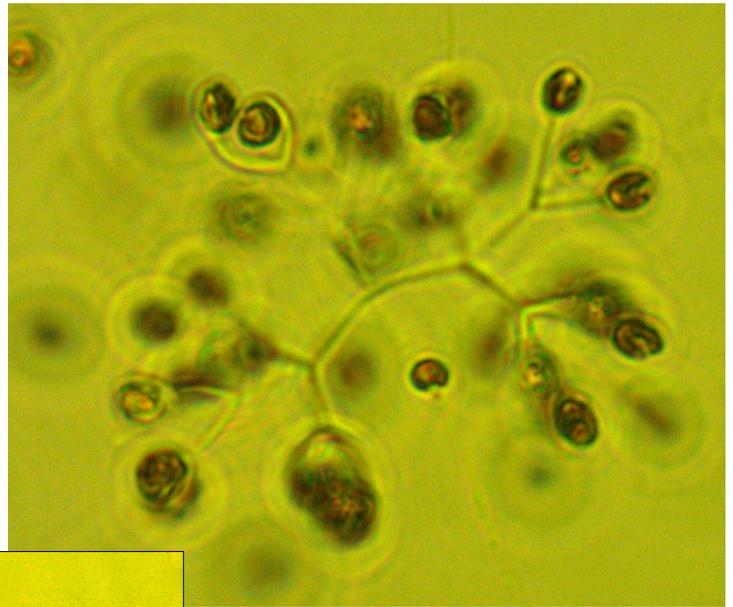




2016 Baseline Algae Monitoring Report

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Summary

Recognizing the importance of understanding algae population dynamics in lakes, LEA began monitoring epilimnetic algae in 2015. The 2016 season began in June, with samples collected on 15 basins within the lakes region. Depending on the basin, 1-5 samples were collected throughout the course of the summer. Basins where multiple samples were collected were sampled roughly every 2 to 4 weeks.

Algae samples were collected from surface waters using Tygon® flexible tubing. Samples were preserved and settled using the Utermöhl technique, then counted under magnification. The genus of each algae and the number of cells present were recorded. Multiple samples were taken from a number of lakes, allowing for comparison of algae populations over time. Studying these seasonal changes in the makeup of algae populations is important to understanding lake ecology.

This study focused on planktonic algae, which are those that are free-floating in the water, rather than attached to rocks or other material.

Summaries of individual lakes' results are available in LEA's 2016 Water Testing Report or in the online lake information pages, both available on our website, www.minelakes.org.



Collecting a core sample from the upper portion of a lake using a Tygon® core tube. The water collected will be settled and counted for algae.

Introduction and Background

Studies of algae populations are one of ways lake managers can determine water quality. Algae are the foundation of lake food webs because they directly and indirectly feed much of the animal life existing in and on a lake. Of course, algae are also the source of algal blooms, which result from an over-abundance of nutrients or a lack of algae consumers. Either way, algal blooms are often a sign of a water quality problem, a situation that is bad for people and for the lakes themselves.

The goal of LEA's algae testing program is to identify the various kinds of algae present in the Lakes Region of western Maine, quantify them, and study how they change over time. Algae populations change in a predictable way over the course of a year, and understanding which algae are present at certain times can tell us about lake conditions. For example, diatoms are common in the early spring because they prefer colder water and well mixed lake conditions. Large diatoms tend to settle out in the summer when waters are calmer.

Certain types of algae are only present when specific water quality conditions exist, which makes them good environmental indicators. Additionally, the dominant algae in a sample can be very informative, since different algae will dominate under varying water quality conditions. LEA identifies algae to genus level, which is one step above species level. Species level identifications can be much more informative about water quality conditions in some instances, but are also much more difficult and time consuming.

