2021 Cyanobacteria Monitoring Report for Bakers, Ice House, and Uncle Harvey's Ponds and Crystal and Pilgrim Lake in Orleans, MA

Prepared for the Orleans Pond Coalition (OPC)
By the Association to Preserve Cape Cod (APCC)

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Summary

APCC began cyanobacteria monitoring of freshwater ponds in Orleans in 2020 with biweekly samples of Bakers Pond, through a partnership with the Brewster Ponds Coalition (BPC). In 2021, at the request of OPC, APCC initiated an official season-long monitoring program at four (4) ponds and lakes in Orleans: Ice House, and Uncle Harvey's ponds and Crystal and Pilgrim Lakes. Bakers Pond also continued to be monitored in a partnership with BPC. The goal of this monitoring was to track and predict the formation of harmful cyanobacteria blooms (HCBs) and expected toxins. In 2021, three of these waterbodies, Ice House and Uncle Harvey's ponds and Crystal Lake were in the Low Warning Tier for the entirety of the season. Pilgrim Lake was in the Low and Moderate Warning Tiers during season. Bakers Pond was in the Low and High Warning Tiers during the season. Over the course of the monitoring season, APCC recommended temporary use restrictions in only Bakers Pond due to cyanobacteria concerns. All monitoring results were shared with OPC, the town, and the public throughout the season. This report summarizes the 2021 cyanobacteria monitoring results for the ponds listed above. This document should be printed in color, as some sections are color-coded.

Background

Cyanobacteria are an ancient group of photosynthetic microorganisms common in freshwater systems on Cape Cod and worldwide. Under the right conditions, they can multiply rapidly and form harmful cyanobacteria blooms. A number of cyanobacteria genera can produce harmful toxins known as cyanotoxins. HCBs have increased worldwide, including in the U.S., because of nutrient enrichment and rising water temperatures due to global warming. These conditions will likely continue to exacerbate harmful cyanobacteria blooms, leading to the need for increased cyanobacteria monitoring and awareness of the extent of the issue.

Cape Cod ponds are commonly used for swimming, boating, paddle boarding, and fishing. Due to the threat of public exposure to cyanobacteria, the Massachusetts Department of Public Health (MDPH) has guidelines for municipal officials to post and remove advisories at ponds for established thresholds for cyanobacteria concerns (MDPH). Frequent cyanobacteria monitoring allows resource managers to track cyanobacteria trends in their ponds throughout the season and

to be proactive about posting and removing recreational advisories. For more information and resources on cyanobacteria, see Appendix 1.

Goals

Over the course of the last decade, APCC has received input from many pond associations, organizations, and local and regional resource managers on Cape Cod regarding concerns about pond health, pond water quality and the need for data received in a timely manner to inform pond protection measures and ensure public safety. In response to these concerns and limited data, APCC established its Cyanobacteria Monitoring Program in 2017 with guidance and input from state and federal agencies and scientists, including Nancy Leland from Lim-Tex, Jim Haney from the University of New Hampshire Center for Freshwater Biology, the U.S. EPA (EPA recreational waters), Massachusetts Department of Public Health (MDPH), and Karen Malkus-Benjamin, formerly with the town of Barnstable's Health Division (Town of Barnstable).

The goals of APCC's Cyanobacteria Monitoring Program are to collect field and lab data on cyanobacteria in Cape Cod ponds in order to improve understanding of cyanobacterial populations in ponds, increase public awareness of harmful cyanobacteria blooms, and communicate results to help promote public health and safety. Cyanobacteria monitoring is conducted on a biweekly basis during the duration of the contract period with weekly monitoring conducted during periods of concern.

Methods

Overview

APCC's Cyanobacteria Monitoring Program uses an EPA-approved monitoring protocol developed by scientists from the University of New Hampshire (Dr. James Haney and Nancy Leland). The monitoring protocol (Quality Assurance Project Plan, or QAPP) was developed by the EPA for the Cyanobacteria Monitoring Collaborative (CMC 2017). It incorporates methods described in published scientific articles (Leland and Haney, 2018; Leland, Haney, Conte, Malkus-Benjamin and Horsley, 2019). The protocol utilizes a combination of field observations, microscopy and fluorometry to analyze samples from freshwater lakes and ponds for cyanobacteria and cyanobacteria pigments. The data collected includes photographs and field observations, digital microscopy to identify composition (type of cyanobacteria present) and dominance, and concentrations of phycocyanin and chlorophyll pigments indicative of the amounts of cyanobacteria vs. general algae and phytoplankton, respectively. APCC tracks changes in cyanobacterial composition, dominance and abundance on a biweekly basis from June to September at a minimum, and a full season of May to November.

At this sampling frequency, APCC is often able to forecast when cyanobacteria blooms may be forming or when toxin concentrations may be approaching harmful levels. These signs instruct APCC to increase the frequency of monitoring and to inform town officials to be aware of potential threats and to plan for proactive management actions to protect public safety.

In contrast to measuring cyanobacteria using cell counts, which is one of the methods listed by the Massachusetts Department of Public Health (MDPH), the method used by APCC is less costly, offers a faster turn-around time for results, and is often useful for predicting imminent cyanobacteria bloom formation. The use of microscopy to determine dominance along with fluorometry of phycocyanin pigments also reveals expected genus-specific toxicity, which is not possible using basic cell counts. Cyanobacteria pigment data and other collected data also support research efforts that will expand our understanding about the health of the ponds.

Sampling

For the four (4) ponds and lakes, water samples were collected by Orleans Pond Coalition citizen scientists, with assistance from APCC interns when necessary, on a biweekly to weekly schedule, depending on cyanobacteria risks, between June and November. BPC citizen scientists also took samples from Bakers Pond throughout the season. At each sampling event, two samples were collected from shore, one using a 1-meter tube called the Whole Lake Water sample and one using a 50-micron (um) mesh student plankton net, called the Bloom Forming Colonies sample. When possible cyanobacteria bloom material was discovered, samples were taken of the material for additional analysis. At least twelve (12) pond water samples were collected at each of the five (5) ponds and lakes for a total of at least sixty (60) samples that were processed and analyzed at APCC's office in Dennis, MA. The types of samples collected are described below.

Whole Lake Water Sample

The sample taken from the 1-meter tube is called the Whole Lake Water (WLW) sample. This is an unconcentrated water sample containing cyanobacteria from the full extent of the 1-meter sampling depth from the surface to just above the bottom near shore. This sample is further processed by APCC staff to obtain data on cyanobacteria size fractions in the water column. This process is further explained in the "Lab Analysis" section. This sample is not used to forecast future bloom accumulations, as it contains cyanobacteria currently in the water column near shore.

