Spring 2021

Zooplankton Community Structure and Grazing within a Stormwater Detention Pond in Coastal South Carolina

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Bachelor of Science, Biology
SUNY Cortland, 2018

Submitted in Partial Fulfillment of the Requirements

For the Degree of Master of Science in

Marine Science

College of Arts and Sciences

University of South Carolina

2021

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ACKNOWLEDGEMENTS

I would first and foremost like to thank my advisor, Dr. Jay Pinckney, for his invaluable guidance and mentorship. He has shown me what it I would also like to thank my committee members; Dr. Josh Stone and Dr. Bill Strosnider for their advice and help. They have both been patient and understanding while I have worked towards building my skills and learning. I want to thank Halley Carruthers for being my field work and sampling buddy as well as a great friend. This work would not have been possible without the help of John Williams, who helped tremendously with lab work as well as Bruce Pfirrman and the Baruch Marine Field Lab who helped me carry out my fieldwork. My mom, dad and sister have also given me unconditional support and love that has helped motivate me to complete my master's degree.

ABSTRACT

Stormwater detention ponds (SDPs) on the coast of South Carolina have become increasingly prevalent as the area experiences rapid urbanization. SDPs are man-made reservoirs implemented to minimize pollution inputs into receiving waters and are home to diverse biota, including zooplankton. Zooplankton are a good water quality indicator due to their quick response times and trophic regulators of phytoplankton through grazing. Zooplankton and phytoplankton interactions in stormwater detention ponds are an essential component for understanding plankton community dynamics in SDPs. This purpose of this study was to determine the seasonal variability in zooplankton community composition and grazing rates at 14-day intervals in one SDP located in Murrells Inlet, SC. Zooplankton samples were collected using a diaphragm pump as well as a 150μm net. Grazing experiments were conducted via 12-hour incubations in the dark. Copepods and cladocerans were the most abundant mesozooplankton groups throughout the sampling period, although nauplii had a large increase in the October months. Temperature and chl *a* were correlated with zooplankton abundance ($p<0.05$), while zooplankton were relatively tolerant to low DO concentrations. Microzooplankton community structure also shifted along with temperature change. *Netzelia* was the most abundant genus followed by *Paramecium* until August 20th. After this date, *Paramecium* was dominant and *Netzelia* was a minor part of the community. Cyanobacteria abundance (based on zeaxanthin concentrations) had no effect on zooplankton abundance, possibly due to low cyanobacteria concentrations. The average amount of phytoplankton grazed

per day for the microzooplankton only treatment was 0.66 (± 0.30) µg chl-*a* L^{-1} d^{-1} and 1.00 (± 1.22) µg chl-*a* L^{-1} d^{-1} for the combined micro and meso treatment. Mesozooplankton contributed to phytoplankton grazing as much as microzooplankton and both groups had low to non-existent grazing on cyanobacteria. Cyanobacteria could be a nuisance to the SDP if they bloom and other management strategies, outside of zooplankton grazing, should be explored to prevent possible cyanobacterial blooms in the future. This is the first detailed study of zooplankton community structure and grazing in an SDP in coastal South Carolina and highlights the need for additional research in this area.

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LIST OF SYMBOLS

T_0	Time point initial.
T_2	Time point two.
T_4	Time point four.
T_{12}	Time point twelve.

LIST OF ABBREVIATIONS

DO.....Dissolved Oxygen

Chl a..... Chlorophyll a

Fuco.....Fucoxanthin

Zea.....Zeaxanthin

CHAPTER 1

INTRODUCTION

The South Carolina coast has experienced rapid urbanization, which has altered many ecological landscapes and expanded impervious surface areas (Lewitus et al. 2008). Impervious surfaces increase runoff to man-made catchments, possibly causing negative ecological impacts on ephemeral aquatic environments. There are multiple different types of infrastructure, such as constructed wetlands, that are used to control runoff. However, stormwater ponds are currently the most widely used management solution to treat runoff (National Research Council (NRC), 2008). Stormwater detention ponds (SDP) are man-made reservoirs of standing water that were first implemented to provide stormwater storage to control runoff. They are beginning to be built to enhance stormwater quality to minimize pollution inputs into receiving waters (Tixier et al. 2011, Vincent and Kirkwood 2014). SDPs dominate the coastal landscape, especially in South Carolina, where a study of 511 ponds found that wet detention basins were the most frequently used structures (Beckingham et al. 2019, Drescher et al 2007). An average of 100 SDPs have been constructed each year from 1994 to 2013 in both the Myrtle Beach and Charleston metropolitan areas (Smith et al. 2018). Population growth in coastal areas of South Carolina is expected to continue, leading to more land use changes and possibly more SDP construction (Beckingham et al. 2019).