One very important indicator is the amount of cyanobacteria in a lake. Cyanobacteria are not technically algae, but rather photosynthetic bacteria. Nevertheless, they are treated as a type of algae because they have many of the same characteristics as algae. High levels of cyanobacteria are often correlated with high phosphorus levels, and cyanobacteria such as *Aphanizomenon*, *Dolichospermum* (formerly *Anabaena*), and *Microcystis* are the most common cause of algal blooms. All surface waters contain cyanobacteria, and they are some of the oldest organisms on the planet. While many types of cyanobacteria are harmless, several kinds are able to produce toxins and large amounts of these toxic cyanobacteria indicate poor water quality. Cyanobacteria tend to be most common in the later part of the summer, when temperatures are warmest.

LEA currently monitors only phytoplankton—algae that are free-floating in the water column. Benthic algae—those that are attached to rocks and substrate or that accumulate in shallow, shoreline areas are not included in this study. Samples are collected from the top 3-10 meters of each lake at the deepest part of the basin. Multiple samples from several basins are collected in order to record seasonal fluctuations in algae populations. This also enables us to record which algae are present under different water quality conditions for a more complete account of each lake's algae population. The record of algae present in each lake can also be used as a baseline to measure change in future research.

Though LEA's algae monitoring program began in 2015, several changes were made in the procedure at the beginning of the testing season in 2016 and again in the middle of the season, which means that sample results obtained by different procedures can not be directly compared. Samples from 2016 were processed using the Utermöhl technique, which involves preserving and settling samples in a special chamber. The samples from 2015 were live samples that were concentrated using a filtering method. In 2016, samples were analyzed using an inverted microscope, however prior to August 1st samples were counted at 200x magnification and after that date they were counted at 600x magnification.

Introduction and Background (continued)

There are over 750 freshwater algae genera present in North America, representing thousands of species. Eighty-four different genera of algae were identified in the lakes region in 2016. These 84 genera were grouped into 7 algae types: Green algae, Cyanobacteria, Golden Algae (a category which includes Chrysophytes, Haptophytes and Synurophytes), Diatoms, Cryptomonads, Dinoflagellates, and Euglenoid algae. Other types of algae that exist in freshwater include red algae, eustigmatophytes, raphidophytes, tribophytes and brown algae, most of which are relatively rare in freshwater and contain only a few genera. None of these types of algae were recorded in algae samples from 2016.

Green algae are the most diverse group of algae present in freshwater habitats, with 302 genera found in North America. Of these, 36 were identified in the lakes region in 2016. Green algae come in a variety of sizes and shapes, and can be round or filamentous, single-celled or colonial, and flagellated or unflagellated. Charophytes, the closest algal relatives to terrestrial plants, are included among the green algae. Green algae can be identified by their deep green-grass color and rigid cell walls.

Cyanobacteria, while commonly grouped in with algae, are actually prokaryotic bacteria that can photosynthesize. Cyanobacteria are also known as blue-green algae. Most forms of cyanobacteria are colonial, and can be round or filamentous. There are 124 freshwater genera found in North America, and of these 17 were counted in the lakes region. While Cyanobacteria are present in all waters and many of them are harmless, there are several species that can produce toxins and will form blooms when nutrient levels are high. In fact, the higher the phosphorus concentration in lakes, the more cyanobacteria dominate that lake's algae population. Cyanobacteria are often brown, olive green, or blue-green in color, and their cells do not contain organelles.

Golden algae are common in lakes with low to moderate nutrient levels, low conductivity and alkalinity, moderate color and slightly acidic pH, and are common in the lakes region. For the purposes of this report, Chrysophytes, Haptophytes, and Synurophytes have been lumped together into this category. In North American freshwaters, there are 72 genera of Chrysophytes and 3 genera of both Haptophytes and Synurophytes. In the lakes region, a total of 10 genera of Golden algae were identified. Golden algae can be identified by their brown to yellow color and the delicate nature of their cells. The most striking genera have silica spines or plates, and many have small flagella. They are often colonial and a few of the common genera are relatively large in size.