Bloom Forming Colonies Sample

The second sample, which is taken through the student plankton net, is called the Bloom Forming Colonies (BFC) sample. This is a concentrated sample taken by towing the student plankton net across a 3-meter cast near the surface. This sample will contain mostly the larger cyanobacteria colonies which tend to form visible blooms and scums. Fundamentally, this sample creates an artificial cyanobacteria accumulation of what the natural cyanobacteria accumulation would contain if the wind condensed cyanobacteria over a distance of 3 meters into a potentially harmful accumulation near shore where children and pets typically interact with the pond.

The concentrations of cyanobacteria in BFC samples can fluctuate dramatically and sudden continuous increases of cyanobacteria concentrations in BFC samples can foreshadow imminent

cyanobacteria bloom formations. Understanding the toxin concentrations of this sample can also inform APCC with knowledge of when a cyanobacteria bloom accumulation, even a narrow cyanobacteria scum, may likely contain microcystin concentrations greater than the thresholds set by MDPH for recreational waters. This concept is discussed further in future sections.

Cyanobacteria Scum Sample

The third sample, taken only when needed, is called the scum sample and may contain cyanobacteria bloom material. Although visual evidence alone of potential cyanobacteria bloom material can be compelling, microscope and fluorometry analysis of the material can confirm whether the material is indeed a cyanobacteria bloom rather than an accumulation of algae, diatoms, etc. Microscope analysis of the bloom material can also inform APCC of the genus of cyanobacteria that composes the bloom, giving an understanding of the types of toxins that the bloom may have produced.

Field Observations

Field observations were documented at each sampling event by completion of a field data sheet with information on weather, visual appearance of pond surface, water temperature, etc. Photographs were taken of the pond's shoreline at each sampling event, providing documentation of pond appearance and visible conditions and evidence of bloom accumulations.

Orleans Sampling

In the Orleans ponds this season, at least twelve (12) samples were collected at each of Bakers, Ice House, Uncle Harvey's ponds, and Crystal and Pilgrim lakes on a biweekly to weekly basis between June and November. Samples were taken at the locations designated in Figure 1.

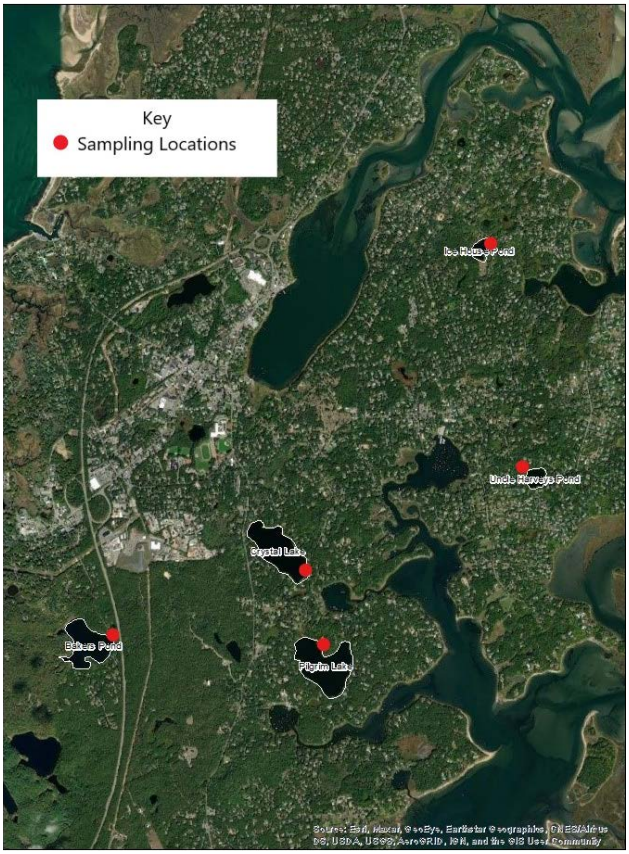

Figure 1. Cyanobacteria sampling stations in Orleans in 2021.

Lab Analysis

Sample processing

On the same day as sample collection, APCC processed and analyzed samples following our protocol. Triplicate 5 milliliter (mL) samples were taken of each of the 3-4 sample types: the WLW sample, the < 50 micron (µm) sample, the BFC sample, and the scum sample when possible cyanobacteria bloom material was discovered. The < 50 µm sample is developed by filtering the WLW sample through a 50 micron (µm) filter, which results in a sample containing only the relatively small colonies of cyanobacteria. Smaller cyanobacteria, known as pico-cyanobacteria, are believed to produce toxins that differ from toxins produced by larger cyanobacteria. APCC has been contributing to Nancy Leland's research on these communities and we hope to apply these findings to inform our program in future seasons. The WLW and scum samples are processed without further action. The BFC sample is further separated through Zapprs, which is explained with pictures in the EPA QAPP (CMC 2017).

Microscopy

Using a microscope, APCC staff and interns counted colonies of cyanobacteria from a 1 mL sample from the BFC sample. The information is used to estimate dominance of different cyanobacteria genera. If one genus is found to be the "dominant genus" (defined as 90% of the cyanobacteria community on the slide), then APCC can target the toxins produced by that genus of cyanobacteria as the toxins of concern for that pond at that time. If a scum sample was taken, APCC also analyzed it under the microscope to inspect genus composition and to confirm whether the scum was indeed composed of cyanobacteria. See Appendix 3 for more information about common cyanobacteria genera APCC has observed in Cape Cod ponds.

Fluorometry to measure phycocyanin pigments

Each triplicate 5 mL sample was frozen and thawed for the purpose of lysing cells to liberate cyanobacteria pigments. Samples were then analyzed for cyanobacteria pigments (phycocyanin) and non-cyanobacteria algal pigments (chlorophyll-a) using a calibrated fluorometer in parts per billion (ppb). APCC uses phycocyanin concentrations in micrograms per liter (ug/L) as an indicator of cyanobacteria biomass rather than cell counts. Understanding cyanobacteria concentrations using fluorometry allows APCC to track cyanobacteria community trends over time. All data was stored on APCC's online server.

When feasible, APCC may conduct a test for microcystin concentrations using an Abraxis test strip for microcystin concentrations in recreational water. However, funding for these kits was limited in the 2021 season, leaving most communication about potential toxin risks coming from regression data, described in the next section, and the presence of confirmed cyanobacteria bloom presence.

