Plans & Citizen Volunteers	Cell Density	ELISA & LC-MS for Microcystin, Anatoxin-a & Saxitoxin	Alum to specific ponds
Rhode Island Department of Health	Waste Water Reduction Plans	Water Chemistry & Cell Density	ELISA & LC-MS for Microcystin, Anatoxin-a, Saxitoxin & Cylindrospermopsin	Alum & Copper Sulfate
Lim-Tex	-	Water Chemistry & Cyanobacteria Growth Curve	ELISA for Microcystin	-

3.4 Provide Recommendations and Alterations to the Current Advisory Thresholds and Monitoring Techniques.

To formalize our suggestions to improve Worcester's HABs management technique, we had to look at all of the information that we organized from our interviews as well as the research that we conducted. We then looked at Worcester's current management techniques to find where improvements could be made based on our new knowledge.

3.4.1 Formulating Suggestions

To formulate our suggestions to improve Worcester's HABs management strategy, we looked at the organized information from our interviews and research and compared it to Worcester's current management techniques. We broke everything down into sections of prevention, monitoring, testing, and treatment. We analysed each section of the information that we gathered and found which approach had the best results for the issues that Worcester has been experiencing. We then compared this to the appropriate section of Worcester's current management strategy. When we compared them, we decided if the information that we gathered could improve the current strategy and if so, we made the appropriate recommendations on how to improve them. Along with the recommendation, we provided an explanation on why we believed our suggestion would be an improvement and the benefits they could expect from it. With some of the sections there were more than one suggestions that we had so we broke down each one and explained how each of them could add further improvement. It was important for us to keep in mind the resources Worcester has available for HABs management when we made our suggestions.

Chapter 4: Findings

4.1 Introduction

Based on Indian Lake's cyanobacteria cell counts, toxin results and interviews with researchers, scientists and DPW&P employees, we dissected and analyzed all information that we collected to help build a set of recommendations on HAB management strategies for the Worcester DPW&P to follow. Our five conducted interviews helped us gather valuable information in monitoring, prevention methods and advisory guidelines that we could take in and compare to Worcester to offer further advice on how to more accurately determine when is the right time to close water bodies. The data from Indian Lake in Worcester guided us in specifically what current and potential toxins Worcester is dealing with and how they should test for these toxins.

4.2 Monitoring and Prevention Methods Used by Worcester DPW&P

It was very important for us to start by understanding current monitoring and prevention methods used by the Worcester DPW&P. We needed to fully understand these strategies in order to compare the other information that we gathered and also to find areas that could be improved upon.

4.2.1 Worcester's Cell Density Threshold and Toxin Thresholds

In our interview with our sponsor Ms. Jacquelyn Burmeister, (Senior Environmental Analyst for Worcester's DPW&P and lead team member for the local Lakes and Ponds program), we discussed the guidelines/thresholds Worcester is currently using to protect their water bodies and their public health. They follow a set of three guidelines created by the MassDPH for the whole state to use as recommended guidelines/thresholds to apply when looking for a HAB. One of the three guidelines is a specific threshold they follow that pertains to the gross cell density of cyanobacteria species in the water body. This threshold is 70,000 cells/mL, meaning when Worcester DPW&P employees conduct their cell density testing and their cell count results break this 70,000 cells/mL threshold, Worcester DPW&P is advised to temporarily close down their water body.(Burmeister, 2020)

The cyanobacteria toxin thresholds that Worcester uses for *Microcystin* follow the MDPH toxin advisory level of 14 µg/L or 14 parts per billion (ppb). In addition to the *Microcystin* toxin threshold, Worcester also uses the EPA recommended threshold values for *Cylindrospermopsin* toxin levels at 15 ppb or µg/L. Tables 4.1 and 4.2 below specify Worcester's Toxin and Cell Density Advisory Threshold Levels.

Table 4.1. Worcester Toxin Advisory Thresholds

Threshold (µg/L)	Microcystin	Cylindrospermopsin
Advisory Closure	≥14	≥15

Table 4.2. Worcester Cell Density Advisory Threshold

Threshold (cells/mL)	Total Cyanobacteria Cells
Advisory Closure	≥70,000

4.2.2 Recurring Cyanobacteria; Aphanizomenon, Limnothrix and Microcystis

The Worcester DPW&P collect samples of water from Indian Lake every other week throughout the summer. They send these samples out to a cell count testing facility and receive the results about seven days after they send the samples out. Once the results surpass the advisory threshold of 70,000 cyanobacteria cells/mL, another sample is taken and sent over for toxin testing. We analyzed the cell count and toxin testing data of Indian Lake from 2017-2019.

Based on our data analysis (Figure 4.1), we noticed that Indian Lake has been dominated by three major cyanobacteria species; *Aphanizomenon flos-aquae* (*AFA*), *Microcystis aeruginosa* and *Limnothrix*. Figure 4.1 displays the dominance of these three species in the cell counts that were taken between 2017-2019 in Indian Lake. *AFA* has accounted for 80.5% of total cyanobacteria cell counts in the past three years, *Limnothrix*, which is a distant second at 10.5%

and *Microcystis aeruginosa* is a close third at 7.9% (Figure 4.1). All the other species that are present in Indian Lake cell counts only consume a small slice of the pie including *Anabaena*, *Anabaena flos-aquae*, *Anabaena planctonica* and

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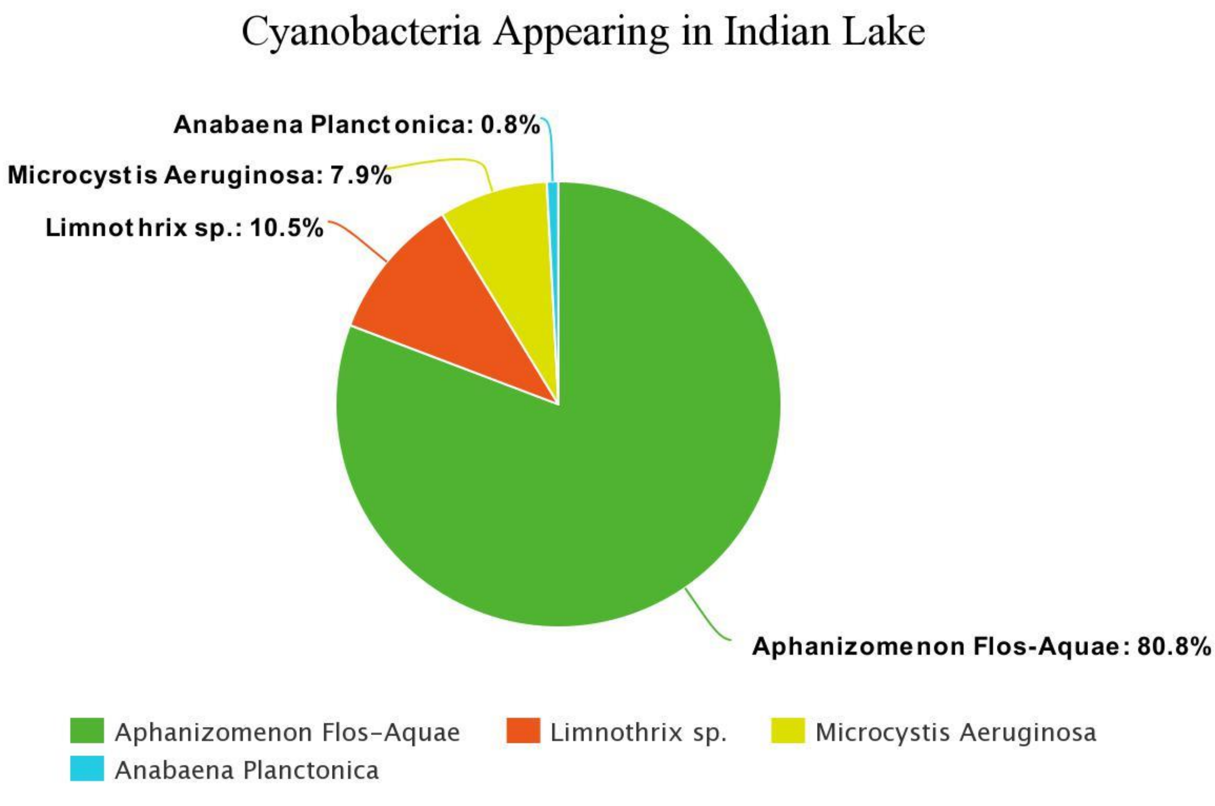


Figure 4.1. Cyanobacteria Cell Count in Indian Lake 2017-2019

In a three year span, shown in our table below (Table 4.3); *Microcystis aeruginosa* appears in 16 out of 21 total samples, *Limnothrix* appears in 14 and although *AFA* is only present in 11 samples but nonetheless *AFA*'s blooms are massive.

