2022 Report of the Assessment Subcommittee to the Wolfeboro Waters Committee

Water Quality Programs with a Long History

Most of the waterbodies in Wolfeboro have had ongoing associated lake associations concerned about their water quality. Over the years, local volunteers recruited by these associations have been sampling their lakes as part of either the UNH Extension Lay Lakes Monitoring Program (LLMP) or the NHDES Voluntary Lake Assessment Program (VLAP). Those statewide programs seek to track long-term trends and determine the trophic status (oligotrophic=good water quality, mesotrophic=medium quality, or eutrophic=overgrown and lower quality) of lakes across the state. Trophic classification for stratified lakes is based upon the concentrations of phosphorus, dissolved oxygen, chlorophyll-*a* (a measure of algal abundance), aquatic vascular plant abundance, as well as the water clarity/Secchi disk transparency in surface waters at deep water sites for three summer samples over three consecutive years.

Five of our local waterbodies are oligotrophic (good-generally quite good):

- Lake Winnipesaukee, Lake Wentworth, Crescent Lake, Rust Pond, and Upper Beach Pond (the Town of Wolfeboro's public drinking water source).

The other two are mesotrophic (medium):

- Mirror Lake and Sargents Pond.

The New Hampshire Department of Environmental Services (NHDES) surveys lakes around the state on a rotating schedule (on a 10-year cycle) to determine their trophic status, ensuring compliance with state water quality standards, and supplementing data used to assess designated use support for federal water quality reporting.

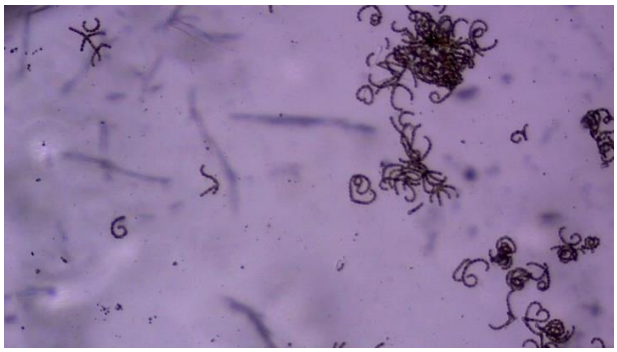
These trophic classifications tend to change very slowly over decades, especially for relatively larger waterbodies, such as Lake Winnipesaukee and Lake Wentworth.

To mitigate and protect local water quality, local public works projects have employed best management practices (BMPs), often with financial support from NHDES grants, that focus on limiting the addition of pollutants, including phosphorus and sediment to our lakes, especially.

Cyanobacteria- A recent growing concern

On a separate track, NHDES hired a cyanobacteria program coordinator who receives reports and analyzes water samples for potential cyanobacteria present in NH lakes. When cyanobacteria is confirmed to be present in a waterbody at concentrations above 70,000 cells/mL and until the concentration goes below that level, the state will issue an advisory warning that the water poses a possible health threat to humans and their pets.

The first such advisory was issued locally in 2008 for Mirror Lake. Since 2018, NHDES advisories have been issued twice more for Mirror Lake and twice for portions of Lake Winnipesaukee in Wolfeboro. In 2022, 5 advisories were issued within the Lake Winnipesaukee watershed, which includes all of Wolfeboro Waters, including an advisory for Jockey Cove at the end of June.

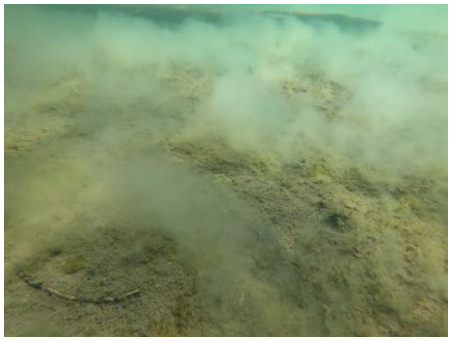


Microscopic picture of Dolichospermum that bloomed in Jockey Cove, Lake Winnipesaukee this past summer just before the 4th of July Weekend

Significant, but not advisory-level, concentrations of cyanobacteria have been observed at other times on local lakes.