Interpretation of Results

APCC staff interpret the results within a guidance framework that incorporates the most recent scientific information as well as existing state and federal guidance (EPA recreational waters, MDPH). APCC uses a 4-tiered system for interpreting cyanobacteria data in order to make recommendations to local officials and inform the public of recent findings. These "Warning Tiers" are used in biweekly reports and the online interactive map. The 4 tiers are "High, Restriction in Place," "High, Restriction Pending," "Moderate," and "Low." A detailed description of APCC's Warning Tiers is provided in Appendix 2.

To estimate toxin levels, measured phycocyanin concentrations in samples are compared to published linear regression relationships between concentrations of phycocyanin in whole lake water and bloom-forming colonies with expected microcystin concentrations for certain genus compositions (Leland and Haney, 2018; Leland, Haney, Conte, Malkus-Benjamin and Horsley, 2019). These regressions are used to estimate expected microcystin concentrations. Direct toxin measurement is expensive and was not funded in the 2021 season. APCC uses three different regression formulae for these purposes with one used for *Microcystis* spp. dominant samples, one for *Dolichospermum* spp. dominant samples, and one for samples of mixed assemblages. The

regressions project higher microcystin concentrations for *Microcystis* spp. dominant samples than *Dolichospermum* spp. dominant samples with similar phycocyanin values (Leland, Haney, Conte, Malkus-Benjamin and Horsley, 2019).

APCC's program seeks to be proactive in identifying potential cyanotoxin production and exposure. The Bloom Forming Colonies sample is a concentrated sample taken through a 3-meter student plankton net tow meant to mimic the natural accumulation of bloom material that can occur if conditions are right. In absence of funding for additional toxin testing, APCC utilizes regressions that convert genus specific cyanobacteria biomass (measured by phycocyanin) into expected concentrations of the microcystin toxin based on historical data. This way, resource managers can be made aware of possible cyanobacteria accumulations and potential toxin exposure allowing proactive rather than reactive responses.

Reporting

Biweekly reports

Results were provided in biweekly reports to local municipal officials and pond associations. Depending on results, reports included recommendations concerning appropriate advisory posting or removal for the public to minimize or avoid risks due to cyanobacteria exposure. During periods of possible harmful cyanobacteria bloom formation requiring weekly sampling, additional reports and updates were sent to officials and pond associations as well. Pond associations play a key role in raising public awareness of cyanobacteria risks and alerting pond residents of cyanobacteria monitoring results throughout the season.

Interactive map

An interactive map is hosted on APCC's website where recent monitoring results were posted throughout the season. Updates were submitted on an automated basis at 7 p.m. on the same day as reports of results were emailed to town officials. In some cases, automated map updates were postponed a day if a town official requested additional time to review results before they would be posted. The interactive map is located at: https://apcc.org/our-work/science/community-science/cyanobacteria/.

Email alerts

APCC provided an email registry signup on our website for an e-blast system designed to update interested residents about recent harmful cyanobacteria bloom discoveries. Residents could sign up for these Cyanobacteria Alerts throughout the season. The link for the Cyanobacteria Alert e-blast signup is located here https://apcc.org/our-work/science/community-science/cyanobacteria/cyanobacteria-alert/.

Results

Cyanobacteria monitoring results are described in this section. For each pond, a table and a graph are provided to delineate results that affected the interpretation of risk in the pond related to APCC's Warning Tier criteria. The full Warning Tier criteria are described in Appendix 2. Descriptions of how data were interpreted, and risks communicated to town officials and the public are provided for each pond in this section as well. A complete table of results is provided in Attachment 1. This table is formatted to be printed on an 11x17 sheet.

Bakers Pond

Bakers Pond experienced instances in the Low and High Warning Tiers during the 2021 monitoring season (Table 1). Bakers Pond was placed in APCC's High Warning Tier late in the sampling season due to cyanobacteria concerns. **Advisory Recommended from 10/13/21 to 11/8/21**.

On 6/9/21, APCC placed Bakers Pond in the Moderate Warning Tier even though it fell just short of the thresholds for that tier. The Bloom Forming Colonies sample taken on 6/9/21 was measured to have a phycocyanin concentration of 1019.6 ppb, just below APCC's Moderate Warning Tier phycocyanin threshold of 1,100 ppb in these samples. Given the close proximity to the threshold and rapid growth since the previous sampling event, APCC elevated the risk level to the Moderate Warning Tier. Following this sampling event, however, APCC decided it best to stick with strict interpretations of our criteria, rather than round up, for the remainder of the sampling season. Results close to increasing to a higher Warning Tier would simply be retested the following week for safety, as occurred for a sample in Uncle Harvey's Pond later in the season, rather than be elevated to the higher Warning Tier. The cyanobacteria biomass in Bakers was found to be significantly lower the following week, sample taken 6/15/21, keeping Bakers in the Low Warning Tier.

Late in the season, the Bloom Forming Colonies sample taken on 10/13/21 was estimated to have a microcystin concentration of 13.2 ppb, drawing cause for concern. This is above MDPH's threshold of 8 ppb microcystin for recreational waters, placing Bakers in the High Warning Tier for concerns with high toxin levels in potential cyanobacteria accumulations. Shortly after, a cyanobacteria bloom accumulation was recorded on 10/29/21, as the sample on 10/13/21 predicted. APCC recommended advisories for these events due first to the likelihood of toxic accumulations and then for cyanobacteria accumulations themselves. APCC recommended that an advisory continue be posted for the duration of the bloom until two weekly tests confirmed it had dissipated, allowing lingering toxins to decay. This event was followed by two consecutive weekly tests with no bloom material and estimated microcystin concentrations below 8 ppb, allowing the advisory recommendation to be rescinded and a return to the Low Warning Tier for the pond for the close of the sampling season.