Table 4.3 displays the cell density count results that the Worcester DPW&P have recorded from 2017-2019. The sample date column represents the time that the cell density tests were taken. Sample ID is the way that the Worcester DPW&P labels each test to keep data organized. The trophic state index column is a 1-100 scale to measure how nutrient rich a water body is. The higher the number the more phosphorus and nitrogen there is in the water. As the value increases, the chances for a bloom increases since cyanobacteria feed and thrive off of nutrient rich areas. The three cyanobacteria species displayed; *AFA, Microcystis aeruginosa* and *Limnothrix* all show their cell density levels per test. The highlighted rows display the dates where testing exceeded the cell count thresholds and temporary advisory closures were administered. Three out of the four blooms that occurred reached into the 150,000 cells/mL level and as shown in Table 4.3, the toxin levels during the same time period as the blooms were miniscule and nowhere near the toxin advisory threshold levels.

Table 4.3. Worcester DPW&P Indian Lake Cyanobacteria Cell Count Test Results from 2017-2019

Sample Date	Sample ID	Trophic State Index	Microcystis aeruginosa	Limnothrix	Aphanizomenon flos-aquae
7/16/17	TG96	45.1	4,536	1546	503
8/10/17	TG97	54.4	8071	11829	--
8/14/18	UD57	52.3	432	9094	988
8/28/18	UD60	67.1	25127	11024	151980
9/6/18	UD73	65.4	923	14350	120062
9/13/18	UD75	56.7	11299	16396	1154
9/17/18	UD76	52.2	8246	19900	631
6/20/19	VY12	55.9	2554	1197	--
6/28/19	VY14	65.6	21498	1203	--
7/3/19	VY15	54.8	3853	2312	--
7/9/19	VY16	50	--	364	--
7/15/19	VY20	57.4	28635	1193	--
7/26/19	VY32	55.6	5412	19449	--
8/1/19	VY34	50.9	1210	637	--
8/8/19	VY36	60.4	4754	--	15440
8/15/19	VY39	56.5	1911	--	1090
8/21/19	VY42	61.5	--	--	70032
8/23/19	VY43	58.6	--	--	44419
9/10/19	VY44	69.9	--	--	226201
9/16/19	VY46	47.6	2176	--	--
9/20/19	VY48	47.8	--	--	--
		Highlight signifies bloom passes threshold			

Worcester's toxin results from the past three years are taken only after a cell count test has exceeded 70,000 cells/mL. Worcester's toxin results that come back only specify that small traces of *Microcystin* are recorded as shown in Table 4.4. This data demonstrates that the blooms that are occurring have such small traces of toxins to the point where they don't exceed the toxin advisory threshold. To put these *Microcystin/Nodularin* toxins results in perspective of the

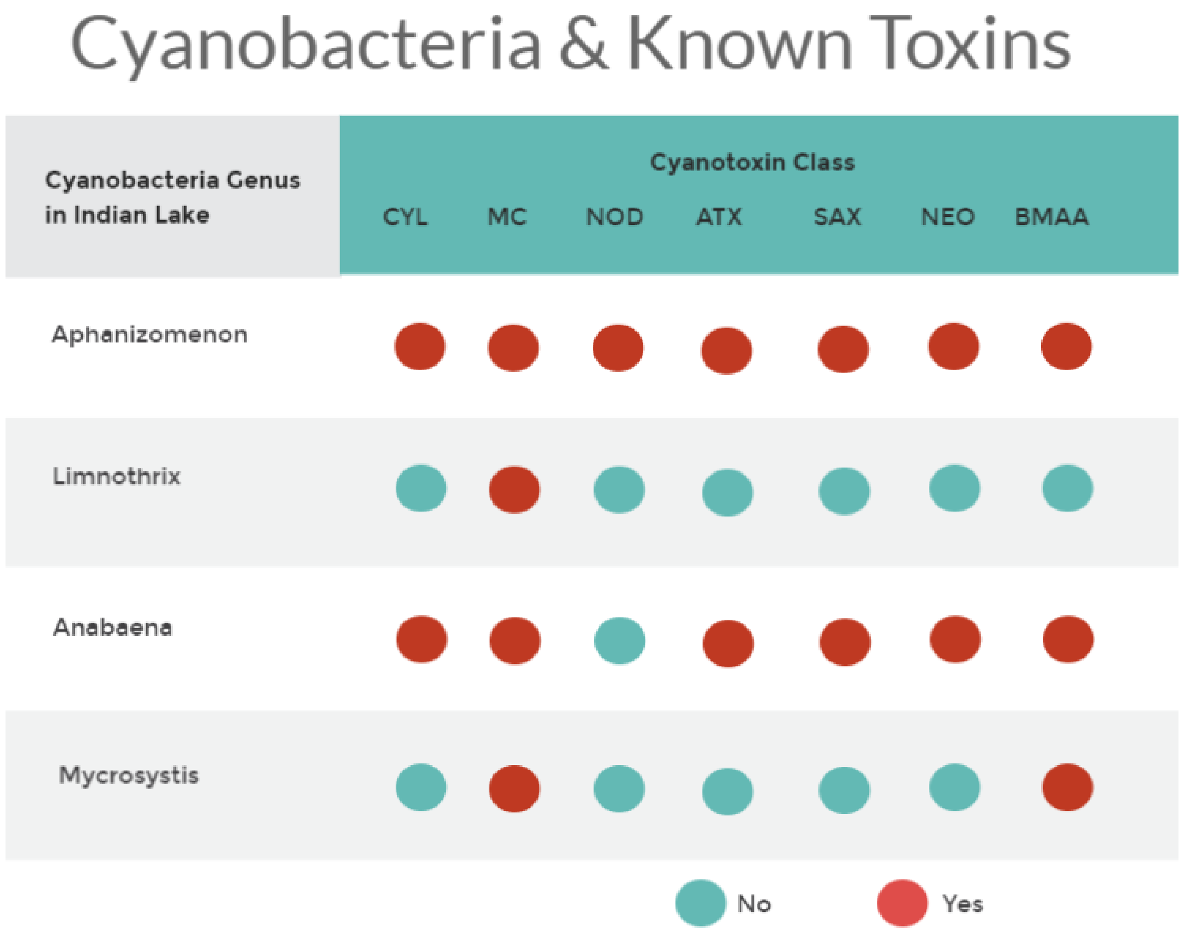
Microcystin threshold levels, the toxin test results need to be multiplied by 40 in order for these four toxins results in Table 4.4 to surpass the toxin advisory threshold of 14 µg/mL (Table 4.1). This poses a question to ask whether or not the cell density threshold is too low because the blooms occurring show minor levels of toxins in Indian Lake (Table 4.4). Worcester DPW&P are pouring loads of time and effort into these tests and findings show that the public's health and safety will not be put into grave danger if they use Indian Lake for recreational use while swimming in toxin levels shown in Table 4.4.

Table 4.4. Worcester DPW&P Indian Lake Cyanobacteria Toxin Results During a HAB

Sample Date	Microcystin/Nodularin (µg/L)	Was Cell Density Threshold over 70,000 cells/mL (Y/N)
8/31/18	0.22	Y
9/6/18	0.18	Y
9/14/18	0.35	Y
9/18/18	0.19	N

4.2.3 Worcester Tests For Toxins; Microcystin and Nodularin

Worcester's toxin data shows that *Microcystin* and *Nodularins* are the toxins that are tested in these water bodies. *Microcystin* is the most popular cyanotoxin that is sheltered in lakes, ponds and oceans around the world so it makes sense that it is the main test for toxin analysis. Nodularin has a similar structure as *Microcystin* so the toxins are paired together when analyzed in a toxin test called the ELISA test. Although *Microcystin* is a popular toxin worldwide, Worcester's main cyanobacteria presence emits more than *Microcystin* toxins.