Diatoms are easily identified by their hard silica-based outer shells. Diatoms are either centric (round) or pennate (long, thin rectangles or canoe-like shapes). Because their shells make them heavy, diatoms often settle out of the water column during the calm summer months, and are most common during periods of mixing the spring and fall, when silica levels rise and waters are colder. There are 118 freshwater Diatoms found in North America, with 16 being identified in the lakes region in 2016. Most diatoms are single-celled, but a few of the common genera are colonial.

Cryptomonads are one-celled algae with two flagella which allow them to move through water. There are 12 freshwater genera in North America, of which two are common in the Lakes Region.

Dinoflagellates are a group made up of large, motile algae. There are 37 freshwater genera in North America, with at least three seen in the Lakes Region. Large numbers of Dinoflagellates indicate high nitrate and phosphate levels. Most Dinoflagellates are covered in armor-like plates that serve as a protective shell.

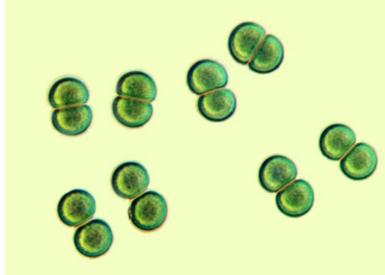
Euglenoids are very common in bogs and ponds with high nutrient and organic matter levels. There are 10 genera found in North America and two genera have been counted in the lakes region.

Introduction and Background (continued)

Pictures of common algae from each group (not to scale)



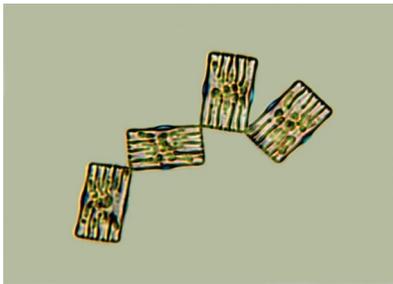
Green Algae: Sphaerocystis



Cyanobacteria: Chroococcus



Golden Algae: Dinobryon



Diatom: Tabellaria



Cryptomonad: Cryptomonas



Dinoflagellate: Ceratium



Euglenoid: Euglena

A Note on Genera Richness

As mentioned, 84 of about 750 different freshwater algae genera were identified in the Lakes Region, which is only about 11% of all genera. Why isn't this number higher? First of all, there are absolutely more than 84 algae genera present in the Lakes Region. The algae collected from the twelve lakes and ponds in this report were all sampled from the open water—meaning that only phytoplankton were included in the samples. Hundreds of algae genera that are primarily benthic would not be included in these samples, as these algae live around the shores of lakes and are usually attached to something, like sediment, rocks, or plants. There are also some algae that are exclusive to running water in streams and rivers.

Another reason that seemingly few algae genera were present is water quality. The 12 lakes that were sampled all have similar water quality, meaning that the same algae dominated many of the samples. Algae that prefer alkaline conditions or high nutrient levels, for example, are not as likely to be found in the Lakes Region. In many lakes, about 6-8 species make up a whopping 90% of the algal biomass at any one time, though the dominant species do change over the course of a year. The other 10% of the biomass may contain dozens of other species, but some of these will be relatively rare and may not make it into an algae sample.

Methods

Algae samples were collected from twelve lakes one or more times between June 21st and September 29, 2016 (Table 1). Samples were taken anywhere from 2-5 weeks apart on lakes with multiple samples. The samples were taken from epilimnetic core samples, meaning they contained a composite of water from the top layer (ranging from 3-10 meters deep) of the water column. The depth of the core was determined by temperature and dissolved oxygen profiles. A total of 49 samples were collected.

Samples were preserved using Lugol's Iodine either at the time of collection, or put on ice and preserved upon return to LEA. Samples were either set immediately or stored at room temperature. Samples were settled using an Utermohl chamber, which consists of a 100 mL tube set over a modified microscope slide. The sample is poured into the tube and algae settles and collects in a well in the slide, then the tube is removed and the slide is sealed. Slides were examined with an inverted microscope at 200x—600x total magnification. Random fields were counted until a total of 400 natural algae units was reached. Algae were identified to genus level (one step more general than species, plural genera) where possible. The number of cells per milliliter (cells/mL) was calculated for each sample.