Sampling Date	APCC Map Warning Tier	Dominant Genus	Bloom Forming Colonies Phycocyanin (ug/L)	Estimated Microcystin Concentrations in Bloom Forming Colonies (ug/L)	Cyanobacteria Bloom Material Presence
5/26/2021	Low	*Dolichospermum* spp.	265.6	0.06	No
6/9/2021	Low*	*Dolichospermum* spp.	1019.6	0.14	No
6/15/2021	Low	Mixed	29.0	0.07	No
6/23/2021	Low	*Dolichospermum* spp.		0.00	No
7/7/2021	Low	NA	2.8	0.01	No
7/21/2021	Low	*Dolichospermum* spp.	64.6	0.03	No
8/4/2021	Low	*Dolichospermum* spp.	673.5	0.11	No
8/18/2021	Low	NA	2.4	0.01	No
8/30/2021	Low	Mixed	16.0	0.04	No
9/15/2021	Low	*Dolichospermum* spp.	715.1	0.11	No
9/27/2021	Low	Mixed	14.4	0.04	No
10/13/2021	**High**	***Microcystis* spp.**	**362.6**	**13.22**	**No**
10/19/2021	**High**	**Mixed**	**154.3**	**0.44**	**No**
10/29/2021	**High**	**Mixed**	**26.9**	**0.07**	**Yes, a Green Line was found on the sand**
11/2/2021	**High**	**Mixed**	**791.6**	**2.51**	**No**
11/8/2021	Low	Mixed	263.9	0.78	No

Table 1: Summary Cyanobacteria monitoring results for Bakers Pond in Orleans, MA. *APCC incorrectly interpreted this sample as registering in the Moderate Warning Tier during the season.

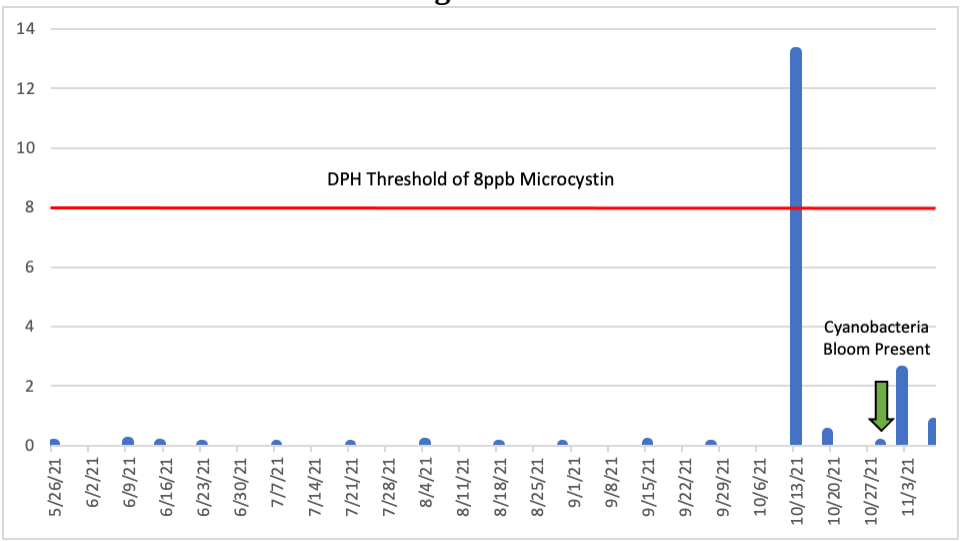

Figure 2. Estimated Microcystin (ug/L) in Bloom Forming Colonies in Bakers Pond. The X-axis contains sampling dates, and the Y-axis contains the estimated microcystin concentrations for each sampling event in parts per billion (ppb). If cyanobacteria bloom material was discovered at a sampling event, it is denoted by a green arrow.

Crystal Lake

Crystal Lake experienced low cyanobacteria concentrations, low estimated microcystin concentrations in the Bloom Forming Colonies sample, and no bloom material accumulations over the course of the sampling season.

Sampling Date	APCC Map Warning Tier	Dominant Genus	Bloom Forming Colonies Phycocyanin (ug/L)	Estimated Microcystin Concentrations in Bloom Forming Colonies (ug/L)	Cyanobacteria Bloom Material Presence
5/28/2021	Low	*Woronichinia* spp.	2.7	0.01	No
6/11/2021	Low	*Woronichinia* spp.	1.4	0.00	No
6/25/2021	Low	Mixed	70.9	0.19	No
7/8/2021	Low	*Dolichospermum* spp.	11.7	0.01	No
7/23/2021	Low	*Dolichospermum* spp.	7.9	0.01	No
8/6/2021	Low	*Woronichinia* spp.	3.4	0.01	No
8/20/2021	Low	*Microcystis* spp.	2.4	0.09	No
9/3/2021	Low	Mixed	4.1	0.01	No
9/16/2021	Low	*Woronichinia* spp.	64.7	0.18	No
9/30/2021	Low	Mixed	2.2	0.00	No
10/14/2021	Low	*Woronichinia* spp.	4.7	0.01	No
11/1/2021	Low	*Woronichinia* spp.	0.0	0.00	No

Table 2: Summary Cyanobacteria monitoring results for Crystal Lake in Orleans, MA

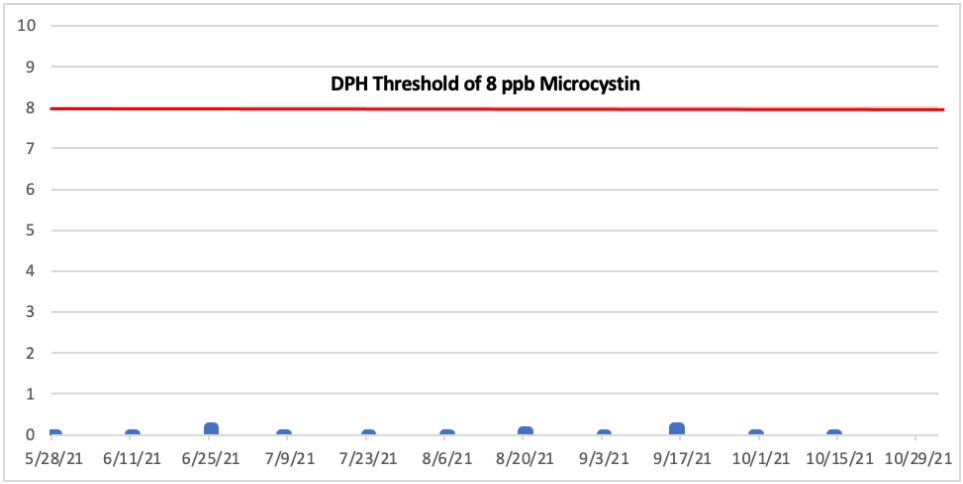

Figure 3. Estimated Microcystin (ug/L) in Bloom Forming Colonies in Crystal Lake. The X-axis contains sampling dates, and the Y-axis contains the estimated microcystin concentrations for each sampling event in parts per billion (ppb). If cyanobacteria bloom material was discovered at a sampling event, it is denoted by a green arrow.

Ice House Pond

Ice House Pond experienced low cyanobacteria concentrations, low estimated microcystin concentrations in the Bloom Forming Colonies sample, and no bloom material accumulations over the course of the sampling season.