Cyanobacteria & Known Toxins

Cyanobacteria Genus in Indian Lake	Cyanotoxin Class						
	CYL	MC	NOD	ATX	SAX	NEO	BMAA
Aphanizomenon	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Limnothrix	No	Yes	No	No	No	No	No
Anabaena	Yes	Yes	No	Yes	Yes	Yes	Yes
Mycrosystis	No	Yes	No	No	No	No	Yes

No   Yes

Figure 4.2. Cyanobacteria and Known Toxins

CYL = cylindrospermopsin

MC = microcystin

NOD = nodularin

ATX = anatoxin-a and homoanatoxin

SAX = saxitoxin and decarbamoylsaxitoxin

NEO = neo saxitoxins

BMAA = β-N-methylamino-L-alanine

AFA produces the toxin *Microcystin* at a very low rate but also produces multiple other toxins; *Cylindrospermopsin, Nodularin, Anatoxin-a, Neosaxitoxin, Saxitoxins and β-N-methylamino-L-alanine (BMAA)* (Figure 2). *Anatoxin-a* and *Saxitoxins* are the two major toxins that *AFA* emits and we believe should be considered in testing for Worcester DPW&P. Also, with the blooms of *AFA* appearing in abundance, this serves as a problem for Worcester because their toxin production rates are very low but their cell counts are usually extremely high. For example four out of eleven cell count detections of *AFA* in Indian Lake were above the advisory thresholds themselves.

Furthermore, *Limnothrix* only emits the *Microcystin* toxin and possibly other toxins that are currently unknown in the cyanobacteria world. *Microcystis aeruginosa* produces *Microcystin* and BMAA. The top three most frequent cyanobacteria species in Indian Lake all produce *Microcystin* toxins. *Microcystins/Nodularins* are the only toxins that are present on the toxin test results gathered after a bloom has surpassed an advisory threshold. Based on our data analysis on the toxin test results, each of the three tests were well below the toxin thresholds for public safety. The toxin threshold level is 14 µg/mL and the toxin results never surpassed 1 µg/mL.

ELIZA toxin tests for Indian Lake are recommended for *Saxitoxins* and *Anatoxins* because *AFA* is the leading producer of these toxins and is the most frequently recurring cyanobacteria species in the water body. Further analysis will be conducted in upcoming sections in 4.3 and 4.4.

4.2.4 Worcester Prevention Treatment Consist of Alum and Copper Sulfate

Worcester has a very effective management plan in place for preventing harmful algal blooms. They begin by performing weekly tests on their bodies of water to test the water parameters and nutrient levels. One indicator Worcester uses to confirm an HAB is the visual identification of a bloom; the green scum mat that forms on the water's surface. If it reaches this point, Worcester DPW&P officials are more than certain that the bloom has surpassed the cyanobacteria cell density count threshold and the water body should be advised for closure immediately. In addition to a visual assessment, Worcester takes samples and orders cyanobacteria cell count tests to determine if the cell count is over the advisory threshold. All testing and monitoring is conducted in the spring through early fall when weather is warm since rising temperature promotes cyanobacteria growth. Worcester DPW&P treats the stream water with aluminum sulfate when nutrient and cell count levels are increased in order to stop the growth of a bloom past the advisory threshold recommending temporary closure. This would allow them to lower the nutrient levels before they are allowed to disperse into the whole lake (Burmeister 2020).

When cyanobacteria cell counts in Indian Lake begin to increase and a bloom is on the horizon, Worcester has the option to implement alum into the lake as well to put a halt to the growth and keep the Lake open. Before implementing their chemical solutions into water bodies such as Indian Lake, Worcester actively implements more eco-friendly methods to reduce cyanobacteria growth as well . They focus on ecological remediation, such as developing vegetation buffers and working with the Indian Lake Watershed Association (ILWA) to work on reducing nutrient loads in local water bodies (Caffrey 2019).

4.3 Information on HABs Management from Researchers and Scientists

Once we fully understood the current management strategies used by the Worcester Department of Public Works and Parks and had analyzed the data that they have collected, we started conducting interviews with researchers and scientists. The goal of these interviews was to collect information about the management strategies used in other parts of the countries as well as to get more insight on harmful algal blooms. It is certain that ensuring public health is one of the targets all our interviewees have in common. In order to provide their communities with the best possible protection from HABs and their impacts on daily life, it requires an effective monitoring plan that offers insight and provides continuous supervision. As we discussed with people in charge of managing HABs response in different areas in the US, we have realized some important components of monitoring plans in use. Funding and crew size do make a difference in implementing a strategy. Thus various monitoring techniques that include ELISA and LC-MS testing for cyanotoxins and cell density count, water quality testing, photosynthetic pigment analysis, thresholds for cyanobacteria density and toxins, as well as prevention plans were included in the responses we received.

4.3.1 Water Chemistry Affects Cyanobacteria Development

When talking about water conditions and the amount of nutrients in the waters, Jacquelyn Burmeister, our sponsor, mentioned that they are major factors affecting cyanobacteria growth. The majority of our interviewees discussed that water quality is an aspect that they pay attention to when it comes to managing cyanobacteria blooms. Worcester DPW&P starting May, conducts a series of indirect testing on a weekly basis, hoping to observe signs favoring the cyanobacteria community. She said these tests provide an insight of possible HABs, giving Worcester a headstart in taking early measures. In addition, she stressed the importance of low price and fast results. A scientist at Lim-Tex, also considers that some factors that are a matter of water quality need to be tested for to predict possible blooms. Lim-Tex checks the temperature and the water clarity, skipping nutrient tests due to budget limitations. Nevertheless, knowing that nutrients need to be taken into consideration, they use a method that they can afford. They analyze the photosynthetic pigments of chlorophyll and phycocyanin and since only cyanobacteria produce phycocyanin (DPW&P Lakes and Ponds Program 2017), they are able to detect possible growth. Utah Department of Water Quality (UDWQ), as one of the employees managing HABs mentioned, only conducts photosynthetic pigment analysis as far as water quality checks for HABs. In addition, when we discussed with an environmental scientist, from the Rhode Island Department of Health, who is working mainly with drinking water and HABs, she informed us that they rely a lot on water quality reports and tests required for drinking water are more intensive and include a wider range of water chemistry tests.

4.3.2 Cell Density Count and Growth Rates Analysis for Cyanobacteria Presence

Most of our interviewees conduct cell density counts to analyze the species and the amount of cyanobacteria present in their water bodies. Based on the cyanobacteria cell count data we received from our sponsor, it seems that the analysis is very informative and provides a lot of useful information about the bacteria contained in the water. It was referred to as the most effective detection method for cyanobacteria presence by most of our interviewees. Another consistency among these interviewees was the long delay, around a week, to receive the sample analysis results. During this period of delay, they would be concerned that people using the water bodies may be risking their health, since it would not yet be known if the count has surpassed the set thresholds.

Furthermore, a researcher working with the University of New Hampshire, introduced us to a different, more cost effective method to foresee possible algal blooms. She said that budget limitations led them to replace the cell density count test, but she seems to advocate for their way to be more accurate and able to predict a bloom a week before occurring. She explained that she uses photosynthetic pigments, phycocyanin specifically to detect the presence of cyanobacteria in combination with size fractionation to "isolate the different communities within the cyanobacteria population". They are able to differentiate and study the different communities that are associated with bloom formation in this way. She emphasized that they are interested in the growth rates and response of these communities to the excess nutrients, rather than the inputs that promote their growth, “that’s the foundation to what we use to evaluate the [community] response”. Her hope is that by better understanding the parameters that cause a bloom to grow, it will help to narrow down the focus on preventing them.

4.3.3 LC-MS and ELISA Testing Techniques for Cyanotoxins Detection and Information on Cyanotoxin Thresholds

LC-MS and ELIZA testing are the most popular testing methods in Rhode Island, Utah and Vermont when it comes to testing for toxins in water bodies. Toxin thresholds account for *Microcystin* in all three states and in Rhode Island, due to their strict drinking water thresholds, they account for *Saxitoxin*, *Anatoxin-a* and *Cylindrospermopsin* thresholds as well.

In Utah, as far as toxin testing goes, they do most of their testing and monitoring in the spring through early fall when the weather is warmer since this type of weather promotes cyanobacteria growth. When they do their toxin tests, they test for multiple types of toxins. The reason for this is they have had multiple dog deaths associated with blooms. They believe that even if a bloom did not exceed the 100,000 cells/mL threshold, a small amount of cyanobacteria can be toxic and pose a serious threat to the public’s health. That’s why they conduct multiple tests to get a better grasp of what is present due to the high toxicity of some toxins even in minor presence. Therefore, they do both ELIZA and LC/MS when it comes to toxin testing to make sure they get as many results as possible. Their advisory level of *Microcystin* is 14 µg/L or 14 parts per billion (ppb).

