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The Impacts of Seasonality and Nutrient Loading on Microcystis Bloom Development in Wall Pond

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By

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ABSTRACT

The harmful cyanobacteria *Microcystis* globally dominates eutrophic freshwater systems. Eutrophication leading to nitrogen and phosphorus loading into aquatic systems is increasing bloom propagation and shifting diatom/dinoflagellate dominated systems to cyanobacteria dominated systems. Understanding seasonal variability and environmental parameters combined with nutrient loading will allow for better understanding of what factors are influencing *Microcystis* blooms. Biweekly plankton samples and environmental parameters were collected from Wall Pond from spring 2022-spring 2023. Results show that yearly plankton samples shifted from *Microcystis* dominated in the early summer to diatom dominated in the late summer-early fall then back to *Microcystis* dominated in late fall-early winter and then became dinoflagellate dominated in late winter-early spring. Results show that summer *Microcystis* blooms dominated due to increased temperatures and phosphorus, even though nitrogen was low. *Microcystis* bloomed in the late fall-early winter due to a hurricane that caused nutrient loading. *Microcystis* blooms dominated when N:P ratios were above Redfield ratio of 16. As N:P ratios and DIN concentrations decreased the system shifted from *Microcystis* dominated to dinoflagellate dominated. Bioassay results showed dual limitation of nitrogen and phosphorus.

INTRODUCTION

Globally, in freshwater ecosystems Harmful Algae Blooms predominated by cyanobacteria are becoming increasingly prevalent. The rise of harmful species is mainly due to anthropogenic nutrient loading as well as climate warming (Jankowiak et al., 2019). *Microcystis* is a cosmopolitan, colony-forming, toxic cyanobacteria that dominates in eutrophic freshwater systems (Moisander et al., 2009) during the summer and fall months (Affan, et al., 2005) and is

commonly associated with nondiazotrophic cyanobacteria (Paerl et al., 2001) and heterotrophic bacteria (Worm and Sondergaard et al., 1998). The production of the hepatotoxin microcystin by *Microcystis* impacts aquatic food webs, causes hypoxia (Leman et al., 2008 and Ghaffar et al., 2017), impedes drinking water, and recreational and commercial water use (Paerl et al., 2016, Shan et al., 2020, and Hark et al., 2016) which impacts millions of people worldwide. In Lake Taihu, China an increase of *Microcystis* blooms due to eutrophication has degraded water quality impacting 10 million people's access to drinking water (Paerl et al., 2011). In Lake Erie blooms of *Microcystis* in 2011 and 2014 costed 136 million total due to ecosystem impairments and water treatment (Paerl et al., 2016).

Eutrophication caused by nitrogen and phosphorus loading into aquatic systems has increased *Microcystis*. Fertilizer runoff leads to the introduction of urea into systems and can quickly cause pervasive blooms in closed water systems (Reed et al., 2016 and Paerl et al., 2016). Globally, nitrogen-based fertilizers have increased nine times and phosphorus-based fertilizers have increased three times since the 1980's (Glibert et al., 2014). Limiting nutrients in water bodies is highly variable but commonly freshwater systems are phosphorus limited (Ghaffar et al., 2017, Paerl et al., 2016 and Paerl et al., 2010). However, Jankowiak et al. (2019) found that *Microcystis* tends to be nitrogen limited because it has luxury uptake of phosphorus and can therefore thrive under lower phosphorus conditions. Similarly, harmful nondiazotrophic cyanobacteria often thrive under enlarged N:P conditions (Glibert et al., 2014 and Harke et al., 2016). Pearl et al. (2016) conducted a whole-lake bioassay study on 20 north-temperate lakes and found that 4 lakes experienced positive growth above the control when nitrogen was added, 9 lakes had positive growth under phosphorus addition, and 12 lakes had positive growth under nitrogen and phosphorus addition. Reed et al. (2016) found cyanobacteria species thrive under

high urea and ammonium concentrations. Specifically, *Microcystis* requires combined sources of nitrate, ammonium, and dissolved organic nitrogen (Paerl et al., 2016). Glibert et al. (2014) and Paerl et al. (2016) suggests that joint control of nitrogen and phosphorus should be utilized to best control harmful cyanobacteria blooms.

Microcystis bloom initiation requires water temperatures above 20°C and nitrogen and phosphorus input (Lehman et al., 2008, Srifa et al., 2016 and Jankowiak et al., 2019). Buoyant blooms of *Microcystis* can quickly rise to the surface and out compete other species in calm vertically stable waters with long residence times (Lehman et al., 2008 and Worm and Sondergaard et al., 1998). *Microcystis* has several adaptations allow for large bloom propagation including gas vacuoles (Ghaffer et al., 2017), luxury uptake of phosphorus (Jankowiak et al., 2019 and Harke et al., 2016), solar radiation tolerance (Kramer et al., 2018), mucus producing large colonies to avoid grazing, and overwintering in sediments (Ghaffer et al., 2017, Kramer et al., 2018, and Harke et al., 2016). Monospecific *Microcystis* blooms thrive in high pH, highly turbid, and low carbon waters. Similarly, microcystin production has been hypothesized to increase under stressful environmental conditions including, oxidative stress (Paerl and Otten, 2013) low nitrogen to phosphorus ratios, alteration of intracellular carbon to nitrogen ratios, increased temperatures, radiation limitation, and iron limitation (Shan et al., 2020). However, toxin production and bloom initiation vary widely in time and space.

Studying how seasonal and nutrient conditions cause shifts from diatom dominated systems to cyanobacteria dominated systems is important to better understand bloom development and ecosystem functions. The objective of this study is to determine how seasonal and nutrient variance impact *Microcystis* bloom development and phytoplankton assemblages on Coastal Carolina University campuses Wall Pond, South Carolina. Specific care will be given to

harmful cyanobacteria species presence (*Aphanizomenon, Dolichospermum, Microcystis, Planktothrix, Raphidopsis*) as they could also cause toxin production in Wall Pond. Wall pond is understudied freshwater system highly impacted by human influence and potentially impacted by point-source fertilizer run-off. Having a better understanding what factors influence bloom initiation in Wall Pond will allow for increased ability to predict and thereafter mitigate when a bloom of *Microcystis* occurs in closed-water systems.

METHODS

Site description:

Wall Pond is an approximately 9,060 m^2 freshwater system centrally located on Coastal Carolina University campus in South Carolina (Figure 1). The pond is a shallow, closed man-made system with potential storm drainage. The blooms are most likely impacted by fertilizer input from adjacent lawns and biological waste products from the ponds resident turtle population.

Field sampling:

Biweekly plankton samples and environmental parameters were collected from Wall Pond from spring 2022-spring 2023. Environmental parameters of temperature (°C), wind speed (mph), wind chill (°C), relative humidity (%), heat stress index (°C), dewpoint (°C), wet bulb temperature (°C), and barometric pressure (lnHg) were collected using a Kestrel instrument model 5500 to establish a seasonal variance in weather. The wind direction and precent cloud cover was also recorded. A YSI Pro2030 was utilized to establish aquatic environmental parameters of O2 (mg/L), O2 (%), Conductivity (mS/cm), salinity, and water temperature (°C). Water was transferred to two 1000 mL bottles via a bucket for future nutrient analysis. Bioassay

experiments for nutrient limitation were conducted seasonally by transferring water from the bucket into an acid washed 20 L caraboid for immediate set-up in the laboratory. Plankton samples were collected by towing a 20-micron plankton net for three minutes and then immediately looked at in lab.

Laboratory:

After collecting water from Wall Pond, the mean in-vivo concentration of phycocyanin (μg/L), phycoerythrin (μg/L), and chlorophyll (μg/L) was measured in triplicate using a Turner AquaFlour and turbidity (NTU) was measured using a Hach 2100Q instrument. 30 mL of water was transferred into 50 mL Blue Falcon tubes in triplicate for Nitrate + Nitrite analysis. 10 mL of water was transferred into 15 mL Blue Falcon tubes in triplicate for ammonium and phosphorus analysis into 50 mL and 15 mL falcon tubes respectively. All samples were frozen for future analysis.

Plankton samples were viewed using an Olympus BX51 microscope. Relative abundance of *Aphanizomenon*, *Dolichospermum*, *Microcystis*, *Planktothrix*, *Raphidopsis*, centric diatoms, pennate diatoms, dinoflagellates, cyanobacteria, cladocera, ciliates, rotifers, green algae, *Ceratium*, and *Peridinium* was noted on a scale of not present to elevated. During large dinoflagellates blooms a hemocytometer was used for cell counts. To preserve samples for plankton counts, 2.25 mL of Lugols solution was added to a 45 mL of sample water.

Seasonal bioassays were conducted to determine if there is a seasonal variation in nutrient limitation in the pond. Triplicate acid cleaned bottles were amended with nitrate (25 μm), phosphate (6 μm) and nitrate and phosphate and incubated for 48 hours in a thermos scientific precision incubator set to an ambient temperature with 12-hour light cycles. After

incubation samples were filtered using a vacuum pump and a 0.7-micron GF/F glass fisher filter for future analysis of chlorophyll production.

