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The Effects of Hydrologic Heterogeneity on Harmful Algal Blooms in Freshwater Reservoir, Lake Sinclair, Georgia

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**The Effects of Hydrologic Heterogeneity on Harmful Algal Blooms in Freshwater
Reservoir, Lake Sinclair, Georgia**

Margaret Beth Blackledge

Submitted to the Department of Biological and Environmental Sciences in partial fulfillment of
the requirements for the degree of Master of Science

Dr. Kalina Manoylov, Faculty Advisor

December 2, 2021

Georgia College & State University



Thesis/Dissertation Signature Request Form

The Effects of Hydrologic Heterogeneity on Harmful Algal Blooms in Freshwater Reservoir, Lake Sinclair, Georgia

Submitted by Margaret Blackledge in partial fulfillment of the requirements for the degree of M.S. Biology.

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Abstract

Aquatic habitats are frequently studied after a major water quality problem like the occurrence of an algal bloom. In this study, A proactive rather than a reactive response was considered, where the complexity of conditions conducive to uncontrolled cell growth were studied before a bloom took place by sampling regularly. This study aimed to monitor water quality by monthly sampling of algal communities for approximately one year. As the base of the aquatic food web, algae are a highly diverse group of organisms with varying sensitivity to physical and chemical changes in the environment. Four shallow sites were monitored at Lake Sinclair, GA once a month from November 2019 to October 2020, and four deep sites were monitored from May to August 2020 for conductivity, DO, pH and water temperature along with the algal community composition. Algal biomass was estimated in the field (analyzing algal mats attached to rocks or sand), and in the lab (analyzing composite samples). Algae that were alive at the time of collection were enumerated and identified to the lowest taxonomic unit. 192 samples were collected at midday and processed within hours. An ANOVA two-factor test was performed on the average chlorophyll-a concentration for each month and each site. The difference in average total chlorophyll-a among sites within habitat (shallow and deep) and through time were not significantly different. Habitat differences for dissolved oxygen (DO) were different for shallow and deep sites. Surface water temperature was not significantly different within the shallow and deep sites. DO was never below 4 mg/L, and chlorophyll-a production was higher in the shallow habitats. A total of 58 algal taxa are displayed in this study. Species richness was significantly higher in shallow sites where there was a maximum documentation of 78 species. There was taxonomic heterogeneity between the sites with toxigenic cyanobacteria documented in 28% of shallow sites and 22.9% of deep sites. The

population density of toxigenic cyanobacteria was never above 5,000 cells/ml in any site, too low to potentially trigger production of cyanotoxins. However, in June, one of the triplicate samples taken at site 5, a deep site, had a toxigenic *Aphanocapsa* sp. 5 experience relative abundance at 29.41% of the total algal community. Chain forming diatoms dominated deep sites, but cyanobacteria were commonly documented in low abundance, represented by filamentous cyanobacteria *Phormidium* sp. The total biomass, measured as total chlorophyll-a, were significantly different between shallow and deep sites. Using the EPA developed tool for linking potential for toxic bloom to chlorophyll-a, concentrations higher than 39 µg/L were considered possible indicators for toxic algal bloom development. The highest chlorophyll-a concentration was documented only in shallow sites at 487.4 µg/L, but blooms were never recorded and cyanotoxins were not detected. Regional consideration of water mixing, siltation, and low light availability despite high temperatures potentially prevented blooms. Generally low nutrient conditions maintained a diverse algal community that did not develop into a monospecific growth.

I. Introduction

Algae, a polyphyletic group of aquatic and photosynthetic organisms, play a great part in earth's biosphere. They produce half of the atmosphere's oxygen and are the major primary producers for aquatic ecosystems (Chapman 2013). When conditions are favorable to a species, algae will grow quickly and develop into a bloom. Some algal species will produce algal toxins during a bloom to cope with overcrowding (Kardinaal et al. 2007). The algal community is monitored to determine the health and safety of aquatic environments due to the integral part algae play in aquatic food webs and nutrient cycling and the potential for harmful algal blooms. However, only sampling one time and one site per lake from the deepest area (collecting within the photic zone) is currently required (U.S. EPA 2019) since most of the variability in physical conditions and water quality in natural lakes occurs in the vertical dimension such as light, temperature (stratification), dissolved substances, productivity, and decomposition (Wetzel 2001). Sampling protocols, driven by resource availability, require on spot collection of physical and chemical data augmented with analyses of biological indicators such as chlorophyll-a, phytoplankton, and/or macrophytes, fauna etc.

This study tests the practice of sampling only one site from a lake as opposed to recognizing heterogeneity in lakes and sampling shallow and deep sites multiple times. Additionally, this study provides predictive data for toxin production based on algal biomass and species composition. In order to save limited resources, the EPA prioritizes recreational waters for monitoring the water for blooms. The study location was Lake Sinclair, Georgia, a popular lake for recreation and a drinking water source for surrounding cities. The EPA monitors cyanotoxins and cell densities. The Microcystin guidelines for recreational water are 8µg/L and

Cylindrospermopsin guidelines are 15µg/L (U.S. EPA 2019) associated with more than 10,000 cells/ml.

1. The study area: Lake Sinclair

This study was conducted on Lake Sinclair which is a sinuous reservoir with an abundance of coves. The reservoir is connected to Lake Oconee by Wallace Dam and flows into the Oconee River via Sinclair Dam. Water is released through the turbine daily from Wallace Dam and pumped back nightly. Lake Sinclair was chosen as the subject of this study due to its status as a reservoir. Under the Clean Water Act of 1972, bodies of water must “maintain chemical, physical, and biological integrity”. When engineering reservoirs, this statement of the law has been interpreted to mean that man made water bodies must behave as natural water bodies and perform the same ecosystem services. Lake Sinclair was designed to create renewable hydroelectricity, is a popular spot for water recreation, it is a drinking water source, and was used as turbine cooling for a local coal plant. Although, the coal plant was closed in 2018 due to thermal pollution documented by the EPA, together with other Clean Water Act (1972) citations (Nolin 2020, Smith and Manoylov 2013). Lake Sinclair is also a source of drinking water for Baldwin and Putnam counties, making it especially important that algal blooms and algal toxins are monitored. As recently as 2015, the EPA developed health advisory guidelines for surface and drinking waters for cylindrospermopsin and microcystins.

2. Current trends in applied ecology

This study examined the accuracy of sampling standards by analyzing the composition of different sites within Lake Sinclair to determine whether sampling for routine algal community monitoring should cover a wider area. The benefit to routinely covering larger areas is greater spatial resolution for the determination of harmful algal bloom risk or health of the ecosystem.

Additionally, within an aquatic habitat, one may be able to detect movement of populations as species disperse from presence at one site to presence at multiple sites over the course of a season. With the importance of using biological traits to plan conservation as described by Miatta et al. 2021, rare species either contribute to functional diversity or functional redundancy within a species pool (Record et al. 2021). There are algal indicators of nutrients, physical and chemical factors. The presence and abundance of toxigenic algae may indicate if algal toxins are being produced. The disappearance of large, high caloric value algal cells indicates feeding patterns of zooplankton, invertebrates, and fish.

Algal work requires species level identification, as species traits and abundance carry ecological meaning (Manoylov 2014). Lack of taxonomic resolution is a problem prevalent in databases involving sightings, especially added to by people who are not taxonomic experts. Like the very diverse group of algae, this is especially a problem within fisheries science. Blasco et al. (2020) suggests that increasing the availability of DNA barcoding technology would improve taxonomic resolution and it would improve the state of many other databases as well. The potential Algae DNA barcode genes code for proteins used in photosynthesis or ribosomes. Still a young technology, DNA-Barcoding has issues such as varying rates of evolution between and within species which may cause an overlap in what should and should not be considered a unique species. Algae require multiple genes for DNA barcoding, and selection of an appropriate gene can sometimes prove a challenge. DNA differences also do not always correspond with the morphological differences on which most of previous literature has been based (Chaudhary and Dahal, 2017). Creating monocultures of algal species to isolate DNA in order to add species to DNA barcode databases, is notoriously difficult. Thus, at the current state, morphological species

level identification is still an acceptable method for algal community monitoring (Manoylov 2014).

3. Predicting algal blooms and water quality

Vogt, Sharma, and Leavitt (2017) found that water quality of eutrophic lakes was mostly regulated by water temperature, DO, nutrients, and pH. Water opaqueness decreased light availability and delayed algal blooms, especially when coupled by nutrient limitations (Wilkinson et al. 2017).

3.1 Affect of HABs on ecosystems - Algal blooms can cause environmental harm in many ways, one of which is through the manipulation of dissolved gasses in the water. Low oxygen stress is a major consequence of these blooms because it alters the suitability of habitats for beneficial organisms and the size of their populations (Breitburg et al. 2003). Initially, algal photosynthesis supersaturates aquatic environments with dissolved oxygen (DO) (Burkholder et al. 2018). When the temperature change occurs rapidly, oxygen dissolved in the water cannot find an equilibrium with the air above the water (YSI Environmental, Inc. 2009). The microbial decomposers spend oxygen to decompose any dying algae along with any other vegetation that died due to being shaded out. The increased respiration of other microorganisms including bacteria, fungi, protozoans and fish result in increased flux of diel DO (Dodds 2006). Additionally, algae release biologically available nutrients during decomposition that can be used to grow other taxa, worsening the bloom conditions (Buchsbaum et al. 1991, Havens et al. 2001, Gao et al. 2013). At night, the high respiration of decomposers, combined with the lack of photosynthesis, leads to hypoxic or anoxic conditions (low DO or no DO, respectively) (Junk 1973, Lyons et al. 2014). At the end of the bloom, a massive amount of biomass sinks to the bottom of the system and depletes the oxygen in the lower water column (Burkholder et al. 2018). The hypoxic conditions

are exacerbated by the bloom, blocking the surface of the water and preventing photosynthesis below the water surface and oxygen diffusion (Havens et al. 2001). The swings between anoxic and oxygen supersaturated conditions eliminate stress sensitive aquatic organisms (Breitburg 2003, Izagirre et al. 2007, Morgan et al. 2006, Wilcock et al. 2010). Another aspect of low oxygen conditions is that the lack of saturation of DO increases solubility of toxic metals, which can be found in lake sediments (Mitch and Gosselink 2007, Stumm and Morgan 1996). This can be a human health risk if fish are eaten out of affected lakes. The ability of sediments to hold phosphorus is also reduced. The loss of nitrogen in denitrification does not occur in anoxic environments, creating a positive feedback loop between blooms and nutrient availability. As oxygen is depleted, decomposition of organic material slows (Wetzel 2001).

Algal blooms increase turbidity and decrease light needed for lake vegetation to survive (Hauxwell et al. 2001, Valiela et al. 1997). In addition, some algae grow epiphytic on plants, leading to their deaths and causing habitat loss for aquatic species that hide in the vegetation (Burkholder et al. 2007, Lembi 2003). Some Aquatic life would otherwise use the vegetated environments for spawning (Pihl et al. 1995, Thomsen et al. 2006, Wennhage and Pihl 1994). Fish and macroinvertebrates have trouble foraging in anoxic environments and prey populations are reduced (Gray et al. 2002, Osterling and Pihl 2001, Pihl et al. 1991, 1995). An increase in pH which is caused by biomass accumulation inhibits the ability of zooplankton grazers to feed (Buskey 2008) and reduces fish growth (Shimps et al. 2005, Wennhage and Pihl 1994). In addition, the prey population declines, and the presence of algal mats also decreases the ability of birds to forage (Cabral et al. 1999, Green et al. 2013).

Toxins produced by the algal bloom damage the livers, hepatopancreases, kidneys, nervous systems, and osmoregulation of the gills in fish. Aquatic species experience reduced

fecundity and spawning success coupled with problems with embryo development and survival. Larvae in these environmental conditions have problems settling and are often deformed. Deformities include thin epithelial layers, large body surface relative to healthy specimens of the species, high metabolic rate, and limited motility. The effects of algal toxins are worse for early life history stages, species composition of affected lakes is altered with algal mats and can cause trophic dysfunction. In addition, toxins heavily impact birds, especially migratory birds which have already spent energy reserves in their journey. Small doses of toxins could impact the ability of these birds to feed, which could potentially lead to starvation and death (Burkholder et al. 2018) Aquatic life can be additionally exposed to toxins via exposure to toxin contaminated wastes (Lehtiniemi et al. 2002, Svensen et al. 2005). The invasive Zebra Mussels, *Dreissena polymorpha* (Pallas), have particularly cyanotoxin-rich feces and move cyanotoxins from the water column to benthic communities (Babcock-Jackson et al. 2002). Blooms of nontoxic cyanobacteria can produce the same trophic changes because they are seen as low-quality food by zooplankton (Laurén-Määttä et al. 1997).

Aquaculture is an important use of inland bodies of water. Many reservoirs like Lake Sinclair are being used for the purpose of aquaculture. Fish raised using inland aquaculture make up 65% of fish farmed for the purpose of human consumption between 2003 and 2013. Inland aquaculture will become more and more common as natural fisheries are depleted and aquaculture as a technology is perfected. The increase in inland aquaculture is unfortunately linked to the spread of freshwater algal toxins globally (Greer et al. 2017). *Prymnesium parvum* (N. Carter) is a planktonic species of alga which blooms worldwide and causes fish kills. The toxins produced by this alga during a bloom cause gill damage. Rainbow trout, *Oncorhynchus mykiss* (Walbaum), were exposed to *P. parvum* during a Svendsen et al. study (2018). Oxygen

intake initially increased, and the fish made attempts to enter high flow areas to receive more oxygen. Then oxygen consumption per breath decreased throughout the procedure. In a second experiment, to assess fish recovery through behavior, it was found that *P. parvum* causes non-recoverable respiratory failure in the trout. Blood samples were taken from the fish after euthanization to test toxicity and five out of six were positive for the toxin (Svendsen et al. 2018). The effects of toxins coupled with the anoxic conditions brought about by algal blooms are a threat to both commercially farmed and wild fish stocks.

4. Algae as nutrient indicators

Algae make useful biological indicators due to their timely reaction to physical and chemical changes in their aquatic habitats. Algae can be used to monitor loading of inorganic nitrogen and phosphorus within the aquatic systems they inhabit because they are limiting nutrients. These nutrients, often running off from agricultural fields, are contributing to increasing rates of harmful algal blooms (HABS). Subbiah et al. (2019) found several direct and inverse relationships between certain cyanotoxins and chemical and physical factors within the environment. Anatoxin concentration was directly correlated with Ca^{+2} concentrations, pH, water temperature, total phosphorus and turbidity and inversely related to conductivity, DO, and the concentrations of F^- , Cl^- , Br^- , NO^{-3} , SO^{-24} , Na^+ , and NH^{+4} . Microcystin-LR concentrations were directly correlated with total suspended solids, turbidity, and total phosphorus. Anatoxin is inversely correlated with alkalinity. Microcystin-LA concentrations were directly correlated with turbidity and total phosphorus and inversely correlated with the concentrations of F^- , Cl^- , SO^{-24} , Na^+ , and NH^{+4} . Microcystin-RR concentrations in water samples were directly correlated with Ca^{+2} , water temperature, and turbidity while inversely related to conductivity, alkalinity, total dissolved solids, and the concentrations of Cl^- , Br^- , NO^{-3} , SO^{-24} , Na^+ , and NH^+ .

Cylindrospermopsin and saxitoxin concentrations were both directly correlated with water temperature and inversely correlated with the concentration of Mg^{+2} . Though the cause of some of these relationships are unclear, it can be inferred that these environmental conditions reflect on the timing of ecological needs within the timeframe of a bloom. Of the chemical and physical factors tested by Subbiah et al. (2019), pH, water temperature, conductivity, and DO, also were measured in this study using a YSI. Subbiah et al. (2019) mentioned turbidity as the best predicting factor of cyanotoxin concentrations with cylindrospermopsin having lower concentrations in highly turbid waters and microcystin and anatoxin having higher concentrations as turbidity increased. Curiously, less consistent in Subbiah et al. (2019) was the relationship between the presence of blooms and the presence of cyanotoxins in the water.

In a case study by Newell et al. (2019), *Microcystis* bloomed annually in Western Lake Erie due to anthropogenic eutrophication. After planting, farmers are encouraged to fertilize, and this leads to increased NH_4 in the Lake Erie system during the months of May and October. The comparison between NO_3 and NH_4 nitrogen is significant because NH_4 comes from commercial farming and thus is likely anthropogenically added to aquatic environments via runoff. NH_4 is correlated with the dominance of cyanobacteria over diatoms. Since *Microcystis* does not fix nitrogen, previous studies have shown that soluble reactive phosphorus loading sourced from the Maumee River is correlated with the *Microcystis* Blooms. Kjeldahl nitrogen, nitrogen that is reduced and bioavailable, has increased over the past decades and has been shown to correlate with cyanobacteria biomass as well. The ratio of chemically reduced nitrogen (TKN) to Nitrate concentrations was compared to chlorophyll and phycocyanin concentrations. TKN: NO_3 was positively correlated with both chlorophyll and phycocyanin. Newell et al. (2019) advised control of Nitrogen loading in addition to Phosphorus control in response to this data.

Due to an increase of dissolved organic carbon (DOC) reported in inland waters of the Northern temperate region, Feuchtmayr et al. (2019) attempted to increase the understanding of the relationship between DOC, nutrient inputs, and warming on the inland lake ecosystems. This study found that Chlorophyll-a concentration, which signals phytoplankton concentration, was not significantly altered by warming. Since intermediate organic matter levels significantly increased Chlorophyll-a concentration in summer, autumn, and winter. Cyanobacterial blooms occurring in the summer increased in intermediate organic matter levels and occurred least often in high organic matter levels. Macroinvertebrate abundance increased in spring and summer with intermediate or high organic matter levels, and fish abundance was not affected by organic matter levels. Fish abundance was higher, and peak abundance occurred two weeks later in ambient mesocosms. It is suggested that organic material concentration has a more pronounced effect in shallow lake ecosystems than temperature or phytoplankton concentrations. Cyanobacterial blooms occurring in intermediate levels of organic matter suggest that HABs may increase in clear, shallow, eutrophic lakes and DOC increases (Feuchtmayr et al. 2019).

In addition to cyanobacteria, diatoms are particularly good indicators because they respond quickly to changing conditions and their silica cell walls can be easily preserved for further studies. Due to this, it is possible to tap into a vast historical record of climate and ecosystem data in an area made possible through diatom microfossils. In a study performed by Hazuková et al. (2019) examining the viability of the PDI (Planktonic Diatom Index), it is used to assess the water quality of a body of water using the species composition of diatoms there. The PDI is based on how eutrophication, specifically high phosphorus, affects the population structures of diatoms. Total phosphorus and PDI scores were significantly correlated, but when measured within each basin, the total phosphorus and PDI scores of the Western Basin was not

significantly correlated, suggesting significant algal community differences. This may be due to the Western Basin being a highly variable environment, even though this basin was considered eutrophic (Hazuková et al. 2019).

Kragh and Sand-Jensen (2018) found that phytoplankton biomass was higher in hard waters during summer than it was in soft waters with the same nutrient load. The more eutrophic the lake, the more hard water stimulated algal growth. Hard water lakes tended to be more basic with a pH range of 7.3 to 9.3 which allowed atmospheric CO₂ to enter the water at an increased rate. They proposed that in a nutrient rich environment, inorganic carbon becomes the limiting factor and higher pools of dissolved inorganic carbon support phytoplankton productivity in high pH, hard waters (Kragh and Sand-Jensen 2018).

Lee et al. (2019) set out to understand how the combined effects of warming and eutrophication affect phytoplankton production and the production of algal blooms. These observations occurred in natural bodies of water. Temperature had a negligible effect if the sites were unenriched, but temperature increased phytoplankton production when the sites were enriched. However, when one site had a low salinity of 6.5 PSU, temperature did not increase production. Nitrate concentration to chlorophyll-a concentration (NCCA) had a clear relationship with algal production. When NCCA was at or above NCCA 1.2, the production of phytoplankton increased. Phytoplankton production decreased at or below NCCA 1.2. This data seemed to be corroborated by similar international and other states' data, from the UK, US (California), Estonia, and Norway, where production increased above and decreased below a NCCA of 1.5. It was suggested that NCCA could become an indicator for changes in algal production (Lee et al. 2019). This research shows that temperature variations, which can occur within the same lake,

combined with local nutrient enrichment, has the capability of increasing or decreasing algal production.

5. Predictive modelling

Great strides have been taken in mapping biological concepts including ecosystem services and health hazards. Much of this advancement is due to the recent popularity of predictive modeling. Record et al. (2021) named the incorporation of data into models as a current issue of the data landscape. Yang et al. (2021) applied the Lambert-Beer's law to rivers by creating the C^NANDY (Coupled Complex Algal-Nutrient DYnamics) model. They use phosphorus, a common aquatic pollutant, as the limiting dissolved nutrient due to it being a required nutrient that no algal group can synthesize on their own. They were able to map out phosphorus levels and layer river size as well. Yang et al. (2021) found that benthic algae outcompeted pelagic in small rivers which are shallower and have better light penetration into the benthic zone, and pelagic algae outcompeted benthic algae in larger rivers where there was less light energy penetrating into the benthic zone. Phosphorus increased as the water flowed downstream, likely because the pollutant was draining from the surrounding watershed, into the Weser river (Yang et al. 2021).

One of the directives of the EPA is to create networks of relationships between water quality variables. This allows researchers to better understand the underlying mechanisms and cuts back on the amount of testing that needs to be done. The EPA created a tool for estimating the concentration of microcystin in the water based on the concentration of chlorophyll-a. It is a simplification of the relationship between chlorophyll-a, phytoplankton biovolume, cyanobacterial relative abundance, cyanobacteria biovolume, and microcystin. The effectiveness of this tool varies based on the confidence level. When confidence levels are low, as they often

are as data taken from natural settings, the tool becomes less effective to the point where the predicted microcystin levels could either be below or well above criteria (EPA 2019).

6. Reservoir systems

Lake Sinclair is a reservoir formed by the damming of the Oconee river. After damming, the functions of the river watershed, like support of floodplain productivity, vegetation recruitment sites, and maintenance of riverbank wetlands, were altered. Through interpretation of the Clean Water Act of 1972, the reservoir should retain some of the same ecosystem services as a natural lake. When rivers become reservoirs, sediment that once flowed downstream instead accumulates in reservoir deltas and upstream backwaters. Natural lakes have minor stage fluctuations, but man-made reservoirs often fluctuate between low pool and high pool to support hydropower generation and to control floods. The vertical hydrologic fluctuation of reservoirs creates an unusual aquatic and riparian environment (Rood et al. 2020). Algarte et al. (2009) found that the flood pulse within reservoir systems increased connectivity which in turn increased species richness and changed the composition of periphyton. In addition, the riparian vegetation around reservoirs must tolerate being both submerged and unsubmerged due to the high levels of fluctuations (Rood et al. 2020).

7. Using fluorometers to determine algal biomass

Poikane et al. (2014) defined the ‘good-moderate’ status boundary as 21-23 $\mu\text{g L}^{-1}$ of chlorophyll-a in shallow lakes (<3m) and 10-12 $\mu\text{g L}^{-1}$ of chlorophyll-a in deep lakes (3-15m), but acknowledged that other factors such as hydrology, turbidity, and lake specific chemical composition make it hard to define boundaries across all lakes. Poikane et al. (2014) model was defined for lakes in central Europe. Fluorometers like AlgaeGuard and Benthotorch (Moldaenke BBE, <https://www.bbe-moldaenke.de/>), approximate biomass by exciting pigments related to

taxonomic groups of algae and measuring the fluorescence that excitation emits. Chlorophyll-a, used by all algal groups, is excited by blue light (400-530 nm), and phycobilins which are present in Cyanobacteria and Cryptophyta are excited by green, orange, and red light (550-630 nm). Since chlorophylls and phycobilins are the only pigments to autofluoresce, narrower wavelength ranges are used to further differentiate taxonomic groups by their pigments. Phycocyanin, one of the two phycobilin types, is the main accessory pigment for most cyanobacteria, fluoresces at 640-660 nm. Diatoms contain chlorophylls a and c and carotenoids have higher fluorescence of chlorophyll-a than other chlorophyll-a containing organisms, green algae have chlorophylls a and b and can be differentiated also (Gregor and Marsalek 2005).

8. Preliminary data

Preliminary data from previous research at GCSU (Dr. Manoylov's Aquatic ecology capstone classes, where available 2014-2016) provided algal species richness, species density, and toxicity analyses. This study lasted three months in early spring and established algal species community indices. Eight locations on Lake Sinclair were analyzed for algal community composition and toxicity analyses. Lake Sinclair was chosen because it is used for cooling by a Georgia Power plant, and this created a unique environment for algae. The toxigenic cyanobacteria genera *Microcystis*, *Aphanizomenon*, and *Anabaena* were found in open waters. *M. aeruginosa* was also found in low abundance in all sampling locations. Most samples were dominated by diatoms, likely due to the high ratios of nitrogen to phosphorus present in the spring months. Microcystins varied from 0-5 ppb. No other algal toxins were documented even though *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya & Subba Raju and *Dolichospermum flos-aquae* (Brébisson ex Bornet & Flahault) P.Wacklin, L.Hoffmann & J. Komárek were common and abundant (Manoylov unpublished data).

In another preliminary study (data collected 2010-2012), high turbidity (12-14 NTU) prevented algal blooms in the shallow coves. Algal community composition, nutrients, and temperature did not vary between the bloom and non-bloom years (Manolov and Mutiti, unpublished data). The current research addresses the following aims: determine the effect flow velocity on algal blooms in shallow water columns and develop a better understanding of current relationships between temperature, pH, DO, and algal community composition. This project will test the hypothesis that differences in shallow coves and deep main channels create distinct regions within a reservoir in which algal community dynamics occur independently from one another. The presence of toxigenic cyanobacteria and relative abundance will be documented and related to toxin production.