A researcher for the VANR said that when it comes to toxin testing they choose ELIZA and LC/MS to test for a wide array of toxins. This allows them to understand what types are present and understand if a specific type keeps reoccuring. The threshold they use for toxins is 6 µg/L for *Microcystin* and varies for the other toxins. They did not have a specific threshold for cell density since they focus more on toxin testing to determine whether or not they will do a closure. All of their testing and monitoring is conducted in the spring through early fall when weather is warm since warmer weather promotes cyanobacteria growth.

At RIDH for toxin testing they use ELIZA as their primary toxin test and they mainly test for *Microcystis*, *Anatoxin-a*, *Saxitoxin* and *Cylindrospermopsin*. In terms of toxin thresholds they are more strict because they are in charge of drinking water reservoirs and the thresholds they use are as follows: 0.3 µg/L total for *Microcystin* toxin level, 20 µg/L for *Anatoxin-a*, 1 µg/L for

Cylindrospermopsin, 0.2 μg/L for *Saxitoxin* total level. However, she believes toxins levels of *Microcystin* and *Saxitoxin* that total 10 μg/L is reasonable for recreational water bodies.

They believe toxin testing should be done weekly if a bloom seems to be appearing. Their testing and monitoring is conducted in the spring through early fall when weather is warm since warmer weather promotes cyanobacteria growth. Rhode Island's testing period is May 1st-November 1st.

4.3.4 Cyanobacteria Thresholds for Closure Range between 70,000 -100,000 cell/mL of Water

Advisory guidelines and thresholds are an intricate part to prevent HABs from harming the public's health and the ecosystems associated with the affected water body. Throughout our interviews, we learned different out of state departments and municipalities vary in their cyanobacteria cell count thresholds from 70,000 cells/mL, which is parallel to Worcester's threshold, to 100,000 cells/mL, the threshold that Utah has implemented into their strategy.

The interviews included a number of questions on proper guidelines and thresholds to use. The interviewees were all located in different parts of the U.S and dealt with many different HABs outbreaks large and small. Therefore, our results vary from region to region based on the sizes and locations of HABs outbreaks along with what the water body was used for. For example, if the water body is a source of drinking water, then, the thresholds/guidelines would be much stricter than if it was a recreational water body.

A scientist working at Lim-Tex talked to us about the kits the company created for testing the water that assist townships in eastern Massachusetts to monitor lakes and ponds in the Cape Cod area. When they test, they do it biweekly and from the results, they use an equation to create a growth rate curve. Meaning, they try to predict when growth in cyanobacteria may occur which will allow them to conduct a closure before the bloom grows to its full form. Lim-Tex does not conduct testing for toxins since they only work with photosynthetic pigment presence and creating a growth curve to make predictions. They do not follow a specific threshold for cell density count because they do not test for cell counts and base their closures on their predictions. But she did state the 70,000 cells/mL is a strong threshold to follow since that is normally a high risk of cyanobacteria presence. She did advise us that using the growth curve has proven to be fairly accurate especially when implementing different aspects like weather, time of year, and stormwater runoff which may feed the growth of the cyanobacteria and speed it up.

While talking with two members of ITRC working in Utah, we learned that the UDWQ deals a lot with HABs and they monitor all the major lakes in the state. They have had a lot of experiences with outbreaks of various sizes which is how their HABs division was formed within the water quality department. We discussed how they use the EPA's advisory threshold for cell density of 100,000 cells/mL, which is higher than the value Worcester is currently using.

An environmental scientist from the RIDH talked about how she does cell counts and microscopy to identify the presence of potential HABs. They have specific guidelines for weekly testing especially for water sources. They have the public water systems to keep logs on characteristics like pH, cell counts, temperature, and different chemicals that may be present in the water. This allows them to stay on top of making sure a HAB cannot grow because a HAB affecting a public drinking source can be a detrimental event. These logs must be detailed and regulated regularly to protect the public's health. The threshold they use for cell count is 70,000 cells/mL. In addition to that, they have visual guidelines as well because if a scum mat has formed that means there is more than likely a bloom that has formed and testing should be ramped up and a closure should probably be put into effect to eliminate public health risks.

4.3.5 Aphanizomenon flos-aquae (AFA) has Large Cyanotoxin Production

Researchers from Utah and Rhode Island, managing and testing their local water bodies deal with *Aphanizomenon* bloom similar to Worcester. Through all the information gathered based on their responses to the cyanotoxins and cyanobacteria they are dealing with, we found that *AFA* not only produces *Microcystin* but many other toxins that are less common among other cyanobacteria species.

Given the importance of specific cyanobacteria genera and their toxic products for this project, the interviews included questions on the cyanobacteria species we encountered in the Indian Lake data analysis. Discussion revealed that *AFA* produces various classes of toxins in addition to *Microcystin* and *Anatoxin-a* for which Worcester tests for. Multiple of the other classes produced, such as *Cylindrospermopsin* and *Saxitoxins* are known to be dangerous to public health. Another observation based on the responses was that these four cyanotoxins mentioned above: *Microcystin* and *Cylindrospermopsin* which are hepatotoxic and *Anatoxin-a* and *Saxitoxins* being neurotoxins, are the most common as well as most tested for cyanotoxins.

4.3.6 Known Information on Limnothrix and Toxic Theory on Microcystis

Because the cell density count data for Indian Lake includes populations of the genera *Limnothrix* and *Microcystis*, these were considered further in the interviews. Interviewees indicated that a lot more is unknown rather than known for *Limnothrix*, but it usually blooms in the lower column of the water and testing samples may need to be taken in deeper depth. Moreover, an interesting theory was discussed regarding *Microcystis'* presence in toxic blooms. An environmental scientist from RIDEM, shared with us a year's worth of data from the RIDEM's cell density and toxin tests, indicating that toxin results may be high when *Microcystis* is present during a bloom. The data included multiple *AFA* dominated blooms with cell density over 200,000 cells/mL and no presence of *Microcystis* resulting in very low toxic levels. On the other hand, when *AFA* dominated blooms occurred counting around 70,000 cells/mL with presence of *Microcystis,* the toxic results were significantly higher. This may indicate that *Microcystis* when interacting with other genera functions as a toxic stimuli. More research is needed to fully understand the process.

4.3.7 Citizens Volunteers Assisting Cyanobacteria Management Teams

While a very simple way of estimating ongoing blooms is visual assessment of the water clarity or the existence of the surface scum, a researcher from Vermont Agency of Natural Resources(VANR) shared an interesting approach they followed to achieve immediate communication of the problem so appropriate measures are taken in place on time. She told us that the few people on her HABs management team were not enough to effectively monitor all their water bodies, so they recruited citizen volunteers to join the team. Training and updates on cyanobacteria is offered every year for the volunteers who would either conduct simple quality assurance tests of the lake comparing them to given reports so that they can report back to the Vermont Watershed Management Division Lakes & Ponds Program and further testing would be done if necessary, or they just report their visual evaluation. Angela and her team incorporating the citizens to this management plan, want the people living around the lakes to understand the science of cyanobacteria, the threats and the importance of the testing process.

4.3.8 Efforts on Preventing Excess Nutrients from Accessing Water Bodies

Prevention is a very important aspect of HABs management. Once a bloom is formed, not only is it very hard to treat it without harming the surrounding environment but also causes it to shut down for recreation. Some cities, like Worcester, put forth great amounts of time and efforts into managing and treating their water to keep them safe so that they can be open as much as possible for their residents. By preventing algal blooms, they achieve less temporary shutdowns of water bodies for health concerns.

Two members of the Interstate Technology and Regulatory Council (ITRC), who monitor HABs on the state level in Utah, focus a lot of their prevention efforts on nutrient reduction plans. Aiming their attention at preventing nutrients from accessing the water to reduce treatments since their lakes are quite large and they believe them to be a temporary solution while nutrient reduction offers better long term results. A researcher of VANR, who does a lot of her research on Lake Champlain also implements nutrients reduction plans. She gave us insight as to her experiences and the prevention methods that she currently uses. Her main focus has been on the watershed around Lake Champlain where she works with a lot of farmers around the lake as the pesticides they use and all of the animals on their land, result in a significant amount of nutrient rich runoff from their property. She also works with landowners to ensure that their septic systems are operating properly and not leaching anything that could reach the water. Road runoff is another concern for her. The salt that is used on roads in the winter can become a huge problem for lakes in the warmer weather. In addition, one of her plans involved cities to implement sewer systems to control wastewater so that it can be treated properly instead of going straight into the water and providing a significant amount of nutrients.