Chlorophyll *a* was measured in the laboratory using fluorometry based on methodology described by Arar and Collins (1997). Nitrate and nitrite were measured colorimetrically in the lab using spectrophotometry based on methodology outlined in Bendschneider and Robinson (1952). Ammonium was measured fluorometrically in the lab using a fluorometer as outlined in Holmes et al. (1999). DIN was calculated by combining nitrate plus nitrite and ammonium concentrations. Phosphorus was measured colorimetrically in the lab using a spectrophotometer as outlined in Murphy and Riley (1962).

RESULTS

Environmental Factors:

The temperature in the pond ranged from 14.1 °C to 28.6 °C with an average temperature of 20.8 °C (Figure 2). The salinity in the pond ranged from 0.1 ppt to 0.5 ppt with an average salinity of 0.38 ppt (Figure 3). In June oxygen concentration was under the biological stress threshold (Figure 4A). Dissolved oxygen concentrations ranged from 4.8-13.1 mg/L (Figure 4A). For most of the year, DO was supersaturated in the winter months and undersaturated in the summer months (4B). However, it was above the biological stress threshold most of the year (4A). Turbidity was largely variable throughout the year and ranged from 2.6 NTU to 14.03 NTU (Figure 5).

Nutrients:

Ammonium concentration peaked at 8.93 μmol/L during the beginning of the fall/winter *Microcystis* bloom then decreased throughout the bloom event (Figure 6, and Table 1).

Ammonium stayed low throughout the rest of the study. Nitrate and nitrite concentrations rapidly increased until peaking on 11/3/22 at 15.64 μmol/L (Figure 7). Then nitrate and nitrite concentrations decreased throughout the rest of the fall/winter *Microcystis* bloom event into the *Peridinium* bloom (winter bloom) and started to rise again during the pollen event on 3/1/23. Phosphate concentration remained low then rapidly increased during the pollen event (Figure 8). Phosphate concentrations ranged from 0.53 to 2.19 μmol/L. Phosphate increased to 0.94 μmol/L during the summer (July) bloom of *Microcystis* but averaged 0.61 μmol/L during the fall/winter bloom of *Microcystis*. Dissolved inorganic nitrogen concentrations ranged from 2.83 μmol/L on 2/1/23 to 22.45 μmol/L on 10/20/22 (Figure 9). DIN increased from 8.64 μmol/L on 10/6/22 to 12.19 μmol/L on 11/17/22 peaking on 10/20/22 at μmol/L. The fall/winter *Microcystis* bloom correlated with high concentrations of dissolved inorganic nitrogen. Dissolved inorganic nitrogen concentrations decreased as *Peridinium* blooms became abundant in the pond. The nitrogen to phosphorus ratio in Wall Pond was above Redfield ratio of 16 during the *Microcystis* bloom event from fall/winter (Figure 10). N:P ratios declined as the plankton assemblages shifted from *Microcystis* to *Peridinium* blooms. N:P ratios ranged from 2.9 during the pollen event to 34.5 during October.

Nitrogen and phosphorus dual treatment had a significant limitation on chlorophyll concentration on 3/1/22 (p-value<0.5, t-test) (Figure 11). The control chlorophyll concentration was 11.3 μg/L compared to nitrogen treatment chlorophyll of 13.1 μg/L, phosphorus treatment chlorophyll of 12.0 μg/L, and nitrogen and phosphorus treatment chlorophyll of 13.82 μg/L.

Chlorophyll:

Chlorophyll concentrations are not significantly correlated to DIN (R^2=0.0095), Phosphate (R^2= 0.0045), or Temperature (R^2=0.0757) (Figure 12). Wall Pond was

hypereutrophic (>56 μg/L) regarding chlorophyll in July and was eutrophic (7.3-56 μg/L) the rest of the year except for October (Figure 13). Chlorophyll concentrations ranged from 4.88 to 61.42 μg/L. Phycocyanin and phycoerythrin concentrations ranged from 21.76 to 95.52 and 0.22 to 0.48 respectively (Figure 14). During the summer bloom chlorophyll and phycocyanin and phycoerythrin concentrations increased to 61.42 μg/L, 95.52, and 0.48 respectively (Figure 13 and 14). At the beginning of the fall/winter *Microcystis* bloom, chlorophyll was low but then increased during the peak bloom on 11/6/22. Phycocyanin and phycoerythrin increased at the beginning of October *Microcystis* bloom and then steadily decreased throughout the bloom event (Figure 14). Phycocyanin and phycoerythrin concentrations increased again from the *Peridinium* bloom to the pollen event.

Plankton assemblages:

Cyanobacteria bloom events occurred in the late fall-early winter and in July (Table 1 and Figure 15). *Microcystis* dominated cyanobacteria bloom events, however other cyanobacteria species both harmful and nonharmful increased during *Microcystis* blooms. Rotifers and centric diatoms increased between the summer and late fall bloom of *Microcystis*. *Peridinium* blooms increased towards the end of the *Microcystis* bloom event and persisted into spring. Hemocytometer counts of *Peridinium* ranged from 23,750 to 77,500 cells/mL with the largest count occurring in February (Figure 16). The *Peridinium* bloom ended before a large pollen event occurred. The pollen event occurred on 3/1/23 and was characterized by large pollen streaks on the pond and the microscopic sample was dominated by pollen molecules (Figure 15E).

DISCUSSION

Environmental Factors:

Findings indicate that Wall Pond experiences seasonal variance in plankton assemblages based on nutrient concentration and environmental parameters. Four major bloom events occurred in Wall Pond (Table 1). The summer *Microcystis* bloom occurred on 7/20/22. The second diatom and rotifer bloom occurred in August (fall bloom). Then another *Microcystis* bloom occurred from October to December (fall/winter) and finally a dinoflagellate bloom occurred during the winter (winter). Finally, phytoplankton assemblages crashed as the incidence of pollen increased in March and created a layer of pollen on the pond.

The summer bloom of *Microcystis* can be correlated with temperatures above 20°C and high turbidity (Figure 2 and 5). Affan et al. (2005) reported incidence of *Microcystis* blooms occurring when temperatures are above 20°C regardless of nitrogen to phosphorus ratio inputs in aquaculture ponds in Bangladesh. Harke et al. (2016) a review, found that incidence of cyanobacteria increased under warmer temperatures compared to eukaryotic phytoplankton. In August the plankton community shifted from a cyanobacteria dominated system to a diatom and rotifer dominated system (Table 1). Similarly, Affan et al. (2005) found that phytoplankton blooms increased from August to September due to an increase in temperature, pH, and light availability. Increases in rotifer abundance could have created a grazing pressure on *Microcystis*. Although temperature stayed consistently above 20°C (Figure 1), salinity and turbidity decreased after the July bloom (Figure 3 and 5). Oxygen also decreased to 5.35 mg/L and became undersaturated in terms of dissolved oxygen percentage (Figure 4). Throughout the study salinity stayed below 1 ppt therefore most likely did not have any impact on plankton assemblages.

Microcystis concentrations increased from October to December following hurricane Ian at the end of September that produced 4.15 inches of rain (NOAA). Although temperatures fell to below the optimal temperature range for *Microcystis*, nutrient inputs could have fueled the fall/winter bloom as DIN and N:P ratios spiked after the hurricane (Figure 9 and 10). Turbidity increased at the bloom peak in December and then decreased as the *Microcystis* bloom shifted to a *Peridinium* bloom (Figure 5). Oxygen concentrations stayed undersaturated but declined after the hurricane event, then increased during the *Microcystis* fall/winter bloom (Figure 4).

During the winter, temperatures were below cyanobacteria optimal temperature regimes (Figure 2). *Peridinium* blooms increased during the winter to concentrations ranging from 27,000 to 82,500 cells/mL (Figure 16). These findings differ from the conclusions by Affan et al. (2005) who found phytoplankton abundances decline during the winter due to a decrease in temperatures, low pH, a decrease in light availability, and a decline in nutrients. Dissolved oxygen became supersaturated during the *Peridinium* bloom and then declined during the pollen event.

Future studies should consider *Microcystis* impacts on pH. *Microcystis* has been reported to increase surface pH to above 9 as they consume inorganic carbon, thus giving *Microcystis* a competitive advantage against other phytoplankton specifically diatoms (Wilhelm et al., 2020). During the fall/winter bloom of *Microcystis* diatom concentrations decreased in the sample (Table 1). Considering CO_2 concentrations can also be important to determine *Microcystis* usage of CO_2 and subsequent impact on plankton assemblages (Wilhelm et al., 2020 and Harke et al., 2016). *Microcystis* has an affinity for both high and low CO_2 concentrations which will allow it to continue bloom development under a variety of conditions and could be specifically harmful as anthropogenic climate change alters CO_2 concentrations (Harke et al., 2016).

Nutrients:

Microcystis blooms occurred when N:P ratios were above 16 in October to December (Figure 10). Similarly, Paerl et al. (2011) reported that N:P ratios in Lake Taihu, China was highest during winter. Cyanobacteria species are favored at high N:P ratios compared to diatoms and dinoflagellates (Hillebrand et al., 2013). Based on experimental modeling Hillebrand et al. (2013) found that diatoms prefer low N:P ratios (14.9), dinoflagellates prefer medium (15.1), and cyanobacteria prefer high N:P (25.8). Liu et al. (2019) found the N:P optimal growth range for *Microcystis* was 32. However, Lehman et al. (2008) reported that *Microcystis* dominated under lower N:P ratios of 10 in August and September in the San Francisco Estuary.