Sampling Date	APCC Map Warning Tier	Dominant Genus	Bloom Forming Colonies Phycocyanin (ug/L)	Estimated Microcystin Concentrations in Bloom Forming Colonies (ug/L)	Cyanobacteria Bloom Material Presence
5/28/2021	Low	NA	6.0	0.01	No
6/11/2021	Low	NA	1.1	0.00	No
6/25/2021	Low	*Microcystis* spp.	1.1	0.04	No
7/9/2021	Low	NA	0.6	0.00	No
7/23/2021	Low	Mixed	18.1	0.05	No
8/6/2021	Low	Mixed	7.0	0.02	No
8/20/2021	Low	Mixed	3.3	0.01	No
9/3/2021	Low	*Microcystis* spp.	3.3	0.12	No
9/16/2021	Low	Mixed	3.9	0.01	No
9/30/2021	Low	NA	2.2	0.00	No
10/14/2021	Low	*Microcystis* spp.	34.3	1.26	No
11/1/2021	Low	*Woronichinia* spp.	8.9	0.02	No

Table 3: Summary Cyanobacteria monitoring results for Ice House Pond in Orleans, MA

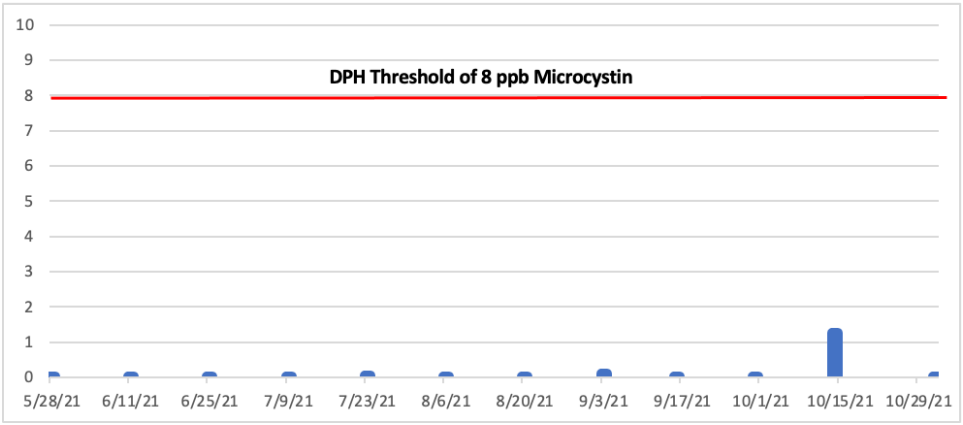

Figure 4. Estimated Microcystin (ug/L) in Bloom Forming Colonies in Ice House Pond. The X-axis contains sampling dates, and the Y-axis contains the estimated microcystin concentrations for each sampling event in parts per billion (ppb). If cyanobacteria bloom material was discovered at a sampling event, it is denoted by a green arrow.

Pilgrim Lake

During the 2021 monitoring season, Pilgrim Lake experienced instances in APCC's Low and Moderate Warning Tiers (Table 4 below).

The Bloom Forming Colonies sample taken on 6/11/21 contained a phycocyanin concentration of 2,454.9 ppb, greater than the APCC threshold for the Moderate Warning Tier of 1,100 ppb for *Dolichospermum* spp. dominated samples**.** As a result, Pilgrim Lake was moved to the Moderate Warning Tier. When sampled again the following week on 6/18/21, the cyanobacteria biomass was found to be much lower, allowing a return to the Low Warning Tier where it remained for the rest of the season.

Sampling Date	**APCC Map Warning Tier**	**Dominant Genus**	**Bloom Forming Colonies Phycocyanin (ug/L)**	**Estimated Microcystin Concentrations in Bloom Forming Colonies (ug/L)**	**Cyanobacteria Bloom Material Presence**
5/28/2021	Low	*Dolichospermum* spp.	142.3	0.04	No
6/11/2021	Moderate	*Dolichospermum* spp.	2454.9	0.23	No
6/18/2021	Low	Mixed	4.7	0.01	No
6/25/2021	Low	NA	2.2	0.00	No
7/9/2021	Low	*Woronichinia* spp.	7.1	0.02	No
7/23/2021	Low	Mixed	6.2	0.01	No
8/6/2021	Low	Mixed	11.2	0.03	No
8/20/2021	Low	Mixed	3.6	0.01	No
9/3/2021	Low	*Woronichinia* spp.	7.7	0.02	No
9/16/2021	Low	Mixed	2.2	0.00	No
9/30/2021	Low	*Oscillatoria* spp.	4.5	0.01	No
10/14/2021	Low	Mixed	13.6	0.03	No
11/1/2021	Low	*Microcystis* spp.	11.0	0.40	No

Table 4: Summary Cyanobacteria monitoring results for Pilgrim Lake in Orleans, MA

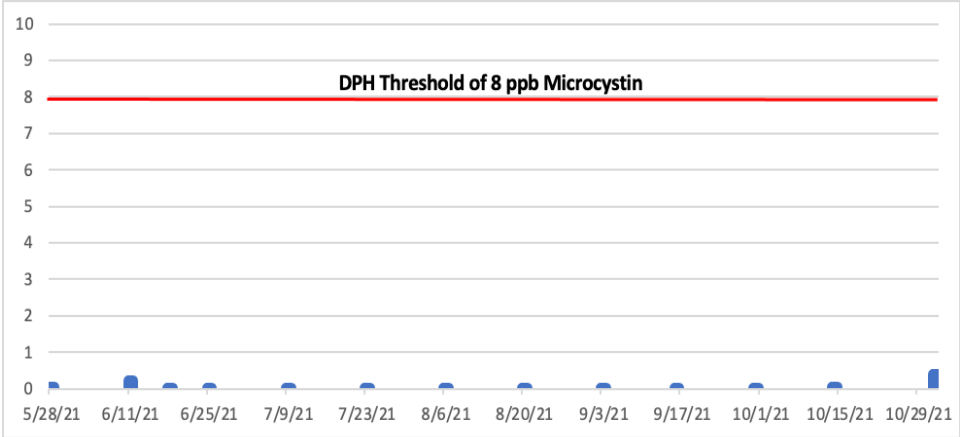

Figure 5. Estimated Microcystin (ug/L) in Bloom Forming Colonies in Pilgrim Lake. The X-axis contains sampling dates, and the Y-axis contains the estimated microcystin concentrations for each sampling event in parts per billion (ppb). If cyanobacteria bloom material was discovered at a sampling event, it is denoted by a green arrow.