An employee of the Rhode Island Department of Health (RIDH), member of the management team for drinking water reservoirs, was able to inform us on some prevention techniques that Rhode Island currently uses. One technique that they adopted is installing aerators into highly prone parts of the lake to increase oxygen levels. This helps prevent nitrogen and phosphorus levels from rising, eliminating the food source of the blooms. They also work with farmers to prevent agricultural runoff and reduce nutrients that reach the water bodies. They do not like to use chemicals at all unless absolutely necessary. That is why they focus a lot of their efforts on prevention.

4.3.9 Guidelines on HABs Prevention Support Existing Management Plans

A researcher for the VANR, said that in Vermont, they have a lot of major lakes and smaller ponds that are affected by HABs. Their biggest lake, Lake Champlain, has had numerous HABs outbreaks in different areas of the lake but It is very hard for them to test multiple locations due to its size. They focus on nutrient reduction so she said that guidelines for creating buffer zones or implementing some sort of nutrient filter would help mitigate the amount of nutrients entering the water. Similarly, UDQW works towards limiting nutrient abundance and they created a set of guidelines to implement buffer zones around lakes and rivers to naturally filter nutrients out before they seep into the water. They believe cutting off the main nutrient source will help mitigate the growth of blooms. To figure out the areas that are known for the most nutrient runoff they conduct studies around the water bodies testing the type of runoff the water body receives.

4.4 Compare and Contrast Researchers'/Scientists' Methods

After conducting interviews, we pulled all the information gathered to begin comparing and contrasting all the responses. As we went through we found many commonalities between interviewees responses and the methods them and their organization uses. Differences were found within the responses which allowed us to begin formulating the best recommendations for Worcester's use.

4.4.1 Monitoring Methods

When it comes to testing, it was clear that ELISA was the typical testing technique for some cyanotoxins detection. All of our respondents told us that they prefer the ELISA test specifically for *Microcystis*. The test is cost effective and relatively fast as our interviewees mentioned. However, its limitations regarding lack of validation to test for specific cyanotoxins and higher detection limits makes it unsuitable and less precise. Some of them also use ELISA to test for *Anatoxin-a* but others have decided to invest a little more money on LC-MS to guarantee better results. LC-MS provides a wider testing range for cyanotoxins such as *Saxitoxins* and *Cylindrospermopsin*, with which the set of 4 most common cyanotoxins is completed.
Refer to Table 4.5. below for a comparison of the responses of each interviewee broken down by testing methods. ELIZA and LC-MS for cyanotoxin presence and cell density and growth rate for cyanobacteria presence. As you can see ELIZA is the most common between the cyanotoxin testing methods due to its cost effectiveness and simplicity. Also as far as cyanobacteria testing, most of our interviewees use cell density count analysis except for one due to budget limitations.

Table 4.5. Comparison of Cyanotoxin and Cyanobacteria Testing Methods among Interviewees.

(Key: Geen=They do use Yellow= to some extent Red=They do not):

	Method	ELIZA Testing	LC/MS Testing	Cell Density Testing	Growth Rate Curve
Organization					
Worcester DPW&P					
LIM-TEX					
Vermont Watershed Management Division					
Rhode Island Department of Health					
Utah Department of Water Quality					

4.4.2 Prevention Methods

After analyzing all of the interviews that we conducted with employees of city's municipalities and members of national HAB research organizations, we noticed a similarity in all of them. Each person that we interviewed mentioned that they focus a lot of their efforts on preventing nutrients from reaching the water bodies in the first place. This can be done by working with landowners to prevent agricultural runoff and to ensure septic systems are working properly. It is also very beneficial to install stormwater management infrastructure near the water bodies to catch road runoff before it reaches the water so that it can be treated properly. There were also other methods that we found that could be used in highly prone areas such as aerators to prevent high nitrogen and phosphorus levels. In addition to the nutrient reduction plans, we also discussed available treatments these HAB management teams apply to prevent a bloom. When the cyanobacteria density counts are getting closer to the thresholds, the departments in charge of HAB need to proceed to effective measures to protect the public's health by applying Alum or Copper Sulfate. Refer to Table 4.6. below for a comparison of the responses of each interviewee broken down by preventive methods.

Table 4.6. Comparison of Prevention Methods among Interviewees.

(Key: Geen=They do use Yellow= to some extent Red=They do not):

	Preventive Method	Stormwater Runoff Prevention	Aluminum-sulfate & Copper Sulfate Treatments
Organization			
Worcester DPW&P			
LIM-TEX			
Vermont Watershed Management Division			
Rhode Island Department of Health			
Utah Department of Water Quality			

4.4.3 Guidelines and Thresholds

After we analyzed all of the responses, we noticed many recurring themes. As for having a cell count threshold it seems having a threshold anywhere between 70,000-100,000 cells/mL is sufficient to detect a bloom preemptively or establish that a bloom may be growing. To continue, toxin thresholds vary but for *Microcystin* it is mainly consistent to establish a threshold for any other toxins we need to be testing for them to establish what types are present and how abundant they are. In Table 4.7 below, you can see the two different thresholds our interviewees use for cyanobacteria density as well as toxin thresholds for advisory closure to protect the citizens. In addition to that, we have included the frequency of the testing procedures in Table 4.7 as well. The testing period for most of the organizations we talked to is late spring to fall, when the weather is warmer.

Table 4.7. Comparison of Thresholds and Frequency of Testing among Interviewees.

(Key: Geen=They do use Yellow= to some extent Red=They do not):

	Guideline/ Threshold	Total ≤70,000 cells/mL	Total ≤100,000 cells/mL	Weekly/ Bi-Weekly Cell Density & Toxin Testing	Daily Testing*	Toxin Thresholds
Organization						
Worcester DPW&P						
LIM-TEX						
Vermont Watershed Management Division						
Rhode Island Department of Health						
Utah Department of Water Quality						

*Daily testing consists of: ph, temperature, nutrient levels, visual look of water and etc.

Finally, guidelines have an important impact on the organizations that use them as they provide specific instruction for dealing with HABs. Most of our interviewees have guidelines on mitigating runoff from the surrounding land that can be very helpful in preventing nutrient runoff which feed cyanobacteria. In addition, in Vermont they have implemented guidelines for citizens volunteers helping with HABs management and have been beneficial to their organization. Refer to Table 4.7. below for a comparison of the responses of each interviewee broken down by guidelines they use.

Table 4.8. Comparison of Guidelines among Interviewees.

(Key: Geen=They do use Yellow= to some extent Red=They do not):

	Guidelines	Use Citizen Volunteers	Buffer Zones For Natural Filtration of Run-off
Organization			
Worcester DPW&P		Red	Green
LIM-TEX		Red	Red
Vermont Watershed Management Division		Green	Green
Rhode Island Department of Health		Red	Green
Utah Department of Water Quality		Red	Green

4.5 Summary

After conducting all of these interviews, we were able to notice a lot of similarities with prevention and monitoring techniques as well as advisory guidelines and thresholds. Currently, Worcester performs cell density tests on their water bodies bi-weekly. If a test comes back and the cell density is above the threshold of 70,000 cells/mL, then they will start to perform toxin tests on the water. From the data that we have on Indian Lake, there are three main species of cyanobacteria that keep appearing in the lake. These are *Aphanizomenon flos-aquae (AFA)*, *Microcystis aeruginosa* and *Limnothrix*. These species of cyanobacteria all produce a cyanotoxin which is *Microcystin*. The city will test for this specific cyanotoxin because of this similarity. The only issue with this is that these species also produce other cyanotoxins that are not being tested for.

The similarities that we found with prevention techniques were very interesting. Almost every person that we interviewed agreed that prevention is the most important part of harmful algal bloom management. They all had the same thought process of attacking the cause of the blooms to have a more long term impact than treating the blooms once they occur which only has a very short term impact. The main focus of their prevention was to eliminate the source of nutrients into the water. The most common practices were working with surrounding landowners to make sure fertilizers aren't being used too close to the water and to also ensure septic systems are working properly. They also encourage cities to install stormwater management infrastructure around the water bodies to eliminate runoff from the roads.

Monitoring techniques is where we found the greatest difference between each person that we interviewed. We found that some cities are utilizing the citizens that live around the lake as monitors. They have been given information on what they should be looking for and if they see anything of concern, then they will report it to the city and they will then conduct tests on that area and decide if it needs to be closed down. Other cities educate lifeguards at town beaches so that they can inspect the water each day to determine if it is safe for people to swim in. This is a very effective way of keeping the public safe as it is a high contact area and it is being monitored every day. When it comes to actually testing the water, the most common tests are the ELISA and LC-MS tests. The only issue with these tests is the high cost and slow turnaround time.