Table 1. Lakes sampled for algae and the number of samples collected from each.

Lake Name	Number of Samples
Hancock Pond	4
Highland Lake	1
Keoka Lake	5
Long Lake	1 from each basin for a total of 3
McWain Pond	4
Moose Pond	5 from the Main Basin and 5 from the South Basin
Peabody Pond	2
Sand Pond	4
Trickey Pond	4
Woods Pond	5
Back Pond	2
Middle Pond	2

Results

Overall, the algae present in each lake were similar. The most common algae in virtually all lakes were either small flagellated algae or *Aphanocapsa*, a small colonial cyanobacterium. Percentages of the seven types of algae varied from lake to lake, but on average cyanobacteria were the most common (primarily due to the *Aphanocapsa*), followed by green algae, golden algae, cryptomonads, diatoms, and dinoflagellates (Table 2). Euglenoids made up 0% of the average sample in each lake, so were left off of table 2. Total cell counts ranged from 2,765 cells/mL in Sand Pond to 33,342 cells/mL in Long Lake's North Basin. The average cell count was 12,271 cells/mL. Because of changes in methodology, only samples collected after August 1st are included in the reporting of type percentages and cell counts.

On average, each lake contained 43 unique planktonic algae genera. Lakes that were sampled four or five times had higher average genera counts than those sampled once or twice. As algae populations change over the course of the summer, different genera will become common and then dissipate. Collecting several samples over time increases the chances of seeing these short-lived genera, as well as the chance of seeing rare genera.

Both the number of cells and the number of natural units of each genus was counted (Table 3). Natural units are simply groupings of cells. For algae that form colonies, one colony is one natural unit, but may be made up of, for instance, 40 cells. Many cyanobacterial genera are large colonies that are made up of many tiny cells. Because of this, cyanobacteria dominate cell counts in many of the samples. Table 3 shows the most common genera of algae based on natural units and cell counts. The four most common algae based on cell counts are colonial cyanobacteria, while only one of the top four algae (and only two of the top ten) are cyanobacteria when looking at natural units. All of the most common algae on a cell count basis are colonial, except for the flagellates. In contrast, seven of the 10 most common algae by natural units are single-celled.

In Table 3, the most common algae by natural units are flagellates. Flagellates are not their own genus, but rather a grouping of different genera that share a similar trait. Because flagellates are often tiny and hard to identify to genus level, they are lumped together in this group. Some flagellates are easier to identify: *Monomastix*, *Cryptomonas*, *Rhodomonas*, and *Chrysochromulina* are all flagellate genera that are listed separately in Table 3. However, in general, these small flagellates are similar in function and ecological role within lakes, so it is acceptable to group them. Because they represent several types of algae, the “flagellate” grouping is not included in the calculation of most common algae types. The only other grouping of genera is *Stephanodiscaceae*, which represents several genera of centric diatoms that are all ecologically similar.

Only four cyanobacterial genera of concern were noted in 2016. These are the algae that are most likely to produce toxins and include *Anabaenopsis*, *Aphanizomenon*, *Dolichospermum* (formerly *Anabaena*) and *Planktothrix*. Of the twelve lakes sampled, two—Middle Pond and Back Pond—did not contain any of these genera. The remaining lakes had *Dolichospermum* in at least one sample. The highest amount recorded was about 400 cells/mL. For comparison, the EPA considers levels of cyanobacteria under 20,000 cells/mL to be low risk. Only one pond, Peabody Pond, had all four genera. *Dolichospermum* was seen in all of the Peabody Pond samples, but the other three genera were only seen in one sample and in very small amounts. It should be noted that *Aphanizomenon* was also found in the sample from Long Lake's North Basin but was not captured in the subsample used for counting. Additionally, the potentially toxic cyanobacteria *Gloeotrichia* is known to be present in Long Lake, Moose Pond, Keoka Lake, McWain Pond, and Peabody Pond.