Uncle Harvey's Pond

Uncle Harvey's Pond experienced low cyanobacteria concentrations, low estimated microcystin concentrations in the Bloom Forming Colonies sample, and no bloom material accumulations over the course of the sampling season. Uncle Harvey's nearly entered the Moderate Warning Tier on 9/3/21 with estimated microcystin concentrations in the Bloom Forming Colonies sample nearly exceeding APCC's Moderate Warning Tier threshold of 4 ppb, but thankfully an exceedance never occurred, including the following week when a sample showed a trend of decreased estimated microcystin concentrations. It was suspected that the removal of plastic currents following the summer alum treatment in the pond influenced the elevated readings on 9/3/21.

Sampling Date	APCC Map Warning Tier	Dominant Genus	Bloom Forming Colonies Phycocyanin (ug/L)	Estimated Microcystin Concentrations in Bloom Forming Colonies (ug/L)	Cyanobacteria Bloom Material Presence
5/28/2021	Low	*Woronichinia* spp.	17.3	0.04	No
6/11/2021	Low	*Microcystis* spp.	1.5	0.05	No
6/25/2021	Low	NA	1.2	0.00	No
7/9/2021	Low	Dolichospermum spp.	1.9	0.00	No
7/23/2021	Low	NA	2.0	0.00	No
8/6/2021	Low	*Microcystis* spp.	26.2	0.96	No
8/20/2021	Low	*Dolichospermum* spp.	48.0	0.02	No
9/3/2021	Low	*Microcystis* spp.	99.6	3.64	No
9/10/2021	Low	*Microcystis* spp.	65.6	2.40	No
9/16/2021	Low	Mixed	0.0	0.00	No
9/30/2021	Low	*Microcystis* spp.	44.8	1.64	No
10/14/2021	Low	*Microcystis* spp.	54.1	1.98	No
11/1/2021	Low	Mixed	264.0	0.78	No

Table 5: Summary Cyanobacteria monitoring results for Uncle Harvey's Pond in Orleans, MA

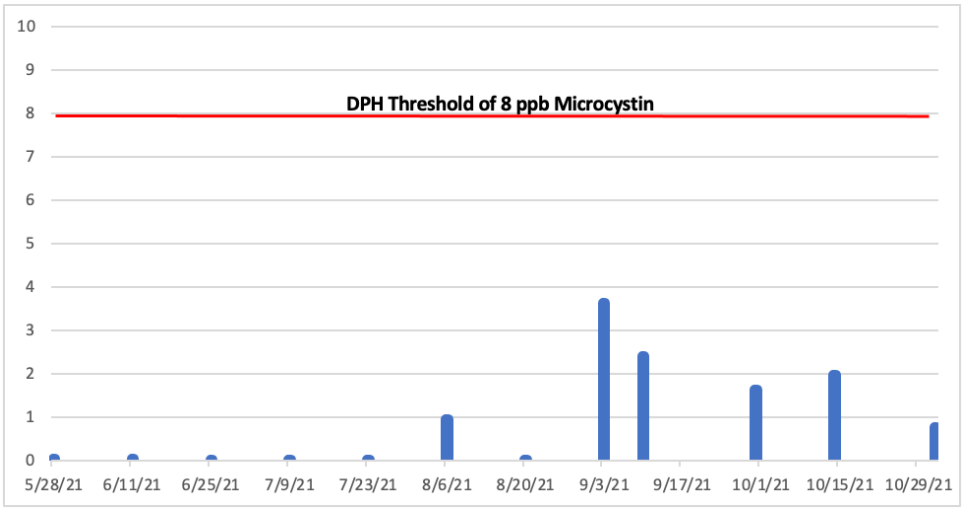

Figure 6. Estimated Microcystin (ug/L) in Bloom Forming Colonies in Uncle Harvey's Pond.

The X-axis contains sampling dates, and the Y-axis contains the estimated microcystin concentrations for each sampling event in parts per billion (ppb). If cyanobacteria bloom material was discovered at a sampling event, it is denoted by a green arrow.

Conclusions

In 2021, the five ponds and lakes that were monitored, Bakers, Ice House, and Uncle Harvey's ponds and Crystal and Pilgrim Lakes experienced cyanobacteria Warning Tier designations ranging from the Low to Moderate to High. Crystal Lake and Bakers, Ice House and Uncle Harvey's ponds were in the Low Warning Tier for the entirety of the season, Pilgrim Lake was in the Low and Moderate Warning Tiers during the season, and Bakers Pond was in the Low, and High Warning Tiers during the season.

With Bakers Pond meeting the criteria for the High Warning Tier during the season, APCC recommended a temporary use restriction for the pond from 10/13/21 to 11/8/21**.**

Of the five ponds, only Bakers Pond was monitored in the 2020 season. In 2020, Bakers Pond was monitored from May to October, reaching Moderate Warning Tier from 6/2/21 to 6/12/21 and staying in the Low Warning Tier for the remainder of the season. In 2021, Bakers similarly experienced elevated cyanobacteria concentrations in early June. Future monitoring of Bakers could help clarify whether this is a common yearly occurrence.

The 2021 cyanobacteria monitoring program resulted in collecting and analyzing samples and documenting results on every intended date throughout the season. With this year being the first formal biweekly sampling of ponds in Orleans, valuable data was collected and communicated properly throughout the season**.** All results throughout the season were promptly shared with the town and the Orleans Pond Coalition via biweekly reports and then entered into the APCC Interactive Map following the completion of sample analysis (APCC Cyanobacteria).

Recommendations

APCC recommends continuing the current full season schedule. Many ponds in APCC's network experience their highest cyanobacteria concentrations in the spring and the fall. Although residents may interact with these ponds less during these early and late season times, there are still dangers posed to pets who may consume or swim in these waters while on walks during colder months.

APCC recommends continued yearly cyanobacteria monitoring. Monitoring over multiple years for full seasons would provide greater understanding of the cyanobacteria community in each of the Orleans ponds. More seasons of data will allow us to draw better predictions year over year. Continued monitoring will also allow for the ability to track degradation in the ponds as increased occurrence of harmful cyanobacteria blooms point to larger issues of pond impairment. Monitoring efforts will shed light on the ponds most in need of protection and restoration.

APCC recommends reducing nutrient loading to freshwater ponds. Residents surrounding vulnerable pond ecosystems should reduce potential nutrient pollution flowing from their property. Excess fertilizer use, septic systems around ponds, poor stormwater management infrastructure, and a lack of adequate vegetation buffers are examples of behaviors that exacerbate nutrient loading of ponds.

