LAGERMAN RESERVOIR:

A LOOK INTO THE FUTURE OF CYANOBACTERIAL FRESHWATER ALGAL BLOOMS

by

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Lagerman Reservoir is a saline water body subject to changing hydrologic regime, periodic algal blooms, and subsequent fish kills. Inflow has decreased and the water level is constantly below the spillway, rendering this system a closed, evaporite basin. The objective of this study is to survey the ecological condition of the reservoir to obtain scientific data for future management decisions and insure the safety of its recreational visitors. Weekly shore samples and monthly depth profile samples were taken, along with data synthesis of the EPA's CyAN satellite monitoring system. Extreme conditions were observed both chemically and ecologically. Cation and anion concentrations were high, with a pH of 8 or higher. Dissolved oxygen was very low, indicating unfavorable conditions for fish and macroinvertebrates. Transparency and nutrient concentrations were also low, with nitrogen limiting the system. The algal biomass was dominated by colonies of Synechococcus, a cyanobacteria typical of marine habitats with a potential to produce toxins and fix nitrogen. In addition, Dolichospermum, Lyngby, Oscillatoria, and Merismopedia are potential toxin producers found in the reservoir. Chlorophyll-a and phaeophytin analysis indicated similar trends to the CyAN satellite data, with a peak of algal growth around mid-June/early July, followed by a die off of algal biomass. Based on Carlson's Trophic State Index, this reservoir ranged from eutrophic to hypereutrophic from June to August 2020. Lagerman Reservoir is considered to be unsuitable for fish and vulnerable to toxic algal blooms that could be hazardous to human health.

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Introduction

Throughout the world, the presence of blue-green algae (cyanobacteria) has been increasing as unprecedented climatic and environmental changes have led to favorable conditions for these algal species. Out of sight, out of mind may work the majority of the time for microscopic algae, but when conditions are favorable, these organisms thrive and become anything but miniscule. Depending on the algal species, expansive blooms are capable of shifting the ecosystem's dynamic, turning the water seemingly unnatural colors, and producing toxins with the potential to kill. Anthropogenic eutrophication, or excessive nutrient enrichment in water, has been noted as the greatest cause of water quality deterioration in aquatic ecosystems globally (Smith, 2009).

Whether they realize it or not, humans have cultivated an intimate and multi-faceted relationship with algae. Through photosynthesis, algae are estimated to be responsible for 50% of all oxygen production worldwide (Chapman, 2013). With the role of primary producers, all aquatic organisms depend on algae as the foundation for the food web, including the fish and seafood we consume. As engineers and scientists search for ways to mitigate the effects of climate change, they're turning to algae to harvest clean and renewable biofuel, sequester carbon, and purify wastewater (Molazadeh, 2019).

While algae offer humanity an array of benefits, they also possess the ability to destroy the very ecosystems they lay the groundwork of. Ecosystems that we, as humans, have commercialized and turned into an industry worth hundreds of billions of dollars. Ecosystems that are at exponentially increasing risk for loss of habitat and species diversity. Several species of cyanobacteria, also known as blue-green algae produce toxins, which can enter our systems directly while swimming, indirectly by consuming exposed shellfish, or even via airborne contact (Trevino-Garrison, 2015). Their microscopic and increasingly widespread occupancy makes them unsuspecting culprits that we can no longer ignore.

What are algal blooms?

In regulated, well-balanced ecosystems, microscopic algae known as phytoplankton are responsible for the base of energy upon which the ecological community is built. With the role of photosynthetic primary producers, they take up light, carbon dioxide, and nutrients such as phosphorus and nitrogen in order to grow, yielding biomass that will then make its way through the food web. Generally, this rate of production is limited by either nutrient availability or adequate light. It also may be kept under control through herbivorous predation or physical variables such as vertical mixing throughout the column or flushing rates of the water body (Paerl, 2001).

A combination of environmental circumstances, such as warm temperatures, increased salinity, changes in rainfall, and excess nutrients can throw off the equilibrium and lead eutrophication, where algae dominate an entire ecosystem (Smith, 2009). Most of these factors are, coincidentally or not, also commonly known impacts of climate change. Nutrient loading is a frequent consequence of the agricultural and livestock industries using fertilizers or producing nutrient-dense animal waste, which can enter aquatic systems directly through point-sources or indirectly through agricultural or urban stormwater runoff (Gatz, 2017). If the growth rate can no longer be controlled by chemical, biological, or physical parameters, biomass will accumulate, preventing light from penetrating below the layer of algal matter and causing discoloration of the water, hence the term “bloom”. Once the nutrients are used up, a massive algal die-off will occur and the decaying biomass will be consumed by bacteria along with available dissolved oxygen. This condition is known as hypoxia or anoxia, depending on the severity (Paerl, 2001). Without oxygen available, all organisms that rely on respiration to live, such as fish or macroinvertebrates, will suffer greatly or even die, resulting in dead zones unable to support life.

To compound the already devastating impacts of harmful algal blooms (HABs), some species of algae can also produce toxins and, in the event of a bloom, concentrations of their toxins become high enough to induce illness or death in a variety of species, including humans. Within four months in 2020, an estimated 350 elephants died in Botswana from water likely contaminated by toxic cyanobacteria. Various algal divisions contain toxin-producing species, predominantly dinoflagellates, diatoms, and cyanobacteria. While the first two tend to proliferate in marine ecosystems, cyanobacterial algal blooms occur in freshwater habitats.

Algal toxins and humans

Cyanotoxins in freshwater may enter human bodies by consuming fish, skin contact, and inhalation, but the most common exposure route is through ingestion, likely during recreational activities. The four key cyanotoxins include microcystins, anatoxins, cylindrospermopsin, and saxitoxins, which are produced by a handful of species within the genera *Microcystis*, *Anabaena*, *Cylindrospermopsis*, and *Planktothrix* (Association of Public Health Laboratories, 2017). These toxins are categorized into hepatotoxins, which target the liver, and neurotoxins, which target the nervous system. The severity of symptoms in animals and humans can vary based on the algal species, method of exposure, and amount to which they have been exposed (Trevino-Garrison, 2015). In hepatotoxins, effects manifest throughout the digestive system, causing vomiting, diarrhea, cough, skin rash, etc. For neurotoxins, symptoms tend to present themselves in the form of muscular paralysis, amnesia, seizures, and more (Brunson, 2018). While death in humans is rare, it is more common in wildlife and dogs.

Alongside health concerns, HABs can harm people financially as well. For the individual exposed, healthcare, loss of income due to illness, and other intangible costs can add up to a hefty expense. A study by Deflorio-Barker et al. from 2018 estimated the costs associated with recreational waterborne illness to range anywhere from \$11 to \$350,370 (in 2016\$) depending on severity. While this study did not look into the percentage of waterborne illnesses caused by HABs, in 2014, Hilborn et al. found 46% of untreated recreational water disease outbreaks to be associated with HABs from 2009-2010. In addition, that single year accounted for 79% of all HAB-related outbreaks reported to the CDC since 1978, suggesting that this concern is rapidly growing. For the US economy, eutrophication of freshwater systems are conservatively estimated to cost 2.2 to 4.6 billion dollars annually; mostly through the loss of lakefront property value and recreational water use (Dodds, 2008). Commercial fisheries also suffer, as both their supply and demand are subject to diminution. Lake Erie, which has repeatedly been subject to blooms, relies on tourism, boating, fishing, and other industries for over \$50 billion annually (Watson et al. 2016). As humanity's actions lead to more frequent and extreme HABs, we will pay the price.

Climate change and cyanobacterial adaptations

Uncoincidentally, the frequency and intensity of algal blooms appear to be increasing alongside the onset of climate change. Given that cyanobacteria have fossil records dating back to at least 2.7 billion years ago, they have had time to adapt to many environmental conditions and survived all mass extinctions (Paul, 2008). While their photosynthetic capabilities evolved, cyanobacteria adapted from a low oxygen environment to an oxygen-rich one. They also were subject to higher temperatures, ultraviolet (UV) exposure, and high levels of iron, sulfide, and methane (Paul, 2008). As cyanobacteria developed, they created an environment with higher oxygen concentrations and, in a way, the world evolved around them.

In today's world, greenhouse gases continue to fill the atmosphere with carbon dioxide and methane, causing Earth's air temperatures to rise. Through conduction, water surfaces also get warmer. Water temperature and dissolved oxygen are inversely related, meaning that the warmer the water gets, the less available oxygen will be to respiration-dependent organisms. Cyanobacteria, on the other hand, are photosynthetic, and raising temperatures allow for faster growth. Increased evaporation accompanies warmer temperatures, leading to lower water levels and increased concentrations of various ions, especially salts. Rising salinity can threaten the world's freshwater supply greatly, but some cyanobacterial species thrive in saline environments such as *Anabaena*, *Microcystis*, and *Nodularia* (Paerl, et al. 2009). Strong winds can benefit toxic blue green species that can become airborne as well as lead to greater mixing of stratified water columns, causing high toxin concentrations to travel deeper and increase the cost of water treatment (Gonzalez-Piana et al., 2018). Droughts also lead to success in blue-greens. With lower water levels, eutrophication is aggravated through higher nutrient concentrations and longer residence times in lakes (Brasil et al., 2015).

In 1994, P. Copper coined the term "disaster taxa" as a way of describing "'simpler', stress-resistant taxa" that may thrive in marginal conditions that more complex species can not. Cyanobacteria may be the model organism for this concept. If their environment has no nitrogen available, species with heterocysts will create their own. Blue-green alga habitats range from freezing arctic temperatures to hot springs or acidic to basic. With an ancient lineage and billions of evolutionary years, cyanobacteria have infiltrated just about every possible niche, causing climate change to seem more like an opportunity for growth rather than a threat.

Anthropological impacts

Beyond the changing variables attributed to anthropogenic climate change, there are a few direct actions that appear to be impacting the intensity and frequency of HABs in freshwater. Increasing population has led to a subsequent demand for food production and urbanization, leading to greater fertilizer usage and wastewater volumes (Drizo, 2019). Nutrient loading is a major catalyst for algal blooms, as algal growth rates are typically limited by quantities of nitrogen and phosphorus. Nutrients may be released from sediments, flow in as agricultural and urban stormwater runoff, or accumulate due to changes in hydrological regimes such as damming (USGS, 2020).