The EPA uses the relationship between DO and chlorophyll-a, using the easy to measure DO variable to predict algal biomass. DO decreases with increasing chlorophyll-a according to the data the EPA has compiled. A second relationship the EPA discovered is that DO decreases from the 150th day of the year and reaches its lowest level around the 200th day of the year (U.S. EPA 2019).

Four shallow sites and four deep sites were monitored monthly to test the hypothesis that heterogeneity between locations within a reservoir and the likelihood of a harmful algal bloom can be predicted using chlorophyll-a. This study was designed to (1) determine if chlorophyll-a is an accurate predictor of the presence of cyanotoxins, (2) if chlorophyll-a can be related to easily measurable variables like Temperature or DO for bloom predictions, and (3) to determine if heterogeneity between locations within a reservoir produce heterogeneity of algal communities between locations.

III. Methods

1. Sampling sites

Four shallow sites from Lake Sinclair were monitored once a month in triplicate for eleven months (Fig 1). Shallow Site 1 (33° 11' 14" N, 83° 11' 14" W) was a boat launching and mooring area. It was a deeper site than the other shallow sites, and the bottom was unreachable. The boat launch area was asphalt, but samples were always taken either from the dock above the muddy lakebed or beside the wooden retention wall where there were piled rocks. Shallow Site 2 (33° 11' 33"N, 83° 17' 37"W) was a fishing nook across from townhomes. It was the quietest area that saw no boat traffic. Shallow Site 2 had a rocky bottom and orchids growing in the riparian zone. Shallow Site 3 (33° 11' 53"N, 83° 17' 45"W) was a boat launch in an RV park across from the now defunct coal power plant, a legacy research site where thermal pollution was evident (Smith and Manoylov 2013). Shallow Site 3 had a concrete boat ramp where samples were taken. The rest of the cove had a muddy bottom. Sediment collected in the ridges of the boat ramp and visible algae, which was collected as part of the composite samples, attached itself to that sediment during the summer months. Shallow Site 3 also had frequent boat traffic. Shallow site 4 (33° 11' 52" N, 83° 17' 41"W) was located at the boat fueling station at the Chevron gas station. It was also a boat launch site, although it had a mud bottom and saw less launching boat traffic than Shallow Sites 1 or 3. All four shallow sites were off a busy road. Four deep sites were monitored from May to August. Along that area of Lake Sinclair, four-way crossroads appear as residential coves align parallel to each other along the length of the lake. The first of these crossroads as one leaves the Sinclair Marina, was Deep Site 1 (33° 11' 11"N, 83° 16' 37"W). Deep Site 2 (33° 11' 4"N, 83° 15' 4"W) was the second crossroad. Deep Site 3 (33° 11' 53"N, 83° 17' 45"W) is located between the offshore 'Goat island' and the

south shore. Deep Site 4 (33° 8' 36"N, 83° 12' 20"W) was from the Sinclair dam. The deep sites do not experience water level fluctuations, sediment loads, and changes in turbidity, and they are colder than the shallow sites.

2. Sampling procedure

At each shallow and deep site, physical and chemical observations were measured in triplicate via YSI (YSI Incorporate, Yellow Springs, OH): 1. dissolved oxygen (DO), 2. conductivity, 3. water temperature, 4. pH. air temperature, water movement, watercolor, water odor, animal and plant life, surface oils and scum, and weather conditions were recorded before sampling at each site. A composite sample of the water column and around 39 cm² of scrapings from every unique epilithic, epidendric, or epipsammic surface that could be reached at each shallow site. Deep site samples were taken at the surface within the photic zone. Samples were collected in 400 ml glass containers.

3. Biomass measurements

In situ estimation of benthic biomass was conducted prior to sampling at the shallow sites, a Benthotorch (Moldaenke BBE, Schwentinental, Germany), readings of the epilithic (epipsammic in the case of Site 3) algae were taken in triplicate.

More precise algal biomass was measured in the lab, as a pigment assays conducted using AlgaeGuard, manufactured by Moldaenke BBE (version 2.6 E1). From each collected sample of 500ml a representative subsample of 300 ml was kept in the dark and cold for AlgaeGuard analyses. The composite sample was mixed before analysis by AlgaeGuard.

The AlgaeGuard plotted a graph of the relative biomass within the taxonomic groups as it continuously measured, 30ml at a time, displaying results in current time via an integrated computer program. The measurements by AlgaeGuard were as follows: Total chlorophyll-a (µg

Chl-a/L), green algae contribution to total chlorophyll-a ($\mu\text{g Chl-a/L}$), cyanobacteria contribution to total chlorophyll-a ($\mu\text{g Chl-a/L}$), diatoms contribution to total chlorophyll-a ($\mu\text{g Chl-a/L}$), Cryptophyta contribution to total chlorophyll-a ($\mu\text{g Chl-a/L}$), and a yellow substances correction in raman units (r.u.). The plotted graph stabilized, and three measurements were taken of each of the previously mentioned tracked measurements. When the plot settled into a logarithmic curve and an average was recorded of these three measurements to produce the biomass measurement per sample. Between each sample, the AlgaeGuard was allowed to reset with DI water until all the pigments readings read as zero. AlgaeGuard analysis was performed within a month of the sampling date.

4. Microscopy

For algal identification, the GCSU algal taxonomic references library (e.g, Krammer & Lange-Bertalot 2007, Prescott 1962), along with a research grade microscope Leica (Leica DMLB, and a Leica DFC7000 GT Camera (Leica Microsystems, Wetzlar, Germany) was used. Soft algae were preserved with 37% Formaldehyde in a ratio of 2 percent per volume Formaldehyde to 100 ml of sample. 200 ml subsamples were concentrated to 40-50 ml and split into two vials. A vial set aside for soft algae was further concentrated to 5 ml. The entire algal community was enumerated and identified to the lowest taxonomic level possible to document taxonomy and species relative abundance data. Soft algae enumeration was carried out using a glass slide and 22X22 coverslip and a 0.2 ml subsample, using transects and fields of view to estimate area viewed at 400x magnification. If a transect had fewer than 20 algal units or the average field of view had fewer than 5 units, the sample was classified as uncountable. A minimum of 100 algal units were enumerated. This study conducted a total of 576 shallow water and 192 deep water analyses.

5. Data analyses

Species richness, Shannon's index, and species evenness were calculated using count data. Shannon's index is calculated as H in the equation: $H = -\sum p_i \ln(p_i)$ Where p_i is the proportion of each species in a sample. Species evenness is calculated as $E = H/\ln(S)$ where H is Shannon's index and S is the total number of unique species in a sample. Samples analyzed with a minimum of 100 algal unit counts starting with June 2020. Uncountable samples were omitted in analyses of Shannon's index and species evenness.

During analysis, biomass data from Two-factor ANOVA without replication was performed on DO, conductivity, pH, water temperature, total chlorophyll-a, green algae biomass, cyanobacteria biomass, cryptophyte biomass, diatom biomass, and yellow substances. The Pearson correlation coefficient was applied to physical, chemical, and pigment assay data. A two-way analysis of variance (ANOVA) was used to assess the significant differences among variables and to assess the significance of their potential interaction. Both Benthotorch and AlgaeGuard biomass data were analyzed with two-factor ANOVA without replication analyses to determine if the datasets between months and sites had significant difference. Boxplots were created considering all measurements for a given site across all months to compare sites (Figs 16-18). Linear regression analysis was performed of every combination of AlgaeGuard analyses, species indices, and physical and chemical data to develop a correlation table (Table 2).

Kruskal-Wallis test and graphs (Systat 13, 2017 Systat Software, Inc.) was performed on all physicochemical water parameter data 2008-2012 for the same locations and 2020 to assess comparisons between the two habitats. This includes data that was collected by Manoylov lab previous research, Georgia Power's available data, and data collected using YSI (conductivity, temperature, DO, pH) as part of this study. Pearson correlation coefficient (r) was performed

on all physicochemical water parameter data and biological data to assess relationships between algal community traits and shallow vs deep lake environments ($r>0.5$ at $p<0.001$ was considered significant).

IV. Results

1. Physical and chemical characteristics

Dissolved oxygen (DO) values were above 5 mg/L in 94.64% of sites sampled (Figs 2 & 3). DO in November was above 8.6 in all sites. In January, DO was lowest at 7.4 mg/L in site 3. The DO of Sites 1-3 in February were between 8 and 8.5 whereas site 4 was higher at 9.49. April DO of sites 1 and 2 were 7 mg/L, Site 3 had the highest DO at 8.18 mg/L, and site 4 had the lowest of 6.3 mg/L. May DO ranged between 6.38 at site 1 and 7.92 at site 8. There was no significant difference between shallow and deep sites in DO during the month of May. June DO is lower at the shallow sites than at the deep sites. Site 1 has the lowest DO. Of the deep sites in June, site 5 had the lowest DO and was close to the level of the shallow sites. In July, site 3 was only slightly above 5 mg/L at 5.2 mg/L while the rest of the sites were in the 6-8 mg/L range. DO in August was lower than 5 mg/L at sites 2,3, and 6. Sites 5 and 7 have DO values of 5.08 and 5.14 mg/L respectively. September DO ran between 5.8 mg/L at site 4 and 8.3 mg/L at site 1. October DO ran between 5.92 mg/L at site 3 and 7.54 at site 1. For all samples DO was significantly different through time (ANOVA, $F_{3,21}=10.27$, $p<0.001$, $F\text{-crit}=3.07$) and was significantly different based on location depth (ANOVA, $F_{7,21}=1.98$, $p=0.02$, $F\text{-crit}=2.49$); In shallow sites, DO increased in colder months (ANOVA $F_{9,27}=11.42$, $p<0.001$, $F\text{-crit}=2.25$), but was not different based on location depths (ANOVA, $F_{3,27}=0.64$, $p=0.60$, $F\text{-crit}=2.96$).

The water temperature in November ran from 14.5 °C to 15.5 °C (Fig 4). The highest November water temperature was at site 4 and the lowest at site 3. Site 2 was the warmest spot in January, at around 15 °C, and Site 3 was the coldest spot, at 12.64 °C, followed closely by site 4. In February, sites 1 and 2 were around the same temperature, at just above 12 °C, and site 3 was the warmest, at 14.85°C. The coldest site of April was site 1, at 20.66 °C, and the warmest was site 4, at 22.8°C. May water temperature stayed between 22 and 27 °C with the highest water temperature, being 26.57 °C at site 3. Sites 7 and 8 had the highest water temperatures in June, at 28.1 °C and the lowest water temperature was at site 4 at 26.87 °C. In July, all the water temperature readings at shallow sites were above 33 °C (Fig 5). In August, all water temperature readings were above 30 °C, with the highest temperatures recorded at sites 1 and 4, 32.77 °C and 32. 63 °C respectively. Site 4 is the warmest site in September at 25.2 °C, while site 2 is the coldest at 24.5°C. In October, water temperature is the lowest at site 3 with 23.16 °C, and the highest at site 1 with 23.5 °C. Water temperature changed significantly following seasonal changes for all sites, June-August through time (ANOVA, $F_{3,21}=96.08$, $p<0.001$, $F\text{-crit}=3.07$) but did not differ with depth (ANOVA, $F_{7,21}=2.48$, $p=0.5$, $F\text{-crit}=2.49$). In shallow sites, temperature decreased significantly compared to deep sites (ANOVA, $F_{9,27}=285.71$, $p<0.001$, $F\text{-crit}=2.25$) but was not different between the 4 shallow sites (ANOVA, $F_{3,27}=0.95$, $p=0.43$, $F\text{-crit}=2.96$).

Conductivity in November was consistently between 67 and 68, except for site 3 which was 64.7 (Fig 6). In January, sites 1 and 2 were around 55 and sites 3 and 4 were 80 and 76 respectively. Sites 1 and 2 were around 40 and Sites 3 and 4 were just above 60 in February. April sites 1 and 2 were between 56 and 60 and Sites 3 and 4 were around 50. Conductivity in May was between 50 and 55 for all sites except 6 which had a conductivity of 44.2. June conductivity ranged between 61 and 65. July conductivity ranged between 62.8 at both sites 7

and 8 and 74.8 at site 2 , but was not different based on site depth (ANOVA, $F_{3,27}=2.26$, $p=0.1$, $F_{\text{crit}}=2.96$, Fig 7). Conductivity in August was significantly higher in the shallow sites than in the deep sites (ANOVA, $F_{9,27}=7.51$, $p<0.001$, $F_{\text{crit}}=2.25$). The highest was at site 1 at 74.6 and the lowest was at site 8 which was 69.36. September conductivity ranged between Site 1, at 65.33, and Site 3, at 69.53. October conductivity ranged between 64.1 at site 1 and 70.5 at site 3. Conductivity was significantly different through time, June-August (ANOVA, $F_{3,21}=112.84$, P $p<0.001$, $F_{\text{crit}}=3.07$), and significantly different in shallow versus deep sites (ANOVA, $F_{7,21}=3.7$, $p=0.009$, $F_{\text{crit}}=2.49$).

The pH in most sampling sites was basic (Figs 8 & 9). In November, Site 2 had a pH of 8.69. Sites 1 and 3 were close to being over 8.5 with some of the replicant pH values being over 8.5. The average of site 1 was 8.47 and site 3 was 8.45. Site 4 was also on the higher end with 8.17. January had the highest recorded pH values with Site 3 having 9.32 and site 4 having 9.05. Site 1 had 8.86. Site 2 had the lowest pH with 8.29. In a change of direction from most other months, February was quite acidic. Site 1 was at 3.78, site 2 was at 5.27, site 4 was at 6.03, and site 3 was at 6.26. The pH in April and May ranged from 7.2 to 7.5. In June, sites 6 and 7 surpassed 8.5 pH. Site 6 with an average of 8.58 and Site 7 at 8.92. Site 8 had a pH of 8.08. The rest of the sites ran between site 1 at 7.04 and site 3 at 7.35. In July, sites 7 and 8 are the most basic at 8.77 and 8.87 respectively (Fig 9). Site 3 has the next highest pH at 8.06 with sites 2, 1, and 4 closely following at 7.92, 7.9, and 7.87 respectively. Site 5 has the lowest pH at 7.34. August pH was highest at site 4 at 8.62 and lowest at site 6 at 7.03. The second highest pH value in August was 7.9 at site 3. September pH varied between 7.83 at site 2 and 9.28 at site 3. October pH ranged from 7.24 at site 2 to 7.73 at site 3. pH was not different between sites 1-8, June-August (ANOVA, $F_{3,21}=2.60$, $p=0.08$, $F_{\text{crit}}=3.07$), or months (ANOVA, $F_{7,21}=1.04$, $p=0.43$,

F-crit=2.49). Shallow sites were significantly different through time, November-October (ANOVA, $F_{7,21}=22.07$, $p<0.001$., F-crit=2.25), and between sites (ANOVA, $F_{3,21}=3.17$, $p=0.04$, F-crit=2.96). DO, Conductivity, and pH had an even spread of data across all sites according to boxplots (Fig 18). Water temperature had a smaller spread of data and was slightly higher in deeper sites compared to shallow sites according to box plots.

2. Algal biomass

Algal biomass measured with total Chlorophyll-a (Fig 10) was highest in August, especially in site 1 where total chlorophyll-a was 487.4 $\mu\text{g/L}$ in one sample. Site 4 had readings over 100 $\mu\text{g/L}$ and site 2 had readings over 50 $\mu\text{g/L}$. October had the second highest biomass, where site 2 had over 100 $\mu\text{g/L}$ and site 3 and 4 had over 50 $\mu\text{g/L}$. Site 1 also had the highest biomass measured with total chlorophyll-a in September, where total chlorophyll-a at the site was over 50 $\mu\text{g/L}$. Shallow sites had similar biomass through time (ANOVA: $F_{4,12}=2.52$, $p=0.10$, F-crit=3.26) and there was no difference between sites with variable human alterations (ANOVA: $F_{3,12}=1.68$, $p=0.22$, F-crit=3.49). Similarly, biomass was not significantly different through time (ANOVA: $F_{2,14}=2.51$, $p=0.12$, F-crit=3.74) or between shallow versus deep sites (ANOVA: $F_{7,14}=2.08$, $p=0.12$, F-crit=2.76).

AlgaeGuard measured the relative biomass of composite samples and showed similarly high biomass readings in August, September and October (Figs 11 & 12). Site 1 showed the highest biomass in August and September and was dominated by cyanobacteria followed by cryptophytes in August and diatoms and cyanobacteria in September. Site 4 in August also showed a green algae reading over 50 $\mu\text{g/L}$. Site 2 in October had the highest biomass readings which were dominated by diatoms followed by Cryptophyta. There was a relatively even biomass distribution of Cyanophyta, Chlorophyta, Cryptophyta, and yellow substances across all

sites, but with a wider spread of data usually present in the shallow areas (Fig 17). Total Chlorophyll-a was strongly correlated with cyanobacteria ($r^2=0.86$) and Cryptophyta ($r^2=0.76$) (Table 2). Cyanophyta was correlated with Cryptophyta ($r^2=0.75$) due to sharing of phycobilin accessory pigments. Green algae were not different through time, June-October (ANOVA, $F_{4,12}=1.26$, $p=0.34$, $F\text{-crit}=3.26$), or sites (ANOVA, $F_{3,12}=1.08$, $p=0.4$, $F\text{-crit}=3.49$); cyanobacteria also did not differ in June-October (ANOVA, $F_{4,12}=1.45$, $p=0.28$, $F\text{-crit}=3.26$), or sites (ANOVA, $F_{3,12}=1.78$, $p=0.2$, $F\text{-crit}=3.49$). Diatoms concentrations were significantly different through time in June-October (ANOVA, $F_{4,12}=4.65$, $p=0.02$, $F\text{-crit}=3.26$), but not between sites (ANOVA, $F_{3,12}=1.18$, $p=0.4$, $F\text{-crit}=3.49$). Diatoms concentrations, which did not significantly differ between June-August (ANOVA, $F_{2,14}=1.62$, $p=0.23$, $F\text{-crit}=3.74$), differed significantly with site depth (ANOVA, $F_{7,14}=4.23$, $p=0.01$, $F\text{-crit}=2.76$). Cryptophytes did not differ significantly through time June-August (ANOVA, $F_{4,12}=0.79$, $p=0.55$, $F\text{-crit}=3.26$), sites 1-4 (ANOVA, $F_{3,12}=0.91$, $p=0.46$, $F\text{-crit}=3.49$), June-October (ANOVA, $F_{2,14}=1.10$, $p=0.36$, $F\text{-crit}=3.74$), or sites 1-8 ($F_{7,14}=1.38$, $p=0.29$, $F\text{-crit}=2.76$).

The in situ Epilithic algae biomass distribution obtained from the Benthotorch measurements showed frequent domination by cyanobacteria and diatoms (Figs 13-15). Cyanobacteria biomass dominates: sites 1, 2, and 4 in April; the same sites in May; sites 1 and 2 in September; and site 2 in October. Diatom biomass dominates: sites 1, 2, and 4 in June; all sites in July; sites 1 and 2 in August; and site 1 in October. Green algae only dominate the epilithic biomass of site 3 in June and site 4 in August. ANOVA without replication for epilithic relative biomass results were as follows: cyanobacteria were not significant through time (ANOVA, $F_{6,18}=1.89$, $p=0.14$, $F\text{-crit}=2.66$), but was significantly higher in shallow sites (ANOVA, $F_{3,18}=3.86$, $p=0.03$, $F\text{-crit}=3.16$); green algae were not significant through time (ANOVA, $F_{6,18}=1.04$, $p=0.43$,

F-crit=2.66), or location (ANOVA, $F_{3,18}=0.8$, $p=0.51$, F-crit=3.16); and diatoms were not significant through time (ANOVA, $F_{6,18}=1.37$, $p=0.28$, F-crit=2.66), but were significantly higher in deep sites (ANOVA, $F_{3,18}=4.74$, $p=0.01$, F-crit=3.16).

For shallow site, the in situ epilithic algae biomass distribution obtained from the Benthotorch measurements showed frequent domination by cyanobacteria and diatoms (ANOVA, $F_{3,18}=3.86$, $p=0.03$, F-crit=3.16, Figs 12-14). Cyanobacteria dominated sites 1, 2, and 4 in April and May; sites 1 and 2 in September; and site 2 in October, but Cyanobacteria contribution to total chlorophyll-a was not different (ANOVA, $F_{6,18}=1.89$, $p=0.14$, F-crit=2.66). Diatom were dominant in sites 1, 2, and 4 in June; all sites in July; sites 1 and 2 in August; and site 1 in October. Green algae only dominated site 3 in June and site 4 in August. Epilithic green algal biomass was not significantly different between months (ANOVA, $F_{6,18}=1.04$, $p=0.43$, F-crit=2.66), or location (ANOVA, $F_{3,18}=0.8$, $p=0.51$, F-crit=3.16). Diatoms were abundant in sites 1, 2, and 4 in June; all sites in July; sites 1 and 2 in August; and site 1 in October, but not significantly different through time (ANOVA, $F_{6,18}=1.37$, $p=0.28$, F-crit=2.66). Diatoms were significantly more abundant in shallow sites (ANOVA, $F_{3,18}=4.74$, $p=0.01$, F-crit=3.16).

Lake Sinclair historically (2014 and 2020) had lower levels of chlorophyll-a until recently (Figs 19-21). Chlorophyll-a levels were significantly higher in shallow sites than in deep sites (ANOVA, $F_{1,295}=23.405$, $p<0.01$, $R^2=0.28$, Fig 22). U.S. EPA suggested that states test the possibility of linking easily measurable variables (like DO) with Chlorophyll-a measured around 175-200 days (recommend unified sampling—day of every year for states) of the year to predict potential algal blooms and toxicity (U.S. EPA 2021). Lake Sinclair historically had lower levels of chlorophyll-a until recently, 2014 and 2020 (Figs 19-21). Chlorophyll-a levels were significantly higher in shallow sites than in deep sites (ANOVA, $F_{1,295}=23.405$, $p<0.01$, $R^2=0.28$,

Fig 22). U.S. Depth-averaged Dissolved oxygen (DO) measured at 2 mg/L at chlorophyll-a of 10 µg/L that kept decreasing with increased chlorophyll-a. DO in Lake Sinclair had no relationship with either chlorophyll-a ($p>0.05$, Fig 23) or day of the year ($p>0.05$, Fig 24).

3. Algal community

Sites were dominated by chain-forming centric and pennate diatoms, green algae, and cyanobacteria. Of the 180 samples collected, only 55 samples had high algal density. 34 species appear in more than 10 samples. Of those 34 species, 17 are diatoms, 12 are green algae, 3 are Cyanophyta, and 2 are Dinoflagellata. Species richness was greater in shallow sites compared to deep sites while species evenness and Shannon index did not differ (Fig 16). diatoms accounted for most of the species richness but were not always the most dominant. As expected, based on the Pearson correlation coefficient test, Shannon's index was correlated with species evenness ($r^2=0.76$) and with species richness ($r^2=0.55$).

Bacillariophyta, Cyanophyta, Chlorophyta, Dinoflagellata, Euglenophyta, and Xanthophyta were present in the taxa Lake Sinclair. Diatoms, cyanobacteria, and Chlorophyta were found in 31%, 30%, and 30.5% of samples respectively. Cyanobacteria genera capable of producing toxins such as *Aphanocapsa* (Plate 1 A-E, G), *Cylindrospermopsis* (Plate 1 F), *Microcystis* (Plate 1 H-I), *Planktothrix* (Plate 2 A-B, F-G), *Anabaena* (Plate 2 C, H), and *Oscillatoria* (Plate 2 E, I) were present in 28% of shallow and 22.9% of deep sites. Toxigenic cyanobacteria was found in many of the sites, but the only time a species of toxigenic cyanobacteria surpassed a 10% proportion of a sample was in June when one in one of the triplicates of site 5, a deep site, had *Aphanocapsa* sp. 6 (Plate 1 G) account for 29.41% of the total population of algae. Cymbelloid diatoms (Plate 3 A, C, E, F, H, J) were found in 18.66% of sites and were not documented in deep sites. Centric diatoms (Plate 3 D, F), and desmids green

algae (Plate 5 A-B, D-E, J, L, P, S) were found in 21.66% and 30.56% of sites respectively. Chain-forming Diatoms, chain forming representatives of *Aulacoseira* (Plate 4 A-B, G) or *Fragilaria* (Plate 4 C, E, F), were present in 26.7% of sites. Dinoflagellata of the genera *Peridinium* (Plate 5 I) and *Parvodinium* species *P. inconspicuum*, Plate 5 O) were found in 17.22% of sites. Euglenophyta, Notably *Euglena* (Plate 5 T) and *Trachelomonas* (Plate 5 M), were found in 15.56% of sites. Synurophyceae were present in 0.56% of sites, and the genus present was *Mallomonas* (Plate 5 M). Xanthophytes were present in 4.4% of sites.

V. Discussion

Algal biomass, measured as Chlorophyll-a, was not a good predictor of toxic algal bloom on Lake Sinclair, Georgia for the period of this study. The EPA tool predicting microcystin levels related to levels of chlorophyll-a was tested for the first time in a man-made reservoir during this study. Although chlorophyll-a levels were high (above Poikane et al. (2014) and Georgia DNR thresholds of 24 µg/L and 14 µg/L as averages or single measurements), no blooms or cell concentrations above 10,000-15,000 cell/ml were recorded. Without dense population numbers, cyanobacteria will not produce toxins (Pearl et al. 2013).