During the summer *Microcystis* bloom, phosphate concentrations increased 0.33 μm/L above the average phosphate concentration of 0.61 μm/L (Figure 8). Phosphate concentrations during this bloom event averaged 0.94 μm/L (Figure 8). Similarly, Lefebvre, C. (2020) found that *Microcystis* reached a maximum growth rate when cultures were spiked with 1 μM of phosphate. After the bloom event nutrient regimes varied as diatoms and rotifers started to increase in the fall. During the fall bloom nitrogen concentrations tended to increase as phosphorus decreased (Figure 6, 7, and 8).

The October hurricane event caused nutrient loading from runoff in Wall Pond favoring a late fall-early winter *Microcystis* bloom. After the hurricane event ammonium concentration increased to 8.93 μmol/L, nitrate and nitrite concentration increased to 15.64 μmol/L, DIN increased to 15.64 μmol/L, and N:P increased to 34.5 (Figure 6, 7, 9 and 10). Nutrient loading could have occurred due to an increase in runoff transporting fertilizer and other nutrients to the pond. Jankowiak et al. (2019) results from the western region of Lake Erie showed that nitrogen loading coupled with high temperatures can increase the impacts of eutrophication and frequency

of cyanobacteria blooms. Contradictory to our study where *Microcystis* dominated at high concentrations of DIN (22.45 μmol/L in October), Jankowiak et al. (2019) reported that *Microcystis* blooms dominated at low DIN concentrations (4 μmol). Phosphorus concentrations remained relatively low throughout the fall/winter bloom and Harke et al. (2016) found that phosphorus limitation often occurs during bloom initiation and that *Microcystis* can readily sequester phosphorus at low levels within eutrophic, turbid, shallow waters (Harke et al, 2016 and Jankowiak et al., 2019). During the fall/winter bloom event Wall Pond experienced an increase in turbidity and was eutrophic based on chlorophyll conditions (Figure 5 and 13). Gilbert et al. (2014) found that *Microcystis* favors reduced nitrogen forms specifically ammonium and urea for growth based on the impacts of fertilizer studies within freshwater systems in China (Gilbert et al., 2014, Harke et al., 2016 and Paerl et al., 2016). Therefore, future studies should include urea as a nitrogen source that could impact phytoplankton assemblages. As climate change increases global temperatures, the incidence of hurricane events will increase and propagate further into the fall-winter season. Eutrophication impacts will also become strengthened when coupled with increased temperatures (Jankowiak et al., 2019). Thus, altering aquatic systems from having a low plankton biomass in the winter to cyanobacteria dominated systems based on nutrient availability and low competition (Jankowiak et al., 2019 and Paerl and Otten, 2013).

Peridinium blooms started propagating at the end of November as nutrients and *Microcystis* started to decline (Figure 15). *Microcystis* blooms collapse as nitrogen decreases (Affan, et al., 2005). At the end of the *Peridinium* bloom to the pollen event the system became eutrophic in relation to phosphate (Figure 8). Nitrogen was relatively low during this period, suggesting nitrogen could be limiting plankton assemblages during this time. The N:P ratio also

declined from January to March. This coincides with the bioassay results which found nitrogen and phosphorus to be dual limiting the system during March 2023.

Chlorophyll:

Chlorophyll concentration during the summer *Microcystis* bloom was hypereutrophic (Figure 13). These results correlate with Lehman et al. (2008) that found that maximum chlorophyll concentrations occurred when temperatures were above 20°C. This bloom was also associated with high concentrations of phycocyanin and phycoerythrin (Figure 14). After the bloom event Chlorophyll concentrations dipped below hypereutrophic levels (Figure 13). Phycocyanin and phycoerythrin concentration also decreased (Figure 14). During October chlorophyll declined below eutrophic conditions (Figure 13). Across the rest of the study chlorophyll stayed at eutrophic concentrations. At the peak fall/winter bloom Chlorophyll spiked to 31.51 μg/L before declining during the *Peridinium* bloom. Phycocyanin and phycoerythrin concentrations increased during the pollen event even though chlorophyll declined (Figure 13 and 14).

Bioassay:

Bioassay results showed that plankton assemblages were dual limited by nitrogen and phosphorus (t.test, p-value=0.053, Figure 10). Harke et al. (2016) also found that co-loading nitrogen and phosphorus increases *Microcystis* bloom events (Harke et al., 2016 and Kramer et al., 2018). Paerl et al. (2016) study on 20 different lakes found in Canada, Sweden, Norway, and Alaska found that 12/20 lakes that were dual limited by nitrogen and phosphorus. Dual nitrogen and phosphorus treatment chlorophyll was 2.70 μg/L above the control, therefore suggesting other factors might be impacting bloom propagation. Light limitation could be a significant

factor limiting phytoplankton. Pollen streaks and an abundance of pollen particles were present during the 3/1/23 sample. Future studies should conduct monthly bioassays to determine monthly nutrient limitation.

CONCLUSION

In this study, seasonality and nutrient parameters were utilized to determine the factors that are impacting plankton assemblages, specifically *Microcystis* in Wall Pond, SC. Biweekly environmental, aquatic, and plankton samples were collected from March 2022 to March 2023. The results demonstrate that *Microcystis* can dominate under low nutrient regimes in the summer due to high temperatures, however, requires large nutrient loading of both nitrogen and phosphorus to dominate during the late fall-early winter. *Microcystis* blooms dominated when N:P ratio is above 16. Once *Microcystis* blooms decline there is an assemblage shift to diatoms and rotifers in the summer and dinoflagellates in the winter. During periods of low plankton biomass nitrogen and phosphorus were dual limiting.

TABLES AND FIGURES

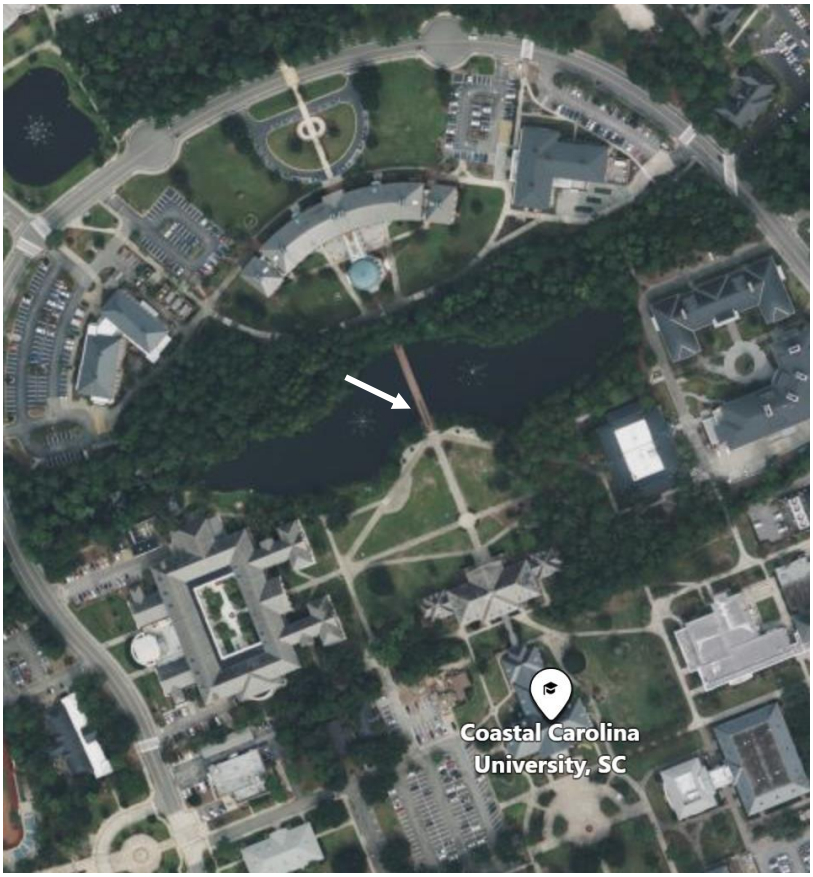


Figure 1: Wall Pond located at Coastal Carolina University Conway, SC sampling site.

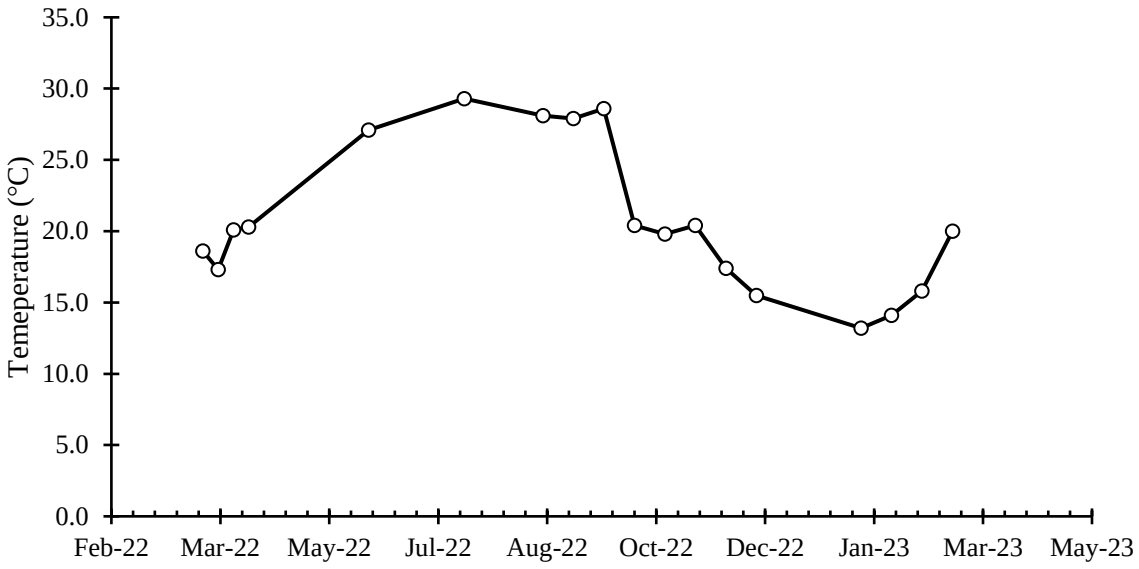


Figure 2: Water temperature (°C) in Wall Pond, SC between March 2022 to March 2023.