The notion that excessive phosphorus is detrimental to aquatic ecosystems is not a recent one. Decades ago, Schindler and his team experimented with lakes in Ontario to determine which nutrients were the greatest cause of eutrophication. The basin fertilized with phosphorus experienced a bloom within two months, and was recovered almost immediately once the phosphorus addition was halted (Schindler, 1974). This concept has been thoroughly researched over the years, and it appears that Schindler was correct and nitrogen reduction is not as effective for preventing or stopping blooms. However, nitrogen concentrations and chemical forms could be key in predicting potential toxicity of these algal blooms. In 1998, Hyenstrand, et al. determined that a low nitrogen to phosphorus (N/P) ratio led to cyanobacterial dominance and increased risk of HABs. A high N/P ratio allowed for green algae (Bulgakov, 1999) or diatoms (McCarthy, 2009) to dominate the ecosystem, which are unlikely to be toxic in freshwater ecosystems.

Existing policy and legislation regarding freshwater HABs

Legislatures do not tend to make HABs a priority for policy as it is not as widely discussed or publically protested like climate change. Congress has been aware of the looming threat of algal blooms since before 1998, when The Harmful Algal Bloom and Hypoxia Research and Control Act of 1998 (HABHRCA) was passed to formulate a task force for research, education, monitoring, etc. (Gatz, 2017). Since then, various forms of legislation have been passed with the goal of addressing HABs and their potential danger to humans. HABHRCA led to the establishment of a National Research Plan for Coastal Harmful Algal Blooms, and by 2004 began to include freshwater HABs as well (Gatz, 2019).

The Safe Drinking Water Act (SDWA) requires the EPA to publish a list of unregulated contaminants in public water systems. These contaminants may occur frequently and/or cause public health concerns, increasing health risk. This list is known as the Contaminant Candidate List (CCL) and also includes maximum contaminant levels (MCLs). Cyanotoxins are on the CCL, but as of 2015 only 3 of over 80 variants of known cyanotoxins are included (CLRMA, 2015). The EPA uses the Unregulated Contaminant Monitoring Rule (UCMR) for contaminants without health-based standards that may be present in drinking water. Unfortunately, since there is not a standardized method of analysis for cyanobacterial toxins, they are not included in the UCMR (CLRMA, 2015). The World Health Organization (WHO) set provisional guidelines for microcystin-LR in 1998 at 1.0 microgram per liter. Additionally, they have set guidelines for cyanobacterial cells, microcystin-LR, and chlorophyll-a levels corresponding to low, moderate, high, and very high risk of health effects. Other states have adapted this method to create their own standards, but this has not been enforced by the federal government. Colorado does not appear to be one of the states voluntarily setting drinking water standards for cyanotoxins.

While the SDWA has been amended to include the assessment and management of algal toxins, less progress has occurred for water sources not used for drinking water, which are regulated in the U.S. under the Clean Water Act (CWA). For recreational water bodies, the WHO, EPA, and 24 states have numeric values for acceptable levels. An additional 11 states have cyanobacterial HAB guidelines based solely on visual inspection. In 2016, the Colorado Department of Public Health and Environment set their lowest recreational water guidelines to 10-20 μg/L for microcystins and 7 μg/L for cylindrospermopsin (US EPA, 2019). Response actions to potential blooms include swimming advisories, warning signs, and closures. Some states have developed total maximum daily load (TMDL) values for impaired water bodies with nutrient parameters (Gatz, 2019), but this is uncommon.

While these are indications of progress, many scientists believe further action is necessary to see tangible results. This would likely be in the form of further research in conjunction with additional regulation on pollution. Current policies may not be stringent enough for significant impact. Polluter Pays Principle, key to multiple environmentally conscious acts from the EPA (Khan, 2015), would be a necessary foundation for any future legislation in this sector of the algal bloom process as it highlights the need for accepted responsibility in these ecological disasters, even from nonpoint sources.

The EPA has emphasized the need to focus on reducing nutrients from point and nonpoint sources; however, the Clean Water Act (CWA) authorizes regulation of point sources only. Point sources, such as wastewater treatment plants, are required to have Federal National Pollution Discharge Elimination System (NPDES) permits. There is not a comparable requirement for nonpoint sources, including lawns, fields, pastures, etc. which may use fertilizers responsible for nutrient loading from runoff (Paerl, 2008). Alternatively, the EPA can influence the management of nonpoint sources through grants and funding (CWA Section 319) for voluntary programs such as the Clean Water State Revolving Fund Program, Section 604(b) planning grants, Wetland Program Development grants, and grants targeted at specific locations such as the Chesapeake

Bay.The NPDES permits specify the amount of pollutant that can legally be discharged. Unfortunately, these permits can be too lenient if a case is made for insufficient limitations due to what is the best "conventional" or "economically achievable" technology, often discounting potentially feasible alternative technologies. In 2017, under the Trump Administration, HABHRCA was amended to require the use of "cost effective methods" when carrying out the law (33 U.S.C. 4002(f)(7)). In order to combat this, technology forcing policies ought to be put into place. This means that regulations may be seemingly infeasible at the current time, but must be implemented in the near future (Gerard, 2004). These types of policies are what will push industries to accomplish the necessary requirements for keeping our environments safe, even if they are challenging and expensive upfront. Evolving businesses, lifestyles, and government takes years, if not decades, to achieve significant results.

Scientific Solutions for Freshwater HABs

As government-run coalitions and task forces are assembled, gaps in research must be acknowledged and filled. It is estimated that at least 46 species of cyanobacteria have been confirmed to be toxic; however, this number is likely an underestimation due to lack of research (WHO, 2003). Topics of these studies could include toxin inhalation exposures, effective nutrient input reduction strategies, benthic toxic cyanobacteria, and freshwater HAB control through methods that do not require repeated application or have the potential to harm aquatic biota beyond the toxic blue green species (Hundell, 2009). Currently, the use of copper sulfate as an algaecide is widespread, despite the fact that its effects are short term and the build up of copper in sediments is toxic to organisms (Dia, 2018). These bloom control methods are short term and retrospective, whereas the need for long term, preventative solutions are necessary.

Laboratory experiments, field simulations, and modeling all have pros and cons, so it is likely that a combination of them all could be most effective. Right now, we have minimal research to build upon, and will need rigorous long-term datasets in order to formulate compelling documentation of climate change and its impact on freshwater HAB frequency and intensity (Wells, 2020). One effective way of accumulating extensive data is through site monitoring via satellite imagery.

Currently, the EPA has chosen to monitor freshwater bodies throughout the U.S. using an application called CyAN. This app uses satellite imagery from the European Space Agency's Copernicus Sentinel-3 Ocean and Land Colour Instrument to estimate concentrations of phycocyanin (the pigment of blue-green algae) and detect potential algal blooms (Boykin, 2019). This data can be synthesized to look at past annual trends of algal populations and predict those of the future. Modeling is a great tool for complex dynamics, as they apply multiple variables and potential outcomes. Unfortunately, there is still a great level of uncertainty in some modeling systems if they do not accurately represent initial conditions or biogeochemical processes. Currently, there are fewer than ten studies that model HAB response to future climate change (Ralston, 2020).

Conducting research on HAB sites

All of this knowledge must be taken into account when attempting to understand the complex and dynamic relationships within an ecosystem subject to eutrophication. Scientifically, there are many biogeochemical and hydrological variables contributing to the set up of a system. Additionally, the legal and financial aspects are just as crucial if the goal is to successfully implement solutions and changes in management for algal bloom mitigation. It is likely that stakeholders, owners, and politicians will have differing priorities, all of which must be considered when making decisions. As scientists, it is important to remain an unbiased source of information, driven by data.

Future goals for HABs

Through all of these forms of legislation and research, the goal remains the same. Prevention, mitigation, recovery, and public education are all necessary components to both adapt to and stop freshwater HABs as they dominate our water resources. As humans, we will need to be well informed about when we may be putting our health at risk by participating in aquatic recreation or simply living downwind of a eutrophic body of water. Our current lifestyles are not sustainable if we wish to have continued access to clean water supplies. With algal blooms in all fifty states, many have the potential to wreak havoc on ecosystems by producing toxins. This ecologically catastrophic mechanism must be looked at in a detailed and dynamic setting in order to gain a full understanding of its looming threat. We must learn how to prevent harmful algal blooms, rather than find solutions after the issue is too far gone.

Lagerman Reservoir Case Study

Executive Summary

The objective of our study is to survey the ecological condition of Lagerman Reservoir, a saline water body subjected to changing water availability and a variable climatic regime. Lagerman Reservoir experiences periodic algal blooms and variable survival of sport fish. Managing the reservoir includes consideration of the quality and quantity of shoreline habitat, crucial for nesting shorebirds. We aim to provide managers with scientific data, so that they are informed when considering the potentially competing needs of wildlife, agriculture, and recreation in their management plans.

In 2020, we observed extreme conditions in Lagerman Reservoir in both water quality and ecological condition, as compared to other lakes and reservoirs in the area. The reservoir is now functioning as a closed basin. That is, water flows into the reservoir, but not out of the reservoir because the water level is continually below the spillway. Over the years, this lack of release leads to evaporative concentration of salts. The concentration of solutes leads to a number of ecological changes, including formation of algal blooms followed by oxygen depletion as the algal material decomposes. Oxygen concentrations were so low that fish populations can not be supported. The reservoir conditions of high solutes, high algal biomass, low water transparency, low dissolved oxygen, and hydrogen sulfide odors are particularly unfavorable for fisheries and recreation. In the current ecological condition, toxic algal blooms are an ongoing concern.