The nearshore regions of lakes are directly connected to the upland watershed. As a result, nearshore environments are influenced by land-use change, pollutant loading, erosion, recreation and resource overuse, all of which can lead to an increase of nutrients in coves (Beeton 2002). Biomass in the shallow sites of Lake Sinclair was 8.9 times higher compared to deep sites. Algal species capable of producing toxins occurred in 88% of countable sites, although not in high concentrations to induce toxin production. Of shallow sites, 28% percent of had toxigenic cyanobacteria genera compared to 22.9% of deep sites. Chain forming

representatives of genus *Fragilaria* dominated shallow sites, and coccoid green like *Golenkinia* sp. (Plate 5 K) dominated deep sites.

1. Physical and chemical water quality parameters

Depth-averaged Dissolved oxygen (DO) measured at 2 mg/L chlorophyll-a of 10 µg/L and kept decreasing with increased chlorophyll-a levels. DO in Lake Sinclair was not significantly correlated with either chlorophyll-a ($p>0.05$, Fig 23) or day of the year ($p>0.05$, Fig 24). Poikane et al. (2014) data looked at shallow and deep lakes in Europe. In Georgia, USA, Lake Sinclair is a unique aquatic habitat where, in the same system, both shallow and deep sites can be seen, and results from this study suggest a chlorophyll-a and bloom relationship. In this study, the 25th percentile of sites had primary producer biomass of above 39 µg/L Chlorophyll-a and possible ecosystem alterations like algal blooms. Probability of a bloom and cyanobacteria dominance were possible in all shallow sites. No blooms or algal toxins were detected. A possible reason for the lack might be the high abundance of the invasive macrophyte Hydrilla. Turbidity of Lake Sinclair is constantly changing due to the dam.

The Georgia Department of Natural Resources states that Lake Sinclair should have a DO “average of 5 mg/L and no less than 4 mg/L” (Chapter 5 of Title 12 of the Official Code of Georgia Annotated). The lowest DO of all samples was 4.12 mg/L in August, site 3. Leaf litter and decaying macrophytes often accumulate and generate anoxic conditions. Though August had the most algal biomass within the water column, the decomposition of which by aerobic bacteria may decrease oxygen in the water, Chlorophyll-a in August at site 3 was relatively low compared to other samples (Fig 10). Špoljar et al. (2017) found that increased flow velocities increased DO and macrophyte growth and proposed hydraulic treatment to regulate macrophyte coverage in shallow reservoirs in response to these results. Based on models, DO was expected to decrease

with increase in chlorophyll-a (U.S. EPA 2019). In this study, DO was found to be unrelated to both chlorophyll-a or day of the year in Lake Sinclair. DO never went below 4µg/L. Mixing and community composition may explain the lack of a relationship between DO and Chlorophyll-a.

Georgia DNR requires Lake Sinclair's water temperature measurements to be below 32.22°C (Chapter 5 of Title 12 of the Official Code of Georgia Annotated). In July and August, that temperature limit is surpassed 6 times: in July all shallow sites were above 32 C; and August, site 4, 32.63°C. Water temperature was significantly different among months, but not significantly different among shallow and deep sites.

There were no conductivity standards presented for Lake Sinclair by Georgia DNR. The conductivity values remained similar through time and differed with depth, but remained consistent with freshwater conductivity values (ranging from 40 to 80). It should be noted that it was difficult to get a stable reading of conductivity at site 3 which was near a power plant and directly below power lines on all occasions when site 3 was sampled.

Water pH did not differ through time but was significantly higher in shallow sites. Georgia DNR considers pH as high as 9.5 as regionally acceptable. None of the samples in this study surpassed that level (Chapter 5 of Title 12 of the Official Code of Georgia Annotated). Of concern, however, are the pH values below 6. Site 1 and site 2 in February are below the lower pH threshold with respective pH values of 3.78 and 5.27. These values were also a major shift from the previous month's site 1 and 2 values of 8.87 and 8.29. Both January and February samples had too few algae units to enumerate, so no algal community change was attributable to this major shift from basic to acidic water. A previous study on Lake Sinclair conducted in 2008 reported a much more neutral average pH of 7.04 with a pH range of 6.82-7.26 (Smith and Manoylov 2013).

2. Boat traffic

Conservation issues resulting from cumulative effects of dams involve declines in native freshwater biodiversity at regional scales (Cantonati et al. 2020). Many large rivers have been transformed into a series of reservoirs (dominated by introduced exotic algal species) (Anderson et al. 2021) connected by highly regulated flows that contain remnant populations of native riverine species. For example, of the 51.2 million km of streams in the lower 48 USA states, only 2% of the rivers remain free-flowing and are relatively undeveloped. Only 42 free-flowing rivers exist that are more than 200 km or more in length (Pringle et al. 2000). This research contributes to the understanding of algal communities within man-made aquatic habitats that is largely missing from the literature.

Three of the four shallow sites tested, site 1, 3, and 4 were common areas to dock, launch, or moor boats. Site 1 and site 3 were dock and launch sites and site 4 was a boat gas station. These areas saw heavy boat traffic, whereas site 2 was tucked away across from a residential area and did not see as much boat traffic. The boat traffic of the sites could have influenced the species distribution in those areas. Lake Sinclair is a popular location for recreation, and there was boat traffic in the main channel where deep sites 5-8 were sampled. Site 5 was just outside of Sinclair Marina and likely saw the most boat traffic of the deep sites.

Turbulence produced by boats tear up vegetation holding sediment in place while stirring up the sediment. The suspended sediment shades out photosynthetic organisms. Fine silt and organic matter, like that at the bottom of Lake Sinclair, are easier to stir up and spend a longer time in the water column compared to coarser sediment types (Sagerman et al. 2019). The shallow areas are where these effects of this study were most visible where increased disturbance was highly possible. Oil spillage and litter are also factors to consider when looking at how boat

traffic affects the ecosystem (Mohammed 2018). Boating is a source of lead and zinc contamination in sediment as well (Gopal et al. 2017).

It is unknown whether the coronavirus pandemic of 2020 changed boating habits of Lake Sinclair residents during this study. It is possible that residents drove their boats more often as it is an activity that does not require contact with other households.

3. Chlorophyll-a

Chlorophyll-a is a photosynthetic pigment found in algae (Wetzel 2001) and other green plants. The concentration of chlorophyll-a, therefore, is commonly used as a measure of the density (biomass) of the algal population in a lake or reservoir. Chlorophyll-a concentrations are generally highest during summer when algal populations are highest. Moderate populations of desirable algae are important in the food chain; however, excessive populations or algal blooms are undesirable. Algal blooms can cause taste and odor problems and limit light penetration needed to support the growth of submerged aquatic plants. Certain species of blue-green algae (cyanobacteria) can produce toxins.

Harmful algal blooms are detrimental to the health of community members who live and recreate on the bodies of water they affect. Neurotoxins, hepatotoxins, and carcinogens produced by algae can make contact with the public through skin, through consumption of affected fish, and sometimes through the air. Algal toxins have been known to make pets ill, sometimes fatally, and are implicated in some human hospitalizations as well. Harmful algal blooms are also detrimental to the economy of a community, as the unsightly, odorous, and dangerous blooms drive tourists, anglers, and prospective lake house owners away. The results of this project may be used to develop more geographically accurate monitoring protocols, which would allow communities to enact targeted bloom advisories, thus limiting the use of affected areas of a lake.

There are two chlorophyll-a boundaries considered. The first is presented for Lake Sinclair by Georgia DNR as not to exceed 10 µg/L upstream from the dam (site 8) and 14 µg/L at all other sites (Chapter 5 of Title 12 of the Official Code of Georgia Annotated). It is specified that these readings must be from photic zone samples, so this boundary only can apply to deep site samples. Only site 7, a deep site in July, which was 24.69 µg/L, surpassed this threshold. Despite being composite samples, June's shallow site 2, July's shallow sites 2-4, and August's shallow site 3 are below the threshold.

The second chlorophyll-a boundary considered was Poikane et al. (2014) where the good-moderate boundary is 22 µg/L for shallow water and 10 µg/L for deep water. 8.33% of deep samples (July – site 7) and 55% of composite samples (June - site 1; July - site 1; August - site 1,2, and 4; September - site 1 and 2; and October - all sites) were above the limit of algal biomass creating conditions for algal blooms and toxicity. According to historical data from Lake Sinclair, chlorophyll-a levels did not surpass Poikane et al's good-moderate boundary for shallow sites until 2014 (Figs 19-21). The good-moderate boundary for deep sites was never surpassed. According to the EPA tool predicting microcystin levels from chlorophyll-a measurements, there is a 50% probability of toxin production above 38.7µg/L of chlorophyll-a. Chlorophyll-a surpassed 38.7µg/L after 2014 as well. There were a few outlier samples with high algal biomass which were driving the high chlorophyll-a levels (Fig 20). The highest algal biomass occurred in August (Fig10). Despite the surpassing of these boundaries there were no blooms recorded during the time of this study, due to the diverse algal community composition and primary production dominated by diatoms. Other literature suggests that regional differences play a factor in the efficacy of using chlorophyll-a to determine presence of toxins. Downing et al. (2001) reported a strong relationship between chlorophyll-a and risk of cyanobacteria

dominance using research from Iowa and Kansas. A study of two lakes in California by Howard et al. in 2021 found varying results where one lake had high chlorophyll-a and high toxin levels, and the other lake had low chlorophyll-a levels yet still had high toxin levels. The two-factor ANOVA test run on Chlorophyll-a revealed that there was no significant difference of Chlorophyll-a among sites or months.

Data from Poikane et al. 2014 and the data from this study was used to model the predictive power of Chlorophyll-a. Based on chlorophyll-a documented values in this study, predictive ability was high in shallow sites. Shallow sites were dominated by invasive macrophyte (Hydrilla) that took up nutrients and prevented toxigenic cyanobacteria growth and probability of toxins. In deep sites the probability of toxins production was low due to different algal composition dominated by diatoms. Available nutrients in shallow sites were low, temperatures were high for temperature, DO stayed high in shallow and deep sites and was not a good predictor of phytoplankton abundance or cyanobacterial dominance, Blooms for both shallow and deep sites were possible, but in diurnal alterations of lake hydrology were not observed.

There are some problems associated with this method of approximating algal biomass. The biggest problem is that excitation light can sometimes not penetrate large colonies, especially colonies of cyanobacteria due to the weak fluorescence of cyanobacterial chlorophyll-a. Samples with low density of algae and high numbers of species experience more variability in chlorophyll-a fluorescence between samples (Gregor and Marsalek 2005). Colored dissolved organic matter can generate background fluorescence that mimics Chlorophyll-a (Cremella et al. 2018). Chlorophyll-a is still widely used as an analog for algal biomass as it is less time intensive

than calculating algal biomass using cell biovolume and density (that requires species level identification using taxonomic literature and research grade microscopy).

4. Algal community analyses

Species richness for both shallow and deep sites. Species richness was significantly higher in shallow sites. Shannon Diversity and Species evenness were not significantly different and indicated a high degree of evenness between sites. There was a marked difference between the algal biomass of the winter months and summer months. From November to May, the concentration of algal cells was too low to enumerate. Much of this, however, was due to the shift in sample type from water column to composite sample which occurred in May. Water column samples were too low concentration to be countable, thus the change to composite samples was made to ensure some documentation of the algal community could be made.

ANOVA two-factor tests on algal biomass data revealed that there was no significant difference between sites or months with regards to relative biomass of Chlorophyta, Cyanophyta, and Cryptophyta. There was a significant difference in Diatom biomass in the summer months between sites when the deep sites were added and among months when testing June-October with just sites 1-4. Among the biomass of epilithic algae, there was a significant difference between relative biomass at sites with regards to Cyanophyta and diatoms, but not Chlorophyta.

Despite the absence of Cryptophyta specimens found in samples, there was presence of Cryptophyta shown in the AlgaeGuard data. The pigment assays use phycobilins to measure the relative biomass of Cryptophyta, which are also used to measure cyanobacteria, and both are excited by green, orange, and red light (550-630 nm) (Gregor and Marsalek 2005). The biomass of cyanobacteria breaking down may be confusing the sensors into attributing some of the phycobilin fluorescence to Cryptophyta representatives. The difference between algal biomass

between samples being insignificant in the composite samples suggests that planktonic algae are well dispersed over the sites tested. The algal biomass of the epilithic community was significantly different among sites, suggesting that the stationary algae, with less ability to disperse, is the most influenced by the natural and anthropogenic influences at each site.

Even with the addition of soft algae in this study, compared to the algal community reported in Lake Sinclair by Smith and Manoylov from 2008-2009, there were far fewer instances where a species was over 25% dominant in samples (6 in this study compared to 23 in Smith and Manoylov (2013). *Achnantheidium minutissimum*, which was over 25% dominant in 2013 (Smith and Manoylov 2013), was not dominant in the current study. Was found in low abundance in 31 samples. *Aphanocapsa* sp. 5 (Plate 1 G), *Golenkinia* sp. (Plate 5 K), *Pseudstaurosira* sp. 1 (chain forming, Plate 4 D), and *Fragilaria* sp. 2 (Plate 4 E) were over 25% in relative abundance in June deep site 1. *Fragilaria* chains (Plate 4) were abundant over 25% in two shallow samples.

5. Invasive macrophyte *Hydrilla verticillata* effects

Hydrilla verticillata (Linn. f.) Royle was present at sites 1-4 from July through October. A methanol extract of *Hydrilla verticillata* was shown by Zhang et al. (2012) to irreversibly damage algal cell membranes. The extract produced O₂⁻ radicals which oxidatively damaged cell membranes and caused the release of proteins and DNA. This, Zhang et al. (2012) hypothesized that the invasive macrophyte growth would terminate an algal bloom. According to the Zhang et al. (2012) experiment which tested the methanol extract on *Dolichospermum flos aquae* (Brébisson ex Bornet & Flahault) P.Wacklin, L.Hoffmann & J.Komárek and *Auxenochlorella pyrenoidosa* (H.Chick) Molinari & Calvo-Pérez which used to be in genus *Chlorella* cyanobacteria were more sensitive to the extract.

However, high amounts of macrophytes in the water will also increase species richness of algae especially when compounded with high water levels. This is because macrophytes provide more substrate for an epiphytic alga to attach to. Macrophytes particularly increase the amount of *Zygnematophyceae* and *Chlorophyceae* (Algarte et al. 2009).

A toxin producing cyanobacterium species called *Aetokthonos hydrillicola* S.B. Wilde & J.R. Johansen which grows epiphytic on *Hydrilla* is linked to Vacuolar myelinopathy, a disease which vacuolizes white matter in the brains of wildlife, particularly in bald eagles and other waterfowl. This disease is caused by a cyanotoxin called aetokthonotoxin (AETX) toxin, produced by *A. hydrillicola*. AETX is only produced when elevated bromide from geologic and anthropogenic sources (e.g., water treatment, power plants, and from spraying chemicals e.g. diquat dibromide used to remove *Hydrilla*) was available to the algae (Breinlinger et al. 2021). *A. hydrillicola* was not documented in this study, though it is possible that the taxon is present as *Hydrilla* made up only a small proportion of the composite samples.

6. Using biological traits in addition to taxonomy to garner further insights

Taxonomy was analyzed in this study to determine the functioning of Lake Sinclair as an aquatic ecosystem, but there is another route one could take in that regard. Biological traits, particularly those which relate to environmental tolerances, may be used to indicate how well an ecosystem is functioning. In 2021, Miatta et al. described five uses of biological traits: (1) to gather information about the “sensitivity of species and communities to disturbance”, (2) to use the same information on sensitivity to predict extinction risk of species, even if not yet threatened, (3) traits which relate to resilience signal the difficulty or ease of which ecosystems recover after a disturbance, (4) conservationists can use the function of biological traits to locate

the most important areas for conservation which incorporate the most functions and ecosystem services, and finally, (5) indicators may be generalized based on biological traits.

When using algae as biological indicators, usually they must be identified to the species level. That does not mean that it is impossible to use sensitivity as Miatta et al. (2021) describe. In fact, there are several instances in this study where physical and chemical properties of the water are above and below what is accepted by the EPA. The issue with using the findings of this study to construct biological indicators based on traits is that the EPA standards for DO, water temperature, pH, and conductivity were not constructed with the preferences of algae in mind, nor was this study designed to test the sensitivity of algae. pH across samples were frequently high. With no significant difference between the groups in both chlorophyll-a and pH, it seems as if the algae present are not sensitive to the high pH. The second and fourth uses of biological traits could be useful to aquatic biologists because microorganisms are not often used in discussions about threatened species or conservation. Algae do, however, serve many of the functions outlined as important by Miatta et al. (2021) including energy and elemental cycling, food supply, productivity, and to a certain extent, habitat creation and modification of physical processes. Algae are essential movers and shakers of any aquatic habitat.

In recovering after a disturbance, traits such as those listed by Miatta et al. (2021): reproduction method, mobility, attachment type, body design, water column migration and horizontal migration, become more important. Ability to produce nearly indestructible reproductive units such as akinetes allow certain kinds of algae populations to go dormant in stressful environments until the habitat becomes hospitable again. Attached algae can resist sudden periods of high flow while ability to migrate in the water column or horizontally allows some species to seek out limiting factors like sunlight.

The fifth point refers to how survival in an ecosystem filters for traits required to survive that ecosystem. These are response guild concepts which allow generalizations across the system without species specific information (Lozanovska et al. 2018). In the example of the riparian response guild concept, species in a riparian ecosystem share hydro-topographic positions and hydrogeomorphic requirements (Merritt et al. 2010). Creating a similar response guild concept for algae, one might think back to Yang et al. (2021) C-ANDY model described above. The aquatic ecosystem will filter algal traits first by their ability to deal with limited nutrients at their respective light levels at their location. Additionally, the water level fluctuations of reservoirs would filter attached algae at the shallow sites by how well they survive periods of desiccation much like how riparian plants are similarly filtered out around a reservoir (Rood et al. 2020). Water from Lake Sinclair is pumped back into Lake Oconee daily. The water intake of dams are usually deep to prevent drought from influencing energy output. During months when stratification is present, cold, hypoxic water is taken from deeper parts of the reservoir and brought to the surface, impacting thermal and low oxygen sensitive species downstream of the dam. The flow of a river slows upon the approach of the reservoir and with it, suspended sediments become unsuspended due to reduced flow. Downstream of a dam, the river is starved for nutrients that once were carried in by way of the suspended sediment. One of the nutrients trapped in reservoirs is Silicon, the limiting nutrient of diatoms that use it to construct their glass cell walls. This leads to a decrease in Silicon downstream of the dam, reducing diatom populations in favor of other algal groups such as cyanobacteria and Chlorophyta. Phosphorus is another nutrient trapped with the sediment and bioavailable phosphorus is released in anoxic waters and during destratification. Phosphorus is a limiting nutrient for all algal groups and can

cause rapid growth (Winton et al. 2019). The entrapment of Silicon and Phosphorus within the reservoir may be the cause of domination by diatoms in Lake Sinclair's algal community.

This study concentrated on shallow vs deep sites within the same aquatic habitat used for hydroelectricity, but in future research turbidity will be incorporated again. During most sampling dates, the water was opaque with suspended clay which may have blocked sunlight and contributed to the low algal densities. AlgaeGuard measurements were impossible until June and water column sampling taken from shallow sites had to be modified to composite in May, again due to low algal concentration. This study is comparatively small both spatially and temporally due to the time constraints of a master's degree, but it is an important one to better understand the differences between sampling sites depth in terms of physical, biological, and chemical characteristics.

VI. Conclusions

This research is part of a new trend of proactive, rather than reactive, primary producer monitoring in aquatic habitats. Cyanobacteria and diatoms dominated this lotic algal community without reaching abundances defined as blooms. Higher algal biomass occurred in high light, nearshore environments. Inputs of sediments and nutrients from widespread land development and insufficient sewage treatment and disposal practices initiated anthropogenic eutrophication of the lake as it has been reported in other low nutrient lakes (e.g., Goldman and De Amezaga (1975). Algal community biomass of composite samples was not correlated with the level of development around each site. Site location did influence the algal biomass of diatoms and cyanobacteria among epilithic algae. Epilithic algae are stationary (unless grazed or scraped) whereas planktonic algae can disperse across a water body. This suggests that multi-site composite monitoring may be useful when planning to monitor the state of algae.

VII. Literature cited

- Algarte, V. M., Siqueira, N., S., Murakami, E. A., & Rodrigues, L. (2009). Effects of hydrological regime and connectivity on the interannual variation in taxonomic similarity of periphytic algae. *Braz Journal of Biology* 69, 609–616.
- Anderson, N. O., Smith, A. G., Noyszewski, A. K., Ito, E., Dalbotten, D., & Pellerin H. (2021). Management and Control Issues for Native, Invasive Species (Reed Canarygrass): Evaluating Philosophical, Management, and Legislative Issues. *American Society for Horticultural Science* 31(4). <https://doi.org/10.21273/HORTTECH04796-21>
- Babcock-Jackson, L., Carmichael, W. W. & Culver, D. A. (2002). Dreissenid mussels increase exposure of benthic and pelagic organisms to toxic microcystins. *Verhandlungen des Internationalen Verein Limnologie* 28, 1082–1085.
- Beeton, A. M. (2002). Large freshwater lakes: present state, trends, and future. *Environmental Conservation* 29, 21–38.
- Blasco, G. D., Ferraro, D. M., Cottrell, R. S., Halpern, B. S., & Froehlich, H. E. (2020). Substantial Gaps in the Current Fisheries Data Landscape. *Frontiers in Marine Science* 7, 612831. <https://doi.org/10.3389/fmars.2020.612831>
- Breinlinger, S., Phillips, T. J., Haram, B. N., Mareš, J., Martínez Yerena, J. A., Hrouzek, P., Sobotka, R., Henderson, W. M., Schmieder, P., Williams, S. M., Lauderdale, J. D., Wilde, H. D., Gerrin, W., Kust, A., Washington, J. W., Wagner, C., Geier, B., Liebeke, M., Enke, H., Niedermeyer, T. H. J., & Wilde, S. B. (2021). Hunting the eagle killer: A cyanobacterial neurotoxin causes vacuolar myelinopathy. *Science* 371, 6536. <http://doi.org/10.1126/science.aax9050>
- Breitburg, D. L., Adamack, A., Rose, K. A., Kolesar, S. E., Decker, M. B., Purcell, J. E., Keister, J. E. & Cowan, J. H. (2003). The pattern and influence of low dissolved oxygen in the Patuxent River, a seasonally hypoxic estuary. *Estuaries* 26, 280–297. <http://doi.org/10.1007/BF02695967>
- Buchsbaum, R., Valiela, I., Swain, T., Dzierzesky, M. & Allen, S. (1991). Available and refractory nitrogen of coastal vascular plants and macroalgae. *Marine Ecology Progress Series* 72, 131–143.
- Burkholder, J. M., Shumway, S. E., & Glibert, P. M. (2018). Harmful Algal Blooms: A Compendium Desk Reference. 243-336. <https://doi.org/10.1002/9781118994672.ch7>. Pro-ed.
- Burkholder, J. M., Tomasko, D. & Touchette, B. W. (2007). Seagrasses and eutrophication. *Journal of Experimental Marine Biology and Ecology* 350, 46–72. <https://doi.org/10.1016/j.jembe.2007.06.024>

- Buskey, E. J. (2008). How does eutrophication affect the role of grazers in harmful algal bloom dynamics? *Harmful Algae* 8, 152–157. <https://doi.org/10.1016/j.hal.2008.08.009>
- Cabral, J., Pardal, M., Lopes, R., Murias, T., & Marques, J. (1999). The impact of macroalgal blooms on the use of the intertidal area and feeding behavior of waders (Charadrii) in the Mondego estuary (west Portugal). *Acta Oecologica* 20, 417–427. [https://doi.org/10.1016/S1146-609X\(99\)00126-5](https://doi.org/10.1016/S1146-609X(99)00126-5)
- Cantonati, M., Poikane, S., Pringle, C., Stevens, L., Turak, E. Heino, J., Richardson, J., Bolpagni, R., Borrini, A., Cid, N., Tvrtliková, M., Galassi, D., Hájek, M., Hawes, I., Levkov, Z, Naselli-Flores, L., Saber, A., Cicco, M., Fiasca, B., Hamilton, P., Kubečka, J., Segadelli, S, & Znachor, P. (2020). Characteristics, main impacts, and stewardship of natural and artificial freshwater environments: Consequences for biodiversity conservation. *Water*, 12(1), 260. <http://doi.org/10.3390/w12010260>
- Chapman, R.L. (2013). Algae: the world’s most important “plants”—an introduction. *Mitigation and Adaptation Strategies for Global Change* 18, 5–12. <https://doi.org/10.1007/s11027-010-9255-9>
- Chapter 5 of Title 12 of the Official Code of Georgia Annotated, GA R&R - GAC - Rule 391-3-6-.03. Water Use Classifications and Water Quality Standards, <http://rules.sos.ga.gov/GAC/391-3-6.03?urlRedirected=yes&data=admin&lookingfor=391-3-6-.03> (accessed July 19 2021)
- Chaudhary, D. K. & Dahal, F. H. (2017). DNA Bar-Code for Identification of Microbial Communities: A Mini-Review *Microbiology* 7(6), 219-224.
- Cremella, B., Huot, Y., & Bonilla, S. (2018). Interpretation of total phytoplankton and cyanobacteria fluorescence from cross-calibrated fluorometers, including sensitivity to turbidity and colored dissolved organic matter. *Limnology and Oceanography: Methods* 16(12), 881-894. <https://doi.org/10.1002/lom3.10290>
- Dodds, W. K. (2006). Eutrophication and trophic state in rivers and streams. *Limnology and Oceanography* 51(1, part 2), 671-680.
- Downing, J. A., Watson, S. B., & McCauley, E. (2001). Predicting cyanobacterial dominance in lakes. *Canadian Journal of Fisheries and Aquatic Sciences* 58, 1905-1908.
- Feuchtmayr, H., Pottinger, T. G., Moore, A., De Ville, M. M., Caillouet, L., Carter, H. T., Pereira, M. G., & Maberly, S. C. (2019). Effects of brownification and warming on algal blooms, metabolism and higher trophic levels in productive shallow lake mesocosms. *Science of the Total Environment* 678, 227-238. <https://doi.org/10.1016/j.scitotenv.2019.04.105>
- Gao, L., Zhang, L., Hou, J., Wei, Q., Fu, F., & Shao., H. (2013). Decomposition of macroalgal blooms influences phosphorus release from the sediments and implications for coastal restoration in Swan Lake, Shandong, China. *Ecological Engineering* 60, 19–28. <https://doi.org/10.1016/j.ecoleng.2013.07.055>