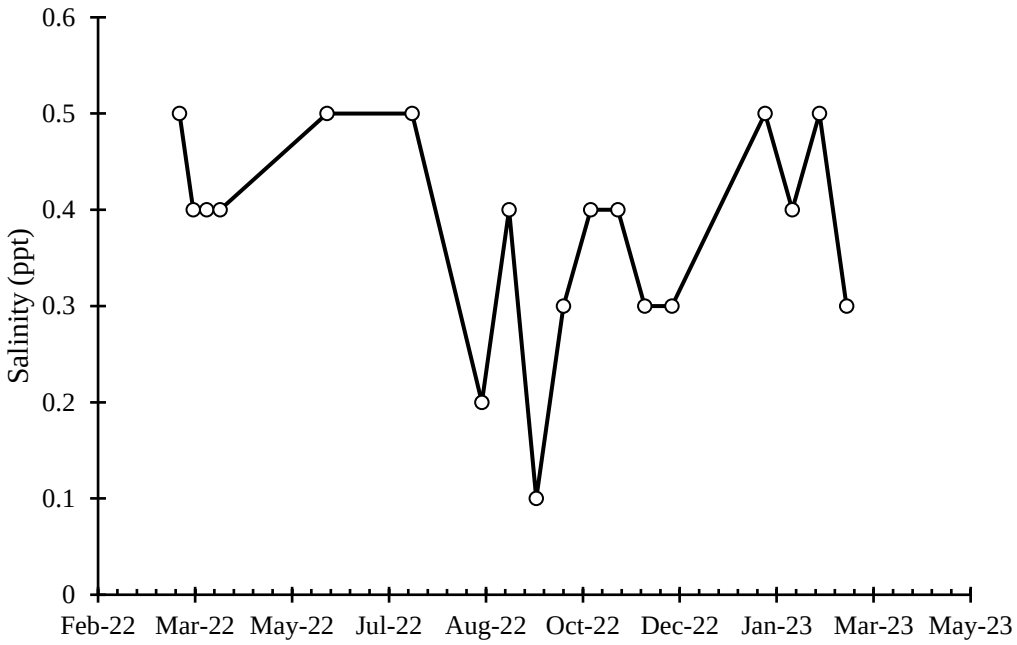
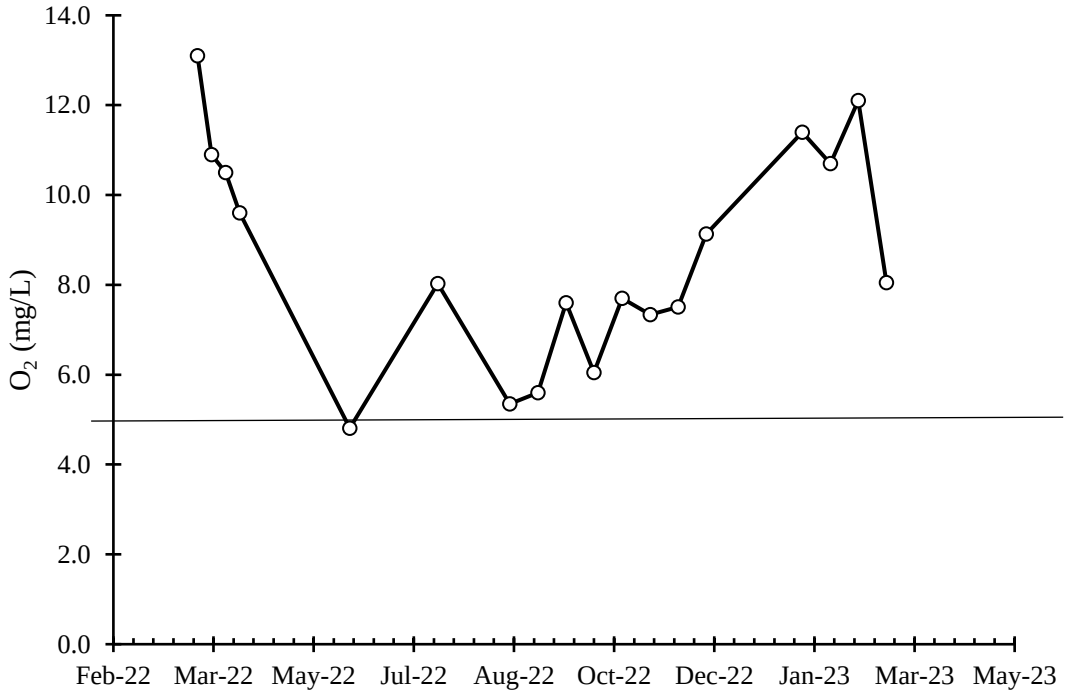
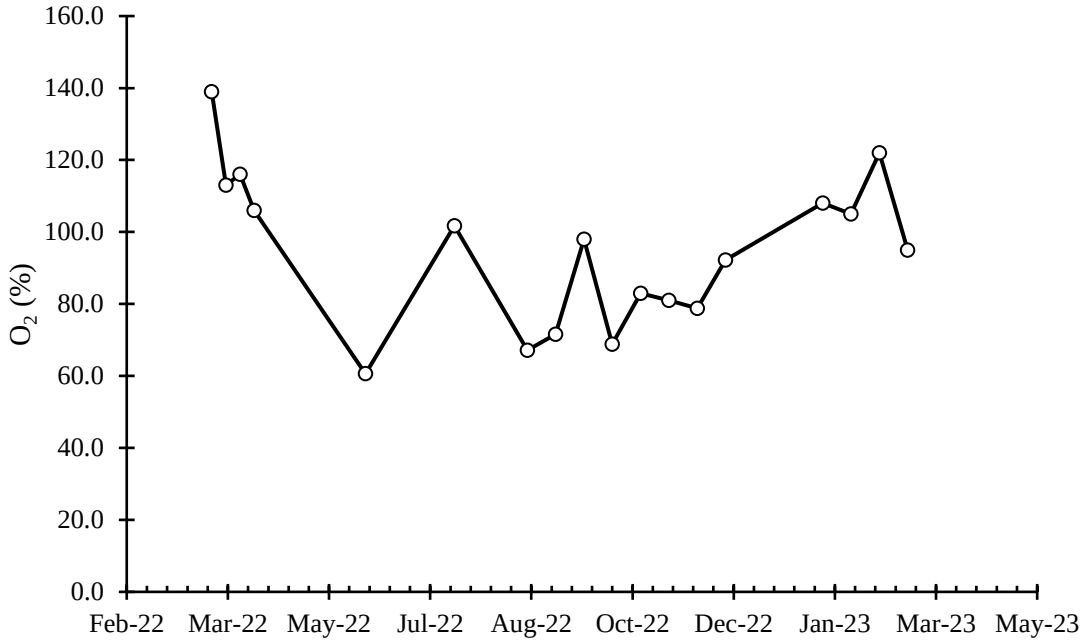


Figure 3: Salinity (ppt) in Wall Pond, SC between March 2022 to March 2023.



A)



B)

Figure 4: A) Oxygen (mg/L) in Wall Pond, SC from March 2022 to March 2023. The horizontal line indicates the biological stress threshold. Oxygen (%) in Wall Pond, SC from March 2022 to March 2023.

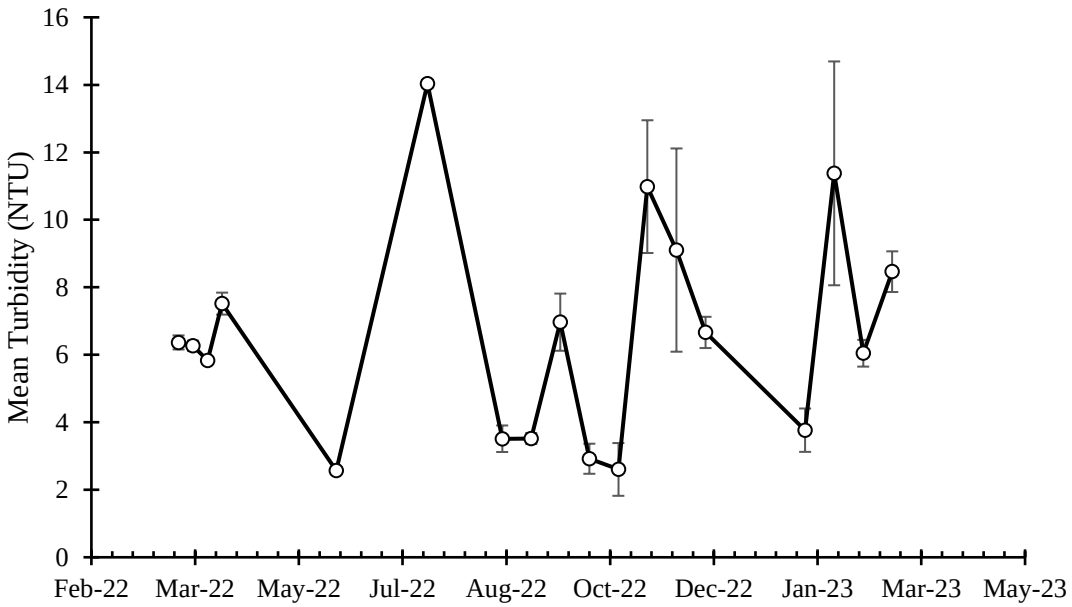


Figure 5: Turbidity (NTU) in Wall Pond, SC from March 2022 to March 2023. Error bars represent standard deviation.