Key Observations

- The reservoir experienced high pH and very high concentrations of major anions and cations. The high concentration of solutes is a result of evaporation in the closed basin.
- Dissolved oxygen concentrations were extremely depleted, and as low as 0.02 mg/L during mid summer. The concentrations of dissolved oxygen were too low to support macroinvertebrates, zooplankton, and fish in the bottom waters. At times, dissolved oxygen concentrations were low throughout the entire water column. As a result, the reservoir is not able to support a fishery, even with frequent restocking.
- Cyanobacterial (blue green) algal biomass was high throughout the year, causing low water clarity.
- Dissolved nutrient concentrations (nitrogen and phosphorus) were low, potentially because of algal uptake and nitrogen-fixing cyanobacteria
- Algal biomass was dominated by colonies of *Synechococcus* sp., a cyanobacteria that is typical of marine and highly saline habitats. It is unusual for a reservoir in Colorado to be dominated by a marine alga. Furthermore, blue-green algae are of concern and species within the genus *Synechococcus* genus include some toxin producing species.

Background

Lagerman Reservoir is located in the plains in eastern Boulder County and is an important recreational area for the local and regional community. For the past several years, Lagerman Reservoir has experienced hydrologic change, as less water has entered the reservoir from Dry Creek and ditch inflows. The reduction in inflow has led to a series of ecological changes. Specifically, since water was no longer released through the outflow (spillway), evaporation has the effect of making the water more chemically concentrated. As a result, the increased concentration of salts, and potentially nutrients, is responsible for the reservoir experiencing decrease in lake level, periodic algal blooms, low dissolved oxygen, and death of fish. All of these changes impact the condition of the site and the public who visit. Furthermore, the shallow, western shore of Lagerman Reservoir has long been valued as a productive habitat for nesting shore and fishing birds. Changes in water level are intimately tied to the amount and quality of habitat for feeding and nesting. Consequently, there is a need to determine the ecological condition and to evaluate potential actions to restore aquatic life.

The urgency of determining potential toxicity of present algal species cannot be overstated. The reservoir is a popular destination for fishing, kayaking, paddle boarding, and bird watching. Dogs may also be put at risk when they drink or swim in the water.

While the frequency of cyanobacterial monitoring is increasing, it is not widespread enough to be completed on all water bodies. Of the sites in Colorado, seven have indicated toxic levels of algae and four have been in the metro-Denver area (Colorado DPHE, 2020). As mentioned previously, the EPA has created the Cyanobacteria Assessment Network Mobile Application (CyAN app). Of the 2,000 or so lakes and reservoirs in the United States, Lagerman Reservoir is included in the CyAN network, and thus the satellite data is an additional resource for monitoring blooms.

Limitations due to COVID-19

Our research plan was altered based on limitations due to timing of approval for fieldwork, safety considerations, and coordination with Boulder County staff. Our original proposal included conducting a bathymetric survey of the reservoir, yet we were unable to perform a certified, engineer-grade survey per Boulder County Open Space's request. Similarly, we were not able to coordinate timely installation of piezometers to monitor groundwater flow. Yet, we were able to take advantage of opportunities to collect important data on the reservoir. For example, in order to obtain water quality and algal samples in a timely manner, we initiated collection of weekly grab samples from the shore, a new task we added to the work plan. We integrated these samples with the water column sampling, and these data gave us a better understanding of the temporal changes in algal composition and biomass. In addition, we were able to submit samples from Lagerman collected in 2019 for gene sequencing to gain understanding of algal populations. Finally, we obtained valuable remote sensing data of cyanobacterial pigments of the reservoir through the EPA Cyanobacterial Assessment Network (CyAN). These data allowed us to calibrate field and remote measurements of algal biomass, extending the period of record for all of 2019 and 2020.

Methods

All of the fieldwork and lab analyses were conducted in accord with University of Colorado (CU) guidelines for COVID-19 safety. All researchers traveled to the research site individually by car and wore masks during the site visit. Shore samples were collected by a single person. Lake sampling was conducted by field crews that were distanced at least 6 feet in a motorboat, or in individual inflatable rafts. PFDs were used by field crews and a person remained on shore as an observer. Lab activities were completed by Maggie Anderson at the CU Sustainability, Energy and Environment Community (SEEC) Building, in communication with Diane McKnight and Sarah Spaulding via zoom.

A. Fieldwork - shore sampling

Bulk water samples were collected from the shore, at the boat dock on the north side of Lagerman Reservoir on 14 dates throughout the summer. On each sample date, two 50 mL vials were filled with surface water. One vial was preserved with 5% Lugol's Solution (potassium iodide) for microscopic analysis of algae. The second vial was filtered in a dark room through a 0.45 µm glass fiber filter. The filter was wrapped in aluminum foil, and frozen until chlorophyll-a analysis in October.

B. Fieldwork - lake sampling and water column profiles

Lake surveys were completed on three dates (June 18th, July 29th, and August 22nd, 2020). For the first collection date, we used a motorboat, while the second collections utilized inflatable Alpaca rafts. Based on a 2004 bathymetric survey by Boulder County Open Space we estimated the deepest point of the reservoir. Depth profiles were conducted using a YSI 556 multiparameter sonde to obtain measurements of temperature, specific conductance, and dissolved oxygen. Measurements were taken every meter (June sampling) or every half meter (July, August). Secchi depth was recorded as a standard measure of water transparency. A Van Dorn bottle was used to collect water samples from the surface (~0.1m), above the thermocline (~2m), and above the bottom sediments (~3m). Samples were collected for algal identification and chlorophyll-a described above. In addition, 50mL aliquots were filtered using a 0.45µm filter for analysis of cations and anions. Samples for nutrient analysis (50mL) were similarly filtered and frozen.

C. Laboratory analyses

a. eDNA sequencing

A raw water sample was collected (October 14, 2019) from the shore of Lagerman

Reservoir and frozen. It was later submitted to Jonah Ventures (jonahventures.com) for analysis of eDNA based on Next Generation Sequencing (23s region).

b. Microscopic imaging and identification of algae

Preserved algal samples were prepared in settling chambers following the Utermohl method and analyzed under a Nikon Diaphot inverted microscope using a 100x oil immersion objective. Samples were analyzed for species composition. Images were captured and assembled into an algal catalog to demonstrate species occurrence and community composition and chloroplast condition, a measure of the health of the algae.

c. Analysis of chlorophyll-a

Calibration:

All work with chlorophyll was conducted in a darkened room. Stock solutions of chlorophyll-a were made by mashing 3 spinach leaves with 100mL of 90% buffered acetone (90% reagent-grade acetone and 10% $MgCO_3$ solution) using a mortar and pestle. The stock solution was used to create two solutions, A and B. Stock A was made by pipetting 10ml of concentrate into a 100ml volumetric flask with 90ml of buffered acetone. Stock B was made by pipetting 10ml of stock A into a 100ml volumetric flask with 90ml of buffered acetone. Diluted standards 1A-6A and 1B-5B were created as serial dilutions from stock A and B using INSTAAR Arikaree Lab protocol (Schein 2017).

A standard calibration curve was obtained by measuring absorbance at 665 nm and 750 nm on an Agilent ultraviolet-visible spectrophotometer (Model 8453). Absorbance values were calculated to chlorophyll-a and phaeophytin concentrations (Equation 1) then plotted against the calculated chlorophyll-a and phaeophytin values (Equations 3, 4) using Horiba FluoroMax-3 fluorescence values. Both chlorophyll-a (unacidified) and phaeophytin (acidified) values were measured. To acidify the sample, 0.1 N HCl was added, gently mixed, and allowed to sit for 60 seconds before re-measuring.

Absorbance was converted to chlorophyll-a concentrations using the following equation:

(1) Concentration Chl-a = [26.7* ((ABS665-ABS665a) - (ABS750-ABS750a)) *1000] / length of cuvette path (in this case, 1)

The concentrations of the stock concentrate, A, and B were well within the target range of 10,000, 1,000, and 100 µg/l, respectively. Using these values and the equation below, the concentrations of the A and B standards were calculated.

(2) Calculated concentration of A/B * ((ml of A/B)/(total volume of dilution))

Next, the diluted standards were measured using the fluorometer for both chlorophyll-a and phaeophytin. The calculation below was used where the calibration factor = 0.0225, r = 8.141, and a band pass of 1.

(3) Chl-a = Calibration factor * (r/(r-1)) * (S b-blank – S a -blank)

(4) Phaeophytin = Calibration factor * (r/(r-1)) * ((r * S a –blank) – S b-blank)

The calculated concentrations were then plotted against the fluorometer measurements to achieve a standard curve.

Analysis of samples:

After calibration, the samples were analyzed using NIWOT Ridge LTER standard operations protocol of monitoring methods for chlorophyll-a and phaeophytin (Pruett 2010). The foil wrapped, frozen filters were removed from the freezer and allowed to thaw to room temperature, then placed in 20mL vials (wrapped in aluminum foil) with 90% buffered acetone. Pigments were extracted over 24 hours in a refrigerator. Then, the Horiba FluoroMax-3 fluorometer was used to measure fluorescence of the samples. The samples were acidified with 0.1 N HCl and measured again. Then chlorophyll-a and phaeophytin concentrations were calculated using equations 3 and 4.

d. Water chemistry analysis

The INSTAAR Arikaree Lab conducted analysis of water samples for cations and anions (chloride (Cl^-), sulfate (SO_4^-), sodium (Na^+), potassium (K^+), magnesium (Mg^+), conductivity and nutrient concentrations (nitrite and nitrate (NO_2+NO_3), soluble reactive phosphorus (SRP)). pH, acid neutralization capacity (ANC), and silica (SiO_2) analyses were also completed. A Lachat 8500 Flow Injection Analyzer was used to measure concentrations of NO_3+NO_2, SiO_2, and soluble reactive phosphorus. Ion chromatography was used to measure standard anions including Cl^- and SO_4^-. The cations Na^+, K^+, Ca_2^+, and Mg_2^+ were analyzed using atomic absorption spectrometry. To measure pH, an Orion pH meter was used. Titration was conducted to complete ANC analysis, and conductivity was measured with a Mettler Toledo conductance meter.

e. Processing CyAN data

In 2019, the EPA publicly released the Cyanobacteria Assessment Network mobile application (CyAN app), a satellite monitoring system used to help track, predict, and monitor cyanobacterial harmful algal blooms. This collaboration involved the National Aeronautics and Space Administration, the National Oceanic and Atmospheric Administration, and the U.S. Geological Survey. It currently provides data for over 2,000 of the largest lakes and reservoirs in the U.S., including Lagerman Reservoir. Using this application, we downloaded data for Lagerman Reservoir (January 2019 – October 2020), synthesized it into plots, and documented trends over the two years.