- Gopal, V., Achyuthan, H., & Jayaprakash, M. (2017). Assessment of trace elements in Yercaud Lake sediments, southern India. *Environmental Earth Sciences* 76(2), <https://doi.org/10.1007/s12665-017-6390-6>
- Goldman, C. R. & De Amezaga, E.(1975). Spatial and temporal changes in the primary productivity of Lake Tahoe, California-Nevada between 1959 and 1971. *SIL Proceedings 1922–2010(19)*, 812–825.
- Gray, J. S., Wu, R. S., & Or, Y. Y. (2002). Effects of hypoxia and organic enrichment on the coastal marine environment. *Marine Ecology Progress Series* 238, 249–279.
- Green, L., Blumstein, D. T., & Fong, P. (2015). Macroalgal mats in a eutrophic estuary obscure visual foraging cues and increase variability in prey availability for some shorebirds. *Estuaries and Coasts* 38, 917–926. <https://doi.org/10.1007/s12237-014-9862-x>
- Greer, B., Maul, R., Campbell, K., & Elliott, C. T. (2017). Detection of freshwater cyanotoxins and measurement of masked microcystins in tilapia from Southeast Asian aquaculture farms. *Analytical and Bioanalytical Chemistry* 409, 4057–4069. <https://doi.org/10.1007/s00216-017-0352-4>
- Gregor, J. & Marsalek, B. (2005). A Simple In Vivo Fluorescence Method for the Selective Detection and Quantification of Freshwater Cyanobacteria and Eukaryotic Algae. *Acta hydrochimica et hydrobiologica* 33(2), 142–148. <https://doi.org/10.1002/ahch.200400558>
- Hauxwell, J., Cebrián, J., Furlong, C., & Valiela, I. (2001). Macroalgal canopies contribute to eelgrass (*Zostera marina*) decline in temperate estuarine ecosystems. *Ecology* 82, 1007–1022.
- Havens, K. E., Hauxwell, J., Tyler, A. C., Thomas, S., McGlathery, K.J., Cebrian, J., Valiela, I., Steinman, A. D., & Hwang, S. (2001). Complex interactions between autotrophs in shallow marine and freshwater ecosystems: implications for community responses to nutrient stress. *Environmental Pollution* 113, 95–107. [https://doi.org/10.1016/S0269-7491\(00\)00154-8](https://doi.org/10.1016/S0269-7491(00)00154-8)
- Hazuková, V., Sgro, G. V., & Johansen, J. R. (2019). Validation of a diatom-based index of water quality confirms its utility in monitoring of the Lake Erie's nearshore area. *Journal of Great Lakes research. International Association for Great Lakes Research* 45(1), 98–108. <https://doi.org/10.1016/j.jglr.2018.10.004>
- Howard, M. D. A., Kudela, R. M., Hayashi, K., Tatters, A. O., Caron, D. A., Theroux, S., Oehrle, S., Roethler, M., Donovan, A., Loftin, K., & Laughrey, Z. (2021). Multiple co-occurring and persistently detected cyanotoxins and associated cyanobacteria in adjacent California lakes. *Toxicon* 192, 1–14.
- Izagirre, O., Bermejo, M., Pozo, J., & Elosegí, A. (2007). RIVERMET (c): an Excel-based tool to calculate river metabolism from diel oxygen concentration curves. *Environmental Modelling & Software* 22, 24–32. <https://doi.org/10.1016/j.envsoft.2005.10.001>

- Junk, W. J. (1973). Investigations on the ecology and production-biology of the “floating meadows” (Paspalo-Echinochloetum) in the Middle Amazon. *Amazoniana* 4, 9–102.
- Kardinaal, W. E. A., Janse, I., Kamst-van Agterveld, M., Meima, M., Snoek, J., Mur, L. R., Huisman, J., Zwart, G., & Visser, P. M. (2007). *Microcystis* genotype succession in relation to microcystin concentrations in freshwater lakes. *Aquatic Microbial Ecology* 48, 1-12. <https://doi.org/10.3354/ame048001>
- Kragh, T. & Sand-Jensen, K. (2018). Carbon limitation of lake productivity. *Proceedings in Biological Sciences* 285(1891), 20181415. <https://doi.org/10.1098/rspb.2018.1415>
- Krammer, K. & Lange-Bertalot. (2007). *Süßwasserflora von Mitteleuropa*. H. (eds H. Ettl, J. Gerloff, H. Heynig, & D. Mollenhauer), Elsevier GmbH, München.
- “Lake Sinclair” 33°10’18”N 83°14’00”W. Bing Maps. November 24, 2019.
- “Lake Sinclair” 33°10’18”N 83°14’00”W. GOOGLE EARTH. July 28, 2021.
- Laurén-Määttä, C., Hietala, J., & Walls, M. (1997). Responses of *Daphnia pulex* populations to toxic cyanobacteria. *Freshwater Biology* 37, 635–647. <https://doi.org/10.1046/j.1365-2427.1997.00189.x>
- Lee, K.H., Jeong, H. J., Lee, K., Franks, P. J. S., Seonge, K. A., Lee, S. Y., Lee, M. J., Janga, S. H., Potvina, E., Lim, A. S., Yoon, E. Y., Yoo, Y. D., Kang, N. S., & Kim, K.Y. (2019). Effects of warming and eutrophication on coastal phytoplankton production. *Harmful Algae* 81, 106-118. <https://doi.org/10.1016/j.hal.2018.11.017>
- Lehtiniemi, M., Engstrom-Ost, J., Karjalainen, M., Kozłowsky-Suzuki, B. & Vitasalo, M. (2002). Fate of cyanobacterial toxins in the pelagic food web: transfer to copepods or to faecal pellets? *Marine Ecology Progress Series* 241, 13–21.
- Lembi, C. A. (2003). Control of nuisance algae. In J.D. Weir and R.G. Sheath (Eds.), *Freshwater Algae of North America – Ecology and Classification* (pp. 805–834). Academic Press.
- Lozanovska, I., Ferreira, M. T., & Aguiar F. C. (2018). Functional diversity assessment in riparian forests - multiple approaches and trends : a review. *Ecological Indicators* 95,781–793. <https://doi.org/10.1016/j.ecolind.2018.08.039>
- Lyons, D. A., Arvanitidis, C., Blight, A. J., Chatzinikolaou, E., Guy-Haim, T., Kotta, J., OravKotta, H., Queiros, A., Rilov, G., Somerfield, P. J., & Crowe, T. P. (2014). Macroalgal blooms alter community structure and primary productivity in marine ecosystems. *Global Change Biology* 20, 2712–2724. <https://doi.org/10.1111/gcb.12644>
- Merritt, D. M., Scott, M. L., Poff, N. L., Auble, G. T., & Lytle D. A. (2010). Theory, methods and tools for determining environmental flows for riparian vegetation: riparian vegetation-flow response guilds. *Freshwater Biology* 55, 206–225. <https://doi.org/10.1111/j.1365-2427.2009.02206.x>

- Manoylov, K.M. (2014). Taxonomic identification of algae (morphological and molecular): species concepts, methodologies, and their implications for ecological bioassessment, *Journal of Phycology*, 50(3), 409–424.
- Miatta, M., Bates, A. E., & Snelgrove, P. V. R. (2021). Incorporating Biological Traits into Conservation Strategies. *Annual Review of Marine Science* 13, 421–443.
<http://doi.org/10.1146/annurev-marine-032320-094121>
- Mitch, W. J., & Gosselink, J. G. (2007). Wetlands, (4th ed.). Pro-ed.
- Mohammed, A. K. (2018). An Assessment of Tourism’s Environmental Impact on the Lake Bosomtwe Basin. *International Journal of Hospitality & Tourism Administration*, 19(3), 259–288. <https://doi.org/10.1080/15256480.2017.1324338>
- Morgan, A. M., Royer, T. V, David, M. B., & Gentry, L. E. (2006). Relationships among nutrients, chlorophyll-a, and dissolved oxygen in agricultural streams in Illinois. *Journal of Environmental Quality* 35, 1110–1117. <https://doi.org/10.2134/jeq2005.0433>
- Nechifor, M., Neagu, T.-M., & Manda, G. (2012). Reactive oxygen species, cancer and anticancer therapies. *Current Chemical Biology* 3, 22–46.
- Newell, S. E., Davis, T. W., Johengen, T. H., Gossiaux, D., Burtner, A., Palladino, D., & McCarthy, M. J. (2019). Reduced forms of nitrogen are a driver of non-nitrogen-fixing harmful cyanobacterial blooms and toxicity in Lake Erie. *Harmful Algae* 81, 86–93.
<https://doi.org/10.1016/j.hal.2018.11.003>
- Nolin, J. (2020) Clock ticking on Georgia Power plan for unlined coal ash storage. *Georgia Recorder*. Clock ticking on Georgia Power plan for unlined coal ash storage - Georgia Recorder
- Osterling, M., & Pihl, L. (2001). Effects of filamentous green algal mats on benthic macrofaunal functional feeding groups. *Journal of Experimental Marine Biology and Ecology* 263, 159–183. [https://doi.org/10.1016/S0022-0981\(01\)00304-5](https://doi.org/10.1016/S0022-0981(01)00304-5)
- Pihl, L., Baden, S. P., Diaz, R. J., & Schaffner, L. C. (1991). Hypoxia-induced structural changes in the diet of bottom-feeding fish and Crustacea. *Marine Biology* 112, 349–361.
<https://doi.org/10.1007/BF00356279>
- Pihl, L., Isaksson, I., Wennhage, H., & Moksnes, P. (1995). Recent increase of filamentous algae in shallow Swedish bays: effects on the community structure of epibenthic fauna and fish. *Netherlands Journal of Aquatic Ecology* 29, 349–358.
- Prescott, G. W. (1962). *Algae of the Western Great Lakes Area*. WM. C. Brown Company Publishers, Dubuque, IA.
- Pringle, C. M., Freeman, M., & Freeman, B. (2000). Regional effects of hydrologic alterations on riverine macrobiota in the New World: Tropical-temperate comparisons. *BioScience* 50, 807–823.

- Poikane, S., Portielje, R., van den Berg, M., Phillips, G., Brucet, S., Carvalho, L., Mischke, U., Ott, I., Soszka, H., & Van, J. (2014). Defining ecologically relevant water quality targets for lakes in Europe. *Journal of Applied Ecology*, 51(3), 592-602. <https://doi.org/10.1111/1365-2664.12228>
- Record, S., Voelker, N. M., Zarnetske, P. L., Wisnoski, N. I., Tonkin, J. D., Swan, C., Marazzi, L., Lany, N., Lamy, T., Compagnoni, A., Castorani, M. C. N., Andrade, R., & Sokol, E. R. (2021). Novel Insights to Be Gained from Applying Metacommunity Theory to Long-Term, Spatially Replicated Biodiversity Data. *Frontiers in Ecology and Evolution* 8. 612794. <https://doi.org/10.3389/fevo.2020.612794>
- Rood, S. B., Scott, M. L., Dixon, M., González, E., Marks, C. O., Shafroth, P. B., & Volke, M. A. (2020). Ecological Interfaces between Land and Flowing Water: Themes and Trends in Riparian Research and Management. *Wetlands* 40, 1801-1811. <https://doi.org/10.1007/s13157-020-01392-4/>
- Sagerman, J., Hansen, J. P., & Wikström, S. A. (2020). Effects of boat traffic and mooring infrastructure on aquatic vegetation: A systematic review and meta-analysis. *Ambio* 49, 517-530. <https://doi.org/10.1007/s13280-019-01215-9>
- Shimps, E. L., Rice, J. A., & Osborne, J. A. (2005). Hypoxia tolerance in two juvenile estuary dependent fishes. *Journal of Experimental Marine Biology and Ecology* 325, 146–162. <https://doi.org/10.1016/j.jembe.2005.04.026>
- Smith, M. E. & Manoylov, K. M. (2013). Changes in Diatom Biodiversity in Lake Sinclair, Baldwin County, Georgia, USA. *Journal of Water Resource and Protection*, 45, 732-742. <https://doi.org/10.4236/jwarp.2013.5707>
- Špoljar, M., Zhang, C., Dražina, T., Zhao, G., Lajtner, J., & Radonić, G. (2017). Development of submerged macrophyte and epiphyton in a flow-through system: Assessment and modelling predictions in interconnected reservoirs. *Ecological Indicators* 75, 145-154. <https://doi.org/10.1016/j.ecolind.2016.12.038>
- Stumm, W., & Morgan, J. J. (1996). *Aquatic Chemistry: Chemical Equilibria and Rates in Natural Waters*, (3rd ed.). Pro-ed.
- Subbiah, S., Karnjanapiboonwong, A. D., Maul, J., Wang, D., & Anderson, T. A. (2019). Monitoring cyanobacterial toxins in a large reservoir: relationships with water quality parameter. *PeerJ*, e7305. <https://doi.org/10.7717/peerj.7305>
- Svendsen, M.B.S., Andersen, N.R., Hansen, P.J., & Steffensen, J.F. (2018). Effects of Harmful Algal Blooms on Fish: Insights from *Prymnesium parvum*. *Fishes* 3(1), 11. <https://doi.org/10.3390/fishes3010011>
- Svensen, C., Strogyloudi, E. Wexels, C. Riser, C. W., Dahlmann, J., Legrand, C., Wassmann, P. Granéli, E., & Pagou, K. (2005). Reduction of cyanobacterial toxins through coprophagy in *Mytilus edulis*. *Harmful Algae*, 4, 329–333. <https://doi.org/10.1016/j.hal.2004.06.015>

- Thomsen, M. S., & McGlathery, K. (2006). Effects of accumulations of sediments and drift algae on recruitment of sessile organisms associated with oyster reefs. *Journal of Experimental Marine Biology and Ecology* 328, 22–34. <https://doi.org/10.1016/j.jembe.2005.06.016>
- U.S. EPA. (2015). Method 545: Determination of Cylindrospermopsin and Anatoxin-A in Drinking Water by Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometry (LC/ESI-MS/MS). EPA 815-R-15-009
- U.S. EPA. (2019). National Lakes Assessment. <https://www.epa.gov/national-aquatic-resource-surveys/nla> (Accessed July 24, 2021).
- U.S. EPA. (2021). Indicators: Chlorophyll-a. <https://www.epa.gov/national-aquatic-resource-surveys/indicators-chlorophyll> (Accessed November 1, 2021).
- Valiela, I., McClelland, J., Hauxwell, J., Behr, P. J., Hersh, D. & Foreman, K. (1997). Macroalgal blooms in shallow coastal estuaries: controls and ecophysiological and ecosystem consequences. *Limnology and Oceanography* 42, 1105–1118.
- Vogt, R. J., Sharma, S., & Leavitt, P. R. (2017) Direct and interactive effects of climate, meteorology, river hydrology, and lake characteristics on water quality in productive lakes of the Canadian Prairies. *Canadian Journal of Fisheries and Aquatic Sciences* 208, 47–59. <https://doi.org/10.1139/cjfas-2016-0520>
- Wennhage, H., & Pihl, L. (1994). Substratum selection by juvenile plaice (*Pleuronectes platessa* L.): impact of benthic microalgae and filamentous macroalgae. *Netherlands Journal of Sea Research* 32, 343–351. [https://doi.org/10.1016/0077-7579\(94\)90011-6](https://doi.org/10.1016/0077-7579(94)90011-6)
- Wetzel, R.G. (2001). *Limnology: Lake and River Ecosystems*. (3rd ed.). Pro-ed.
- Wilcock, R.J., Nagels, J. W., McBride, G. B., Collie, K. J., Wilson, B. T., & Huser, B. A. (2010). Characterisation of lowland streams using a single-station diurnal curve analysis model with continuous monitoring data for dissolved oxygen and temperature. *New Zealand Journal of Marine and Freshwater Research* 32, 67–79. <https://doi.org/10.1080/00288330.1998.9516806>
- Winton, R. S., Calamita, E., & Wehrli, B. (2019). Reviews and syntheses: Dams, water quality and tropical reservoir stratification. *Biogeosciences* 16, 1657-1671. <https://doi.org/10.5194/bg-16-1657-2019>
- Wilkinson, G. M., Carpenter, S. R., Cole, J. J., Pace, M. L., Batt, R. D., Buelo, C. D., & Kurtzweil, J. T. (2017). Early warning signals precede cyanobacterial blooms in multiple whole-lake experiments. *Ecological Monographs* 88(2), 188-203. <https://doi.org/10.1002/ecm.1286>
- Yang, S., Bertuzzo, E., Büttner, & Borchardt, D. (2021). Emergent spatial patterns of competing benthic and pelagic algae in a river network: A parsimonious basin-scale modeling analysis. *Water Research* 193(24), 116887. <https://doi.org/10.1016/j.watres.2021.116887>

YSI Environmental, Inc. (2009) The Dissolved Oxygen Handbook. YSI Environmental, Inc., Yellow Springs.

Zhang, T. T., He, M., Wu, A. P., & Nie, L. W. (2012) Inhibitory Effects and Mechanisms of *Hydrilla verticillata* (Linn.f.) Royle Extracts on Freshwater Algae. *Bulletin of Environmental Contamination and Toxicology* 88, 477-481.
<https://doi.org/10.1007/s00128-011-0500-z>

VII. Tables

Table 1. Summary of data per site for all sampling times. GPS coordinates and dissolved oxygen (mg/L, DO), pH, water temperature (°C), and conductivity (µS/cm, Cond.), and average \pm standard deviation.

Site	GPS	DO	pH	Water Temp.	Cond.
1	33° 11' 14" N, 83° 11' 14" W	7.27 \pm 1.29	7.43 \pm 1.41	22.6 \pm 7.27	60.59 \pm 9.88
2	33° 11' 33" N, 83° 17' 37" W	6.95 \pm 1.4	7.46 \pm 0.92	23.08 \pm 7.2	61.4 \pm 10.22
3	33° 11' 53" N, 83° 17' 45" W	6.88 \pm 1.52	7.93 \pm 0.92	23.16 \pm 7.2	66.32 \pm 9.18
4	33° 11' 52" N, 83° 17' 41" W	7.17 \pm 1.36	7.75 \pm 0.9	23.1 \pm 7.39	65.12 \pm 8.79
5	33° 11' 11" N, 83° 16' 37" W	6.11 \pm 0.96	7.25 \pm 0.08	27.58 \pm 3.46	62.77 \pm 8.78
6	33° 11' 4" N, 83° 15' 4" W	6.69 \pm 1.68	7.6 \pm 0.7	27.84 \pm 3.51	59.88 \pm 11.34
7	33° 11' 53" N, 83° 17' 45" W	8.06 \pm 0.92	8.06 \pm 0.92	28.22 \pm 3.29	61.47 \pm 8.48
8	33° 8' 36" N, 83° 12' 20" W	7.96 \pm 0.68	7.96 \pm 0.68	28.43 \pm 3.11	61.41 \pm 7.87

Table 2. Pearson Correlation Matrix of correlation of water parameters and biological data during 2019 and 2020 (correlations that are significant at the 95% level are shown in bold) total chlorophyll-a ($\mu\text{g/L}$, Chl-a), Chlorophyta chl-a contribution to total ($\mu\text{g/L}$, green algae, Gr.), Bacillariophyceae chl-a contribution to total ($\mu\text{g/L}$, diatoms, diat.), Cyanophyta chl-a contribution to total ($\mu\text{g/L}$, Cy.), Cryptophyta chl-a contribution to total ($\mu\text{g/L}$, Cpt), yellow substances, dissolved oxygen (mg/L , DO), pH, water temperature ($^{\circ}\text{C}$, WT), conductivity ($\mu\text{S}/\text{cm}$, Cd.), species richness (SR), species evenness (SE), and Shannon's index (SI).

	Total Chl-a	Gr.	Diat.	Cy.	Cpt.	Yel. Sub.	DO	pH	WT	Cd.	SR	SE	SI
Tl Chl-a	1												
Gr.	0.07	1											
Diat.	0.16	0.09	1										
Cy.	0.86	0.01	0.01	1									
Cpt.	0.76	0.01	0.01	0.75	1								
Yell. Sub.	0.02	0.01	0.04	0.01	0.04	1							
DO	0.01	0.01	0.01	0.01	0.01	0.03	1						
pH	0.01	0.04	0.02	0.01	0.01	0.02	0.3	1					
WT.	0.01	0.01	0.24	0.01	0.01	0.01	0.13	0.01	1				
Cd.	0.06	0.1	0.01	0.05	0.03	0.01	0.36	0.01	0.22	1			
SR	0.12	0.02	0.3	0.04	0.04	0.02	0.05	0.01	0.32	0.01	1		
SE	0.03	0.11	0.01	0.01	0.01	0.04	0.1	0.01	0.14	0.24	0.11	1	
SI	0.01	0.08	0.04	0.01	0.01	0.05	0.08	0.02	0.3	0.15	0.55	0.76	1

VIII. Figures

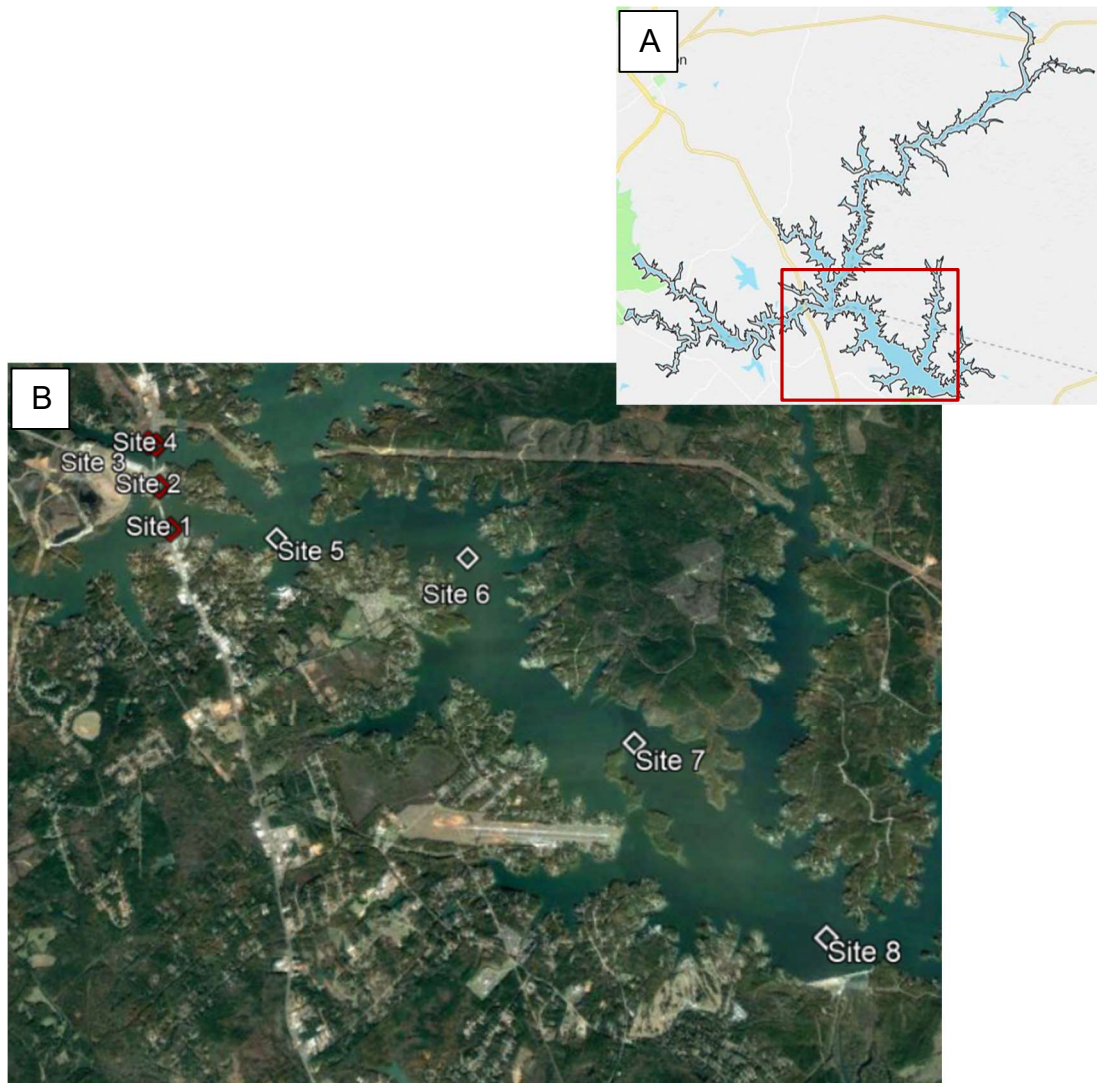


Figure 1: Map of sampling sites. A. Overhead view of Lake Sinclair. B. Close up of sampling sites. Shallow sites are color coded red while deep sites are color coded white. (GOOGLE EARTH Accessed July 28, 2021; Bing Accessed November 24, 2019).