Table 1: Plankton assemblage relative abundance in Wall Pond, SC from 7/20/22 to 3/1/23. Green boxes indicate the species wasn't present. Yellow boxes indicate the species is present in the sample. Red boxes indicate the species was abundant in the sample.

	7/20/2022	8/25/2022	9/8/2022	9/22/2022	10/6/2022	10/20/2022	11/3/2022	11/17/2022	12/1/2022	1/18/2023	2/1/2023	2/15/2023	3/1/2023
Aphanizomenon spp.													
Dolichospermum spp.													
Microcystis spp.													
Planktothrix spp. spp.													
Raphidiopsis spp													
Centric Diatoms													
Pennate Diatoms													
Dinoflagellates													
Cyanobacteria													
Cladocera													
Ciliates													
Rotifers													
Green Algae													
Peridinium													
Ceratium													

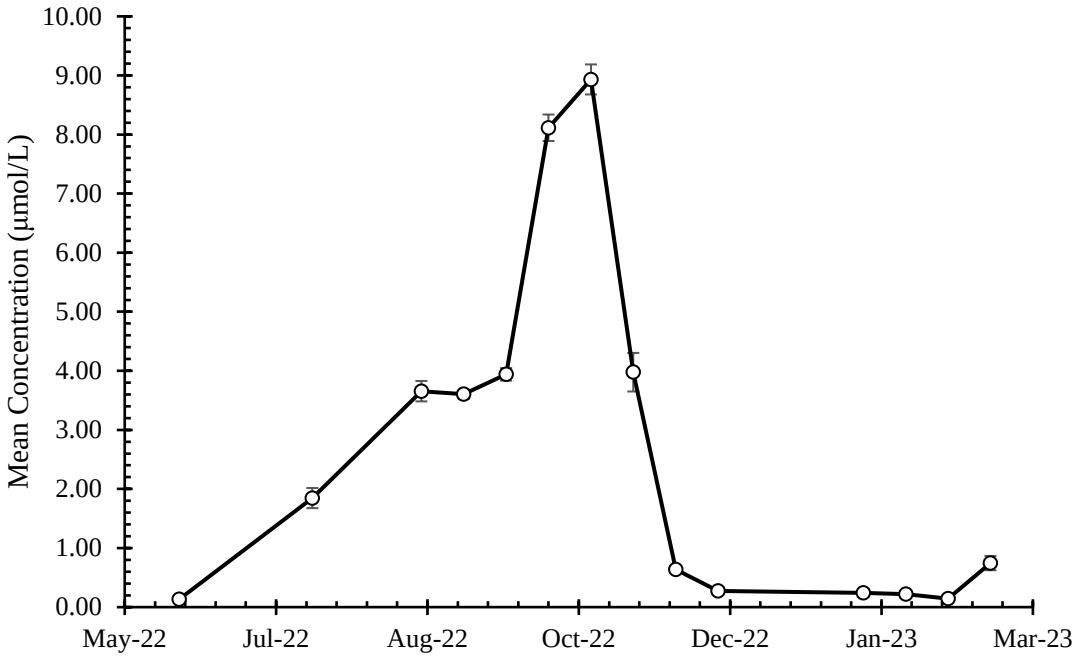


Figure 6: Mean Ammonium concentration (µmol/L) in Wall Pond, SC from June 2022 to March 2023. Error bars represent standard deviation.

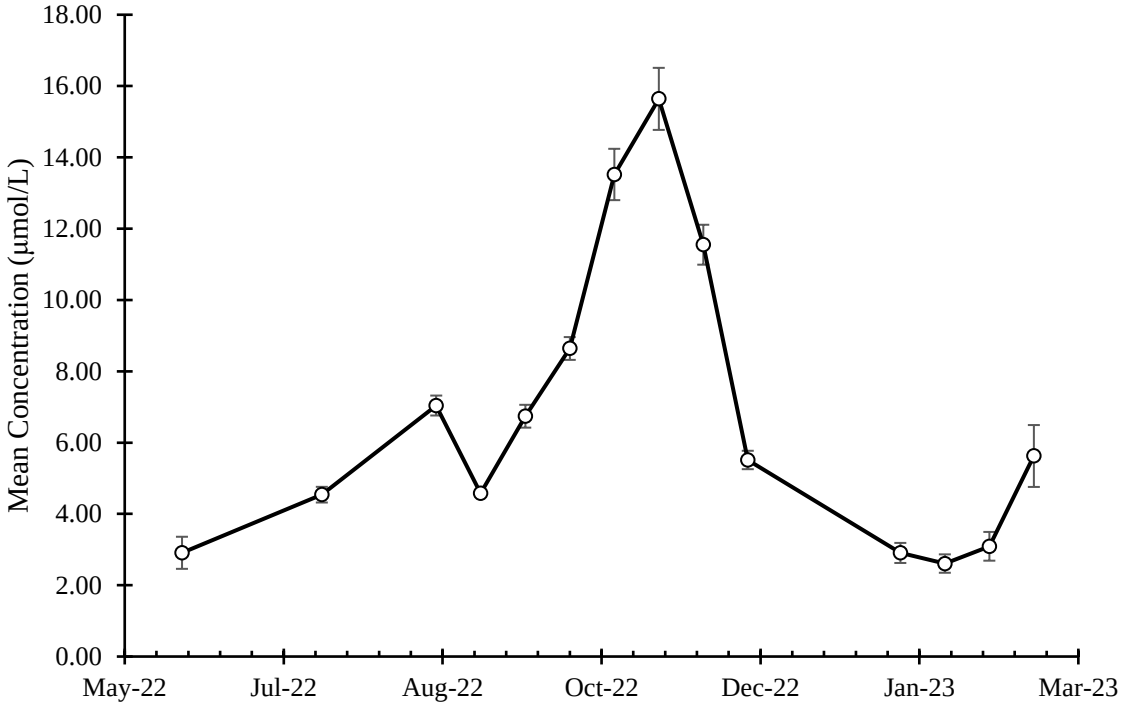


Figure 7: Mean Nitrate + Nitrite concentrations (µmol/L) in Wall Pond, SC from June 2022 to March 2023. Error bars represent standard deviation.

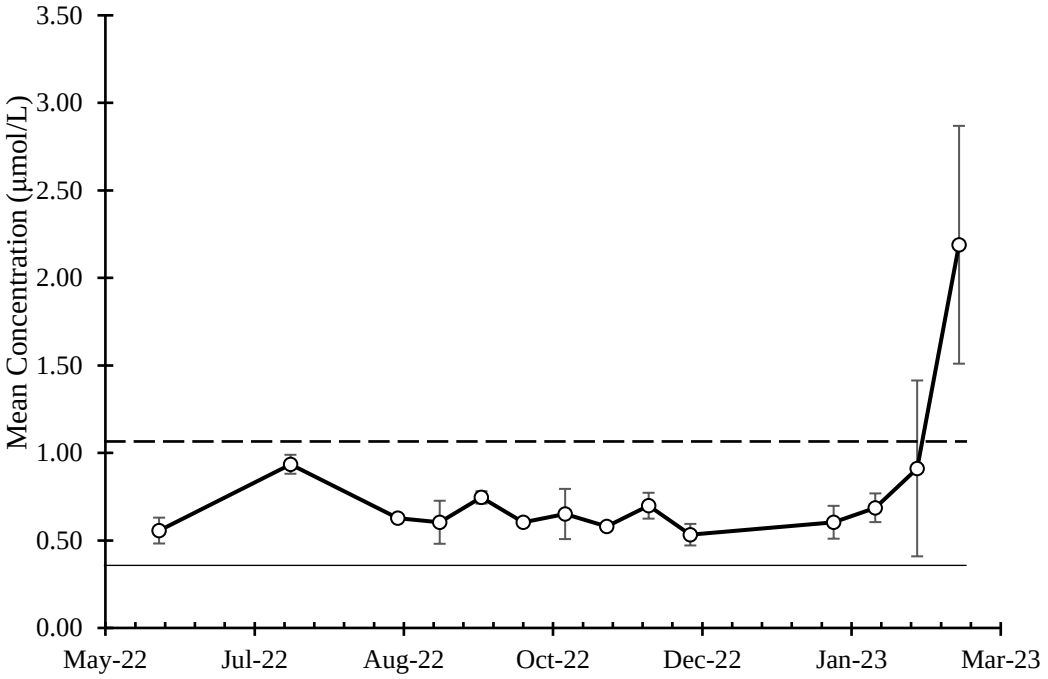


Figure :8 Mean Phosphate concentration (µmol/L) in Wall Pond, SC from June 2022 to March 2023. The horizontal line indicates eutrophic conditions (>025µmol/L). The dashed line indicated hypereutrophic conditions (>1.01 µmol/L). Error bars represent standard deviation.