The CyAN application uses Ocean Land Colour Instrument (OLCI) and Medium Resolution Imaging Spectrometer (MERIS) on-board of a combination of satellites: Sentinel-3, Sentinel-2, Landsat (Latham, 2019). After scanning a 3x3 raster cell matrix (900m x 900m), the Cyanobacterial Index algorithm (Mishra 2019; Ogashawara 2019) is used to estimate biomass (cell concentrations) from spectrometer absorption readings of phycocyanin (the photosynthetic compound in cyanobacteria). MERIS analyzes the bands at 620, 665, 681, 709, and 754 nm. This cross-satellite analysis allows for greater evaluation, validation, and refinement of modeling.

In 2019, when the CyAN application was first launched, data was collected at Lagerman Reservoir less regularly, sometimes multiple times a month, and sometimes not for two months. Since 2020, data has been collected weekly, with values indicating the average cell concentration for that week. To download this data, enter the URL

https://cyan.epa.gov/cyan/cyano/location/data/40.1340063/-105.1884808/all

into a JavaScript Object Notation (JSON) formatter (Crockford 2007), and the coordinates, date, and cell concentrations for each date will be returned. These data were then compiled into graphs (Figures 1 and 2), indicating the trend in phycocyanin concentrations from January 2019 to the present.

Results

a. Water column profiles

In June, the water column was stratified by temperature (Table 1), and correspondingly, dissolved oxygen was variable with depth. While dissolved oxygen was supersaturated in the surface waters, the concentration at 3 m was below 4 mg/L. In late July, the surface waters had not warmed as much compared to the June sampling. The temperature was nearly constant throughout the water column, indicating well-mixed conditions. Dissolved oxygen concentrations, however, were even lower than in June. The percent O_2 saturation was extremely low (24% at the surface down to 2% at 3 m depth). In August, the surface temperatures had not increased appreciably. However, the bottom waters were cooler, showing that some stratification was present. While the percent O_2 saturation was greater in the surface waters than the previous month at nearly 90%, the bottom waters continued to be depleted (at 20% O_2 saturation).

Conductivity levels were very high, reaching up to 9889 µS. This indicates extreme concentrations of dissolved ions.

b. Water column chemistry

Similar to the water column profile values for 2020, stratification was more apparent in June than July and August, especially for Cl^- and SO_4^-. Na^+, pH, ANC were exceptions to this trend, as their range of values grew throughout the summer. Nitrogen to phosphorus ratio (N:P) was always below 3 (Figure 6), indicating that nitrogen is limiting to growth. All organisms require both of these nutrients and the ratio is an indication of the nutrient most controlling for growth. Ratios less than 14:1 indicate limited nitrogen and greater than 16:1 indicate limited phosphorus (Tessier, 2003). This ratio plays a crucial role in understanding characteristics of algal blooms as it is a factor in the dominance of cyanobacteria over other algal microorganisms (Levich, 1996). Generally, as depth increased, the ratio decreased. Overall, the average N:P ratios for the water column did not vary significantly between months.

April 2019 sample analysis (Table 2) indicated higher concentrations of chloride, sulfate, magnesium, sodium, and pH than our 2020 collections (Table 3). These levels were 2.8, 1.2, 1.3, 1.3, and 1.1 times greater, respectively. In addition, total phosphorus levels from 2019 were 0.09 mg/L, over 10 times the concentration of 2020, which had an average of 0.00867 mg/L for soluble reactive phosphorus.

Table 1. Depth profiles for temperature, conductivity, and dissolved oxygen (DO, both concentration and % saturation) in Lagerman Reservoir on sampling dates in 2020.

Sample Date	Depth (m)	Temperature (℃)	Conductivity (µS)	DO (mg/L)	DO (%)
18 June	0.1	23.5	9889	16.5	110
	1.0	23.2	9858	16.9	113
	2.0	19.4	9115	7.1	47
	3.0	17.6	8759	3.6	24
29 July	0.1	23.5	9164	2.0	24
	0.5	23.5	9163	1.5	19
	1.0	23.5	9136	1.3	16
	1.5	23.4	9127	1.2	14
	2.0	23.4	9125	1.1	14
	2.5	22.6	8974	0.2	3
	3.0	21.5	8877	0.2	2
22 August	0.1	24.0	9663	7.1	88
	0.5	24.0	9578	7.0	94
	1.0	23.2	9437	6.0	75
	1.5	23.0	9396	5.0	68
	2.0	22.7	9321	3.0	36
	2.5	22.0	9200	2.0	25
	3.0	21.5	9108	1.6	20

Table 2. Summary of water chemistry parameters of Lagerman Reservoir surface water. Data are from samples collected in April 2019 and analyzed by Colorado Analytical Laboratories. Data were obtained from Colorado Parks & Wildlife (Ben Swigle).

	pH	Cl mg/L	SO_4 mg/L	Na mg/L	Mg mg/L	Total P mg/L
April 2019	8.97	402.8	8477.0	9.7	1304.0	0.09

Table 3: pH, acid neutralizing capacity (ANC), anions, cations, nutrients, and dissolved silica concentrations of samples from the shore and water column of Lagerman Reservoir from three sample dates in 2020.

		pH	**ANC µeq/L**	**Cl mg/L**	**SO_4 mg/L**	**Na mg/L**	**K mg/L**	**Mg mg/L**	**NO_3+ NO_2 mg/L**	**NO_3 mg/L**	**SRP mg/L**	**SiO_2 mg/L**
18 June	Shore	8.16	1709.9	138	6480	1315	33	975.3	0.018	<1	0.0077	3.245
	0.1m	8.2	2200.4	146	6940	1362	45	1060	0.02	<1	0.0082	3.214
	2.0 m	8.21	2352	151	7110	1323	33	973.4	0.013	<1	0.0093	3.684
	3.0 m	8.24	2640.8	193	9130	1395	40	1070	0.017	<1	0.0116	4.005
29 July	Shore	8.16	3220.6	138	6670	1321	34	968	0.047	<1	0.0083	3.769
	0.1m	8.00	3245.8	139	6790	1351	39	986.2	0.016	<1	0.0084	3.954
	2.0 m	8.01	3236.8	138	6680	1310	33	971.6	0.014	<1	0.0082	3.838
	3.0 m	8.02	3534.7	139	6730	1287	33	959.1	0.015	<1	0.0083	4.66
22 Aug	Shore	8.06	1964.9	141	6830	1332	34	973.4	0.013	<1	0.0083	4.455
	0.1m	8.05	2895.6	145	7000	1369	36	993.5	0.011	<1	0.0086	4.47
	2.0 m	8.12	2450	146	6890	1233	36	934.3	0.018	<1	0.0085	4.312
	3.0 m	8.22	3113.1	142	6830	1334	39	986.2	0.014	<1	0.0086	4.572

c. Water column algal results

The June 22nd sample showed greater algal abundance at the surface, with fewer cells at 2 and 3 meter depths. In July and August, the opposite pattern occurred and biomass was greater with depth, indicating a movement of algae from the surface to the depth at a time that aligns with a decrease in chlorophyll-a concentrations (Figure 3). While abundance varied with depth, the species composition appeared to be rather consistent throughout the column. Blue-green algae *Synechococcus*, *Merismompedia* and various genera of filamentous bacteria (some nitrogen-fixing species), were all present and dominant. *Euglena*, various diatoms, and *Cosmarium* were also commonly found. With cyanobacteria being the main concern for toxin production, it is crucial to note the presence of the following genera which have some toxin producing genera: *Dolichospermum*, *Lyngbya*, *Oscillatoria*, *Synechococcus*, and *Merismopedia* (Jakubowska, 2015).

d. Shore algal results

In June, algal chloroplasts were largely intact and full, indicating that the cells were thriving and undergoing cell division. Cells had greater pigmentation, larger colonies, and significantly greater abundance. By July and August, the most species were senescent, indicating unfavorable conditions. Pigmentation was lost, which was clear in diatom species that had minimal oil production. *Chaetoceros*, a species of diatom, as well as dinoflagellates were found to be producing resting spores. Over the season, the species distribution appeared relatively constant, with *Synechococcus* and other cyanobacteria dominating the water body consistently. Primary cyanobacterial genera included: *Synechococcus, Merismopedia, Dolichospermum, Lyngbya, Oscillatoria,* and *Spirulina*. Green algae were also present with *Cosmarium, Oocystis, Ankistrodesmus,* and *Scenedesmus* present. *Euglena* was very common, especially towards the end of the summer. Various dinoflagellates and diatoms were also present.

e. Chlorophyll-a and phaeophytin concentrations

On all three lake sampling dates, as depth increased, there was not a significant change or consistent differences in chlorophyll-a or phaeophytin concentrations (Figure 1). Consistent with the healthy appearance of algae in June, the chlorophyll-a was much greater than the phaeophytin at the time. On the following dates, chlorophyll-a began to decrease as phaeophytin increased. The shore samples indicate a peak in chlorophyll-a levels in mid-June (Figure 2).

The CyAN data indicated a peak in phycocyanin in late-April as well as a smaller peak in early July (Figure 3). When comparing the 2020 to the 2019 data, phycocyanin levels are two to four times higher (Figure 4). The CyAN data should continue to be monitored for ongoing changes in the concentration of cyanobacteria.

A linear regression analysis was performed to compare the corrected chlorophyll-a and phaeophytin concentrations to the CyAN application data by plotting the two variables against each other in several contexts. The weekly chlorophyll-a shore samples versus the CyAN data yielded an R^2 value of 0.1996 (Appendix H). When adding in values from the monthly surface samples, they tended to occupy the area underneath the linear regression line, indicating higher levels for the chlorophyll-a analysis than what the CyAN app estimated.

Figure 1: Chlorophyll-a (filled circles) and phaeophytin (hollow circles) concentrations from the water column in Lagerman Reservoir on sampling dates in 2020.