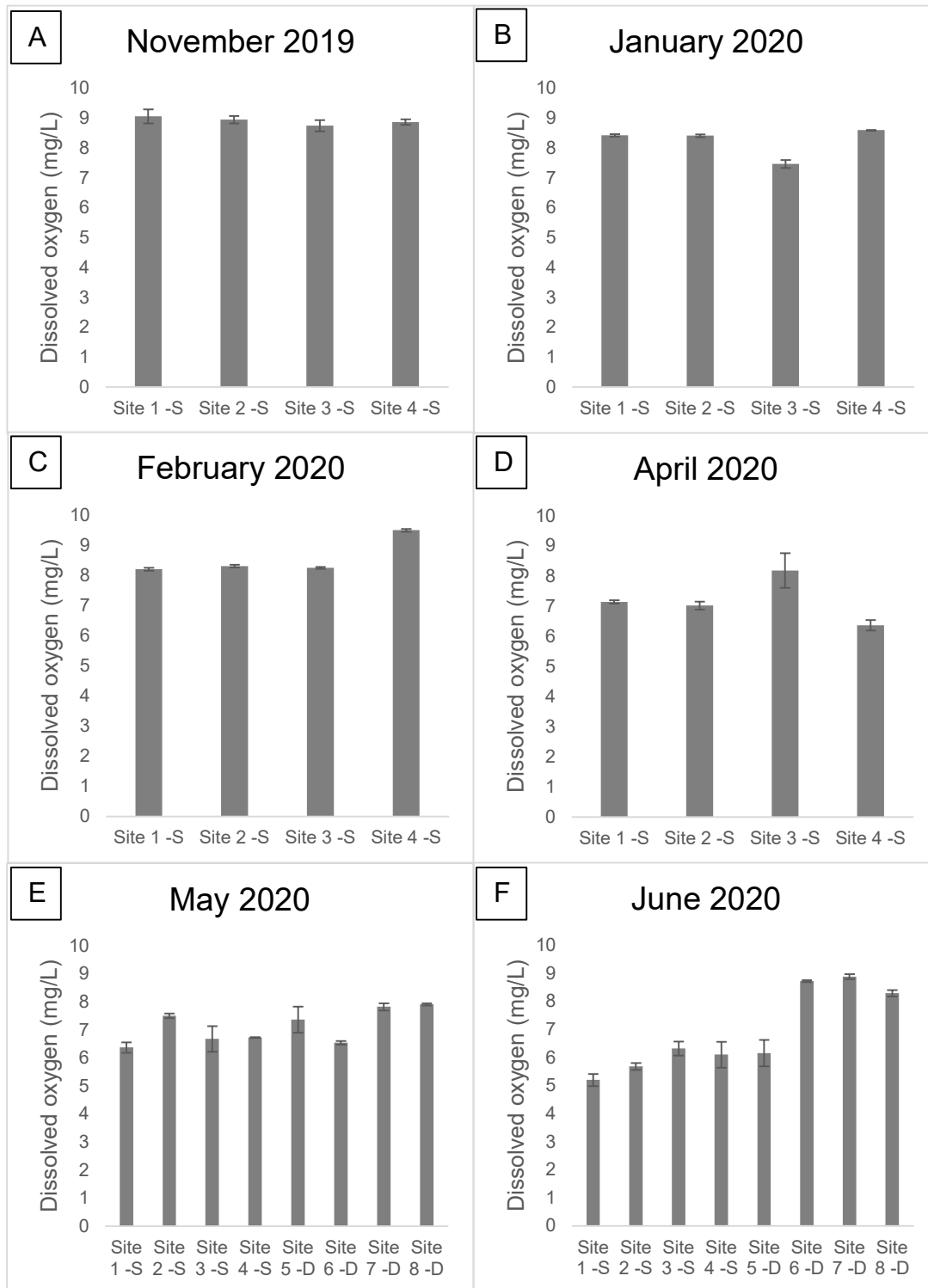


Figure 2: Dissolved oxygen averages per site and month with standard deviation from November 2019 to June 2020.

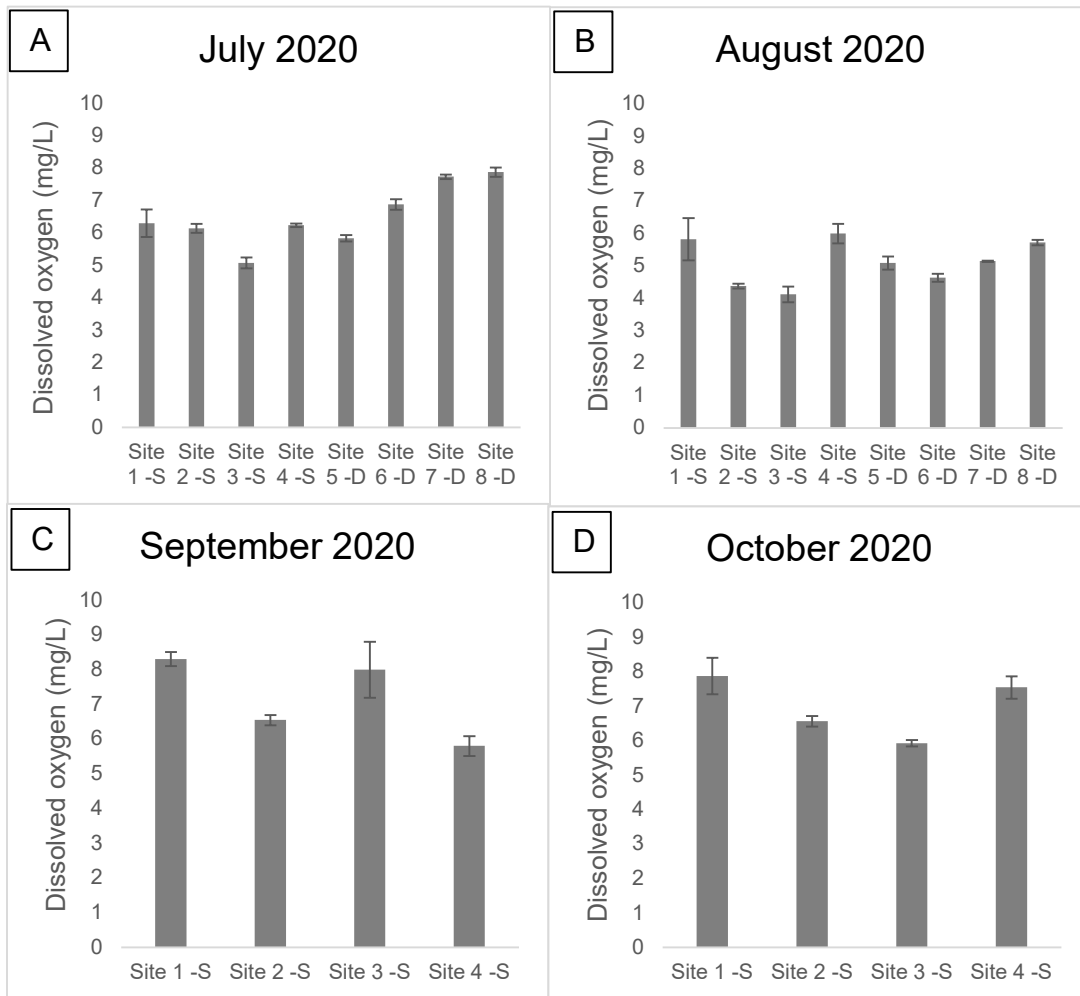


Figure 3: Dissolved oxygen averages per site and month with standard deviation from July 2020 to October 2020.

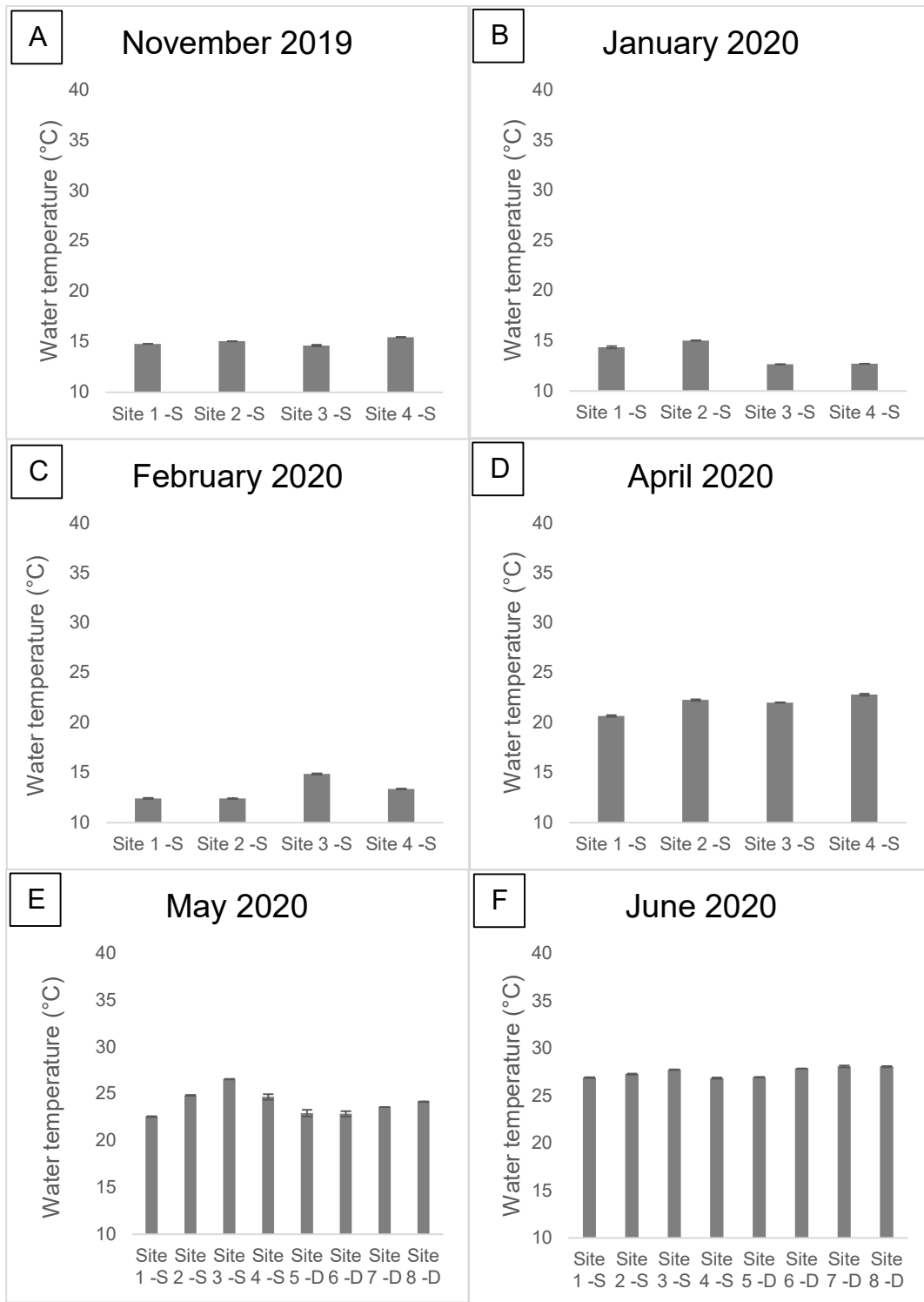


Figure 4: Water temperature averages per site and month with standard deviation from November 2019 to June 2020.

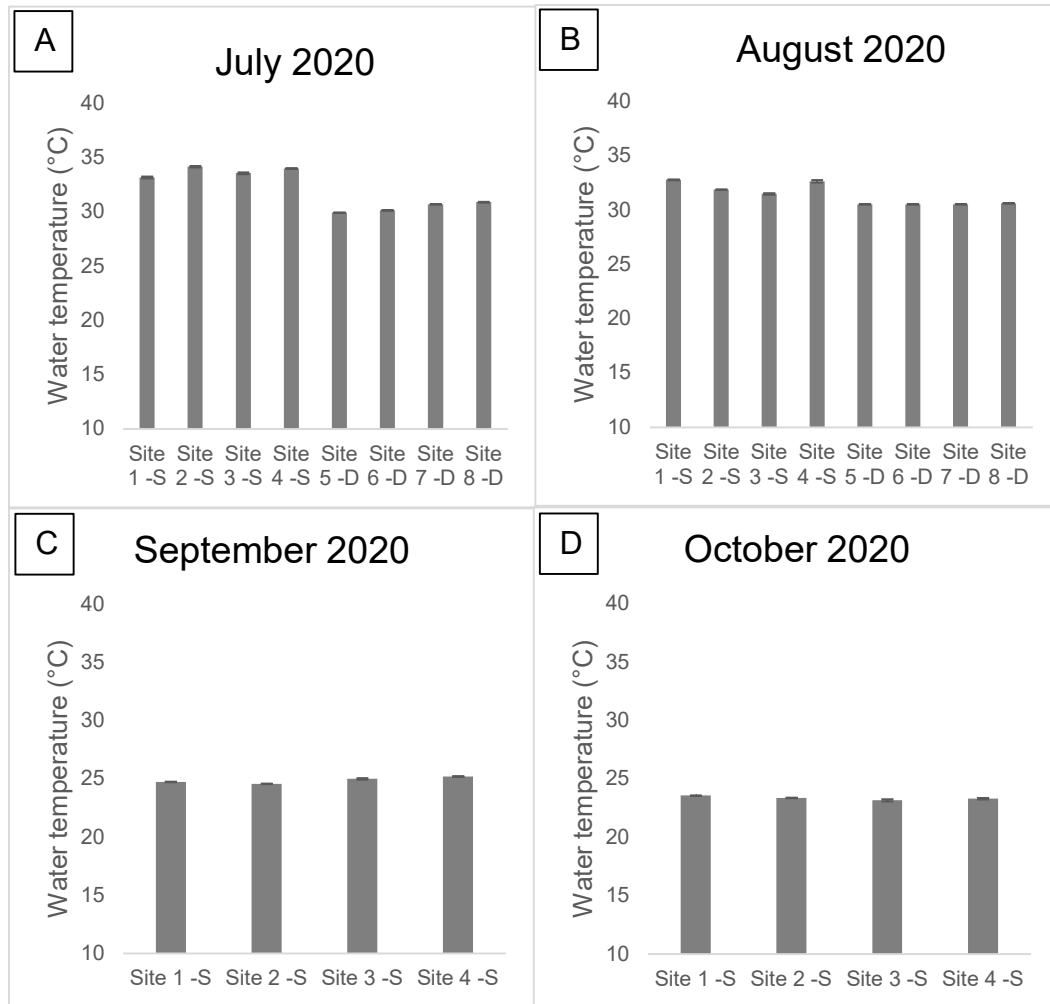


Figure 5: Water temperature averages per site and month with standard deviation from July 2020 to October 2020.

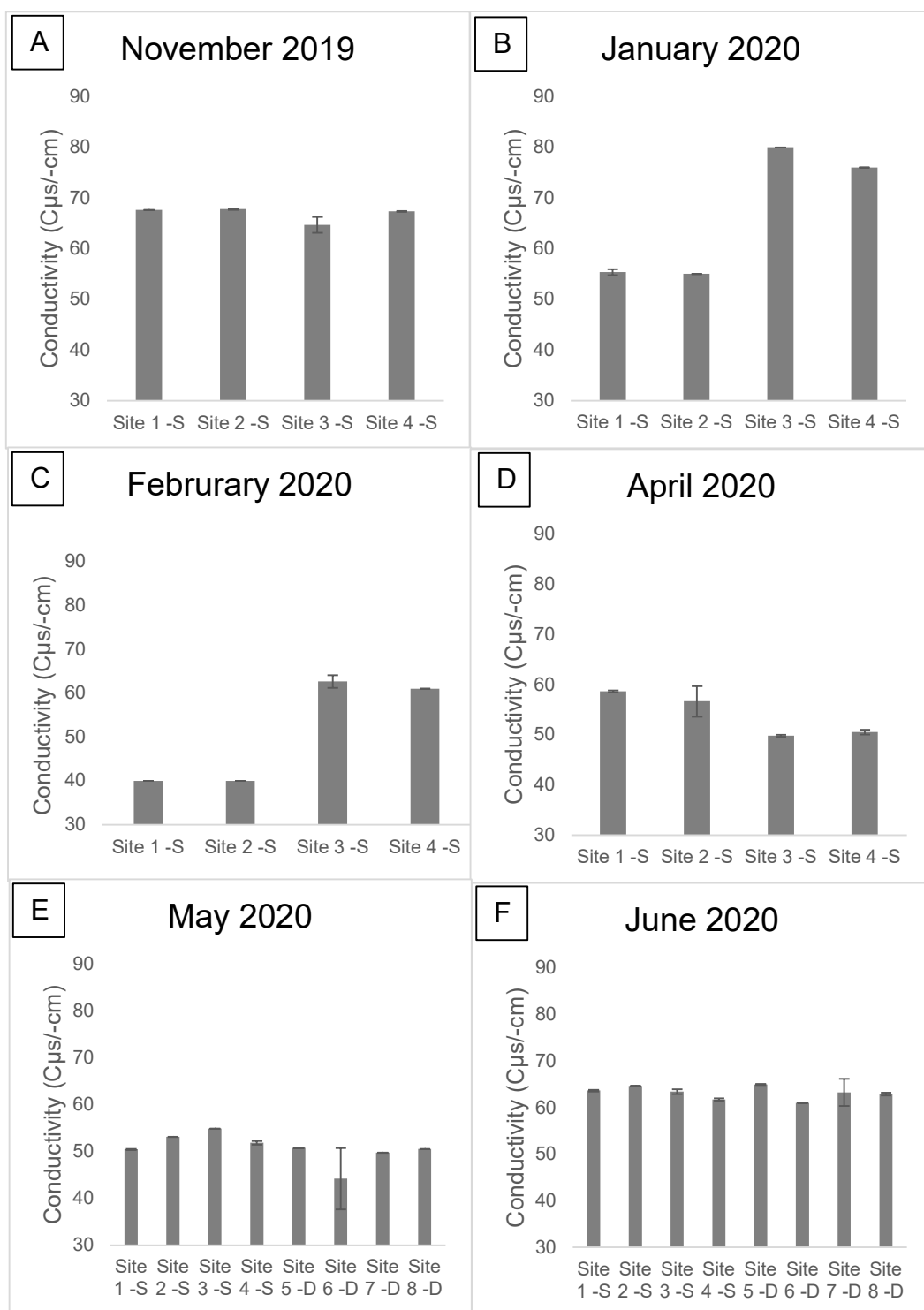


Figure 6: Conductivity averages per site and month with standard deviation from November 2019 to June 2020.

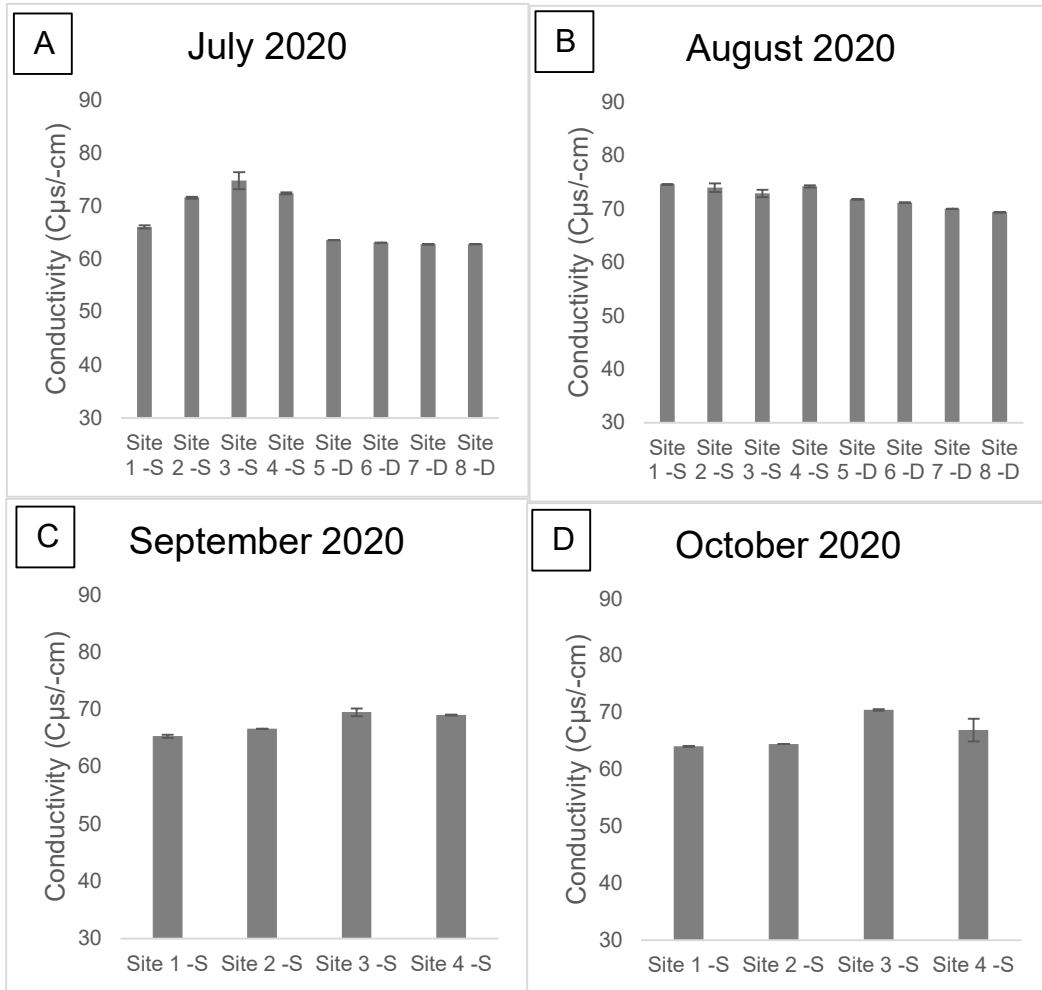


Figure 7: Conductivity averages per site and month with standard deviation from July 2020 to October 2020.

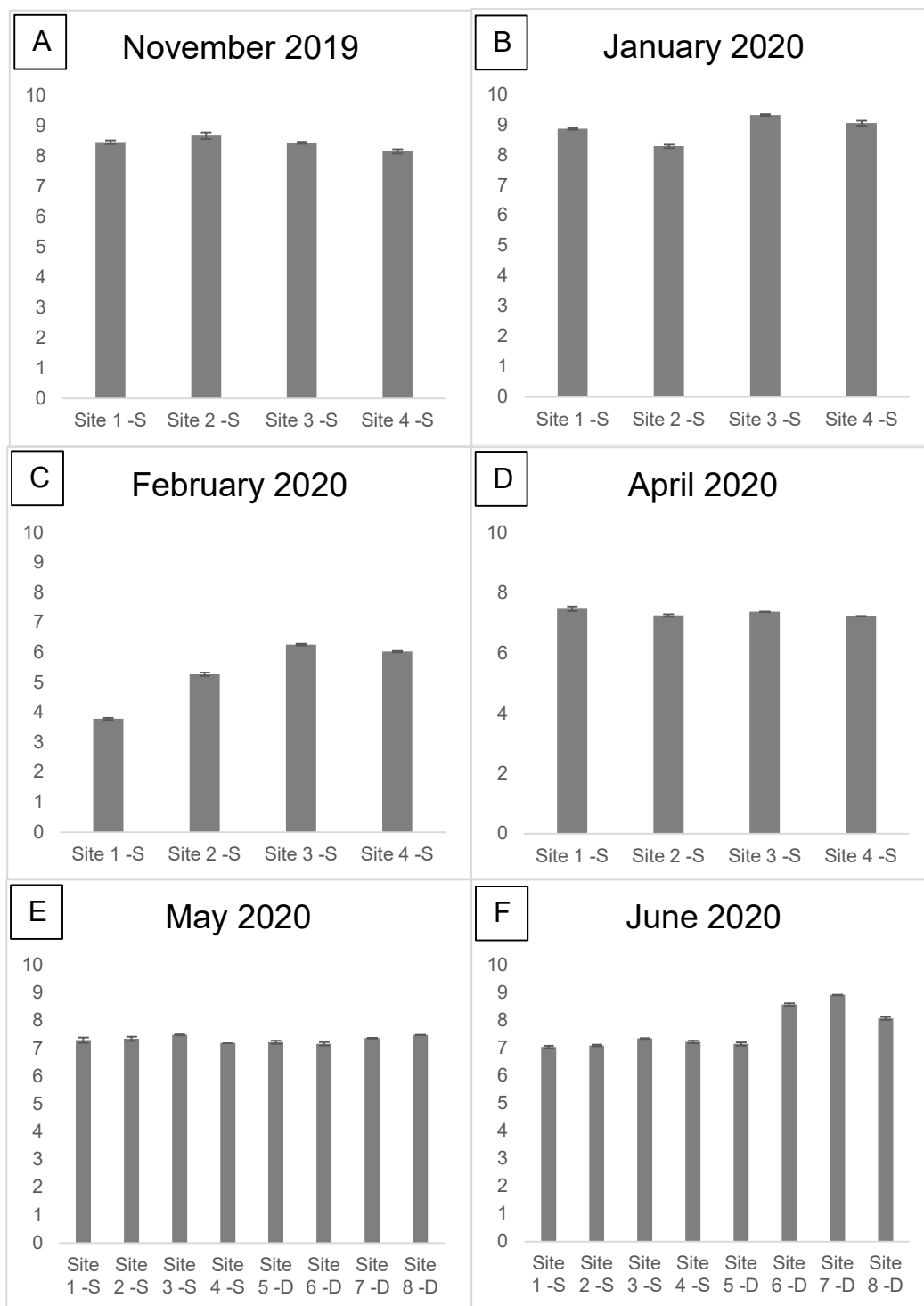


Figure 8: pH averages per site and month with standard deviation from November 2019 to June 2020.