Increasing Biological Growth is being Observed on the Bottom Along Local Shorelines

While water quality measures reported by LLMP and VLAP for our lakes have not changed significantly over the years, many people who have been enjoying our lakes over decades have witnessed significant increases in biological growth on shallow bottoms, particularly during the warmer summer months. Rocks on the bottom are becoming coated with slippery slime. In some locations, muck is starting to grow on the sandy bottom each summer. Plants are growing where there used to be none. In addition, there are numerous observations of large "cotton candy-like" fuzzy balls floating along bottoms in the summer. The increases in biological activity are believed to be associated with increased nutrients and sediments due to growing recreational pressures, watershed development, and climate change.



Metaphyton a.k.a. "Cotton Candy" being seen in our lakes in recent summers

Wolfeboro Waters

In 2019, the Wolfeboro Board of Selectmen established an ad-hoc Cyanobacteria Task Force that gave way a year later to a standing committee called Wolfeboro Waters. Wolfeboro Waters complements the longer-term efforts of the local lake associations (Wentworth Watershed Association, Rust Pond Association, Mirror Lake Protective Association, and Lake Winnipesaukee Association) and collaborates with them to assess and address local water quality. Wolfeboro Waters has a particular focus on the risks of cyanobacteria blooms and means to prevent and mitigate future ones. (Assessing E. coli at public beaches and invasive species are being addressed by NHDES and other town bodies.)

Wolfeboro Waters involves a large and growing number of volunteers involved in water sampling, measurement, and analysis that supplement and complement those of the many volunteers in our local lake associations. Observations, sampling, and analyses continue throughout the calendar year. In addition, many hours are being devoted to attending relevant conferences and to obtaining and reading relevant reports and scientific literature, and to sharing such knowledge.



Wolfeboro Waters has been fortunate to

- have benefited from a large body of previous and ongoing water quality assessments done for the local lake associations on their waterbodies,
- have identified and worked with a group of senior scientists at Bigelow Laboratory for Ocean Sciences in East Boothbay, ME (two of whom have links to our area),
- have developed close working relationships with the cyanobacteria, microbiology, and aquatic geochemist experts at NHDES, UNH and USEPA,
- have participated in the Cyanobacteria Monitoring Collaborative, and the Interstate Technical and Regulatory Council,
- have scientists to consult at Dartmouth College, Colby-Sawyer, and numerous other organizations that complement those of local lake associations.

As a result, we have had a great deal of assistance in keeping abreast of new scientific insights, helping us to design data and information collection, accessing advanced analytical capabilities, training us in various laboratory techniques, and interpretating our results.

We are collaborating with all the local lake associations to learn about their concerns and priorities and to share data, knowledge, and analytical capabilities. We seek to be a valuable resource to the local community on water quality.

Cyanobacteria are Complex

Cyanobacteria have been present in our lakes and other parts of our environment since long before humans existed. Many different types of cyanobacteria can produce and potentially release a wide variety of toxins to the water. Cyanobacteria issues are complex because the types of cyanobacteria present in our lakes depend on species-specific combinations of physical (e.g., temperature and sunlight), chemical, and biological conditions, all of which are complicated and variable. Moreover, the concentrations of cyanobacteria can respond by multiplying hundreds of times within hours in optimal conditions. Another reason for the complexity is that these cyanobacteria risks are being studied by numerous different disciplines (e.g., agriculture, drinking water treatment, public health, limnology, toxicology, and various other subdisciplines of biology) often with different approaches and using different terminology.

However, in recent years more serious cyanobacteria blooms are being observed in freshwater lakes and oceans in recent years resulting in growing attention by scientists from many different disciplines. New insights are being gained into their causes. Among the factors almost certainly contributing to the increase in cyanobacteria concerns are global climate changes that include

- warming of the water,
- fewer days with ice and snow on lakes limiting the penetration of sunlight through the water, and
- more violent storms causing increases in nutrient-runoff into waterbodies.

These are factors that we have limited ability to control locally.

The two elemental nutrients that seem to be the most important in controlling the amount of biological growth in water are **nitrogen** and **phosphorus.** As total nitrogen is about 30 times higher in Winter Harbor (e.g., 178-194 ug/L total nitrogen vs. 5.3-6.7 ug/L of total phosphorus), the concentration of available phosphorus is likely the most important determinant of how much cyanobacteria and most other biological growth we have in local waters. However, each type of cyanobacteria must contend with the presence of phages (bacterial diseases) and predators and must compete with organisms for nutrients, sunlight, and habitat.