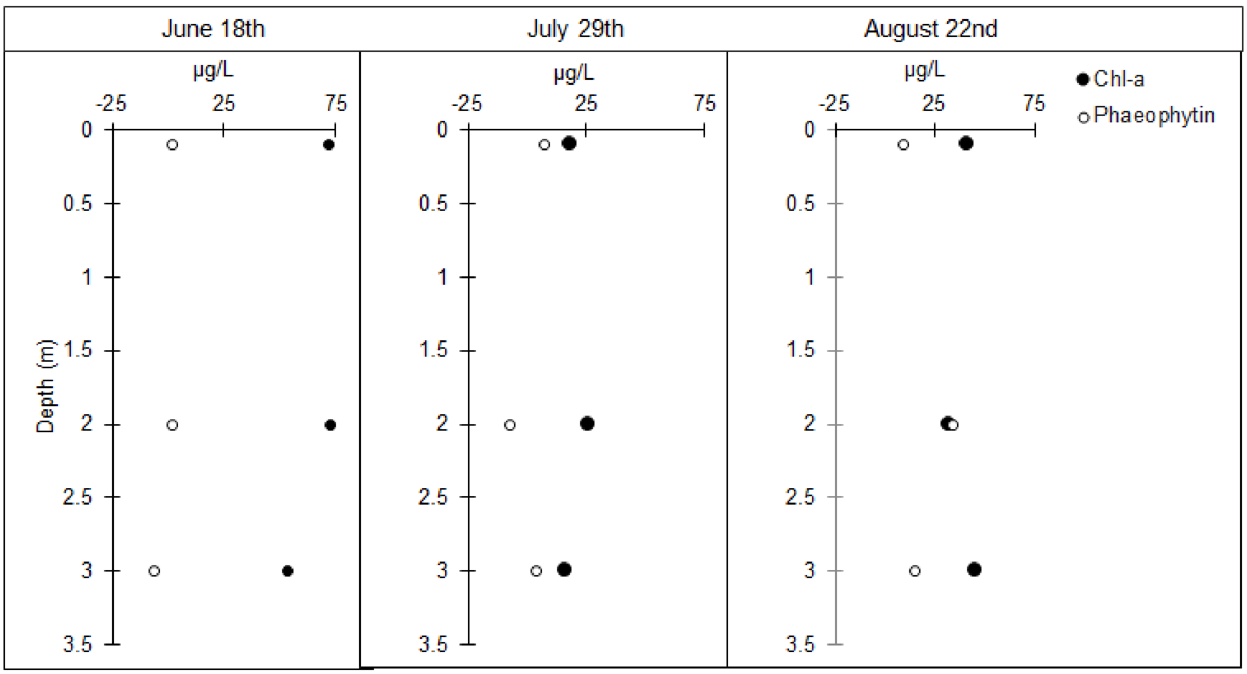


Figure 2: Chlorophyll-a and phaeophytin concentrations of shore samples in Lagerman Reservoir on sampling dates in 2020.

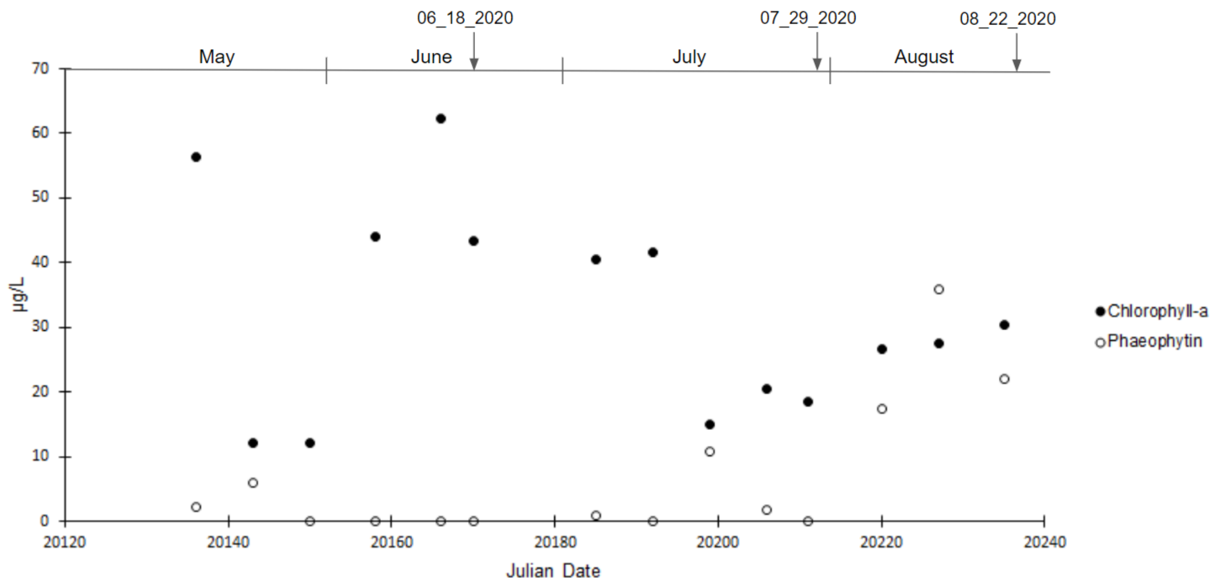


Figure 3: Cyanobacterial cell concentrations in Lagerman Reservoir in 2020 as measured by remote sensing of algal pigments (EPA CyAN).

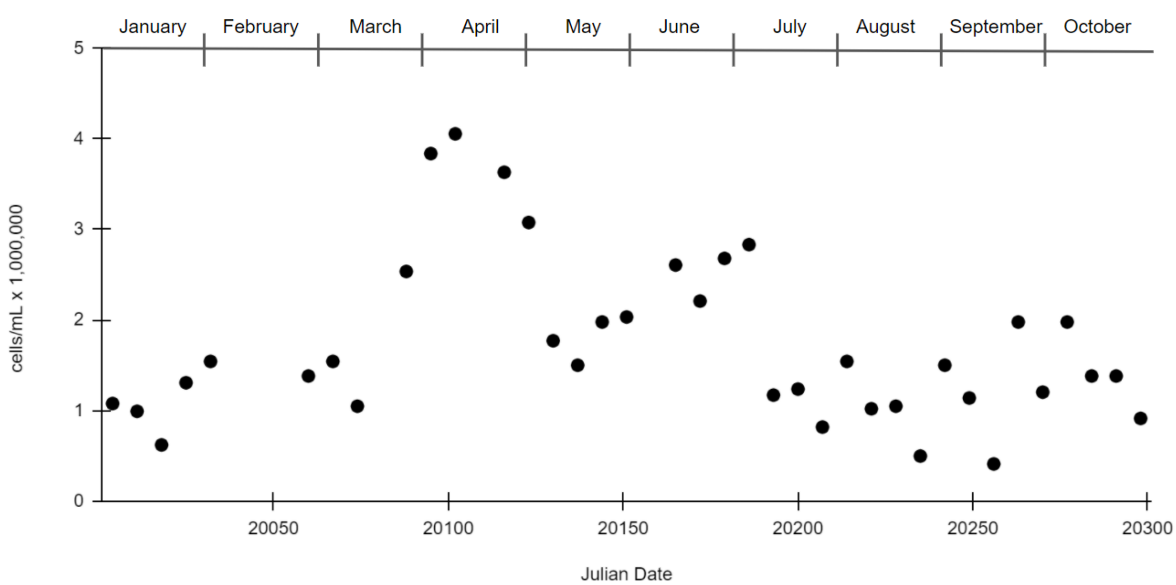
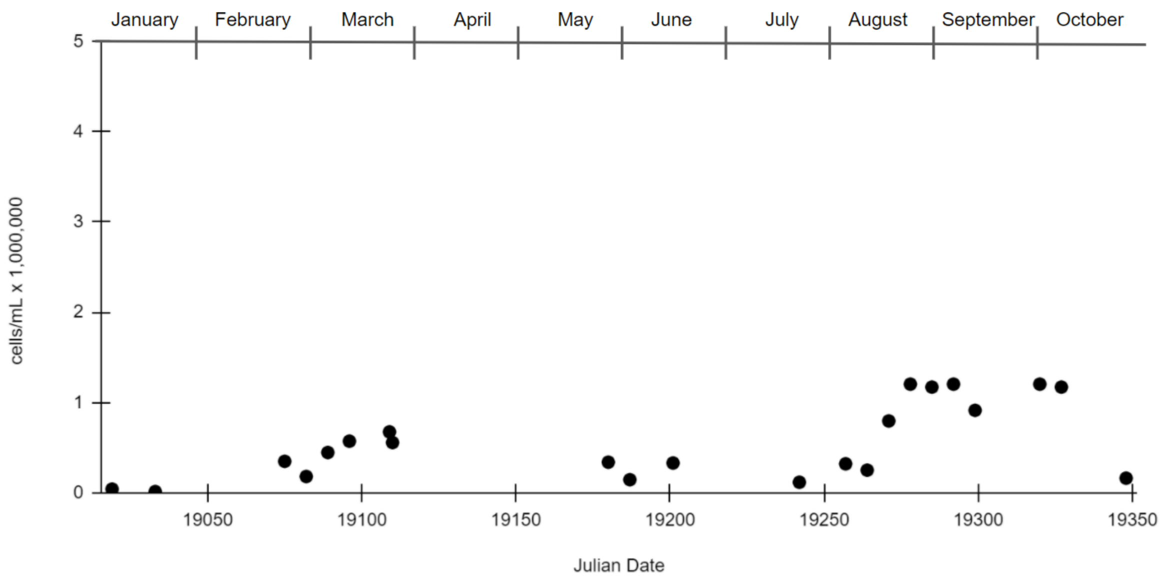


Figure 4: Cyanobacterial cell concentrations in Lagerman Reservoir in 2019 as measured by remote sensing of algal pigments (EPA CyAN).



e. DNA

April 2019 DNA sequencing analysis indicated high diversity and many diatom species that thrive in highly saline waters. In addition, the cyanobacteria *Anabaena, Dolichospermum, Oscillatoria, Phormidium* were confirmed, some of which have been known to potentially produce toxins.

f. Secchi Depth

Secchi depth is used as a method of determining transparency of the water column. Sufficient light is required for algal growth as it can greatly affect photosynthetic rates. The secchi values ranged from 0.2 to 0.3 meters, indicating very low water transparency and values that were nearly constant throughout the summer (Figure 5).