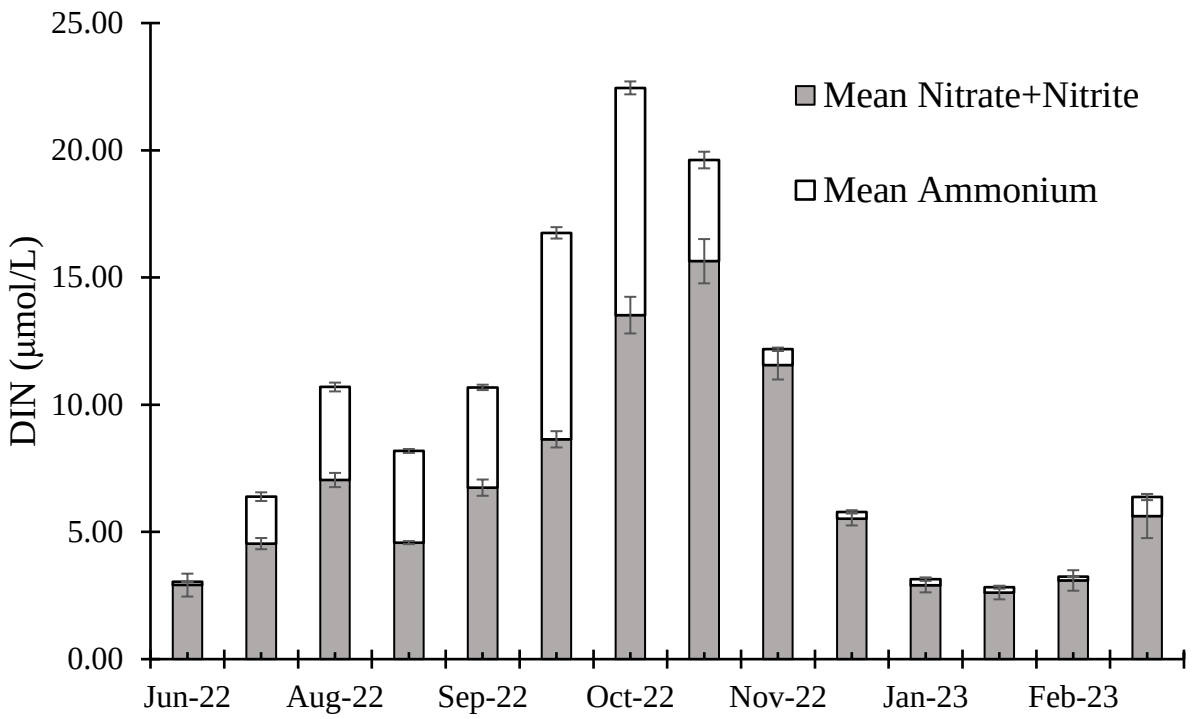


Figure 9: Dissolved inorganic nitrogen concentration (µmol/L) in Wall Pond, SC from June 2022 to March 2023. Grey bars represent Nitrate + Nitrite. White bars represent Ammonium. Error bars represent standard deviation.

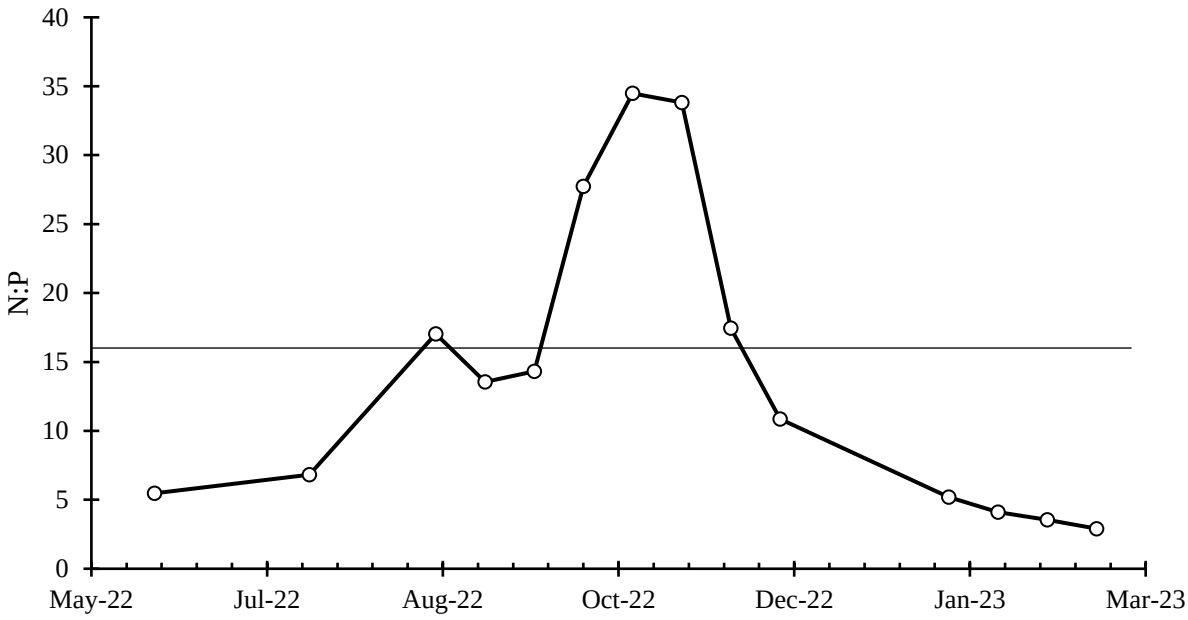


Figure 10: Nitrogen to Phosphorus ratio in Wall Pond, SC from June 2022 to March 2023. The horizontal line indicates a Redfield ratio of 16.

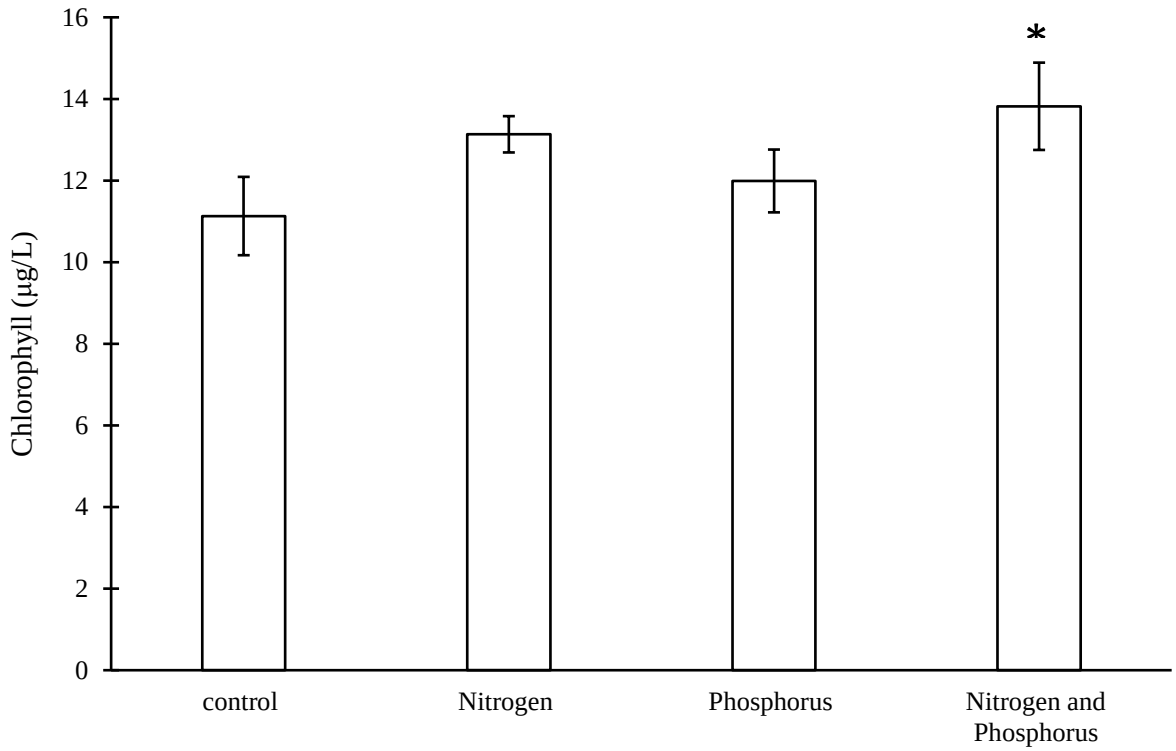
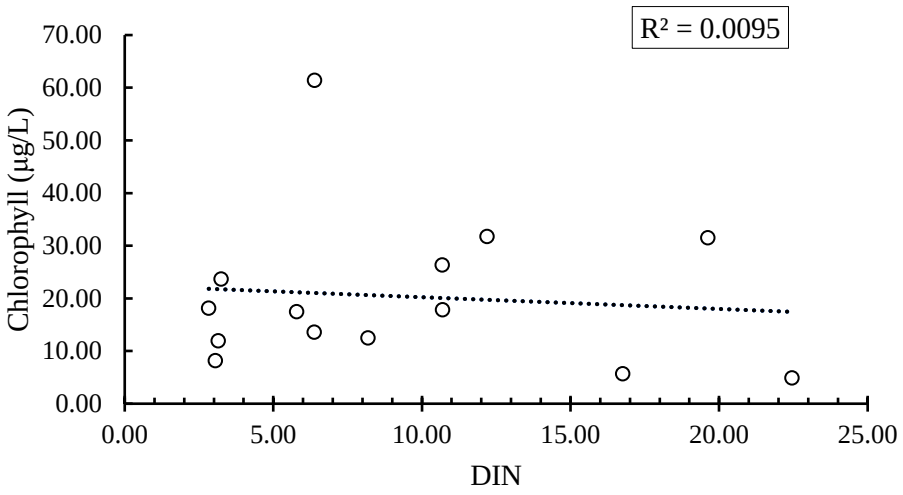
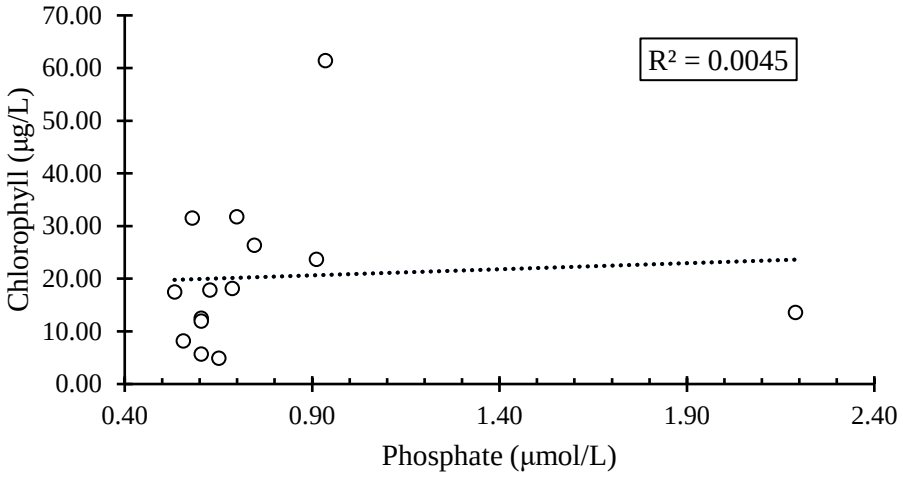