When it comes to advisory guidelines and thresholds, we also found a lot of similarities. For the cities and states that test for cell density, the threshold ranges were 70,000-100,000 cells/mL. Having a threshold within this range allows them to detect a bloom very effectively. When it comes to toxin testing, we found that almost everyone tests for *Miicrocystins* as they are the most common cyanotoxin found across all cyanobacteria species. It is still unsure which other cyanotoxins are necessary to test for as a lot of this information is unknown so it is hard to establish a standard set to test for.

We were able to gather a tremendous amount of information from the interviews that we conducted. By conducting interviews with municipality employees as well as researchers we were able to see the challenges that are faced when dealing with harmful algal blooms all around the country. We were also able to see the passion that these people have for the subject and the extraordinary effort that they put in.

Chapter 5: Recommendations and Conclusion

5.1 Key Findings Summary

Summarizing our results throughout our research, interviews and comparisons, we ended up with 3 major findings. First, Worcester's Indian Lake cyanobacteria species over the last three years (2017-2019) revealed that *Aphanizomenon flos-aquae (AFA)* accounted for 80% of the total cell count of cyanobacteria species in that water body. *AFA* emits toxins such as *Saxitoxin and Anatoxin*. Worcester only receives toxin test results on *Microcystins/Nodularins.* Secondly, over the last three years, Indian Lake has had four blooms exceed the cell count threshold according to the data we received in table 3. All of these blooms have had minimal traces of toxin cells which can conclude two options; Worcester's cell count threshold should be raised and/or other toxins such as *Saxitoxin and Anatoxin* should be tested for. Based on our interviews, Utah as a threshold of 100,000 cells/mL implemented that Worcester could definitely benefit from using. Lastly, throughout our interviews we learned and found that aerators and a great HAB prevention technique that does not affect the surrounding ecosystem and stunts HAB growth at the same time.

5.2 Toxin Testing for Saxitoxin and Anatoxin

Indian Lake's most popular cyanobacteria species *AFA* emits many toxins. According to interviews with researchers from Utah, Rhode Island and Vermont, based on their experience with *AFA* blooms, *Saxitoxin and Anatoxin* are the two most popular toxins that *AFA* produces. We recommend the Worcester DPW&P should begin toxin testing for *Saxitoxin* and A*natoxin*. Based on Worcester DPW& P's Indian Lake toxin analysis papers from GreenWater Laboratories, it states that the analyte in their ELIZA toxin testing is *Microcystin/Nodularin*. An analyte is a subject of chemical analysis and in the toxin cases for Indian Lake the testing analyte was *Microcystin* and *Nodularin* from the 2017-2019 data we analyzed. *Microcystin* is the most recurring and popular toxin that appears in water bodies worldwide so it makes sense that Worcester DPW&P test for them. Despite this toxin's popularity, Worcester's Indian Lake is made up of a majority cyanobacteria species called *Aphanizomenon flos-aquae* (*AFA*). *AFA* produces minor traces of *Microcystin* during its blooms. They produce *Microcystin* but at a very low rate compared to the main toxins that they emit. AFA blooms also produce *Saxitoxins,* *Anatoxins*, *Cylindrospermopsin*, *Microcystin* and the list goes on (Refer to Appendix). After further research we learned that this cyanobacteria species produces *Saxitoxins* and *Anatoxins* more frequently than any other toxin.

To further investigate *AFA*, our team asked HAB researchers and officials from out of state (MA) to give their take on the cyanobacteria and what they know about it. Throughout our interviews we learned that researchers and officials from Utah, Vermont and Rhode Island dealt with *Aphanizomenon* blooms and analyzed what toxins they emitted. *Saxitoxin* and *Anatoxin* were the two popular answers among the interviewees. We asked one researcher about what toxins do they emit the most? We would give the researchers our situation with Indian Lake on what cyanobacteria have been found and how many cells/mL of each species is in the water body and then ask if they were in the Worcester DPW& P's shoes, What toxins would they test for on top of *Microcystin* and *Nodularin*? They said they would test for *Saxitoxin* and *Anatoxin*.

We recommend that these toxin thresholds should follow Ohio's *Saxitoxin* and *Anatoxin* thresholds. There are twenty one states throughout the US that have guidance threshold values and guidelines used for managing HABs and Ohio is one of only two states that include thresholds for *Saxitoxin* and *Anatoxin.* Ohio is one of the most proactive states in the country in monitoring, preventing and managing HABs in water bodies. Ohio's *Microcystin* and *Cylidndrospermopsin* thresholds are similar to ours as their toxin thresholds values are right around Worcester's (Appendix G) (EPA 2017). Toxin thresholds of .8 micrograms/L for *Saxitoxin* and 80 micrograms/L for *Anatoxin* are safe and cautious measures Worcester can consider implementing that mirror Ohio's threshold guidelines.

These S*axitoxins* and *Anatoxins* thresholds are tentative recommendations in which further research and analysis is needed in order to find the optimal threshold for these toxins. Based on our Interviews and overall research and data analysis, we came to conclude that *Saxitoxins* and *Anatoxins* should be tested for in Indian Lake.

5.3 Cyanobacteria Cell Count Advisory Threshold Raised to 100,000 cells/mL

Worcester's cell count advisory thresholds have been exceeded in Indian Lake all while there are small traces of toxins nowhere near the toxin thresholds. The cell count threshold for cyanobacteria could be raised after future testing on *Saxitoxin and Anantoxin* are completed.

We recommend that Worcester considers raising the threshold to 100,000 cells/mL. This will make sure that water bodies around Worcester aren't closed prematurely, maintains public safety and will give Worcester more time to treat blooms before crossing the cell count threshold.

Before considering increasing the cell count threshold to 100,000 cells/mL. We advise Worcester to conduct testing for *Saxitoxin* and *Anatoxin* in addition to *Microcystin* during the first bloom of the spring and summer to analyze those toxin levels and from there determine whether it is safe based on the presence the three toxins in Indian Lake and if necessary other water bodies around the city.

Worcester's cyanobacteria cell count threshold has been exceeded frequently throughout the summer and fall seasons throughout the last couple of years. Surpassing this threshold has temporarily closed down the recreational water body Indian Lake for extended periods of time during seasons where the Worcester community relies on this water body to be open. The advisory closures are currently occurring at a threshold that, based on data analysis of 2017-2019 Indian Lakes toxin and cell count results, poses little to no threat towards the public health and safety as the toxin values are roughly 40 times below the toxin advisory level thresholds.

The EPA and WHO recommend cyanobacteria advisory threshold levels to be 100,000 cells/mL so this is not just a number picked out of a hat. The WHO specifies that as the 100,000 cells/mL threshold has been met the health risks of going in the water are "moderate" (WHO 2016). The EPA specifies that as the 100,000 cells/mL threshold has been met or passed, the water's, "relative probability of acute health effects" are "high" and anything below between 20,000-100,000 is moderate and 20,000 and below is low (EPA 2019). For Indian Lake, since the *Microcystin* toxin levels have been so low (highest recorded in the last three years was .35 µg/L) compared to the corresponding estimated *Microcystin* levels from the EPA of 10-20 µg/L

(Appendix E and F). This displays that our blooms on Indian Lake are notably less toxic compared to EPA's conservative estimates.

Additionally, after interviewing two HABs researchers and officials at Utah Department of Environmental Quality, we learned that they have employed the 100,000 cells/mL cell count threshold throughout their state. Since they dealt with common cyanobacteria species in their blooms such as *Microcystis* and especially abundant amounts of *Aphanizomenon*.

The cell count advisory raise to 100,000 cells/mL feels like a great option for Worcester to look into to further preserve the public health and safety but at the same time ensure that water bodies are not experiencing premature closure.

5.4 Continue Working Through Current Prevention Strategies

We recommend that the Worcester DPW&P continue to work through their current prevention strategies in addition to hearing out some ideas and methods that other out of state departments use to combat HABs. Worcester DPW&P puts massive time and effort in working through generating the best strategy for prevention, treatment and monitoring methods. Working with the Indian Lake Watershed Association (ILWA), they are working towards dredging the Lake and removing excess sediments and cleaning the bottom of the lake's floor. Also, they partake in semi-annual cleanups of the areas around Indian Lake. Worcester tracks the nutrient runoff all the way to the sources that are generating high TPI(Trophic State Index) levels. In addition when in need of a last option in order to cleanse the water body of cyanobacteria and their toxins and in order to prevent the cell count threshold from being passed, Worcester implements chemical input options such as copper sulfate or aluminum sulfate (alum).