Figure 5: Secchi depth measured in the water column in Lagerman Reservoir on sampling dates in 2020.

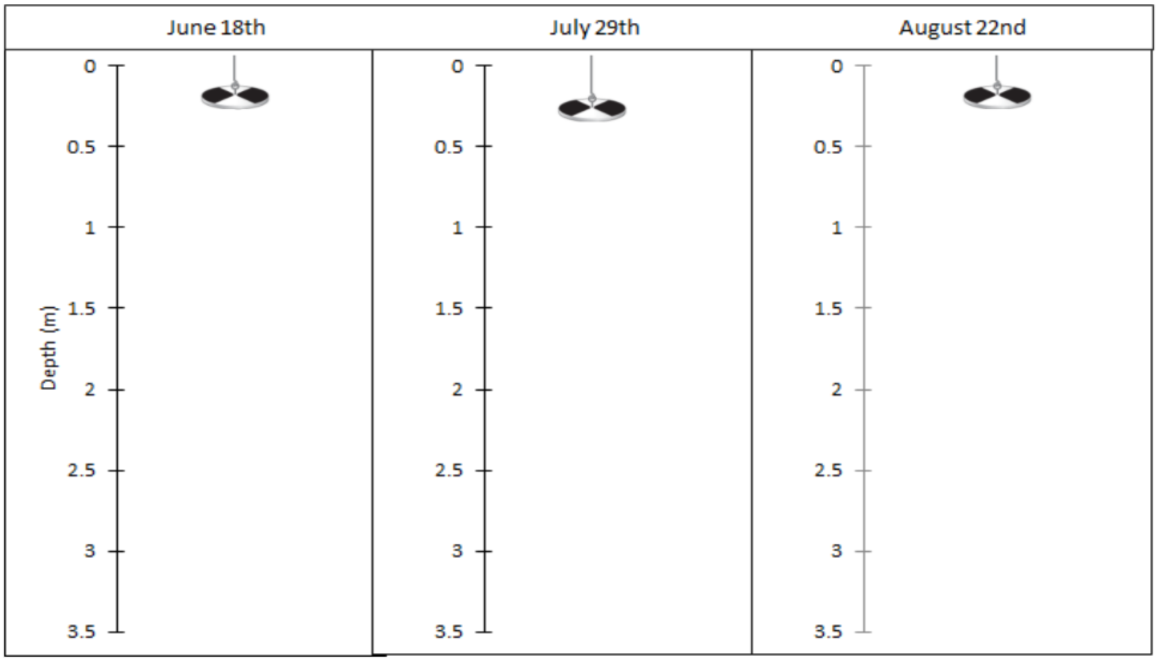
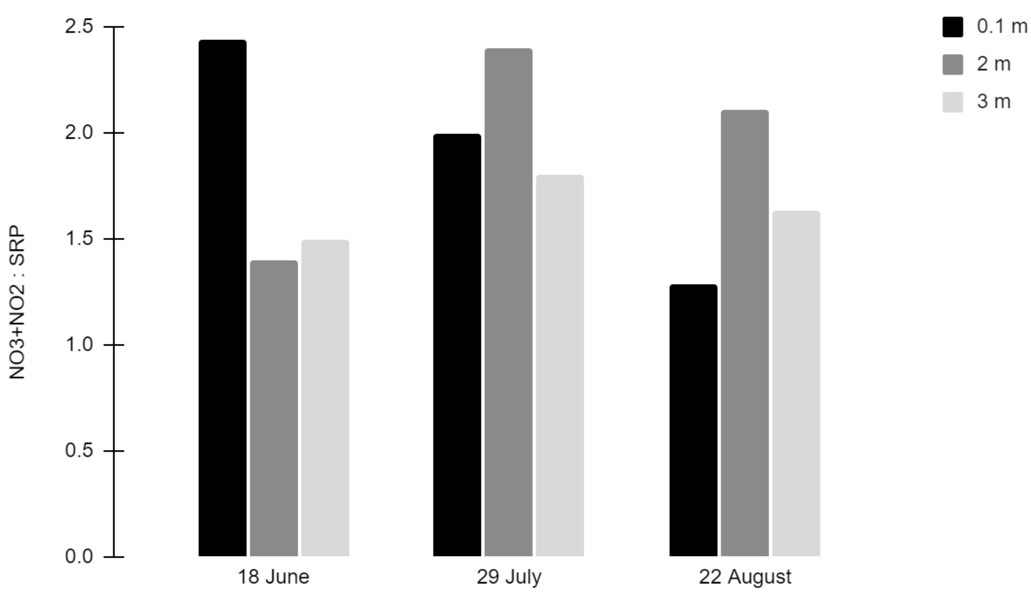


Figure 6. Ratio of nitrogen to phosphorus in Lagerman Reservoir on sampling dates in 2020.



Discussion

Overall, the reservoir is shallow and exhibited only slight stratification in the early summer and was well-mixed by July. Langmuir spirals, a phenomenon that causes circulation of water and accumulation of foam and debris on the surface, were present in June while sampling. This wind-induced mixing resulted in a nearly constant surface temperature throughout the summer months.

Chlorophyll-a concentrations decreased as phaeophytin concentrations increased throughout the summer, which represents a peak in algal growth and biomass followed by subsequent decay. As cells become senescent, chlorophyll-a degrades to phaeophytin (phaeophytin is not photosynthetically active). This observation is supported by the CyAN app satellite imagery. Our data indicated a peak in mid-June, whereas the CyAN data showed a peak slightly later, in early July. Additionally, the linear regression resulted in low R^2 values. This difference could be due to the type of pigment that is analyzed in each process. Chlorophyll-a represents a measure of the algal biomass as a whole; however, the CyAN app focuses on phycocyanin, a pigment specifically found in blue-green algae. Also, some of the weekly shore sample dates were one or two days before the CyAN data collection. When comparing the EPA CyAN app data from 2019 to 2020, it appears that the phycocyanin concentrations have increased drastically, by roughly two to four times.

In June, dissolved oxygen concentrations were greatly stratified, resulting in a narrow zone in the water column that a fish could survive for a limited period of time, as the bottom waters for feeding would be toxic to fish. By the next sampling date, the oxygen concentration further declined, making virtually no part of the water column capable of supporting fish, even temporarily. By August, both the concentration and stratification appear to be recovering, yet with minimal likelihood for success of fish populations. Boulder County Open Space conducted a sampling in early fall 2020, resulting in no live fish detected (M. Kobza, personal communication). This fluctuation of DO was likely due to the algae actively photosynthesizing and producing oxygen early in the year. Once the nutrients were depleted, algae died and were consumed by respiring bacteria, leading to the decline in DO.

Throughout the year, the algae were composed of genera that are indicative of marine ecosystems. This aligns with the high salinity and conductivity of Lagerman.

The secchi depth was extremely low, indicating poor water transparency. Indeed, the low transparency is primarily due to the high abundance of algal cells. Such low transparency could lead to limitation of photosynthesis in the water column, however, as the water experiences high mixing by wind, the high algal biomass can be maintained.

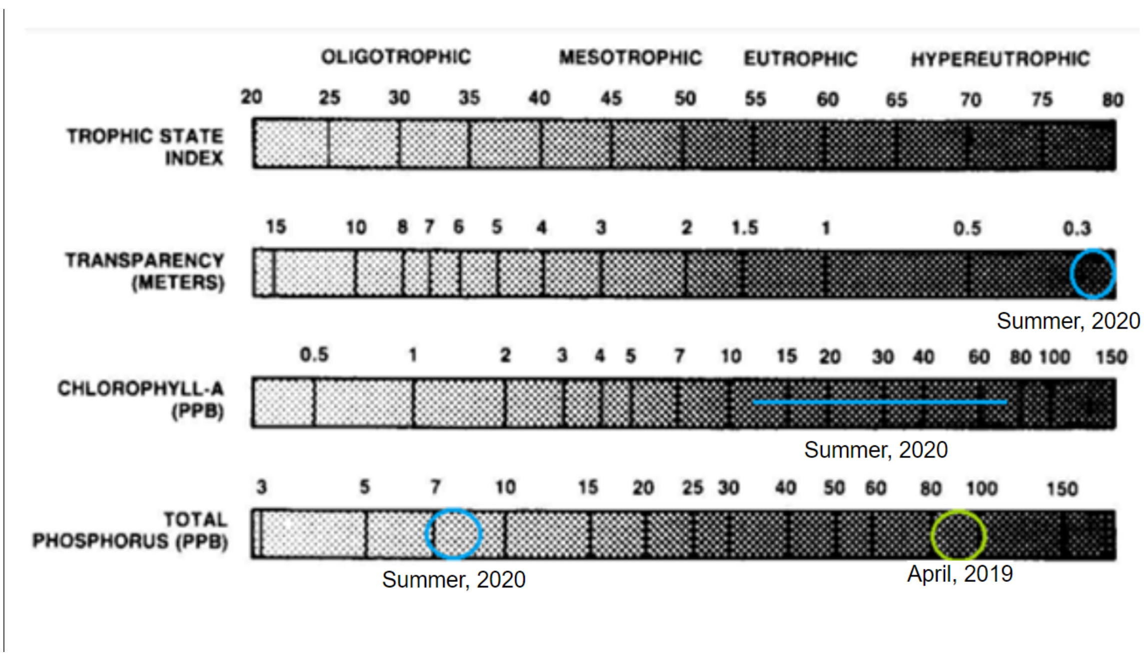
Both nitrogen and phosphorus concentrations were low in 2020. Based on the N:P ratios, nitrogen is the limiting nutrient of this water system. Nitrogen, however, can be obtained from atmospheric N_2 by the diazotrophic cyanobacteria, capable of nitrogen fixation. Multiple species of the colonial picocyanobacteria *Synechococcus* have been shown to fix nitrogen in lab settings, including Miami BG 043511 (Ikemoto, 1994) and SF1 (Spiller, 1987). In Spiller, et al., ammonia, nitrate, and N_2 appear to be the preferred sources among the tested compounds.