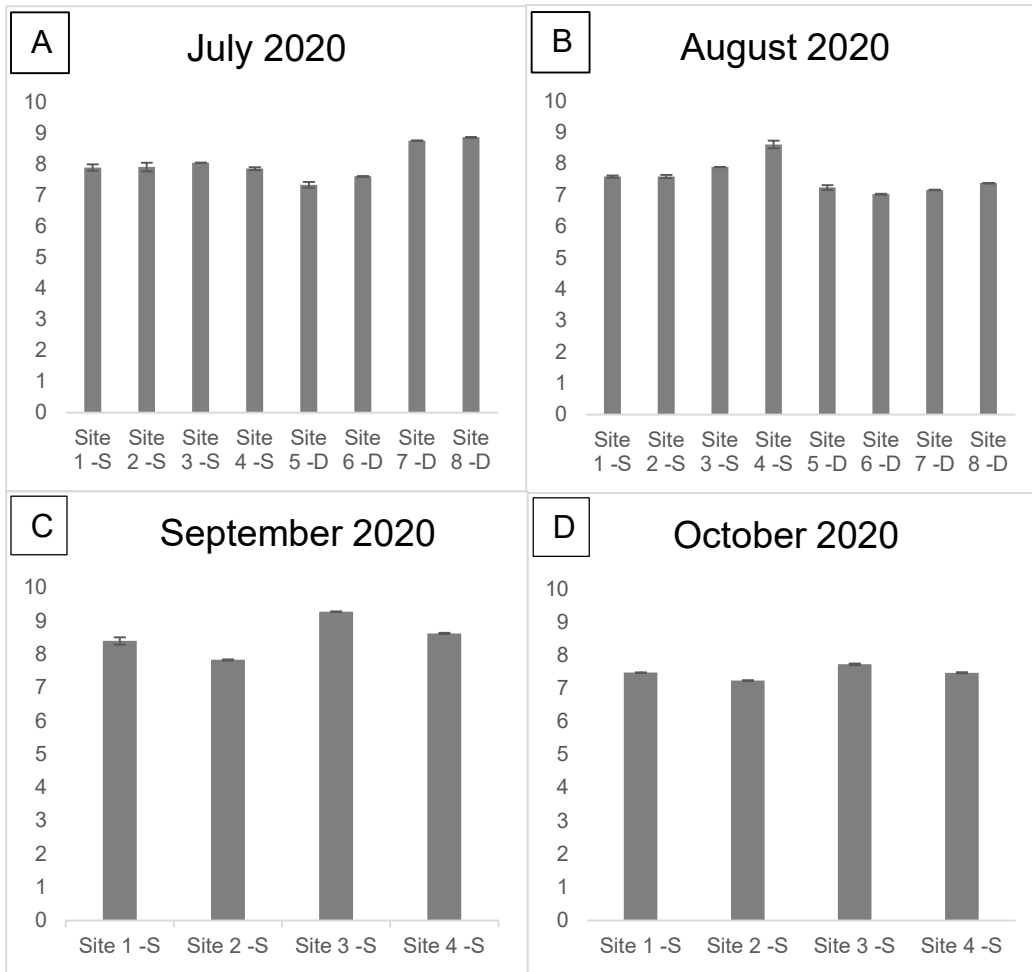


Figure 9: pH averages per site and month with standard deviation from July 2020 to October 2020.

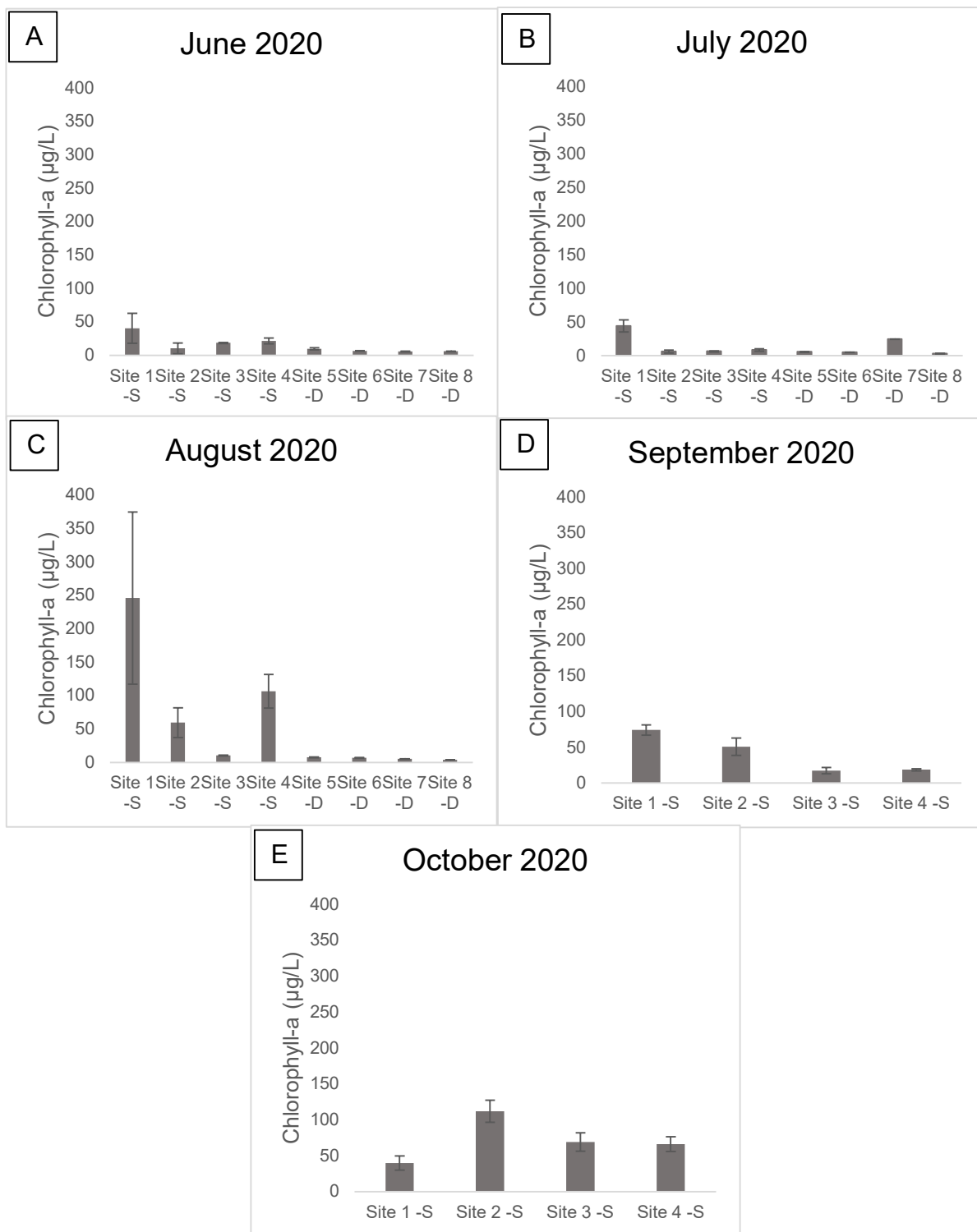


Figure 10: Average total chlorophyll-a with standard deviation by month and site for 5 months from June 2020 to October 2020.

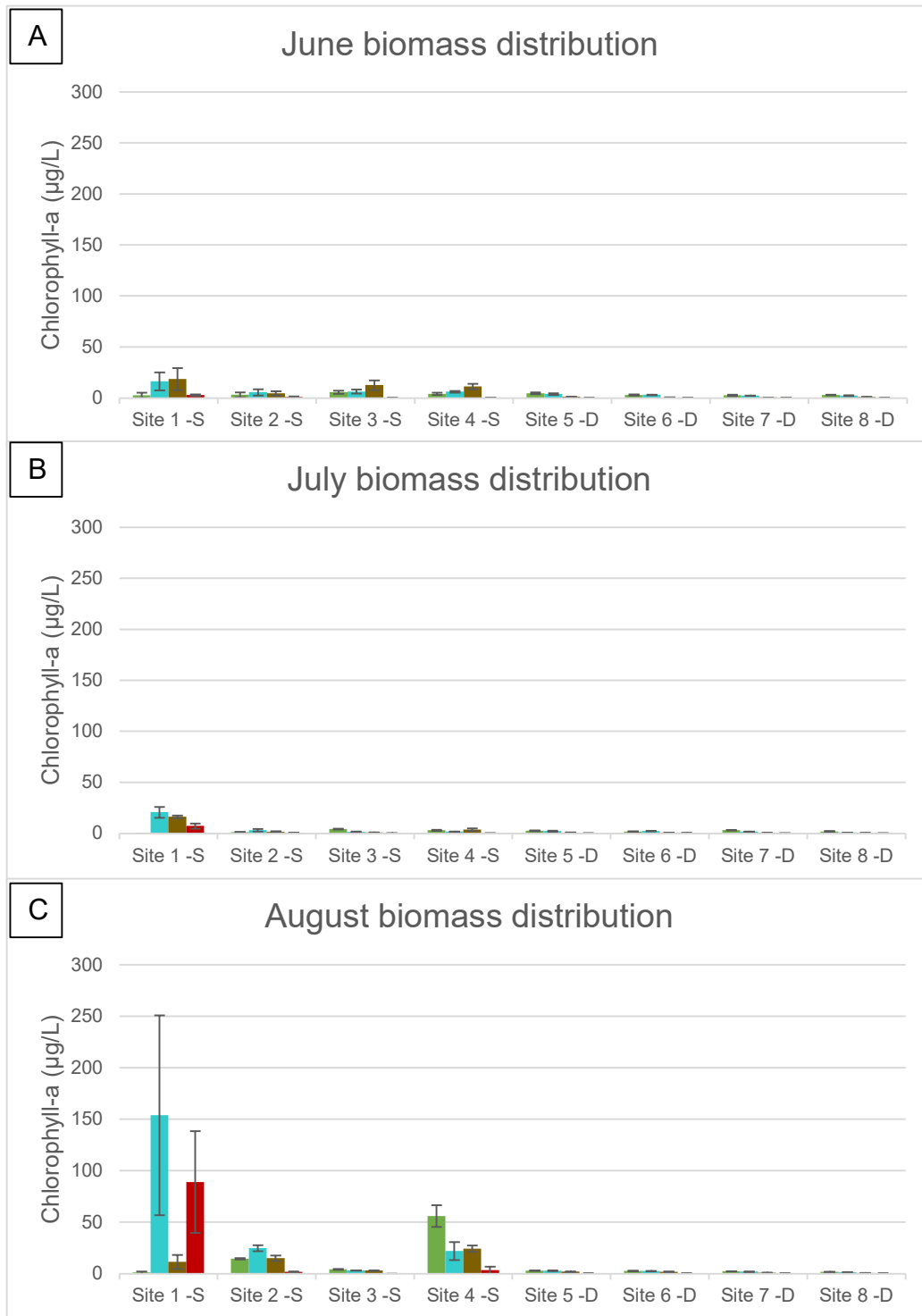


Figure 11: Average relative biomass of green algae, diatoms, and cyanobacteria with standard deviation in chlorophyll-a $\mu\text{g/L}$ per site and per month and site from April 2020 to August 2020. The legend below graph A, applies to all individual bar graphs above.

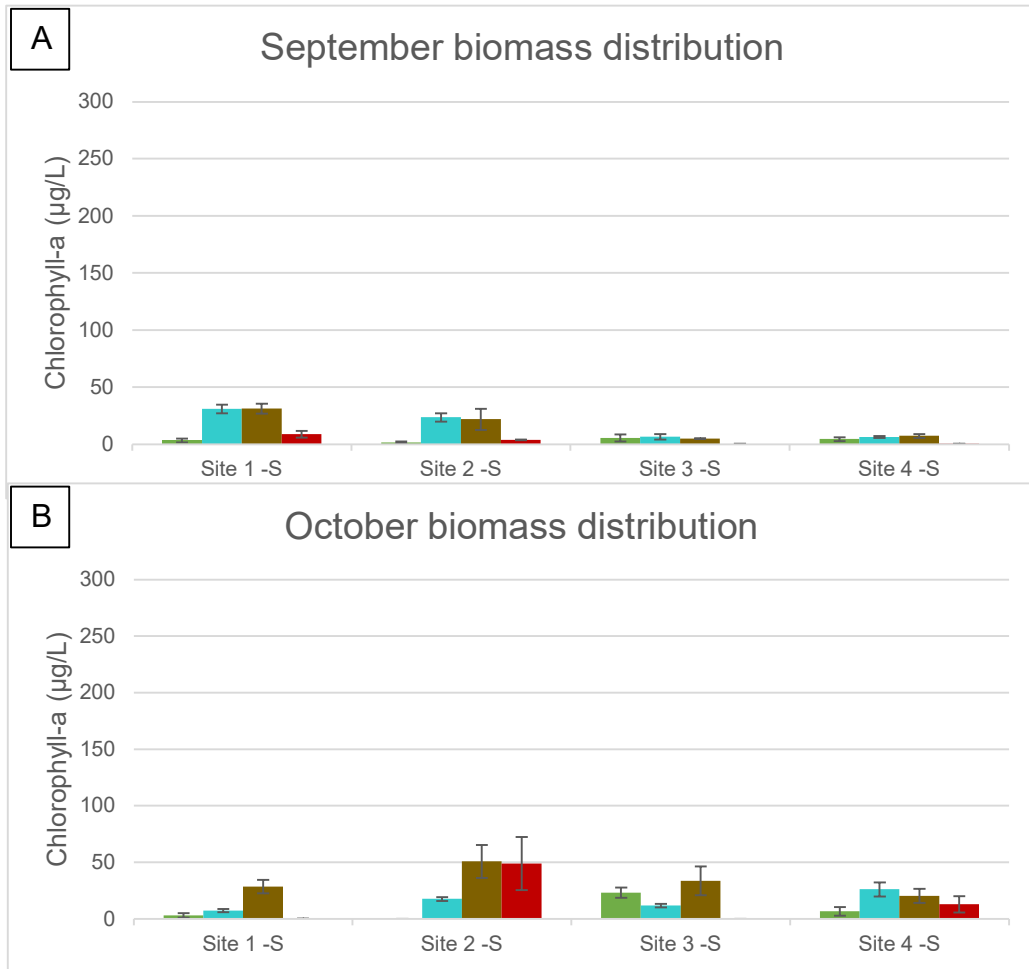


Figure 12: Average relative biomass of green algae, diatoms, and cyanobacteria with standard deviation in Chlorophyll-a $\mu\text{g/L}$ per site and per month and site of September 2020 and October 2020. The legend below graph A, applies to both individual bar graphs above.

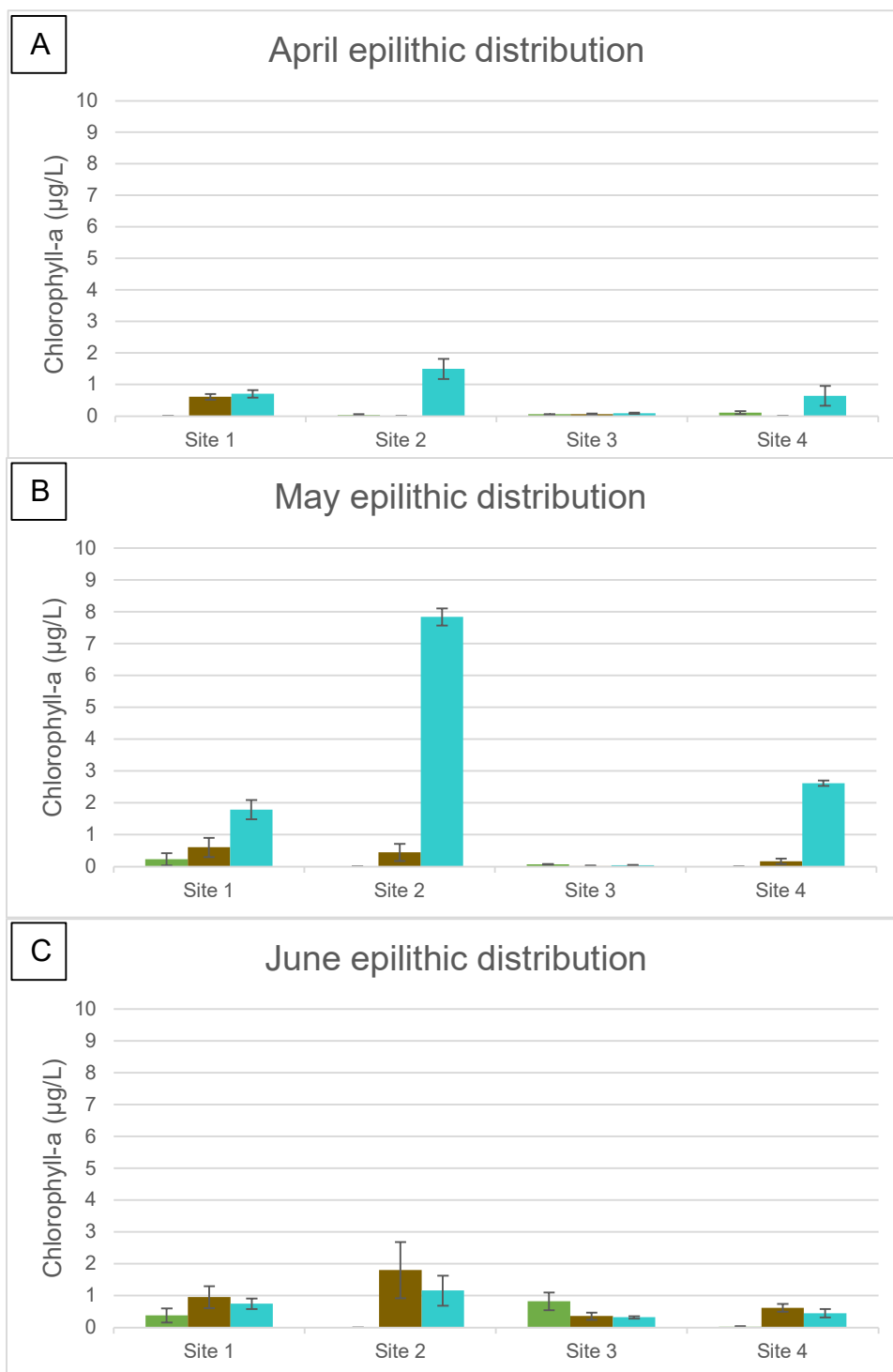


Figure 13: Average biomass of algae groups with standard deviation by site and month from April 2020 to June 2020. The legend below graph A, applies to all individual bar graphs above.

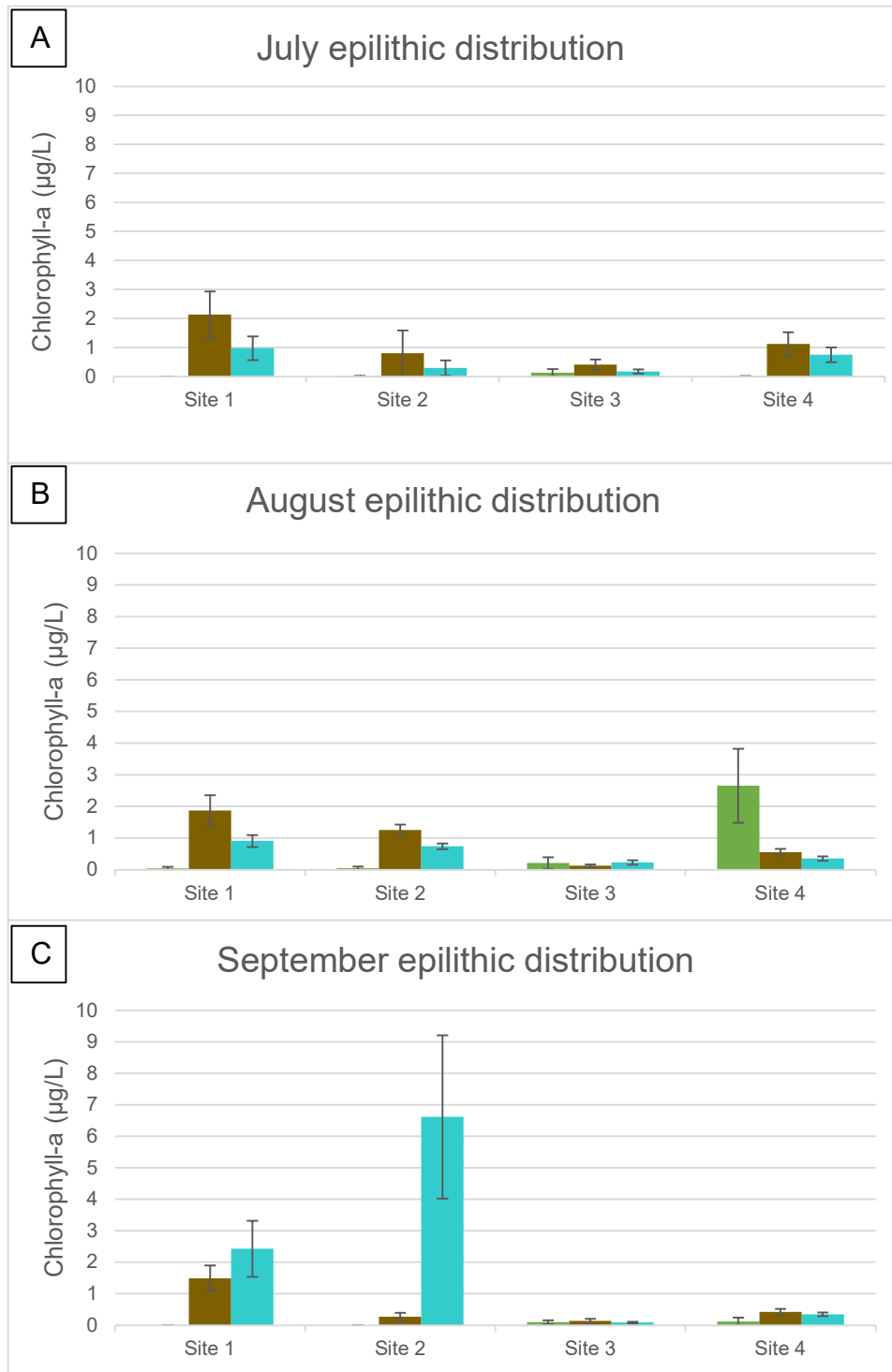


Figure 14: Average biomass of algae groups with standard deviation by site and month from July 2020 to September 2020. The legend below graph A, applies to all individual bar graphs above.

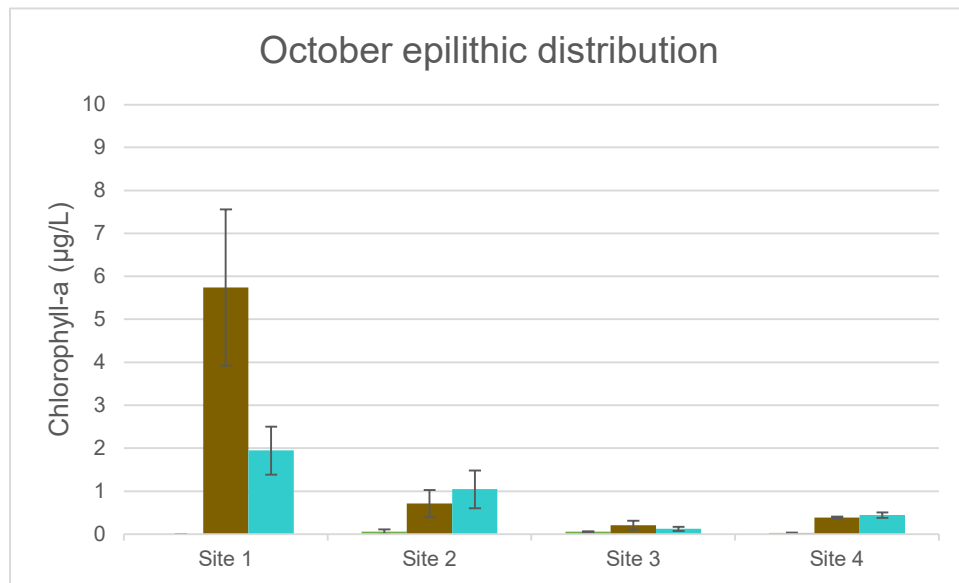


Figure 15: Average biomass of algae groups with standard deviation by site and month for October 2020.