Worcester has been continuously working and partnering with ILWA to clean Indian Lake by conducting sediment analysis of the bottom of Indian Lake to begin to take steps towards cleaning out the floor of the Lake. On top of that they are working through managing the nutrient intake the Lake is consuming from the runoffs and waste water throughout the city and backyards and treating the water with chemical solutions like copper sulfate and alum to keep the cell counts below thresholds levels and keep water bodies open.

Some new prevention techniques to think about are placing aerators in areas throughout Indian Lake where there are the most prone locations for attracting high levels of nutrients. We had two researchers mention this idea and could be a helpful addition to Worcester's current tactics. Also, sending out flyers and informational brochures to landowners around Indian Lake informing them on the harm that fertilizer runoff and excess nutrient runoff causes to the Lake and ways that they can help fix the problem. These are ideas that can be added on to the current plan that Worcester uses for prevention of HABs.

Worcester has many limitations as well in the prevention of HABs in Indian Lake. Worcrester identifies areas where nutrient runoff is coming from and leaking into the Lake but most of these areas are privately owned property where the City of Worcester cannot force these landowners to add vegetation buffers and better manage their nutrient runoffs. The fact that Indian Lake is surrounded by family's private property backyards is a barrier that Worcester can't cross into and have to work in managing areas that are more public. Another limitation for Worcester is their HAB budget. They do not have a bottomless budget where they can spend their money on any and every prevention strategy available for managing and treating blooms.

In summary, compared to most if not all municipalities in Massachusetts and throughout New England, Worcester is one of the most proactive cities in implementing prevention, monitoring and management strategies that work to catch and treat a bloom before they occur and expand rather than respond to green scum mat sightings that cover lakes and are already well over cyanobacteria cell count thresholds. Worcester should maintain their current strategies in prevention of HABs in lakes around Worcester specifically Indian Lake as well as considering new dimensions to their prevention including aerators and sending flyers out to landowners living on water bodies around Worcester..

5.5 Conclusion

After doing all of our research, conducting all of our interviews, and speaking with the Worcester Department of Public Works and Parks, it is clear that everyone wishes that they could do everything possible to manage harmful algal blooms. The only problem is that it all comes down to the resources that are available to them. Water tests can be very expensive and can add up very quickly. When treatment is required, it uses up a tremendous amount of the available resources. There are many options for testing and treatment that have a wide range of costs so there is always a decision to be made on which options to choose.

Our goal was to make suggestions to help improve Worcester's harmful algal bloom management strategy. With our suggestions, we kept in mind the resources that are currently available in Worcester. We do not know the additional resources that will be required to implement our suggestions so it will be important for the Department of Public Works and Parks to decide whether our suggestions will be worth the added resources and will be feasible for Worcester to implement.

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Appendix

Appendix A Worcester Full Cyanobacteria Cell Count Data From 2017-2019

Sample Date	Sample ID	Trophic State Index	microcystis aeruginosa	Limnothrix	Anabaena	Anabaena flos-aquae	Anabaena planctonica	Aphanizomenon flos-aquae	Anabaena circinalis
7/16/17	TG96	45.1	4,536	1546	--------	503	129	503	--------
8/10/17	TG97	54.4	8071	11829	4747	771	1365	--------	--------
--------	--------	--------	--------	--------	--------	--------	--------	--------	--------
8/14/18	UD57	52.3	432	9094	--------	--------	--------	988	--------
8/28/18	UD60	67.1	25127	11024	--------	--------	--------	151980	--------
9/6/18	UD73	65.4	923	14350	--------	--------	--------	120062	--------
9/13/18	UD75	56.7	11299	16396	--------	--------	--------	1154	--------
9/17/18	UD76	52.2	8246	19900		--------	--------	631	--------
--------	--------	--------	--------	--------	--------	--------	--------	--------	--------
6/20/19	VY12	55.9	2554	1197	--------	778	6146	--------	--------
6/28/19	VY14	65.6	21498	1203	--------	902	12448	--------	--------
7/3/19	VY15	54.8	3853	2312	--------	--------	2920	--------	--------
7/9/19	VY16	50	--------	364	--------	--------	--------	--------	--------
7/15/19	VY20	57.4	28635	1193	--------	11633	--------	--------	--------
7/26/19	VY32	55.6	5412	19449	--------	--------	--------	--------	--------
8/1/19	VY34	50.9	1210	637	--------	--------	--------	--------	--------
8/8/19	VY36	60.4	4754	--------	--------	--------	--------	15440	--------
8/15/19	VY39	56.5	1911	--------	--------	--------	--------	1090	--------
8/21/19	VY42	61.5	--------	--------	--------	--------	--------	70032	--------
8/23/19	VY43	58.6	--------	--------	--------	--------	--------	44419	511
9/10/19	VY44	69.9	--------	--------	--------	1751	--------	226201	--------
9/16/19	VY46	47.6	2176	--------	--------	--------	--------	--------	--------
9/20/19	VY48	47.8	--------	--------	--------	829	--------	--------	--------

Appendix B

1. **Interview Questions for Acquiring Information from scientist/researchers and other states**

   a. *Introductions*
      i. Could you tell us a little bit about yourself (What are you involved in, where do you work)?
      ii. How did you start your research in Harmful Algal Blooms (HABs)?
      iii. What does your research focus on (Prevention, Cyanobacteria Growth, Treatment)?

   b. *Prevention for HABs management teams*
      i. What methods/tests do you use to monitor cyanobacteria in your water bodies?
      ii. How often do you practice these methods?
      iii. What toxins do you test for? What testing techniques do you prefer?
      iv. Based on our cyanobacteria data analysis of Worcester's main water body, Indian Lake, we have frequently come across a specific species called Limnothrix that we have found limited information on. If you have encountered this cyanobacteria species before, could you elaborate on what you know about Limnothrix?
      v. The three main cyanobacteria species that we encounter in our cyanobacteria cell count data analysis are; microcystis aeruginosa, Limnothrix sp. and Aphanizomenon flos-aquae. Are these species that you frequently come across in analyzing cyanobacteria in water bodies?
      vi. Have you noticed what the most frequent cyanobacteria species in your cell density tests results are?
      vii. What treatments do you apply to prevent outbreaks(alum, copper sulfate)?
      viii. Why did you choose these methods?
      ix. How often do you practice these methods?
      x. Which one do you believe to be the most effective?

xi. What do you think the criteria should be for closing down water bodies?

c. *Treatment for HABs management teams*

i. What treatment methods do you use when there is an algal bloom outbreak?

ii. How do you decide which treatment method to use?

d. *Guidelines for DEP/DPH employees*

i. How did you determine the current thresholds regarding algal blooms?

ii. What are the threats if the cyanobacteria cell count exceeds 70,000 cells/milliliter per water?

e. *ITRC group*

i. How was the group form?

ii. How did you get involved?

f. *Cyanobacteria Management for researchers*

i. What are the best parameters for determining risk to public health when it comes to cyanobacteria?

ii. Does testing the lake for nutrient level (Phosphorus and Nitrogen), temperature, pH, clarity of water and dissolved oxygen offer useful insight of cyanobacteria presence?

iii. Is testing for cyanobacteria cell density the most accurate way to determine the possibility of an outbreak? If not, what do you think is?

g. *Toxins Management for researchers*

i. Have you determined any of the most frequent toxin species?

ii. Worcester has been using the ELIZA testing method to monitor toxins in their water bodies. Would you recommend any other ways to more effectively test for toxins?

iii. Worcester has only been testing for Microcystins/Nodularins toxins, what other toxins should they be getting tests for in order to protect public health?

iv. Assuming the toxin levels advisory suggested by MDPH of 14 ng/mL are indeed a public health risk, what’s a better way to use cyanobacteria counts as a predictor?

v. At what stage do you think that cyanotoxins threaten public health?