Results (continued)

Table 2. Average percentage of different algae types on each lake in samples taken after August 1st. Percentages are based on natural units. *Long Lake percentages are an average of samples taken in each of three basins.

	Green Algae	Cyanobacteria	Diatoms	Golden Algae	Dinoflagellates	Cryptomonads
AVERAGE	18%	39%	11%	15.5%	1.5%	15%
Hancock Pond	9%	30.5%	19%	24.5%	1.5%	15.5%
Keoka Lake	18.75%	43.5%	4.75%	18.5%	0.25%	14.25%
Long Lake*	10.5%	51%	5.75%	6.75%	0%	26%
McWain Pond	16.25%	52.5%	15%	8.5%	0.25%	7.5%
Moose Pond (North)	34.75%	21%	11.75%	21.75%	0.75%	10%
Moose Pond (Main)	26%	34.75%	5.5%	13.5%	0%	20.25%
Peabody Pond	9.75%	32%	10%	18.5%	0%	29.75%
Sand Pond	10.5%	23.25%	22%	24.5%	8%	11.75%
Trickey Pond	19.5%	47%	3%	9.75%	2.25%	18.5%
Woods Pond	15%	44.5%	11%	13.5%	2.5%	13.5%
Back Pond	22.25%	40%	12.75%	13.75%	2.75%	8.5%
Middle Pond	28.25%	43.5%	3.5%	11.75%	0.25%	12.75%

Table 3. Most common phytoplankton seen in algae samples, based on natural units and cell count data. Genera are listed except in the case of flagellates and Stephanodiscaceae. These rankings are based on data from the entire 2016 season.

Top 10 phytoplankton genera in the Lakes Region				
	Natural Unit based		Cell Count based	
#	Genera	Type	Genera	Type
1	Flagellates	(Multiple)	Aphanocapsa	Cyanobacteria
2	Aphanocapsa	Cyanobacteria	Aphanothece	Cyanobacteria
3	Rhodomonas	Cryptomonad	Merismopedia	Cyanobacteria
4	Chrysochromulina	Golden Algae	Chroococcus	Cyanobacteria
5	Cryptomonas	Cryptomonad	Dinobryon	Golden Algae
6	Asterionella	Diatom	Flagellates	(Multiple)
7	Merismopedia	Cyanobacteria	Coelosphaerium	Cyanobacteria
8	Monomastix	Green Algae	Sphaerocystis	Green Algae
9	Mallomonas	Golden Algae	Asterionella	Diatom
10	Stephanodiscaceae	Diatom	Tabellaria	Diatom

Discussion

The results of algae sampling in 2016 give LEA a baseline of algae conditions in 12 lakes over the months of June, July, August, and September. This baseline sampling is important because it provides a record of conditions to which future data can be compared. This data will help in assessing changes over time and determining what a typical algae population looks like in each lake. Because these lakes currently have good water quality, knowing which algae are present, and in what concentrations, is especially important. Any water quality changes in the future will be easier to assess if current water quality conditions are understood.

While cyanobacteria were the most commonly identified type of algae in most of the samples collected, the instance of toxic or nuisance cyanobacteria was very low. Cyanobacterial genera such as *Aphanocapsa* and *Aphanothece* are often common in low-nutrient lakes. These genera are colonial and are made up of very small cells, so cell counts are often high, but the overall volume the algae takes up is low.

Algae concentrations, measured in cells/mL, varied by over one order of magnitude (about 12x) between lakes. Compared to 2015 data, cell concentrations are much higher because of changes in methodology that have resulted in more accurate cell counts. However, cell counts are still low compared to high-nutrient systems. Because cell count numbers can vary widely depending on counting methodology, there are no established criteria linking cell counts with lake trophic status. However, counts can be compared between lakes and within lakes month-to-month and year-to-year.