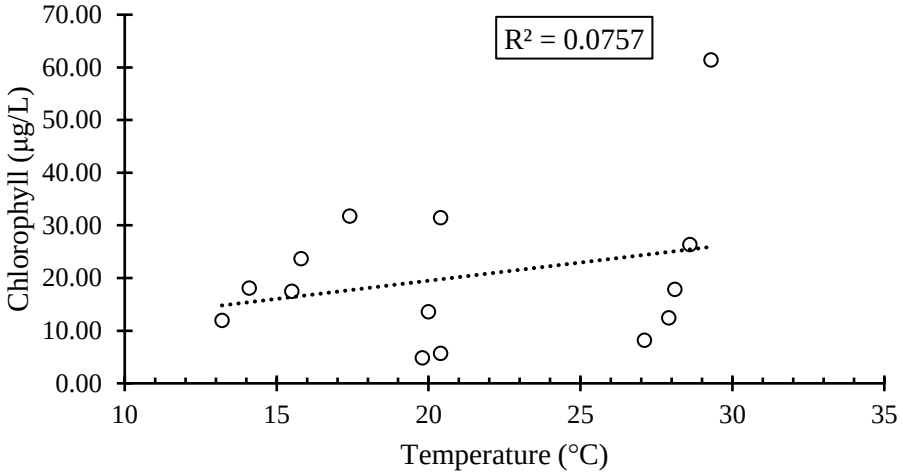
Figure 11: Chlorophyll (µg/L) concentration from a bioassay conducted on 3/1/23. Nitrogen and phosphorus dual treatment had significant difference in chlorophyll concentration. Significant differences are indicated by * ($p<0.05$, t-test). Error bars represent standard deviation.



A)



B)



C)

Figure 12: Chlorophyll concentrations (µg/L) based on DIN (A), phosphate (B), and Temperature (C). There was no significant correlation between DIN, phosphate, and temperature on chlorophyll concentrations.

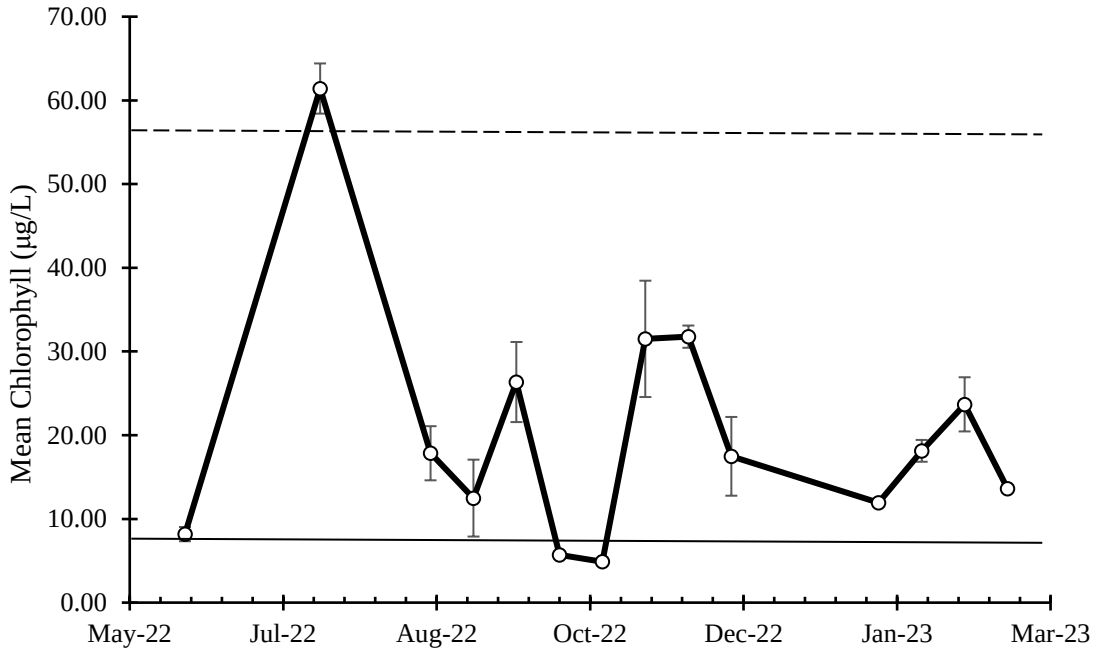
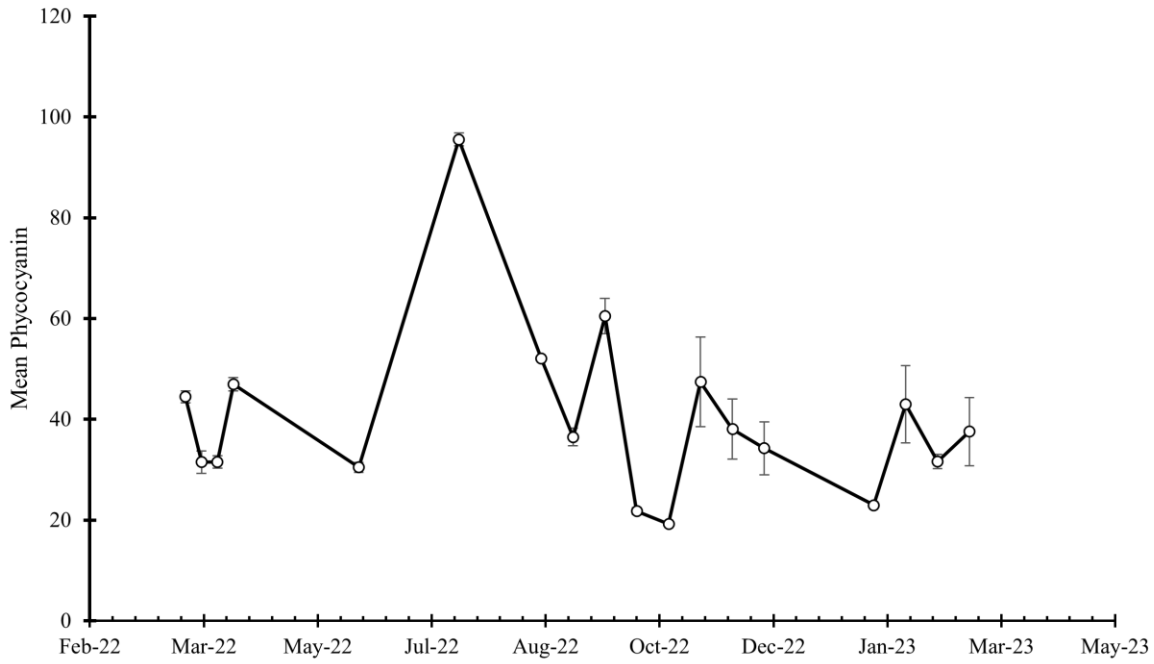


Figure 13: Chlorophyll concentration (µg/L) in Wall Pond, SC from June 2022 to March 2023. The solid line indicates eutrophic conditions (7.3-56 µg/L). The dashed line indicates hypereutrophic conditions (>56 µg/L). Error bars represent standard deviation.