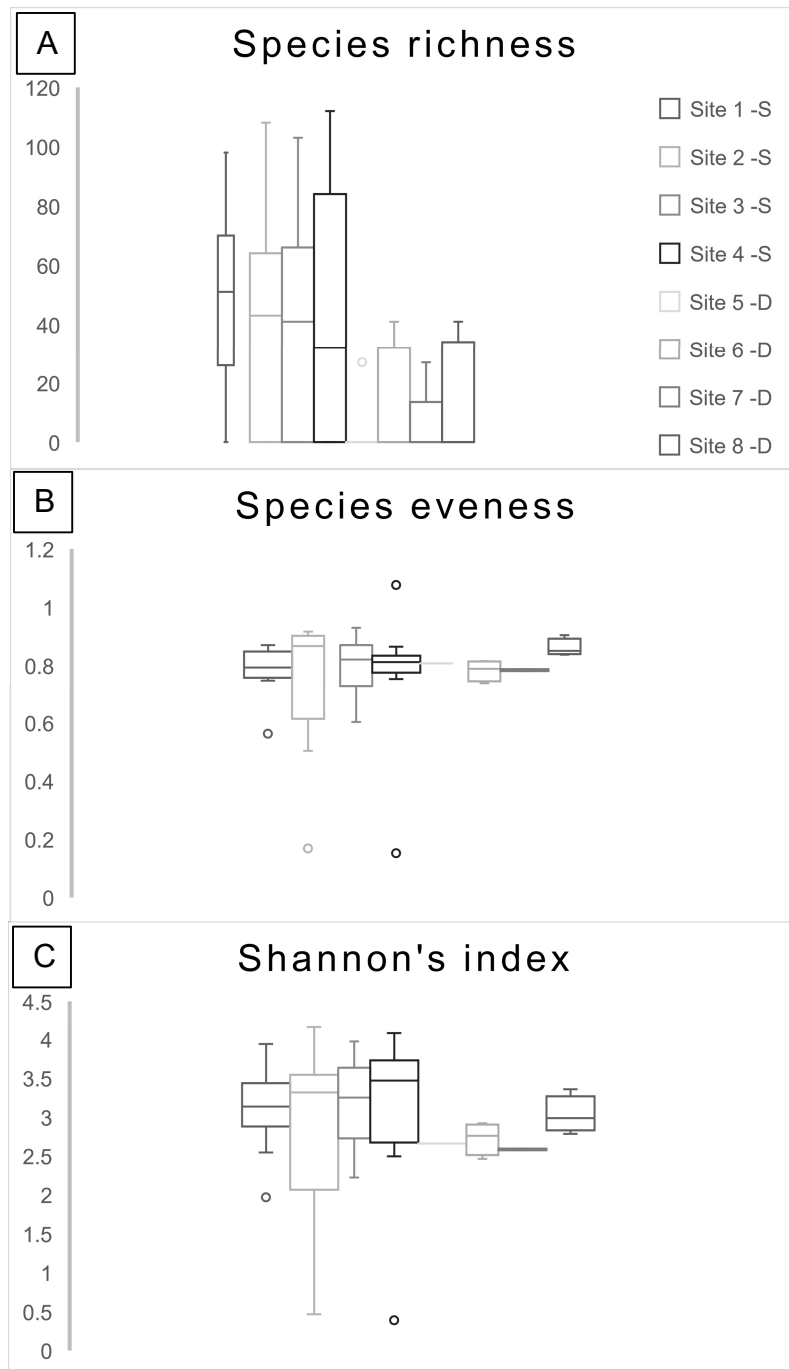


Figure 16: Boxplots describing community indices throughout the year. Boxes are 1-8 in order of sampling site. 1-4 are shallow sites and 5-8 are deep sites.

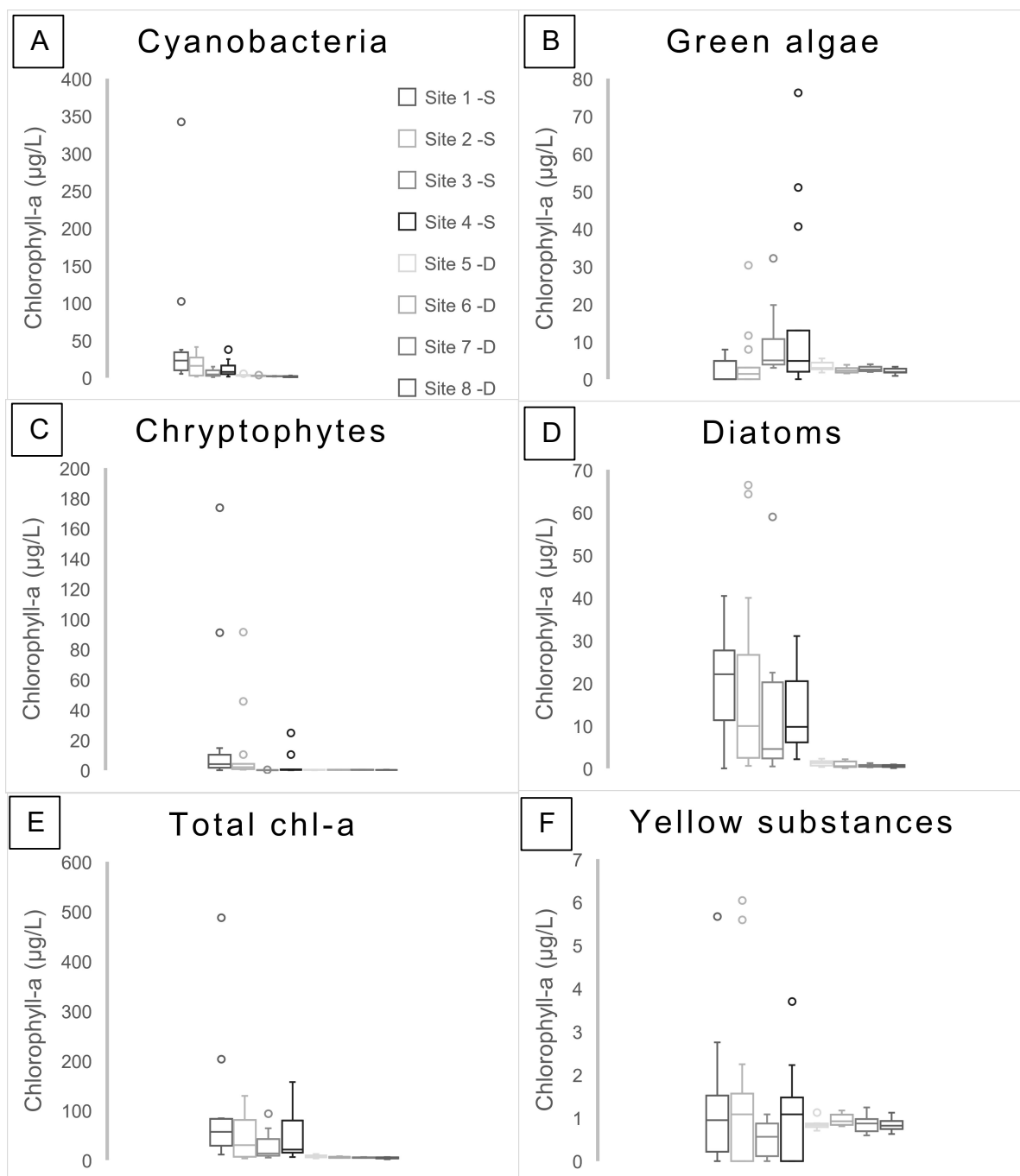


Figure 17: Total chlorophyll-a and algal group biomass along with yellow substances throughout the year. Boxes are 1-8 in order of sampling site: 1-4 are shallow sites and 5-8 are deep sites. Legend in graph A applies to all above.

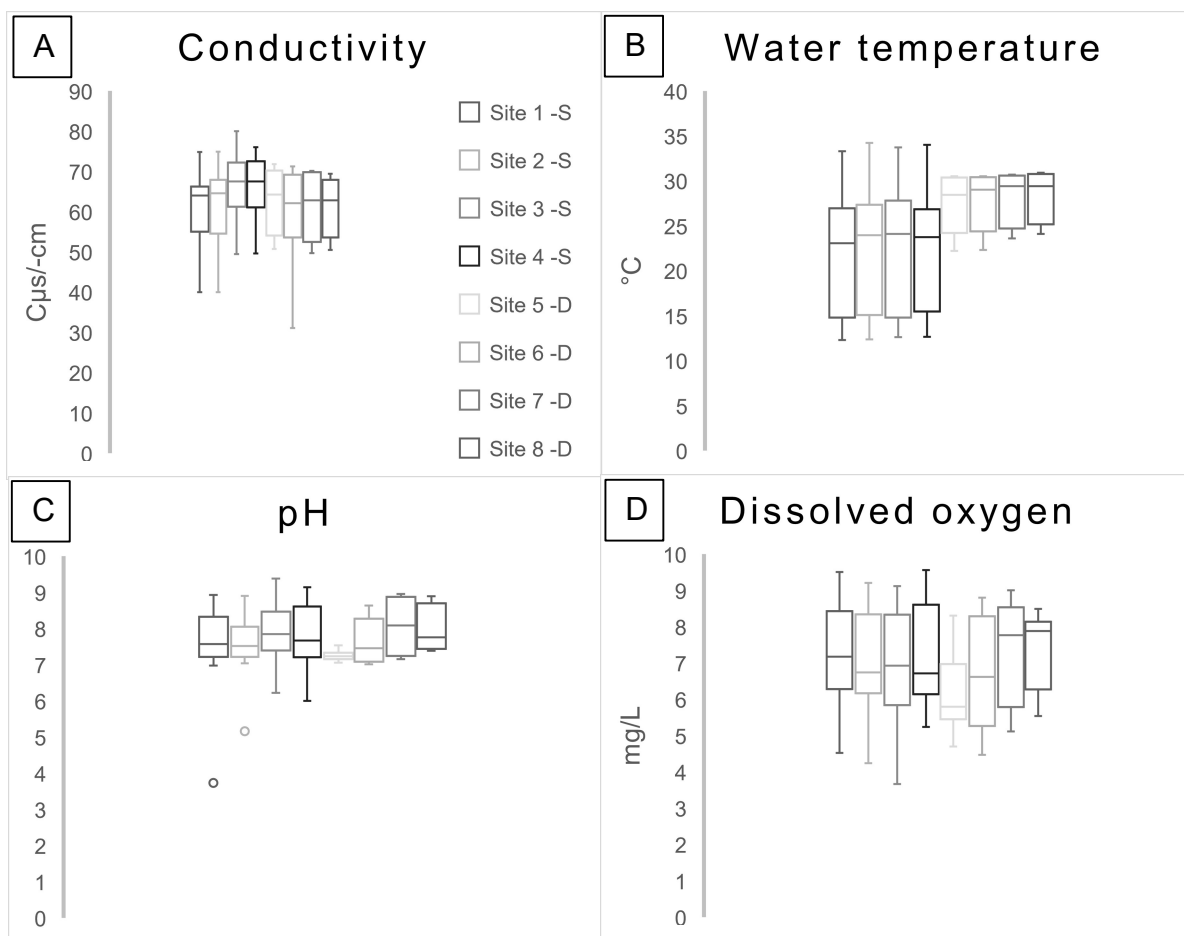


Figure 18: Boxplots describing the spread of physical and chemical data throughout the year. Boxes are 1-8 in order of sampling site: 1-4 are shallow sites and 5-8 are deep sites. Legend in graph A applies to all above.

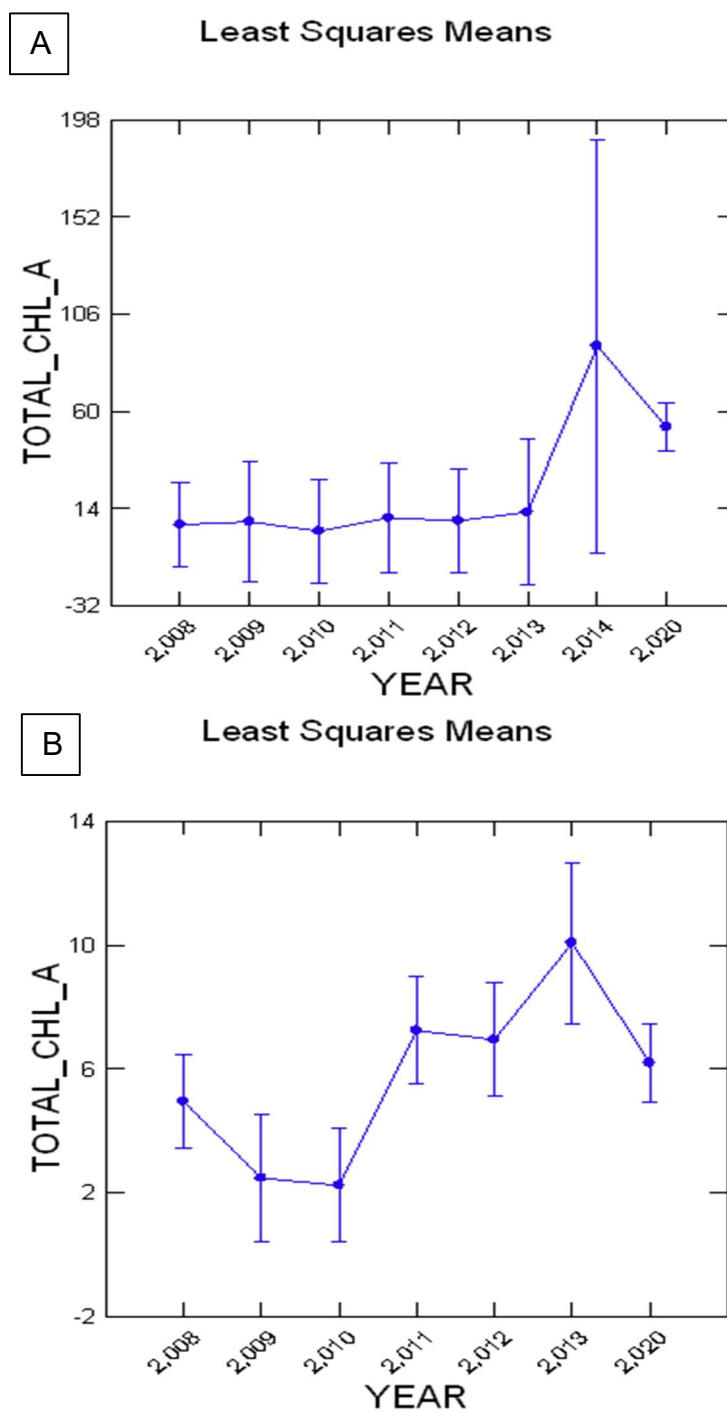


Figure 19: Historical chlorophyll-a ($\mu\text{g/L}$) data for Lake Sinclair according to previous studies done in the Manoylov lab. Graph A tracks shallow sites and graph B tracks deep sites. Chlorophyll-a recommendations for shallow sites are 21-23 $\mu\text{g/L}$. Chlorophyll-a recommendations for deep sites are 10-12 $\mu\text{g/L}$.

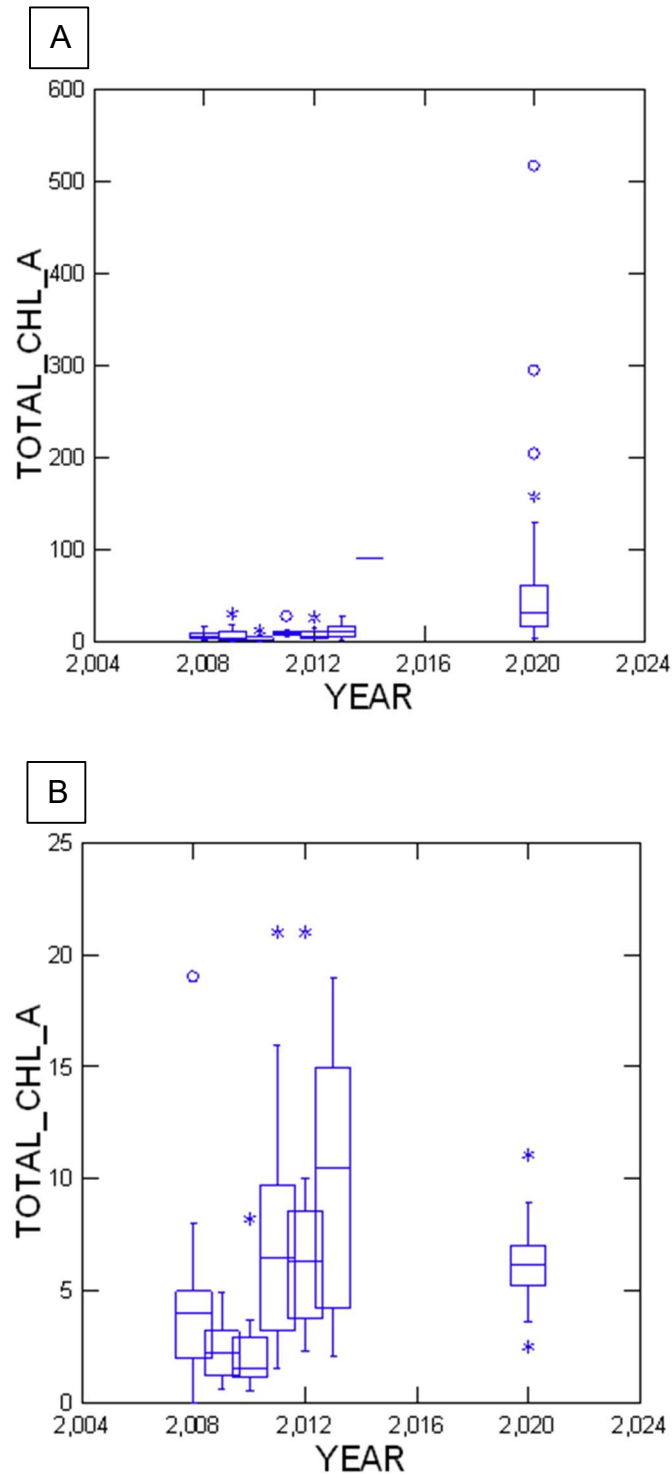


Figure 20: Historical chlorophyll-a (µg/L) data for Lake Sinclair according to previous studies done in the Manoylov lab as boxplots. Graph A tracks shallow sites and graph B tracks deep sites. Chlorophyll-a recommendations for shallow sites are 21-23 µg/L. Chlorophyll-a recommendations for deep sites are 10-12 µg/L.

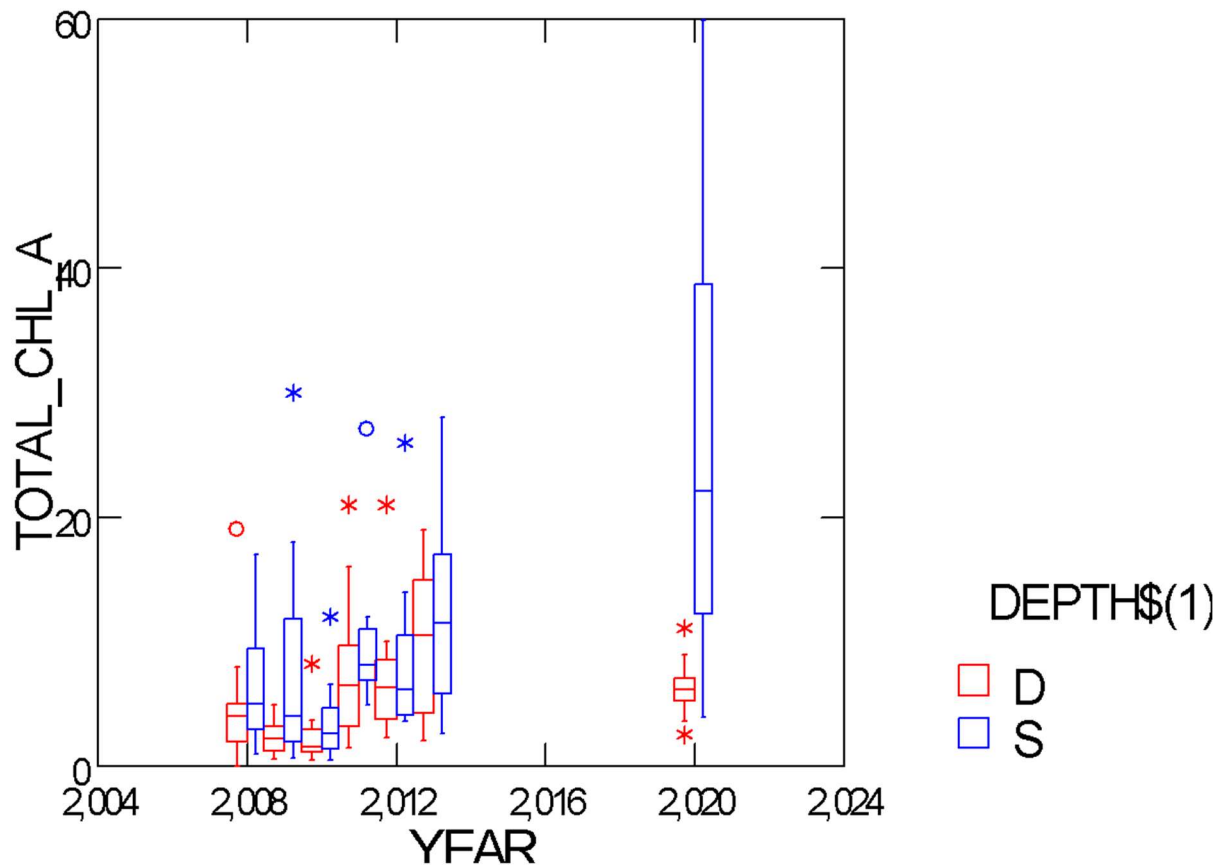


Figure 21: Historical chlorophyll-a ($\mu\text{g/L}$) data for Lake Sinclair according to previous studies done in the Manoylov lab. Shallow and deep site box plots are placed on the same scale for greater clarity. Red indicates deep sites and blue indicates shallow sites. Chlorophyll-a recommendations for shallow sites are 21-23 $\mu\text{g/L}$. Chlorophyll-a recommendations for deep sites are 10-12 $\mu\text{g/L}$.

Least Squares Means

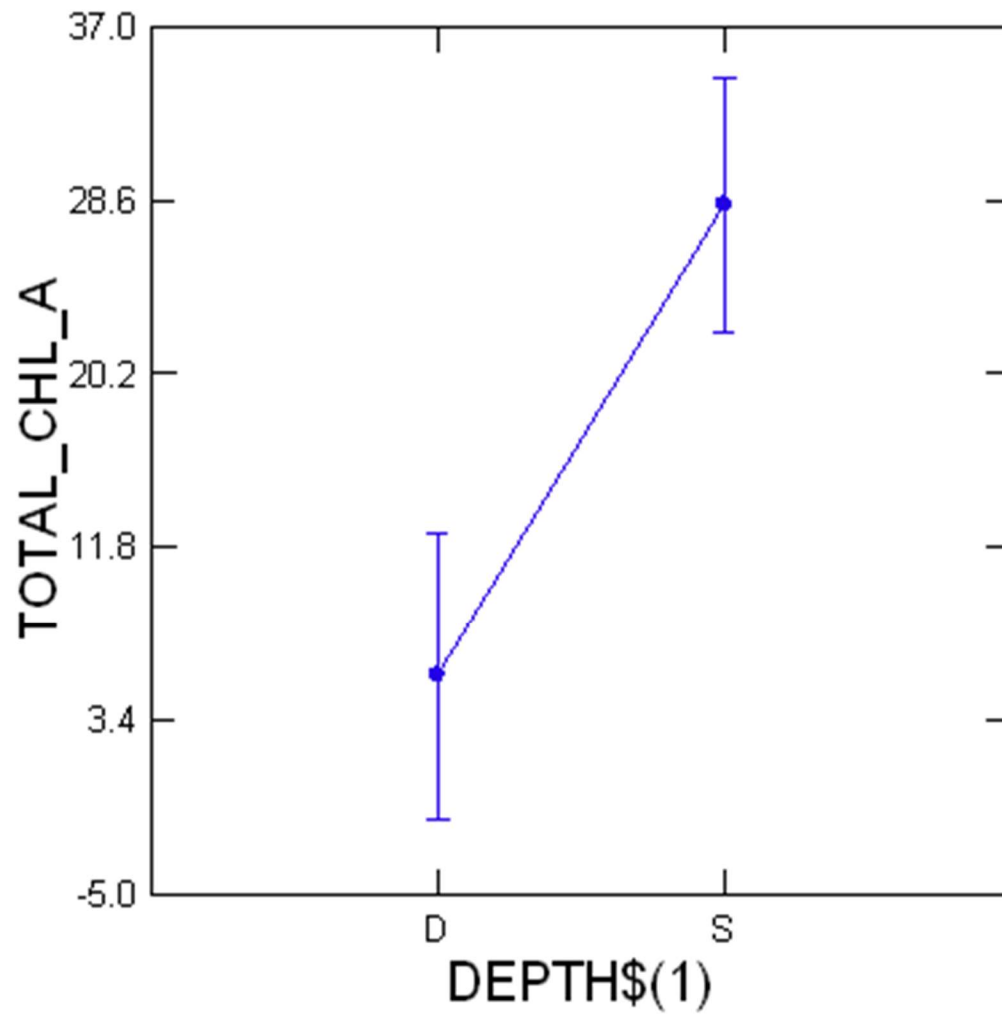


Figure 22: Difference between shallow (S) and deep (D) sites of total chlorophyll-a ($\mu\text{g/L}$) concentrations.