For a comprehensive list of actions that residents, municipalities, and state agencies can take to promote pond health, visit APCC's State of the Waters: Cape Cod website (State of the Waters), specifically, the Action Plan for ponds. For additional restoration information for impaired ponds, APCC recommends reaching out to restoration agencies and organizations for suggestions. There are a number of pond restoration efforts occurring across the region including weed harvesting, alum treatments, aeration, and more. Any of these options will be much more effective when coupled with action for reducing nutrient inputs. Each pond is also unique, and restoration technology that works for one pond may not work well for another. Protection of pond and ecosystem health must also be prioritized.

In addition to managing nutrients, changing climate conditions, including the currently warming atmosphere and altered rainfall patterns, are believed to play a significant role in the increasing frequency and intensity of harmful cyanobacteria blooms (Paerl et al., 2019). Residents and officials should understand that there may be many factors leading to cyanobacteria issues on Cape Cod. Continued work around cyanobacteria and water quality will lead to increased awareness, a safer public, and hopefully improved health of our freshwater ponds.

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Appendix 1. Resources on Cyanobacteria

Harmful cyanobacteria blooms in freshwater bodies are the subject of numerous reports published by scientists, state and federal agencies, and organizations, some of which are listed here:

- The World Health Organization recognized the public health consequences of cyanobacteria in water in 1999 (WHO[1]).
- The Centers for Disease Control (CDC) call cyanotoxins "among the most powerful natural poisons known" (CDC Fact Sheet on Harmful Algal Blooms). The CDC's Physician Card on Harmful Algal Blooms (HABs) states that swallowing water containing cyanobacteria can damage the central nervous system, liver or kidneys; skin contact can cause allergic dermatitis and conjunctivitis; and inhalation of aerosols containing cyanobacteria or their toxins can cause wheezing, coughing, chest tightness, and shortness of breath.
- New England Interstate Water Pollution Control Commission (NEIWPCC) is an interstate commission that helps the states of the Northeast preserve and advance water quality. NEIWPCC's webpage states that "the frequency of HAB occurrence is on the rise and cyanobacteria toxicity has been associated with human health impacts including skin rashes, gastrointestinal and respiratory disease, and liver damage. Effects can be even more pronounced (potentially even fatal) in animals ranging from cattle to dogs. HABs have direct implications to the use of recreational waterbodies for contact recreation, the susceptibility of public water supplies to toxins, and the overall degradation of our aquatic resources."
- U.S. Environmental Protection Agency (EPA):
    - "Monitoring and Responding to Cyanobacteria and Cyanotoxins in Recreational Waters." (EPA recreational waters)
    - EPA Office of Ground Water and Drinking Water webpage. Managing Cyanotoxins in Public Drinking Water Systems. (EPA drinking water)
    - EPA webpage on nutrient pollution and HABs. (EPA and nutrient pollution)
- State agencies, including New York (NY), Rhode Island (RI), and New Hampshire (NH) have cyanobacteria monitoring programs and provide guidance concerning public health and environmental risks posed by cyanobacteria.
- Commonwealth of Massachusetts:
    - Cyanobacteria webpage: (Massachusetts)
    - Massachusetts Department of Public Health (MDPH) website on "Guidelines for cyanobacteria in freshwater recreational water bodies." (MDPH)

Appendix 2. APCC's Cyanobacteria Warning Tiers

Warning Tiers

High, Restriction in Place: Monitoring results indicate high levels of cyanobacteria concentrations detected. Health risk to adults is high and is especially dangerous for children and pets when ingested. APCC found cyanobacteria concentrations near or exceeding state recreational standards with potential for exponential growth rates of cyanobacteria. Any accidental consumption of pond water is considered dangerous and interacting with the pond in general carries risk for adverse health effects.

High, Restriction Pending: Monitoring results indicate high levels of cyanobacteria concentrations detected. We have recommended a restriction and the decision to issue a restriction is pending with the town/DCR. APCC recommends the same level of caution as the "High, Restriction in Place" Warning Tier. Note: the distinction between the "High, Restriction in Place" Warning Tier and the "High, Restriction Pending" Warning Tier is that the former indicates APCC has received confirmation of a posted advisory for cyanobacteria concerns.

High Warning Tier criteria:

- If the regressions for the BFC or WLW give a microcystin concentration greater than or equal to 8ppb. If the sample is 90% or greater *Microcystis* spp. (based on dominance), the 100% *Microcystis* spp. regression is used. If it's between 90% and 10% *Microcystis* spp., the Mixed regression is used. If it's less than 10% *Microcystis* spp., the 100% *Dolichospermum* spp. regression is used.
- If an Abraxis test strip gives a reading of 8ppb or greater microcystin.
- If a cyanobacteria bloom is found and verified. (For this to happen on a scum sample, it must be analyzed under the microscope and confirmed that the bloom is composed of cyanobacteria. If the sample is mostly green algae with a normal amount of trace cyanobacteria, it would not be deemed a cyanobacteria bloom or scum. Confirmed cyanobacteria blooms and scums are accompanied by picture documentation of the scum itself and the microscopy of the scum.)
- A pond should remain in the high tier until two sampling events a week a part both yield results lower than the high tier following the timeline set by the Massachusetts Department of Public Health (MDPH).
- Ponds in the High Warning Tier are sampled weekly.

Moderate: Monitoring results indicate moderately high levels of cyanobacteria concentrations detected. While these conditions pose low to minimal health risks to adults, they can be dangerous for children or pets if water is ingested accidentally or incidentally during recreational activities. Pet exposure can be from drinking pond water or grooming after swimming. Due to lower body masses, children and pets are more susceptible to impacts at lower concentrations than adults. This tier is consistent with the town of Barnstable's "Pet Advisory." Map color is yellow.

Moderate Warning Tier criteria:

- If the regressions for BFC or WLW are 4ppb or greater microcystin.
- If the Abraxis test strip comes up as around 4ppb or greater.
- If the phycocyanin value is greater than 1100 (indicating increased cyanobacteria biomass).

Ponds in the Moderate Warning Tier should be test weekly if possible.

Low: Monitoring results indicate no or low concentrations of cyanobacteria detected. To the best of our knowledge at the time and location of sample collection, regular recreational usage of the pond is safe with respect to cyanobacteria and toxins. Map color is blue.

Low Warning Tier Criteria:

- If none of the conditions are met for the High Warning Tier or the Moderate Warning Tier, the pond is designated in the Low Warning Tier.
- Ponds in the Low Warning Tier should be tested on a biweekly to monthly basis.