Synechococcus sp. Strain SF1 was sensitive to both oxygen and light intensity. Optimum nitrogen fixation occurred at lower levels of light in anaerobic conditions. This could correlate strongly to Lagerman, as the water was very turbid with low DO levels throughout the summer. Furthermore, it is possible that significant amounts of nitrogen were taken up within the high algal biomass. Silica concentrations were also low in the water column, and at concentrations that are limiting to diatom growth.

In April 2019, sample analysis indicated higher levels of chloride, sulfate, magnesium, and sodium (Boulder County data) than any of the 2020 samples. The largest difference in concentration was for phosphorus, which phosphorus was over ten times the concentration in 2020. It is difficult to compare the two years directly, but it appears that in 2020, algal growth was many times greater and available nutrients were taken up within algal biomass. This interpretation is supported by the CyAN data, indicating the highest peak of phycocyanin concentrations in April 2020.

Carlson's Trophic State Index is a measure used to compare multiple parameters of aquatic ecosystems on a common scale. Figure 7 shows the values for Lagerman Reservoir in relation to the range of freshwater systems. It shows total phosphorus levels of 8.67 ppb (0.00867 mg/L, Summer 2020) to be mesotrophic, but 90ppb (0.09 mg/L, April 2019) are hypereutrophic. This index also shows that the Lagerman Reservoir chlorophyll-a concentrations indicate a eutrophic to hypereutrophic ecosystem (12.2-62.2 ppb (or µg/L). Secchi depth (transparency) of 0.2-0.3 m also indicates hypereutrophic conditions. Based on these measures, Lagerman Reservoir is considered to be eutrophic, is unsuitable for fish, and is vulnerable to toxic algal blooms that could be hazardous to human health.

Figure 7: Values for trophic state, transparency, chlorophyll-a, and total phosphorus for Lagerman Reservoir placed on Carlson's Trophic State Index (Stednick 2003).



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Appendices

Appendix A. Results of analysis of eDNA based on Next Generation Sequencing of the 23s nuclear region. Sample was collected October 14, 2019 from the shore of Lagerman Reservoir and analyzed by Jonah Ventures (jonahventures.com). The identifications are based on the internal reference library, which may be misleading for some genera. For example, *Emiliania* is not known to occur in inland waters and may represent lack of resolution in the Jonah reference library. Likewise, the diatom genera *Hydrosera* and *Skeletonema* were not confirmed be visual identification.

Division	**Genus**	**% Abundance**
Cyanophyta	*Synechococcus*	79.0
Bacillariophyta	*Nitzschia*	9.9
Bacillariophyta	*Sellaphora*	2.5
Haptophyta	*Emiliania*	2.4
Cyanophyta	*Tolypothrix*	1.9
Bacillariophyta	*Cylindrotheca*	1.0
Bacillariophyta	*Navicula*	0.7
Bacillariophyta	*Halamphora*	0.6
Cyanophyta	*Lyngbya*	0.3
Euglenozoa	*Euglena*	0.3
Cyanophyta	*Prochlorococcus*	0.3
Bacillariophyta	*Hydrosera*	0.3
Bacillariophyta	*Skeletonema*	0.2
Cyanophyta	*Cyanobium*	0.1
Cyanophyta	*Limnospira*	0.1
Cyanophyta	*Cyanobacterium*	0.1

Appendix B. List of genera, by division, from samples collected from Lagerman Reservoir in 2020. Taxa are illustrated in Appendix Figures 1 and 2.

Division: Cyanophyta

Genera: *Synechococcus* (Plate 1 Figs A, B)*, Dolichospermum* (Plate 1 Figs C, D)*, Merismopedia* (Plate 1 Fig. E)*, Spirulina* (Plate 2 Fig. C)*, Lyngbya* (Plate 2 Fig. E)*, Oscillatoria* (Plate 2 Figs H, I), unknown filamentous cyanobacteria (Plate 2 Figs A, B, D, F), unknown nitrogen-fixing cyanobacteria with heterocyst (arrow) (Plate 2 Fig. G)

Division Bacillariophyta

Species: *Chaetoceros coloradensis* (Plate 1 Figs F, G), *Plagiotropis* (Plate 1, Fig. H), *Surirella* (Plate 1, Fig. I), *Cyclotella* (Plate 1, Fig. J), *Mastogloia* (Plate 1, Fig. K), *Cymbella* (Plate 1, Figs L, M).

Division Chlorophyta

Genera: *Oocystis* (Plate 2 Fig. J), *Cosmarium* (Plate 2 Fig. K)*, Scenedesmus* (Plate 2 Fig. L)*, Ankistrodesmus* (Plate 2 Fig. M)

Division Euglenophyta

Genus: *Euglena* (Plate 2 Fig. N)

Division Pyrrophyta

Unknown dinoflagellates (Plate 2 Fig. O)

Appendix C. Results for chlorophyll a and phaeophytin from Lagerman Reservoir in 2020. Water column and shore samples are reported in the two tables, below.

Water Column Samples	Julian Date	Chlorophyll-a µg/L	Phaeophytin µg/L
June 18 surface	20170	71.99	1.98
June 18 2m	20170	72.44	2.12
June 18 3m	20170	53.43	0
July 29 surface	20211	18.56	7.09
July 29 2m	20211	26.03	0
July 29 3m	20211	16.07	3.92
August 22 surface	20235	41.35	9.35
August 22 1m	20235	32.01	34.20
August 22 2m	20235	45.34	14.62

Shore Samples			
May 15	20136	56.42	2.34
May 22	20143	12.21	5.99
May 29	20150	12.21	0
June 6	20158	44.09	0
June 14	20166	62.27	0
June 18	20170	43.47	0
July 3	20185	40.60	0.86
July 10	20192	41.72	0
July 17	20199	15.07	10.88
July 24	20206	20.55	1.82
July 29	20211	18.56	0
August 7	20220	26.65	17.49
August 14	20227	27.52	36.00
August 22	20235	30.39	22.10

Appendix D. Results for average algal cell concentrations of Lagerman Reservoir from January 2019 to October 2020, using data provided by the EPA CyAN project (https://www.epa.gov/water-research/cyanobacteria-assessment-network-cyan).

Date	Julian Date	Average concentration (cells/mL)
January 19, 2019	19019	39084
February 2, 2019	19033	11588
March 16, 2019	19075	346737
March 23, 2019	19082	178649
March 30, 2019	19089	444631
April 6, 2019	19096	570164
April 19, 2019	19109	672977
April 20, 2019	19110	554626
June 29, 2019	19180	337287
July 6, 2019	19187	143219
July 20, 2019	19201	328095
August 31, 2019	19242	114815
September 14, 2019	19257	319154
September 21, 2019	19264	248886
September 28, 2019	19271	794328
October 5, 2019	19278	1202264
October 12, 2019	19285	1169499
October 19, 2019	19292	1202264
October 26, 2019	19299	912011
November 16, 2019	19320	1202264
November 23, 2019	19327	1169499
December 14, 2019	19348	159956
December 21, 2019	19355	691831
January 4, 2020	20004	654636
January 11, 2020	20011	990832
January 18, 2020	20018	1076465
January 25, 2020	20025	990832
February 1, 2020	20032	619441

February 29, 2020	20060	1306171
March 7, 2020	20067	1541701
March 14, 2020	20074	1380384
March 28, 2020	20088	1541701
April 4, 2020	20095	1047129
April 11, 2020	20102	2535129
April 25, 2020	20116	3837073
May 2, 2020	20123	4055086
May 9, 2020	20130	3630781
May 16, 2020	20137	3076097
May 23, 2020	20144	1770109
May 30, 2020	20151	1499685
June 13, 2020	20165	1976970
June 20, 2020	20172	2032357
June 27, 2020	20179	2606154
July 4, 2020	20186	2208005
July 11, 2020	20193	2679168
July 18, 2020	20200	2831392
July 25, 2020	20207	1169499
August 1, 2020	20214	1235948
August 8, 2020	20221	816582
August 15, 2020	20228	1541701
August 22, 2020	20235	1018591
August 29, 2020	20242	1047129
September 5, 2020	20249	496592
September 12, 2020	20256	1499685
September 19, 2020	20263	1137627
September 26, 2020	20270	409261
October 3, 2020	20277	1976970
October 10, 2020	20284	1202264
October 17, 2020	20291	1976970
October 31, 2020	20298	1380384

Appendix E. Environmental Protection Agency (EPA) remote sensing of Lagerman Reservoir for phycocyanin, a pigment specific to cyanobacteria (Latham, 2019).

As of October 2020, satellite imagery is collected on a weekly basis for Lagerman Reservoir (https://www.epa.gov/water-research/cyanobacteria-assessment-network-cyan). Data are collected at a 900m x 900m pixel resolution. Data are collected as spectra for the wavelengths of phycocyanin and converted to “cell” concentration. Note that this conversion to concentration may vary depending on the size of the cyanobacteria, so it should be used as a general value in trends.

To download these data directly, the URL https://cyan.epa.gov/cyan/cyano/location/data/40.1340063/-105.1884808/all is entered into the “JSON Data/URL” text box of the JavaScript Object Notation (JSON) formatter (Crockford 2007). The website https://jsonformatter.curiousconcept.com/ was used for this report.

After pressing “process”, the location name, coordinates, image date, satellite type and frequency, and cell concentrations for each date are provided under the “outputs” section, dating from the present back to the first sample record from 2019. These data were then compiled into graphs (Main Report Figures 1 and 2), indicating the trend in phycocyanin concentrations from January 2019 to the present. This process can be adapted to other sites that are analyzed by the CyAN project by changing the longitude and latitude values in the URL.

The CyAN application is designed for Android phones. With the application, the user can enter latitutude and longitude coordinates for a site. The application will return a graphic expression of the data, but not the raw data values.