Biomass, a measure of the amount of space and weight taken up by algae cells, was not measured on any lakes. This is because measuring biomass is complicated and time intensive, requiring measurements of the volume of each algae genus present in a sample. Because cell and colony size vary so widely, algae that have a high cell count may not have a high biomass if the cells are very small. Conversely, large cells may make up the majority of algal biomass even if they are not the most commonly counted algae in a sample. Incidentally, lab-based measures of chlorophyll-a concentrations are not substitutes for biomass either, although they are sometimes presented this way. Lab-based chlorophyll-a measurements are not directly comparable to cells/mL concentrations because of differences in the amount of chlorophyll-a in algae cells of varying types and conditions.

Algae samples were counted as both natural units and individual cells. Natural units give a clearer picture of which algae are present, regardless of whether they are colonies or individual cells. For instance, *Rhodomonas* was common in several lakes sampled, but is not on the top 10 algae based on cell count (Table 3) because it is a single-celled algae. Cell counts allow for a better understanding of the relative influence of colonial algae and for better comparison between lakes and years. Colonies often vary greatly in the number of cells they contain – one natural unit of dinobryon could be two cells or one hundred, and knowing the difference is important.

Direct comparisons of data collected in 2015 and the beginning of 2016 is impossible due to changes in methodology adopted in August of 2016. Samples are now counted at 600x total magnification, as opposed to 200x. Smaller algae are more likely to be counted at 600x because they are more visible and identifiable. The 200x magnification is biased toward larger cell sizes, while the 600x magnification is biased toward small cells. Cryptomonads are a good example of the difference between magnifications. In the 2015 report it was noted that there were likely cryptomonads in the algae samples but none were identified due to the low magnification, which made them hard to see. In 2016, they were among the most common algae identified.

Discussion (continued)

Algae assemblages are often used to infer and assess water quality conditions. Good ecological indicators should quickly respond to change, be very sensitive to certain conditions, and be common and easily recognizable. Algae such as *Dolichospermum* and *Aphanizomenon*, two cyanobacterial genera which form algae blooms, are good indicators of eutrophication. In lower nutrient waters, however, it becomes more difficult to reach specific conclusions about water quality. This is because indicators often have to be dominant in the samples. Most common algae occur over a wide range of water quality conditions. *Euglena*, for instance, is an indicator of organic nutrient pollution. However, having one or two *Euglena* cells in a sample does not provide evidence of nutrient pollution in that water body.

Another issue is that some algal indicators are species-specific, meaning that only certain species within a genus are useful for making conclusions. However, since samples are measured to genus level only, this level of detail is unavailable.

General, qualitative assessments can be made based on the relative concentrations of algae and the overall assemblage, but these tend to be less specific. Golden algae are much more common in lower-nutrient, northern latitude lakes. Moose Pond's North basin, Hancock and Sand Ponds, Keoka Lake, and Peabody Pond had the most golden algae on average.

Small flagellated algae were very common in all the lakes sampled. The flagellates *Cryptomonas* and *Rhodomonas* are good quality food for zooplankton and were common in many lakes. This indicates a good basis for lake food webs that support fish and macroinvertebrates. Picoplankton, algae so small that they are generally not identifiable with a regular microscope, were also quite common in the lakes sampled. The abundance of picoplankton and small flagellates is an indication of low nutrient levels.

2017 Sampling

LEA will continue sampling algae monthly between May and September of 2017. We anticipate studying the same lakes in order to establish typical algae assemblages and patterns in algae abundance. The focus for 2017 will be to standardize sample settling times and preservation so all samples are treated equally. Using the microscope camera that was purchased in 2016, we will compile a library of algae images to use for identification and educational purposes as well as a record of which genera are common in our lakes.

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Trickey Pond Association

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