Massachusetts Department of Public Health (MDPH) Guidelines for Cyanobacteria

The MDPH cyanobacteria webpage describes guidelines for cyanobacteria in recreational freshwater bodies that are described as follows ("Guidelines for Cyanobacteria at Recreational Freshwater Locations") (MDPH).

"DPH recommends issuing a public health advisory for HABs at recreational freshwater locations when at least one of the following criteria is met:

1. ***A visible cyanobacteria scum or mat is evident;***
2. ***Total cell count of cyanobacteria exceeds 70,000 cells/mL.***
3. ***Concentration of the toxin microcystins exceeds 8 µg/L; or***
4. ***Concentration of the toxin cylindrospermopsin exceeds 15 µg/L***

Guideline values are based on US Environmental Protection Agency (US EPA) and World Health Organization (WHO[1]) (WHO[2]) recommendations. When issuing an advisory, signage should be posted at each access point at the waterbody warning against any contact with the water.

Rescinding a Public Health Advisory
Cyanobacteria cells can release cyanotoxins into the water when they die. Therefore, algal toxins may be present when a visible scum or mat is no longer evident. DPH recommends the rescinding of a public health advisory after two successive samples, collected a week apart, demonstrate cell counts or toxin levels below the quantitative guideline values." (MDPH).

APCC Warning Tier Development and Connection to MDPH Thresholds

APCC's Cyanobacteria Monitoring Program bridges the gap between these guidelines from MDPH with the metrics and forecasting abilities of the method used. APCC's Warning Tier method and MDPH both recommend advisories for the presence of cyanobacteria bloom material. APCC's Moderate Warning Tier contains a phycocyanin threshold of 1100 ug/L as a metric of biomass, similar to MDPH's threshold of 70,000 cells/mL. These thresholds should not

be compared, but APCC's use of a phycocyanin threshold is used to indicate increased cyanobacteria biomass that could perhaps lead to the formation of a cyanobacteria bloom.

For toxin concentrations, APCC does not have a threshold for cylindrospermopsin due to recommendations at the inception of the program that cylindrospermopsin was not relevant for analysis in the region due to lack of presence. However, MDPH's 8 ppb threshold for microcystin concentrations is used as a core component of APCC's Warning Tiers. It is important to note that this threshold from MDPH is meant for samples that aren't artificially concentrated, as the Bloom Forming Colonies sample is. However, given that the Bloom Forming Colonies sample can project accumulations of cyanobacteria near shore as well as the likely toxicity of that bloom material, these signs of likely imminent cyanobacteria accumulations with natural exceedances over 8 ppb for microcystis concentrations are included in the criteria for APCC's High Warning Tier. Exceedances of microcystin concentrations over 8ppb for Whole Lake Water samples and the results from Abraxis Test Strips are also included in the High Warning Tier. APCC's Moderate Warning Tier uses similar reasoning for the microcystin concentrations for the Moderate Warning Tier threshold of 4 ppb. Although MDPH doesn't have criteria for this threshold or anything similar to a Moderate Warning Tier, APCC includes the Moderate Tier as an alert not just for adult recreation, but more specifically as a warning to parents of young children and pet owners as children and pets are more susceptible to toxin exposure. This Moderate Warning Tier was inspired by the Pet Advisory tier used in the town of Barnstable, developed by Karen Malkus-Benjamin.

APCC uses the Bloom Forming Colonies data as an important metric for forecasting concerns and exceedances. Although not every warning from the Bloom Forming Colonies sample will end in a bloom accumulation or microcystin exceedance, APCC does not wish to wait for one to occur and then alert clients and the public about the issue. This is to say that APCC's Cyanobacteria Monitoring Program is a proactive program rather than a reactive one. With powerful forecasting tools available, health officials and the public are best served when an advanced warning is given to promote public health.

Similar to the criteria for lifting an advisory from MDPH, APCC also requires two weekly consecutive sampling events with results below the High Warning Tier, before removing the pond from the High Warning Tier and recommending the removal of advisories.

Appendix 3. Common Cyanobacteria on Cape Cod

Commonly, APCC see two genera of cyanobacteria quite commonly across Cape Cod. Those two genera are *Dolichospermum* spp. and *Microcystis* spp. Although there quite a few other genera observed in each pond including *Woronichinia* spp., Most sightings and recommendations are made with *Dolichospermum* spp. and *Microcystis* spp. as the genera causing the concern.

Image 1: *Microcystis* spp.

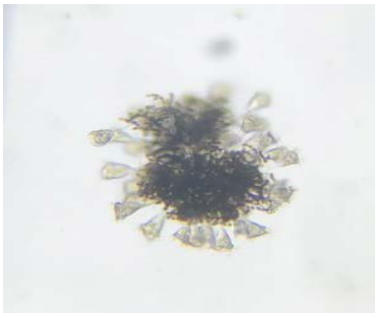

Image 2: *Dolichospermum* spp.

Image 3: *Woronichinia* spp.

Microcystis spp. can produce the hepatotoxin microcystin in relatively high concentrations. It produces other toxins as well, including anatoxin-a and endotoxins. However, it is understood that it produces anatoxin-a, in particular, in relatively low concentrations, but microcystin at relatively high concentrations among genera in the region. Microcystin is a hepatotoxin believed to induce damage to liver and other internal organs, often from ingestion and chronic exposure. MDPH of course has a standard for the microcystin toxin. Interestingly, high *Microcystis* spp. biomass tend to give a much lower phycocyanin reading than cyanobacteria of other genera. Care should be taken not to understate the severity of *Microcystis* spp. biomass concerns strictly due to low phycocyanin concentrations.

Dolichospermum spp. is believed to produce the neurotoxin anatoxin-a at relatively high concentrations. It can produce microcystin and endotoxins as well, but it is understood that it produces microcystin, in particular, in relatively low concentrations, but anatoxin-a at relatively high concentrations among genera in the region. Anatoxin-a can cause a host of neurological symptoms that can occur from acute exposure. APCC is currently working with Nancy Leland on anatoxin-a related projects to better understand the neurotoxin and its presence in the region. Although MDPH does not have an anatoxin-a standard, they recommend posting advisories for *Dolichospermum* spp. blooms, and other states in the U.S. do have standards for anatoxin-a. *Dolichospermum* spp. as well as other cyanobacteria genera in the region, such as *Woronichinia* spp., produce higher phycocyanin readings than *Microcystis* spp.