A)



B)

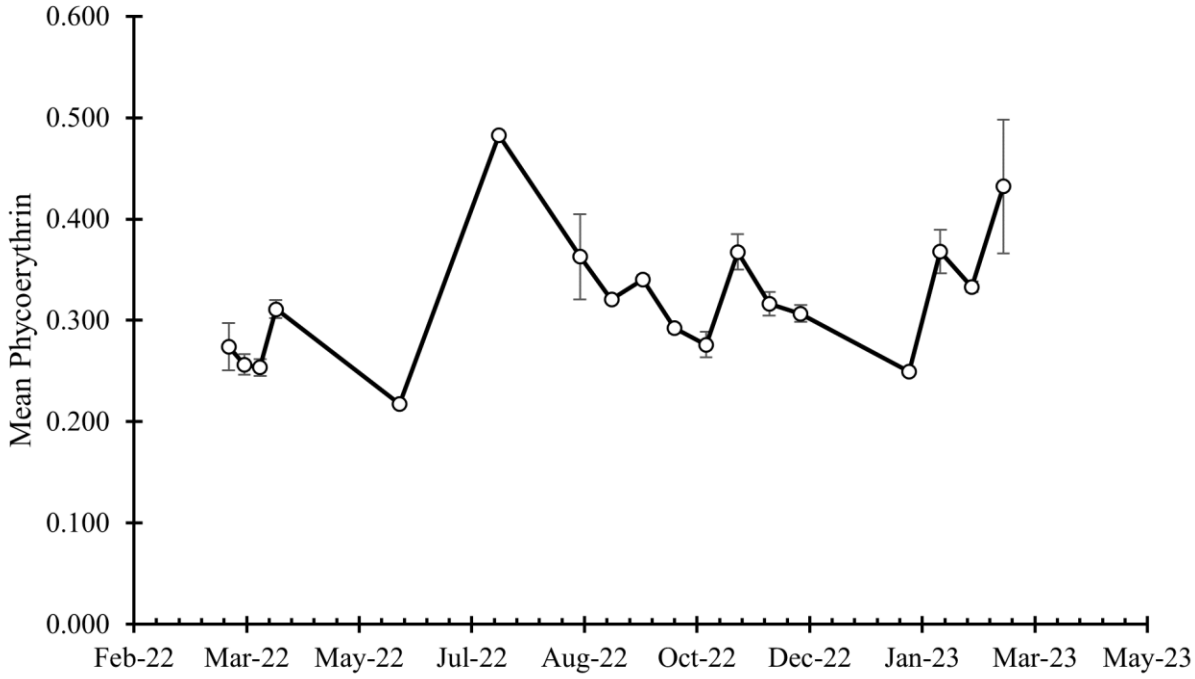
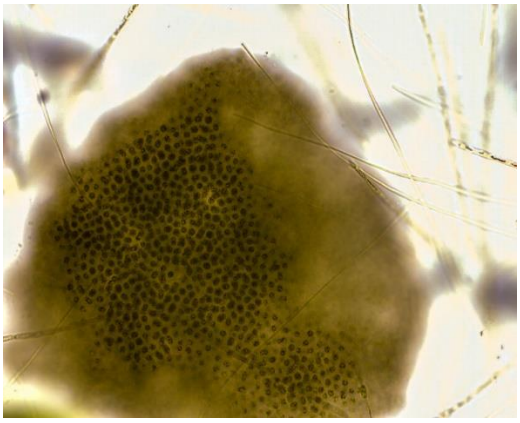
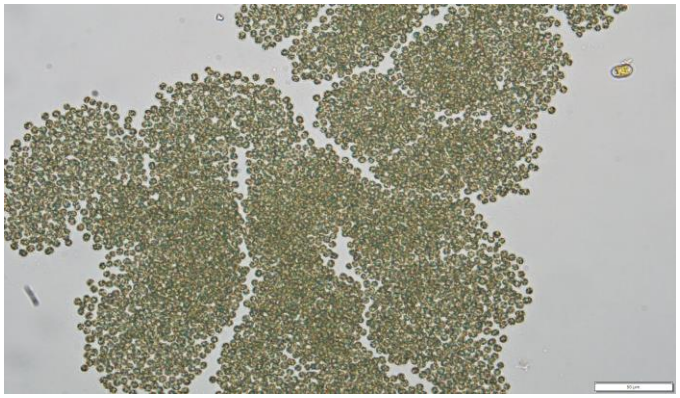


Figure 14: A) Phycocyanin concentrations in Wall Pond, SC from March 2022 to March 2023. B) Phycoerythrin concentration in Wall Pond, SC from March 2022 to March 2023. Error bars represent standard deviation.

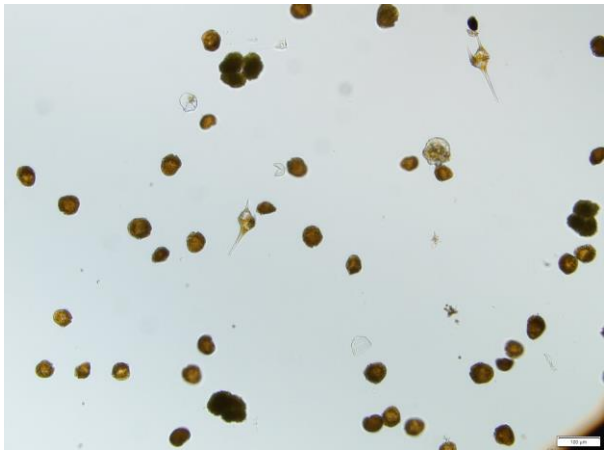


A)

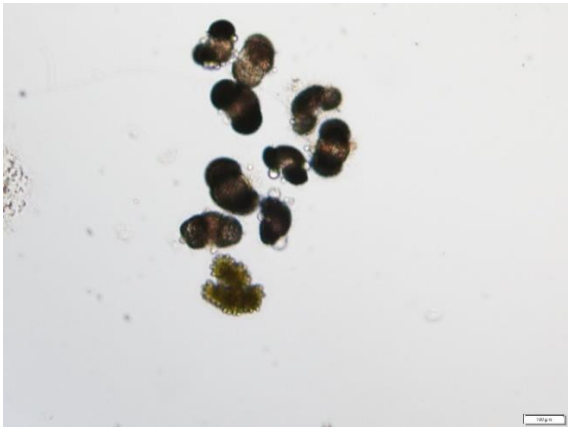


B)

C)



D)



E)

Figure 15: Plankton assemblages during bloom events in Wall Pond, SC. A) Microscopic observation of *Microcystis* on 7/20/22 B) Microscopic observation of *Microcystis* on 10/20/22 C) Visible *Microcystis* bloom on 11/3/22. D) Microscopic observation of *Peridinium*, *Ceratium*, and *Microcystis* on 2/1/23 E) Microscopic obervation of Pollen and *Microcystis* on 3/1/23.

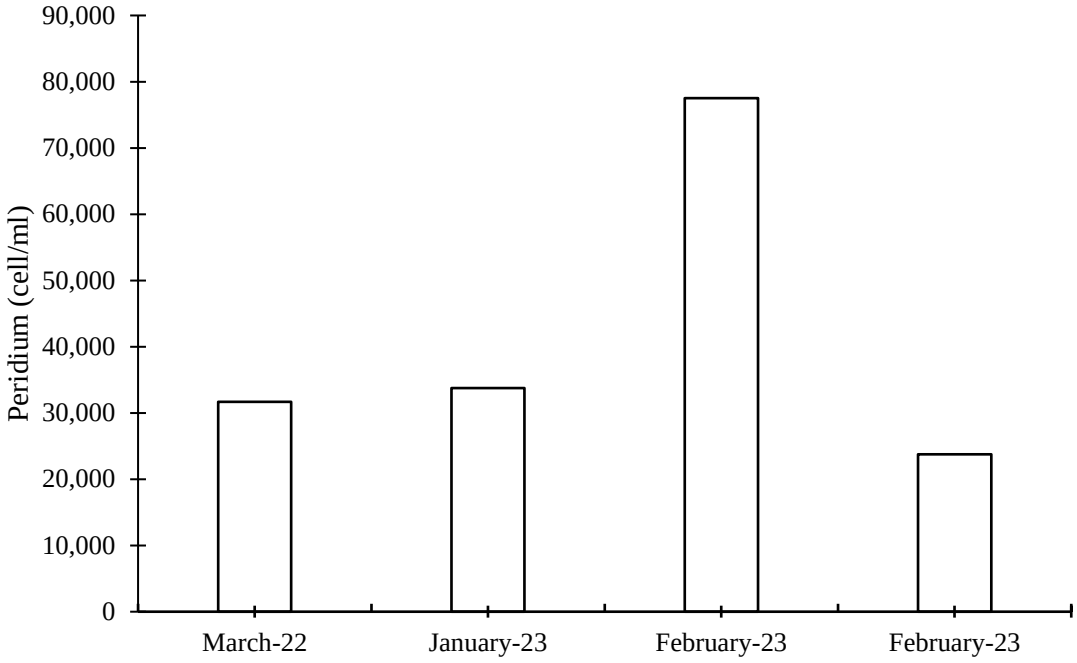


Figure 16: *Peridinium* hemocytometer cell counts (cells/mL) from Wall Pond, SC between March 22 to February 23.

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