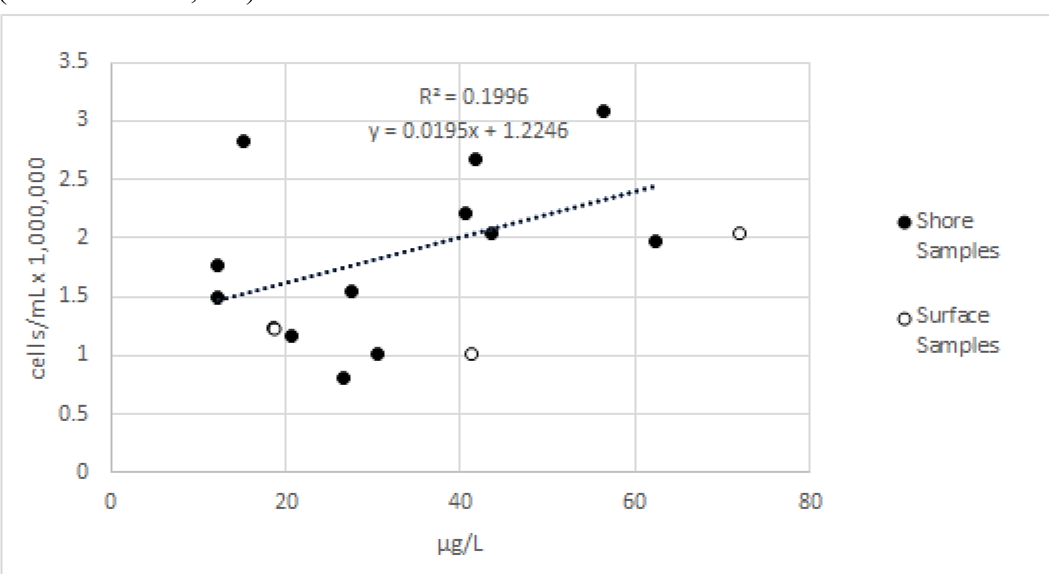
Appendix F. Nitrogen to phosphorus ratios of Lagerman Reservoir during the three profile sampling dates during the summer of 2020. The ratio is an indicator of the nutrients may be limiting in the water system. In this case, the N:P is extremely low, indicating that N is limiting to algal growth.

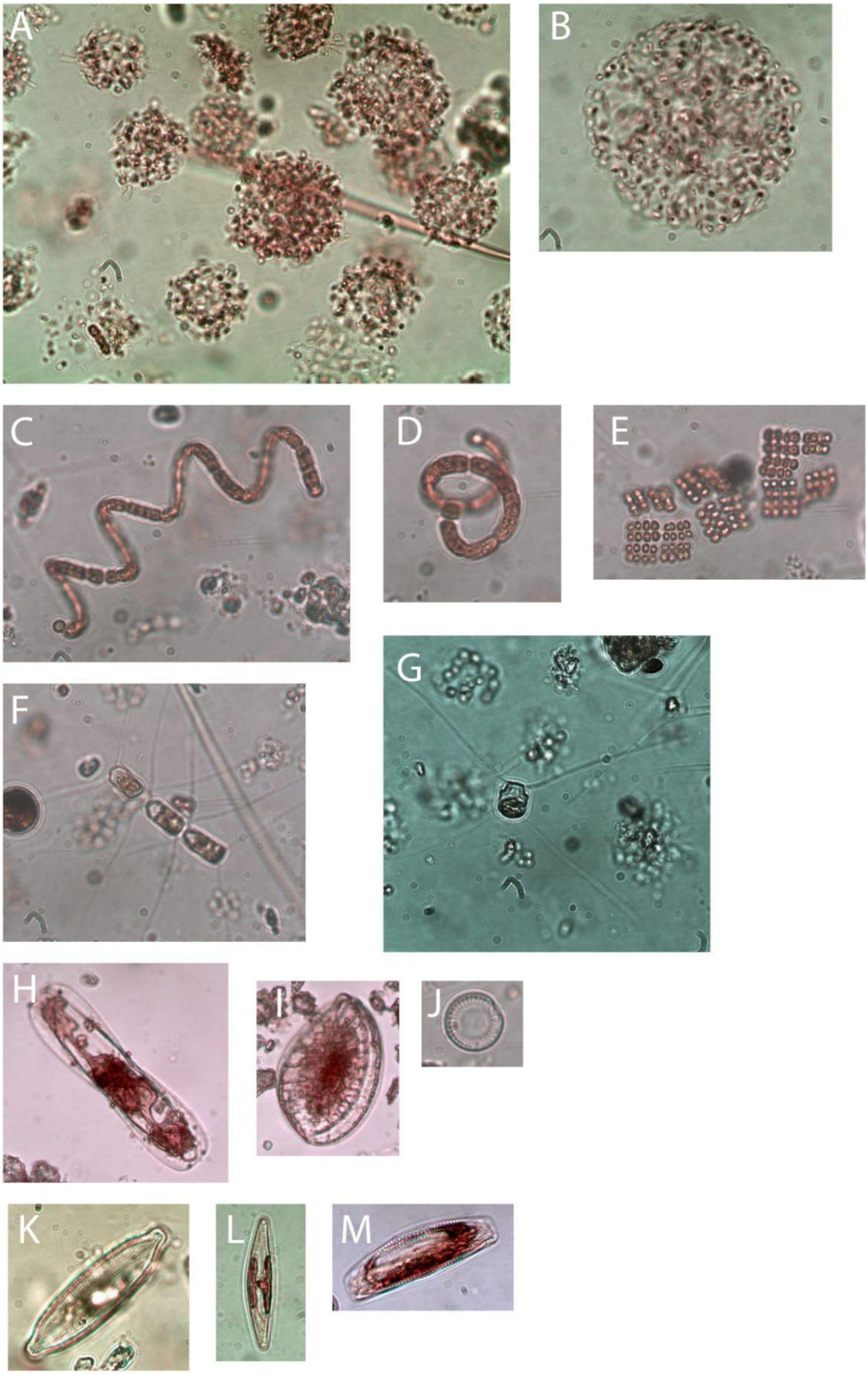
Sample date	Depth (m) 0.1	Depth (m) 2.0	Depth (m) 3.0
June 18	2.4	1.4	1.5
July 29	2.0	2.4	1.8
August 22	1.3	2.1	1.6

Appendix G. Secchi depth of Lagerman Reservoir during the three profile sampling dates during the summer of 2020. The secchi depth is a measure of water transparency and corresponds to the depth at which approximately 1% of the incident light reaches. The secchi depths of 0.2 - 0.3 m are extremely low and indicate poor water transparency. In this case, phytoplankton biomass is so great that the reservoir lacks transparency.

Sample date	Secchi depth (m)
June 18	0.2
July 29	0.3
August 22	0.2

Appendix H. Linear regression analysis of corrected chlorophyll-a (µg/L) versus CyAN app data (cells/mL x100,000)

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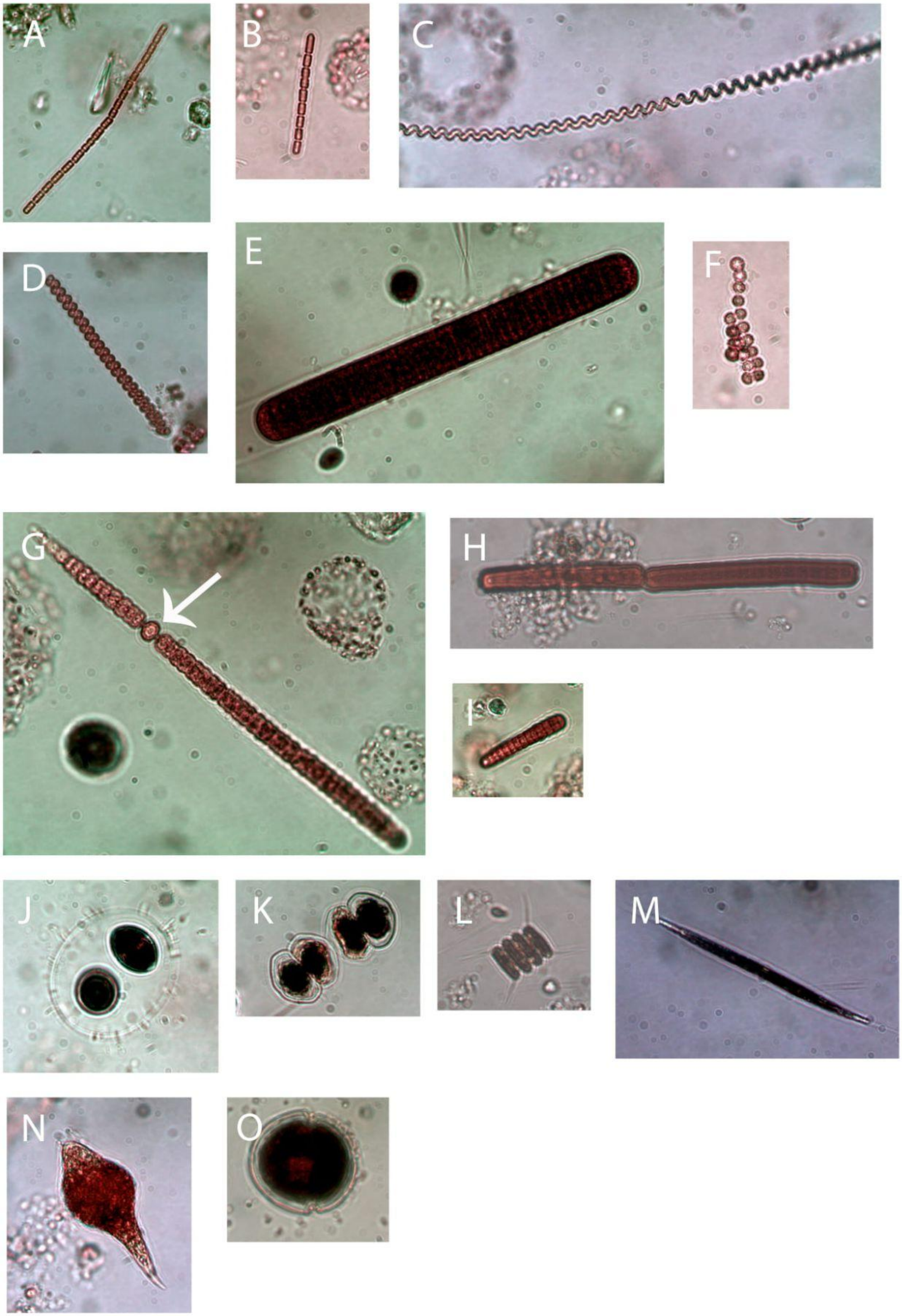
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