<u>Past Measurements Don't Reflect Observed Biological Growth or Risks from Cyanobacteria</u>

LLMP and VLAP measures of trophic status of lakes are important, especially in the context of prioritizing water quality management across a thousand waterbodies in the state. There is no doubt that risks of cyanobacteria are dramatically higher in eutrophic waters than oligotrophic ones. So, total phosphorus concentrations in the surface layer at deep water sites of lakes over the summer months have been a focus of LLMP and VLAP. However, these characterizations may not apply to shallow shoreline waters.

Shoreline Growth on the Bottom of Lake Winnipesaukee
2020 P. Goodwin drone photo in Winter Harbor

Total phosphorus concentrations and other standard LLMP and VLAP measures did not predict (or measure) the three-week-long Gloeotrichia cyanobacteria bloom nor the significant biological growth on the bottom in Winter Harbor in 2018. Analyses of the volunteer-collected surface water samples during the bloom all indicated very good water quality, yet the bloom was severe enough for NHDES to issue an Advisory that lasted over three weeks urging people to avoid contact with the water. Clearly, the parameters that have been the focus of LLMP and VLAP deep site summer sampling programs have not been measuring what is driving the observed biological growth on the shoreline bottoms nor the increases in cyanobacteria blooms

While there has been little change in the total phosphorus concentrations at deep water sites in our deep freshwater lakes, there has been significant biological growth on and in sediment along shallow shorelines. A large portion of added nutrients to our lakes comes from stormwater runoff along their shorelines. We have measured greater nutrient-rich runoff along shorelines occurring outside the summer months during spring snow melt and autumn storms.

Phosphorus

Phosphorus is a key nutrient. However, it is present in several different forms, only some of which are readily biologically available.

- Dissolved inorganic and organic phosphorus are the most biologically available
- Particulate organic phosphorus in living organisms (tiny to large) or those formerly living (e.g., dead leaves and humic matter) is less available but can be converted to dissolved forms
- Many inorganic phosphorus compounds are insoluble and unavailable biologically. Aluminum and ferric iron phosphorus compounds are examples. However, in oxygen depleted water, unavailable ferric iron phosphorus compounds can be reduced to ferrous iron forms that are quite soluble and readily available.

We measured the concentrations of different forms of phosphorus in several sediment samples in Winter Harbor and found that the concentrations in the sediment are much higher than those in the water above. Moreover, measurements in Winter Harbor in 2020 showed that phosphorus was coming out of the sediment over the summer and adding to the concentration in the water. So, the phosphorus in shallow water sediments along shorelines are sources of phosphorus to the overlaying water and certainly a major driver of the observed biological growth on and in bottom sediment.

The 800+ page 2021 Second Edition of WHO's Cyanobacteria Guide indicated that, being very biologically available, any dissolved phosphorus in surface waters would be rapidly taken up by organisms. However, UNH analyses of our local water samples indicate that a large fraction (+/-50%) is dissolved phosphorus. We are trying to understand the significance of this unexpected finding.

Clearly limiting further additions of nutrients, particularly of phosphorus, are needed to limit further degradation of our lakes and of future cyanobacteria blooms. A USEPA webinar this fall featured ongoing research by the University of Vermont that found most current BMPs focused on limiting particulate phosphorus to Lake Champlain are not very effective on reducing dissolved phosphorus flowing into the lake. The researchers found that some BMPs even

become sources of phosphorus within a few years. The university has identified some relatively simple and low-cost alterations to the design of BMPs (e.g., incorporation of some aluminum-containing spent drinking water treatment agents) that make them much more effective in removing all forms of phosphorus for decades. We will seek to get more information on this for our Public Works Department and for local contractors and engineering firms involved in such projects.

Fluorometry

For the past several years Wolfeboro Waters has had access to a Fluoroquik fluorometer that can determine rapidly the concentration of Chlorophyll-a (Chl-A), associated with green algae, and of Phycocyanin (PC) associated with some cyanobacteria, in water samples. In early 2022, Wolfeboro Waters purchased a second Fluoroquik fluorometer that can determine rapidly the concentration of PC and of Phycoerythrin (associate with other cyanobacteria) in water samples. These have been helpful in quickly informing us when samples contain significant concentrations of cyanobacteria and helping us distinguish samples primarily of green algae from ones containing significant cyanobacteria.