Appendix C

2. **Interview Questions for Sponsor Interview**
   a. *Testing Questions for sponsor*
      i. What are the tests that you conduct to monitor cyanobacteria?
      ii. Why did you choose these tests?
      iii. Do you think that cell density test is the most effective?
      iv. Do you think other tests may be necessary?
      v. Are there any difficulties or problems conducting these tests or adding more?
      vi. During what period do you conduct tests and how often?
      vii. Do you take samples from specific spots within the lake and why?
      viii. How do you analyze the data?
      ix. Are Microcystins the only toxin that is tested for in Worcester?
      x. Do all tests results take a week to process? If so, that would raise problems if there was a bloom during that week and kids were still swimming in the water, no?
      xi. Limnothrix sp. Pops up often in cell density data that you sent out to us yet it doesn't seem to be a Microcystin species. Does this cyanobacteria species emit toxins, if so, are there current testing accounting for this species?
   b. *DPH Advisories Questions for sponsor*
      i. What treatment methods do you use when there is an algal bloom outbreak?
      ii. How do you decide the treatment approach to take if a problem is detected in the test?
      iii. Do you think the recommended advisories are helpful?
      iv. What do you think should be necessary to monitor the water sources regarding harmful algal blooms?
      v. Was it your interest that led the Worcester DPW&P to practice such proactive measures to protect the lakes?
      vi. What do you think is the most important factor that prevents other municipalities from proactively monitoring their water bodies?
      vii. Once a bloom outbreak is detected what are the first immediate steps taken afterward?

Appendix D Cyanobacteria Genus and Toxin Chart

Cyanobacteria Genus	Cyanotoxin Class										References
	CYL	MC	NOD	ATX	SAX	NEO	LYN	BMAA	DAT	APL	
Anabaenopsis		✓									Lanaras and Cook, 1994; Graham et al., 2010
Aphanizomenon	✓	✓	✓	✓	✓	✓		✓			Graham et al., 2010; Jacoby and Kann, 2007; Pilotto et al., 1997; Vezie et al., 1998; Graham et al., 2008
Aphanocapsa		✓									Graham et al., 2010
Calothrix		✓						✓			Mohamed et al., 2006; Paerl and Otten, 2013
Coelomoron		✓									Dos S Vieira et al., 2005
Coelosphaerium		✓									Graham et al., 2010; Jacoby and Kann, 2007
Cylindrospermopsis	✓	✓		✓	✓			✓			Graham et al., 2010; Griffiths and Saker, 2002; Woods and Sterling, 2003; Graham et al., 2008; Paerl and Otten, 2013
Cylindrospermum		✓		✓	✓						Borges et al., 2015; Pandey and Tiwari, 2010; Sivonen et al., 1989
Dolichospermum (Anabaena)	✓	✓		✓	✓	✓		✓			Bruno et al., 1994; Graham et al., 2010; Harada et al., 1991; Jacoby and Kann, 2007; Mohamed et al., 2006; Pilotto et al., 1997; Sivonen et al., 1989; Spoof et al., 2006; Vezie et al., 1998 ;Graham et al., 2008
Fischerella		✓									Otten and Paerl, 2015
Geitlerinema		✓			✓						Aboal et al., 2005; Borges et al., 2015; Myers et al., 2007
Gloeotrichia		✓									Carey et al., 2007; Graham et al., 2010; Jacoby and Kann, 2007
Hapalosiphon		✓									Prinsep et al., 1992
Limnothrix		✓									Graham et al., 2010
Lyngbya	✓			✓	✓	✓	✓	✓	✓	✓	Berry et al., 2004; Dos S Vieira et al., 2005; Foss et al., 2012; Harr et al., 2008; Onodera et al., 1997; Stewart and Falconer, 2008; Paerl and Otten, 2013
Microcystis		✓						✓			Botes et al., 1982; Graham et al., 2010; Jacoby and Kann, 2007; Miller et al., 2010; Oberholster et al., 2006; Pilotto et al., 1997; Ueno et al., 1996; Vezie et al., 1998; Graham et al., 2008
Nodularia		✓	✓					✓			Carmichael et al, 1988; McGregor et al., 2012; Pilotto et al., 1997; Graham et al., 2008
Nostoc		✓						✓			Mohamed et al., 2006; Sivonen and Carmichael, 1990; Sivonen et al., 1992 ;Paerl and Otten, 2013
Oscillatoria (Planktothrix)	✓	✓		✓	✓		✓	✓		✓	Brittain et al., 2000; Carmichael and Li, 2006; Graham et al., 2010; Jacoby and Kann, 2007; Luukkainen et al.,1993; Mazmouz et al., 2010; Mez et al., 1997; Sivonen et al., 1989; Graham et al., 2008
Phormidium	✓	✓		✓	✓			✓			Borges et al., 2015; Gugger et al., 2005; Harland et al., 2013; Izaguirre et al., 2007; Mez et al., 1997; Mohamed et al., 2006; Skulberg et al., 1992; Smith, 2012
Planktolyngbya					✓		✓				Graham et al., 2010
Prochlorococcus								✓			Paerl and Otten, 2013
Pseudanabaena		✓		✓							Graham et al., 2010
Raphidiopsis	✓			✓	✓						Graham et al., 2008; Otten and Paerl, 2015
Rivularia		✓									Aboal et al., 2005
Schizothrix				✓						✓	Sivonen and Jones, 1999; Paerl and Otten, 2013
Scytonema								✓			Smith et al., 2011; Otten and Paerl, 2013
Synechococcus		✓						✓			Carmichael and Li, 2006; Graham et al., 2008
Synechocystis		✓						✓			Graham et al., 2008
Trichodesmium								✓			Paerl and Otten, 2013
Tychonema				✓							Shams et al., 2015
Umezakia	✓										Paerl and Otten, 2013
Woronichinia		✓		✓							Oberholster et al., 2006; Paerl and Otten, 2013

Appendix E Tables from EPA Recommendation for Cyanobacteria and Cyanotoxin Monitoring in Recreational Waters

Table 2. WHO (2003) Recreational Guidance/Action Levels for Cyanobacteria, Chlorophyll *a*, and Microcystin

Relative Probability of Acute Health Effects	Cyanobacteria (cells/mL)	Chlorophyll *a* (µg/L)	Estimated Microcystin Levels (µg/L)[a]
Low	< 20,000	< 10	< 10
Moderate	20,000–100,000	10–50	10–20
High	>100,000–10,000,000	50–5,000	20–2,000
Very High	> 10,000,000	> 5,000	> 2,000

Table 1. EPA Recommended Values for Recreational Criteria and Swimming Advisories for Cyanotoxins

Total Microcystins Magnitude (µg/L)	Cylindrospermopsin Magnitude (µg/L)	Duration	Frequency
8	15	1 in 10-day assessment period across a recreational season	Not more than 3 excursions in a recreational season in more than one year [b]

Appendix F WHO Cyanobacteria Cell Count Threshold

TABLE 8.1. CYANOBACTERIAL TOXINS AND THEIR ACUTE TOXICITY[a]

Cyanotoxins	LD_{50} (i.p. mouse)[b] of pure toxin (µg/kg)	Taxa known to produce the toxin(s)	Mechanism of toxicity
Protein phosphatase blockers (cyclic peptides with the amino acid ADDA)			
Microcystins in general (~60 known congeners)	45–>1000	*Microcystis*, *Planktothrix*, *Oscillatoria*, *Nostoc*	all block protein phosphatases by covalent
Microcystin-LR	60 (25–125)	*Anabaena*, *Anabaenopsis*	binding and cause
Microcystin-YR	70	*Hapalosiphon*	haemorrhaging of the
Microcystin-RR	300–600		liver; cumulative damage
Nodularin	30–50	*Nodularia spumigena*	may occur
Neurotoxins			
Anatoxin-a (alkaloid)	250	*Anabaena*, *Oscillatoria*, *Aphanizomenon*, *Cylindrospermum*	blocks post-synaptic depolarization
Anatoxin-a(s) (unique organophosphate)	40	known only from two species of *Anabaena*	blocks acetylcholinesterase
Saxitoxins (carbamate	10–30	*Aphanizomenon*,	block sodium channels
alkaloids)		*Anabaena*, *Lyngbya*, *Cylindrospermopsis raciborskii*	
Cytotoxin			
Cylindrospermopsin (alkaloid)	2100 in 1 day 200 in 5–6 days	*Cylindrospermopsis raciborskii*	blocks protein synthesis; substantial cumulative toxicity

Appendix G Ohio Toxin Threshold Table from EPA website

Ohio	Microcystin-LR: PHA: 6 µg/L; NCA: 20 µg/L Anatoxin-a: PHA: 80 µg/L; NCA: 300 µg/L Saxitoxin: PHA: 0.8 µg/L; NCA: 3 µg/L Cylindrospermopsin: PHA: 5 µg/L; NCA: 20 µg/L	Public Health Advisory (PHA) - swimming and wading are not recommended, water should not be swallowed and surface scum should be avoided. No Contact Advisory (NCA) -recommend the public avoid all contact with the water