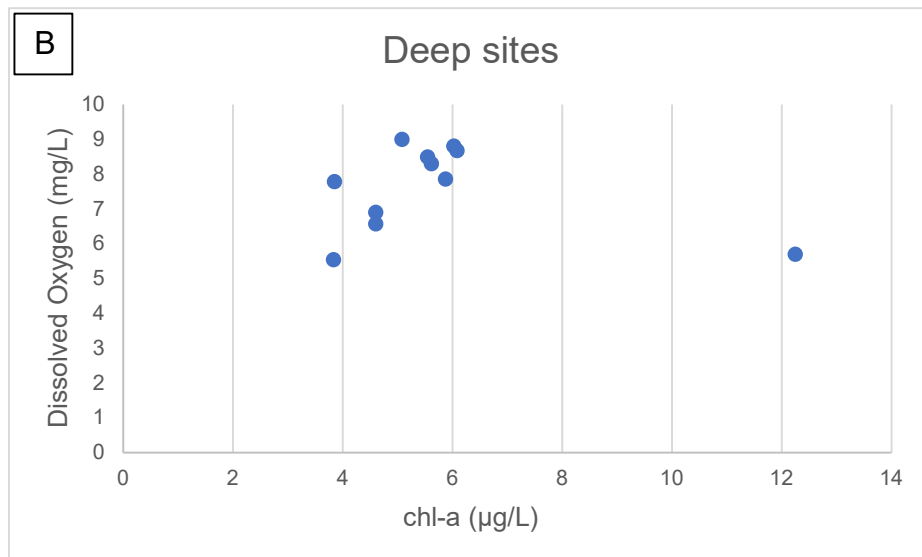
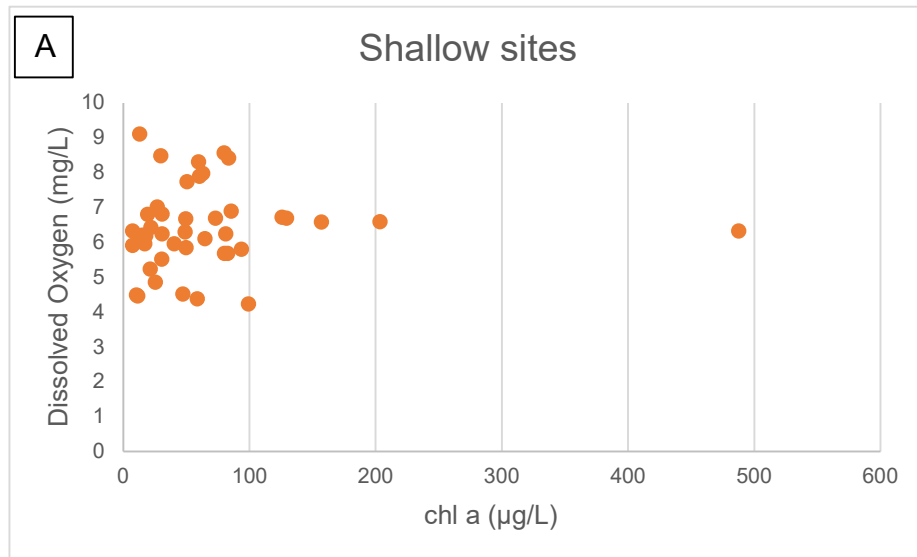


Figure 23: Relationship between dissolved oxygen and chlorophyll-a. Graph A is of shallow sites and graph B is of deep sites.

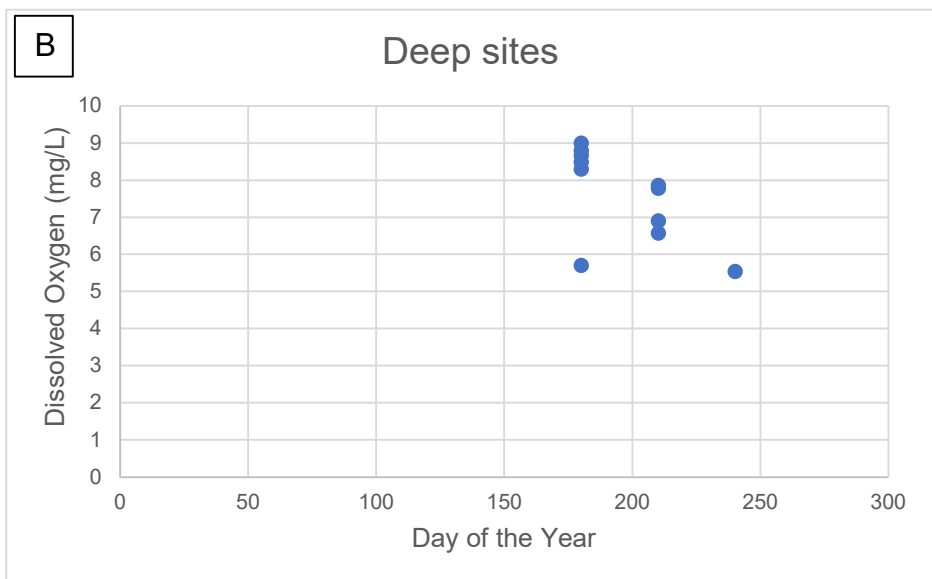
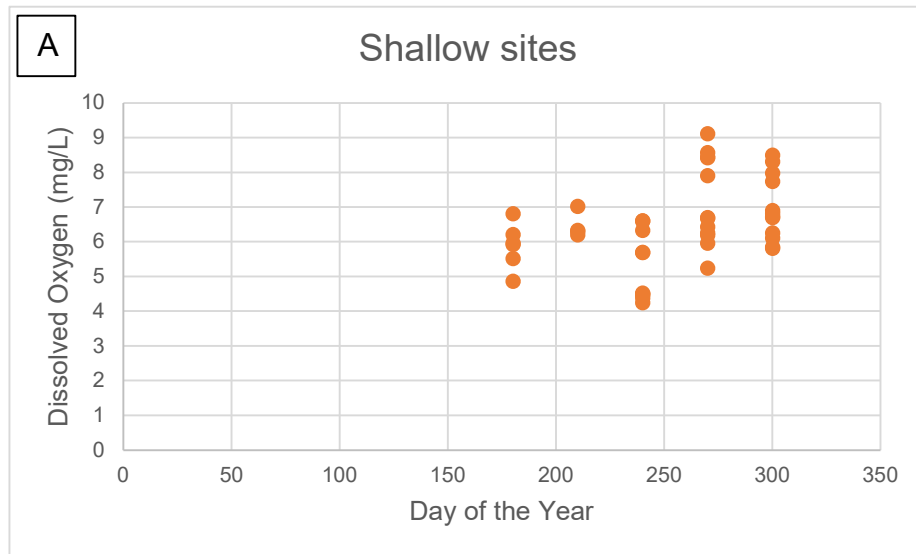


Figure 24: Relationship between dissolved oxygen day of the year. Graph A is of shallow sites and graph B is of deep sites.

IX. Plates

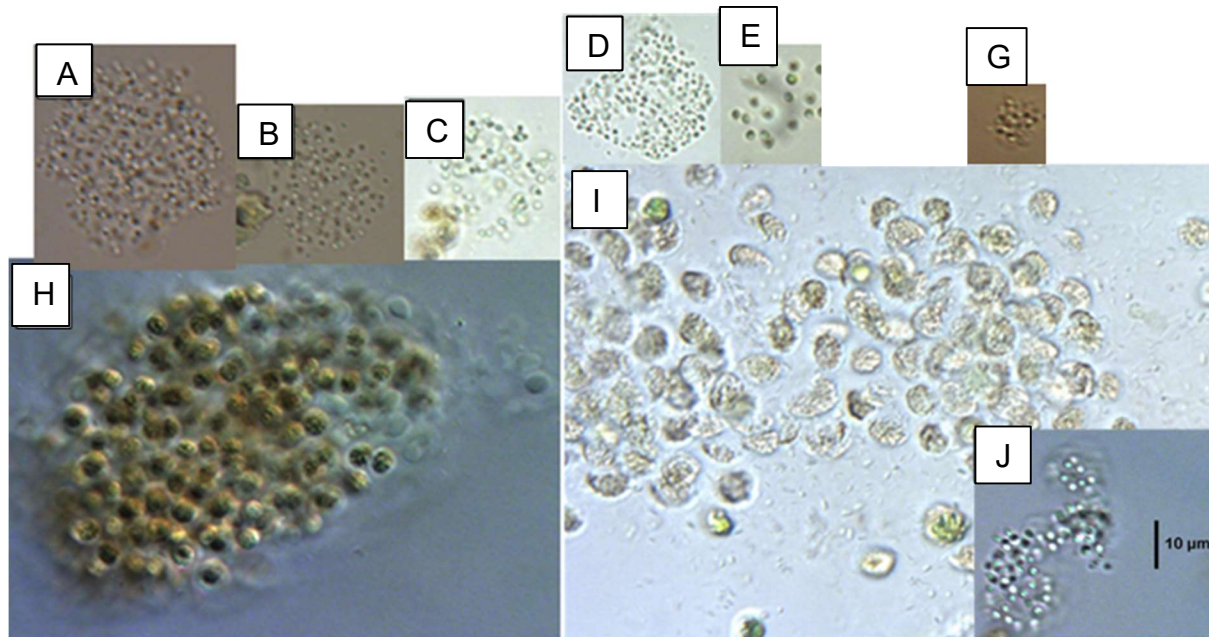


Plate I: Toxicogenic coccoid cyanobacteria A. *Aphanocapsa* sp. 1; B. *Aphanocapsa* sp. 2; C. *Aphanocapsa* sp. 3; D, J. *Aphanocapsa elachista* var. *conferta* (West) G.S. West; E. *Aphanocapsa* sp. 4; F. *Aphanocapsa* sp. 5; G. *Microcystis aeruginosa* (Kützinger) Kützinger; H. *Microcystis* sp. 2; I. *Aphanocapsa* sp. 6. Scale bar in I of 10 µm applied to all images.

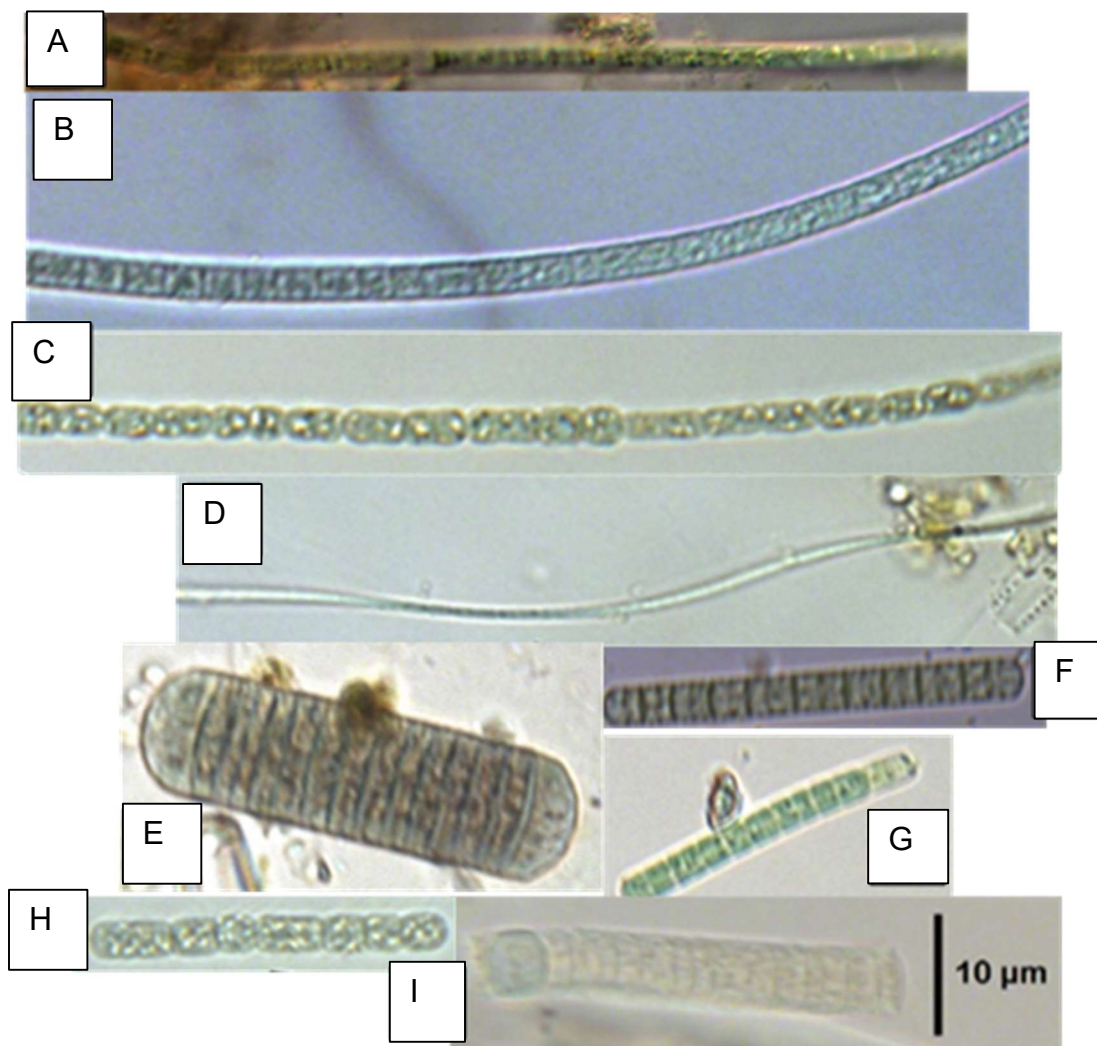


Plate 2: Filamentous cyanobacteria – A-C & F-I are toxigenic A. Planktothrix sp. 1; B. Planktothrix sp. 2; C. Anabaena sp. 1; D. Leptolyngbya sp. 1; E. Oscillatoria sp. 1; F. Planktothrix sp. 3; G. Planktothrix sp. 4; H. Anabaena sp. 2; I. Oscillatoria sp. 2. Scale bar in I of 10 μm applied to all images.

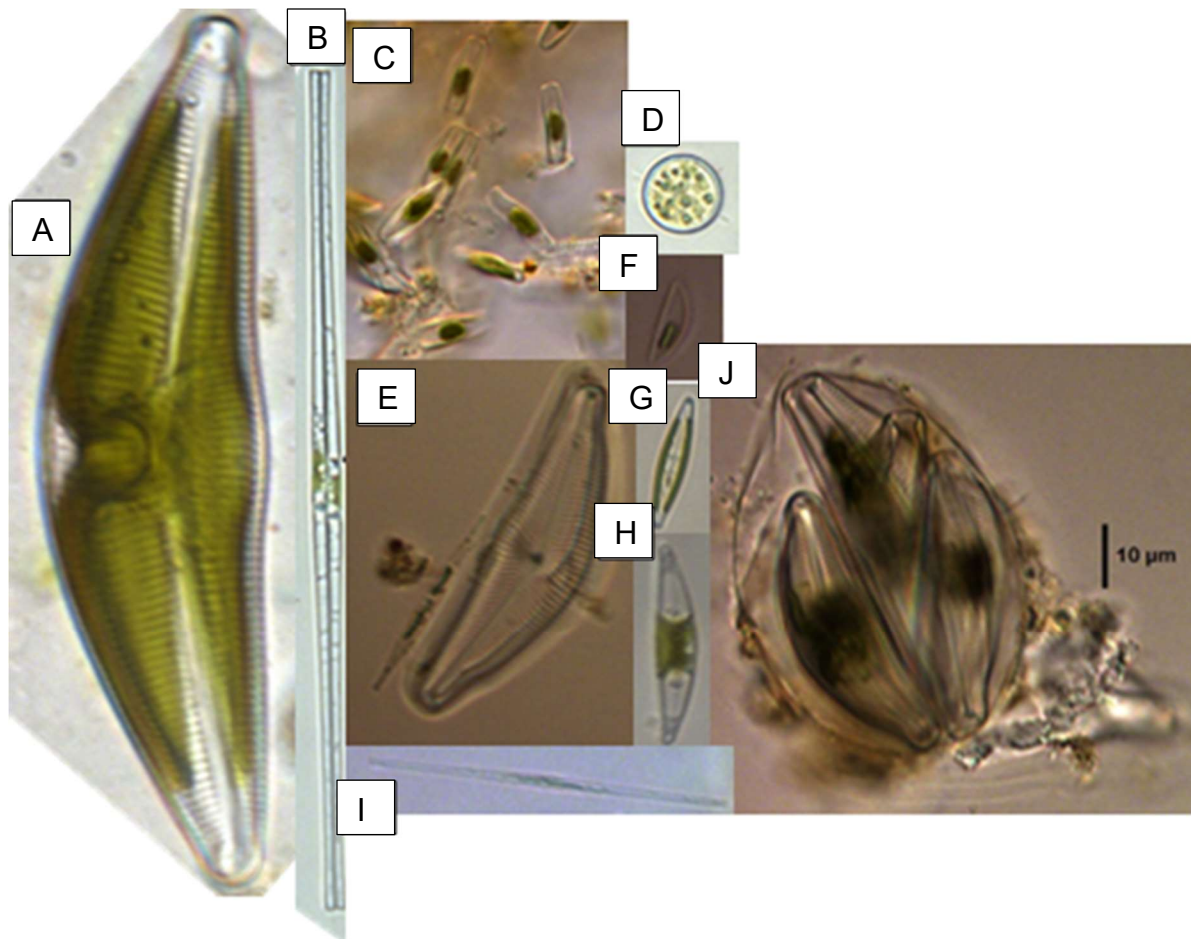


Plate 3: Diatoms, Bacillariophyta – A, E-F, H, & J cymbelloid diatoms (indicators of low nutrients) A. Cymbella aspera (Ehrenberg) Cleve; B. Synedra sp. 1; C. Achnantheidium minutissimum (Kützing) Czarnecki multiple cells in valve view; D. Cymbella sp. 1; E. Cymbella sp. 2; F. Encyonema minutum (Hilse) D.G. Mann; G. Navicula; H. Encyonema sp. 1; I. Synedra sp. 2; J. Encyonema sp. 2. Scale bar of in J 10 μm applied to all images.

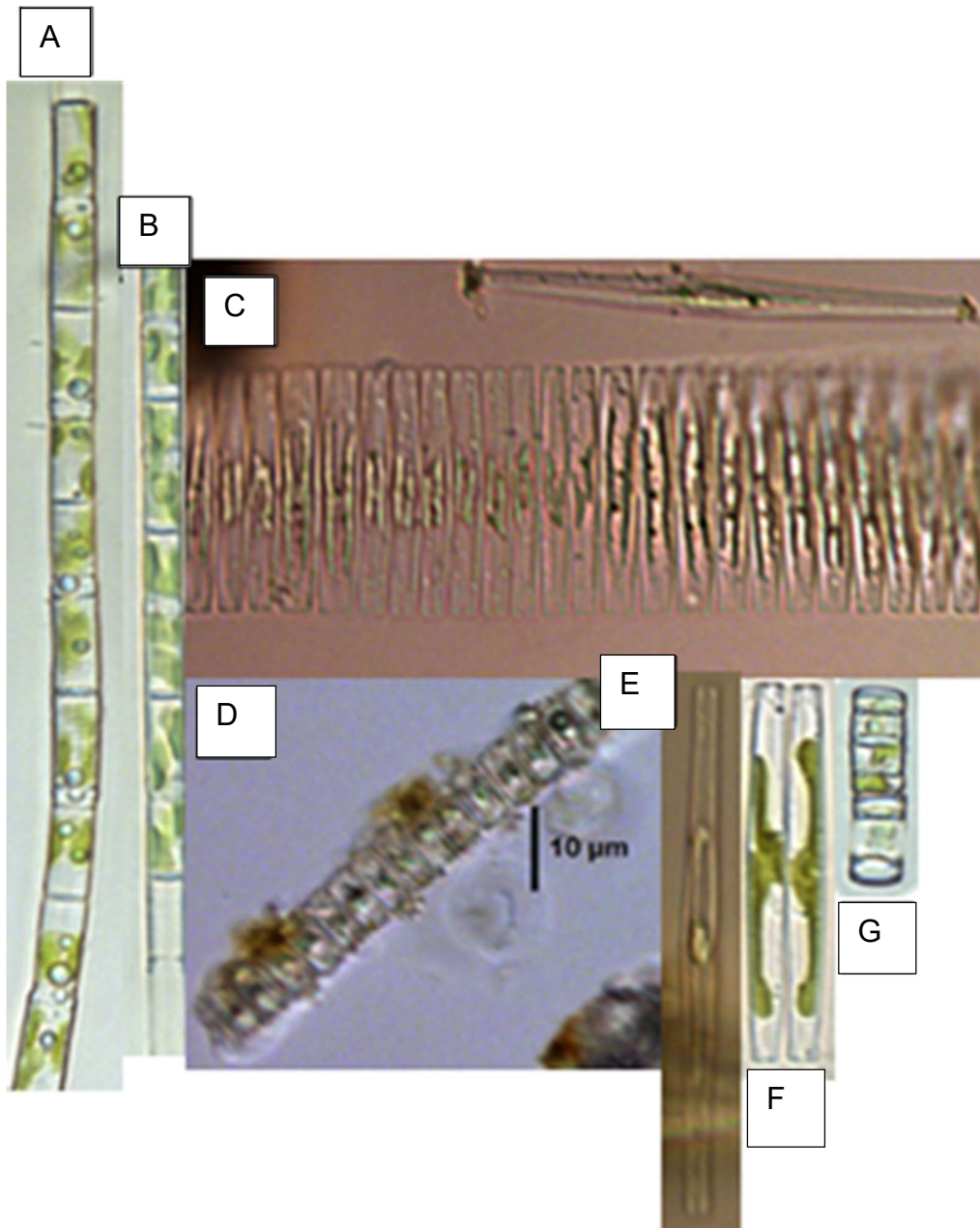


Plate 4: Diatoms, Bacillariophyta – A-B, G, Centric diatoms, C-F Araphid diatoms. A. *Aulacoseira* sp. 1; B. *Aulacoseira* sp. 2; C, F. *Fragilaria* sp. 1; D. *Staurosira* sp.; E. *Fragilaria* sp. 2; G. *Aulacoseira pusilla* (Meister) A.Tuji & A.Houki. Scale bar in D of 10 µm applied to all images.

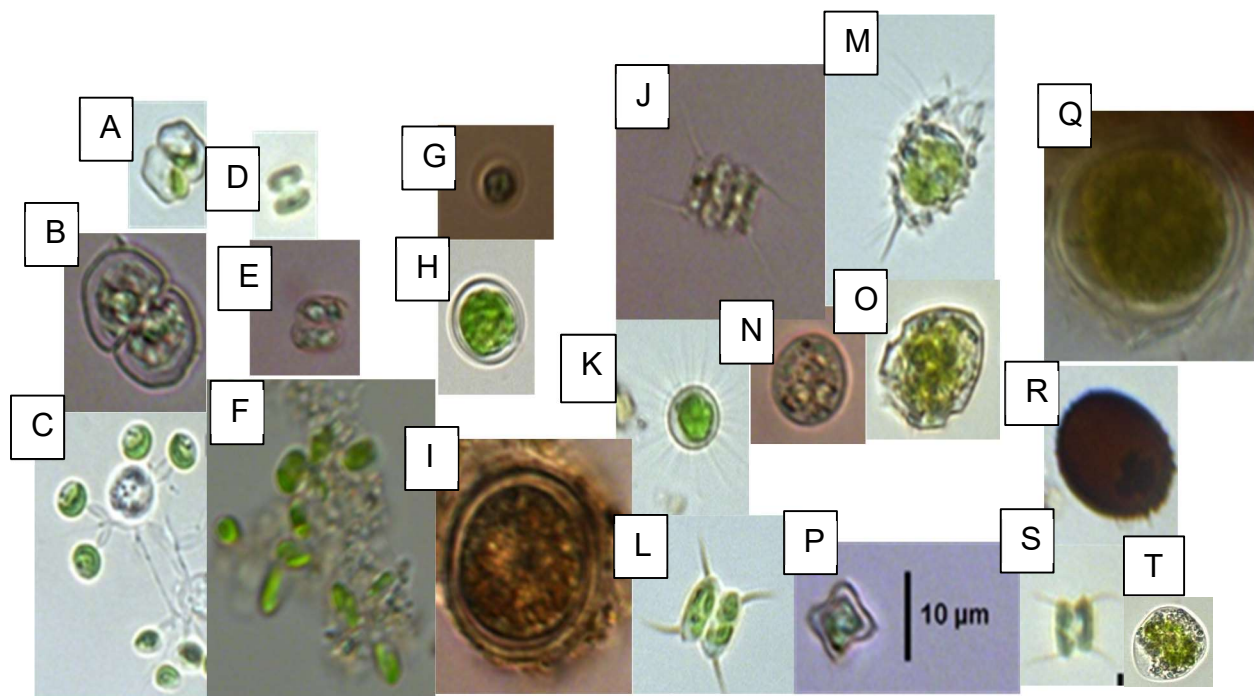


Plate 5: Other algae: Chlorophyta (A-H, J-L, & S-T), Dinoflagellata (I, O, & Q), Euglenophyta (H & R), and Synurophyceae (M). A. Euastrum sp.; B. Cosmarium sp.; C. Dictyosphaerium ehrenbergianum Nägeli; D. Scenedesmus ecornis (Ehrenberg) Chodat; E. Scenedesmus sp. 1; F. Chlorophyta spp.1; G. Gleocystis sp. 1; H. Trachelomonas sp. 1; I. Peridinium sp. 1 ; J. Desmodesmus quadricauda (Turpin) Brébisson ; K. Golenkinia sp. ; L. Desmodesmus sp. 1 ; M. Mallomonas sp. ; N. Chlorophyta spp.1; O. Parvodinium inconspicuum (Lemmermann) Carty; P. Tetraëdron sp. ; Q. Peridinium sp. 2 ; R. Trachelomonas sp. 2; S. Desmodesmus sp. 2 ;T. Chlorophyta spp.2. Scale bar in P of 10 μm applied to all images.