Picocyanobacteria

Over the past two-year Wolfeboro Waters fluorometric results on several water samples from Winter Harbor indicated the presence of cyanobacteria. However, when we put the samples under the microscope, we could see no common blooming types of cyanobacteria-only lots of tiny spots. After several such occurrences, we discussed the problem with Amanda McQuaid (at the time the NHDES Cyanobacteria Program Coordinator) who mentioned that picocyanobacteria exist that are less than a hundredth the size of the bloom forming cyanobacteria.

The scientists at Bigelow Laboratory confirmed the existence of picocyanobacteria. They had processed several of our water samples (including ones from Winter Harbor, Lake Wentworth, and Sargents Pond) and sent them to a lab in Nova Scotia for e-DNA analysis. Because of the Covid-19 pandemic, it took a long time for those samples to be completed. However, when they came back all of them were dominated (80-95%) by Cyanobium, a type of picocyanobacteria. Our Cyanobium is associated with phycocyanin. Most of the scientific reports about Cyanobium are for a marine form that is associated with phycoerythrin.

Cyanobium and picocyanobacteria generally have been less well studied that the larger bloom forming cyanobacteria. However, as our finding is clearly not rare, more scientific attention is now being given to these tiny cyanobacteria.

As picocyanobacteria, because of their small size, are at the bottom of the biological food chain, there are reports that they emit chemicals into the water that deter, at least to some extent, their predators. Some reports suggest that Cyanobium concentrations are nitrogen-limited, in contrast to most other freshwater cyanobacteria, which are phosphorus limited. Other reports suggest that picocyanobacteria can release cyanotoxins like those from larger cyanobacteria. Some reports suggest that they may have wide swings in their concentrations each year. Others suggest that there are important concentration differences (and maybe even picocyanobacteria types) depending upon the depth of the water.

We will be seeking more information about picocyanobacteria generally and Cyanobium more specifically. We know little about the risks or possible benefits of these tiny cyanobacteria that dominate our waters.

Benthic cyanobacteria

Most of the attention on cyanobacteria has been on cyanobacteria in the water column that can form visible blooms and that may release harmful cyanotoxins. The picocyanobacteria discussed above are another form of cyanobacteria that live in the water column.

However, there is increased concern about "benthic" cyanobacteria, ones that live on or in the sediment on the bottom of lakes and rivers. Some of the surface-bloom-forming cyanobacteria live part of their life cycle on or in bottom sediments, particularly over the winter. However, numerous other types of cyanobacteria, often living in mixtures, can also produce harmful cyanotoxins, live and grow into mats on the bottom. These benthic cyanobacteria are common in rivers and lakes that have clear water allowing sunlight to reach the bottom.

Benthic mat washing ashore in Winter Harbor likely containing cyanobacteria

USEPA and the Interstate Technical and Regulatory Council recently held a webinar and released a guidance document on benthic cyanobacteria. They express concerns over such bacteria, as they can grow into thick mats of cyanobacteria mixtures and can often dislodge themselves from the sediment and float ashore, releasing a wide range of cyanotoxins in concentrations harmful to humans and animals such as dogs.

To date there are no federal or international standards for benthic cyanotoxins, but there are serious concerns about them. Undoubtedly, some of the biological growth that we observe on the bottoms of our lakes contain benthic cyanobacteria and may pose a threat to humans and their pets.

Polymerase Chain Reaction (PCR)

"Polymerase chain reaction (PCR) is a fast and inexpensive technique used to "amplify" - copy - small segments of DNA. Because significant amounts of a sample of DNA are necessary for molecular and genetic analyses, studies of isolated pieces of DNA are nearly impossible without

PCR amplification." *NIH National Human Genome Research Institute* (This is the analytical technique that has been used as the most reliable means of test people for the presence of the Covid-19 virus.)

Bigelow Lab is known for its microbiological genetic expertise. Some of their senior scientists have been collaborating with Wolfeboro Waters and have been conducting e-DNA and metagenomic analyses on several of our samples. They have also introduced us to PCR to track real-time (within hours) concentrations of selected cyanobacteria of concern to anticipate and monitor blooms.

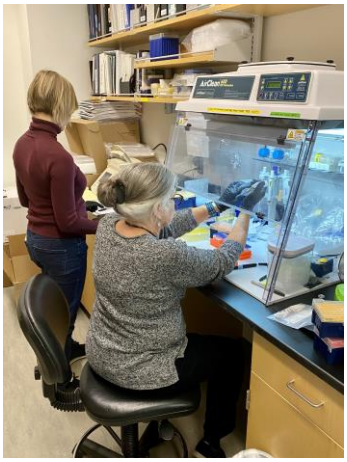
Wolfeboro Waters has purchased a portable three-channel PCR thermocycler and several members of the Assessment Subcommittee are being trained by the Bigelow Lab senior scientists. Bigelow scientists have also helped supply Wolfeboro Waters with the highly specialized primers and probes and the master mixes needed for the analyses. We have collected, processed, and frozen numerous samples over the past two years and continue to add to our inventory. So, there is a significant repository of samples that can be analyzed to help answer several of the questions that we have above. (For example, we should be able to learn more about the Cyanobium picocyanobacteria that we have discovered to be present in large concentrations in our local lakes.)

2023 UNH Summer Intern

To enable us to obtain more PCR results faster from our large and continually growing inventory of PCR samples and to conduct derivative analyses (e.g., fluorometric measurements) we are hoping to share a scientific summer intern for six weeks with the UNH LLMP program in Summer 2023. Through such a collaboration with UNH, we expect to gain more of their expertise in guiding our efforts and in interpreting the results.

Bigelow Lab Scientists

Wolfeboro Waters' knowledge of cyanobacteria and water quality in our lakes has advanced enormously over its short existence in large part through the efforts, expertise, and analytical capabilities of the Bigelow Laboratory for Ocean Sciences in East Boothbay, Maine and its senior scientists. We cannot overstate their contributions to our efforts and to our town.



Two of the Wolfeboro Waters Volunteers Being Trained by Bigelow Lab Scientists to Run PCR Analyses

In addition to the many things mentioned above that Bigelow and its staff have been doing for us, they have conducted analyses on samples across several of our lakes that demonstrate how much they have in common. So most findings from one lake are likely to apply to the others.

We have discussed many different things about cyanobacteria above. However, it is not the cyanobacteria themselves that are of concern but the cyanotoxins that they may or may not release to the water. Bigelow Lab can analyze the most important cyanotoxins for us in the event of a serious local bloom. More importantly, they are evaluating quick, relatively inexpensive cyanotoxin analytical capabilities that we might be able to use to rapidly assess cyanotoxin risks in Wolfeboro.

In addition, Bigelow is processing samples of cyanobacteria from our lakes and others in nearby Maine to determine whether the local forms of particular cyanobacteria have the genes which would enable them to produce various cyanotoxins. Such knowledge can inform public health advisories and/or guide which cyanotoxins to test for.

Communications

We will continue to work with the Wolfeboro Waters Communications Subcommittee on ways to share what we are learning with those most affected (e.g., to inform those living on, fishing in, drinking from, and swimming in our lakes and those living across the watershed how they can limit their risks and how they can help protect their water quality.)

Mitigation and Prevention

We are prepared to support the upcoming Wolfeboro Bay Watershed Management Plan, sharing the knowledge that we have gained and the relevant data we have collected. The watershed management plan seeks to provide a roadmap for individuals and communities to reduce pollutants from entering Lake Winnipesaukee.

There is ongoing research on the prevention and mitigation of cyanobacteria within our waterbodies. In 2021, legislation was enacted to create the Cyanobacteria Plan Advisory Committee. HB1066 mandates NHDES to prepare a plan to prevent the increase of, and eventually control, cyanobacterial blooms in NH's lakes and other waters by Nov. 1, 2023. The plan will be supported by scientific data and include measurable milestones. The committee consists of 17 individuals with expert representatives from different disciplines such as: health and human services, veterinarians, lake associations, and various other fields.

We will continue to work with the Wolfeboro Waters Mitigation and Prevention Subcommittee to help prioritize needs and to help future public works and private projects to cost-effectively limit and reduce nutrient runoff to our lakes.

Respectfully Submitted,

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