SDPs are engineered environments that may host a variety of plant and animal biota, such as invertebrates and reptiles, and can be a large reservoir of biodiversity

through rare species (Scher and Theiry 2005, Brand 2010). Zooplankton and its community structure play an important role in aquatic food webs and ecosystems by grazing phytoplankton, regenerating nutrients through fecal excretion, and providing food for upper trophic levels (Gonzalez 2000). Water quality changes in SDPs, caused by pollutants such as excess nutrients, can affect zooplankton community structure with cascade effects on phytoplankton. These factors can affect water quality parameters and thus affect zooplankton communities. Zooplankton, especially rotifers and crustaceans, make particularly good water quality indicators due to their quick response times via abundance and community composition shifts, sometimes on the time scale of just days, to changes in the environment and effective dispersal (Gannon and Stemberger 1978). Therefore, the assessment of biodiversity variability of zooplankton in SDPs is valuable and can possibly lead to better management practices. Biodiversity is important for providing many ecosystem services such as natural ecosystem sustainability in regard to disturbances, stabilization of the food web, and an increase in ecosystem productivity that could lead to a breakdown of some pollutants (Ortelli and Parris 2019). Stormwater pond management could optimize biodiversity to help maximize the degradation of downstream pollutants as well as prevent eutrophication and nuisance algal blooms.

Salinity, pH, temperature and dissolved oxygen concentration are some of the environmental factors that can influence zooplankton community structure (Van Meter et al 2011). For example, temperature can affect generation times in rotifers and copepods as well as body size of cladocerans and copepods (Gillooly 2000, Havens et al. 2015). In addition, zooplankton can be affected by bottom-up controls such as, changes in nutrients, phytoplankton communities, and aquatic vegetation (Pinel-Alloul and Mimouni

2013). They can also be regulated by top-down controls through fish and shellfish predation (Pinel-Alloul and Mimouni 2013).

These abiotic and biotic controls shift naturally with a change in the seasons. Water chemistry can vary seasonally within SDPs, especially in the summer, due to eutrophication and stratification (Lewitus et al. 2008, Rettig et al. 2005). In addition to natural changes these ponds can experience anthropogenic effects as well, such as increased runoff importing more pollutants. For example, De Lorenzo et al. (2012) and Goel et al. (2005) suggest that pesticide concentrations found in SDPs is correlated with temperature and rainfall. The phytoplankton community in these SDPs may also experience seasonal changes, such as blooms in the summer, having a direct effect on zooplankton feeding and growth, as they are a primary food source for zooplankton.

SDPs receive high levels of organic and inorganic nutrients in runoff. In many aquatic habitats, nutrients, such as fixed nitrogen, often limit phytoplankton growth (Siegel et al. 2011). Excessive inputs of limiting nutrients can result in rapid phytoplankton growth and the formation of blooms. These blooms often serve as hotspots for harmful algal blooms (HABs) (Siegel et al. 2011). Phytoplankton communities can often be regulated by zooplankton grazing and nutrient regeneration, while the phytoplankton community composition can determine grazing rates due to selective grazing (Gonzalez 2000).

As a result of their influence on lake food webs, zooplankton can strongly affect water quality, algal densities, fish production, and nutrient cycling, and may play an important role in regulating phytoplankton biomass and community composition in SDPs. Zooplankton have been well documented in trophic interactions, through grazing

experiments, with phytoplankton in freshwater ecosystems, including ponds (Van Meter et al. 2011). Phytoplankton communities can be both bottom-up limited, through nutrients like nitrogen and phosphorus, and top-down limited, by zooplankton grazing (Gonzalez 2000, Peretyatko et al. 2007, Sitta et al. 2018). Both bottom-up and top-down controls have different degrees of regulation depending on the type of ecosystem. For example, Vanni (1987) explains that bottom-up controls could be more prevalent in nutrient depleted environments due to nutrient limitations affecting growth. Meanwhile grazing could play a more important role in nutrient replete environments (Frost 1991). Zooplankton communities can help decrease HABs in these environments through grazing and have an indirect effect on human health (Pal 2020). Zooplankton and phytoplankton interactions in stormwater detention ponds are an essential component for understanding ecosystem structure and function in SDPs.

The phytoplankton community itself has a large effect on the influence of grazing within an environment. Ghadouani et al. (2003) documented a decrease in zooplankton abundance with an increase in cyanobacteria abundance, while Jang et al. (2003) documented cyanobacteria negatively affecting zooplankton diversity and abundance through the release of a variety of toxins. Size, morphology and feeding strategies determine grazing impacts of zooplankton on certain phytoplankton communities (Ye at al. 2013, Brett et al. 1994).

There have been mixed results in grazing experiments, with some experiments finding grazing to heavily limit phytoplankton communities while others have found little to no effects on phytoplankton communities (Berquist et al. 1986). Both microzooplankton and mesozooplankton can limit phytoplankton communities in some

systems (Dagg et al. 1995). Mesozooplankton can play a particularly important role in productive ecosystems, or ecosystems where nutrients are replete (Calbet 1991). Grazing can often be insufficient to control phytoplankton growth, possibly leading to algal blooms. Different zooplankton species will have different levels of success grazing on certain phytoplankton species, but many cyanobacteria are thought to be resistant to zooplankton grazing (Tillmanns et al. 2008). Multiple hypotheses have been proposed to explain why cyanobacteria may be particularly good at circumventing grazing. For example, toxic cyanobacteria can interfere with zooplankton grazing assemblages due to their size or the formation of filamentous colonies (Tillmanns et al. 2008). The cyanobacteria community can decrease zooplankton biomass and cause a shift in community composition thus leading to reduced grazing pressure. Grazing experiments using SDP communities are nearly nonexistent, leaving the question of what role zooplankton play a role in phytoplankton biomass regulation in SDPs.

In this study, the zooplankton community was characterized in a SDP in coastal South Carolina, at 14-day intervals over the summer months, to determine the short-term changes in community structure. Grazing experiments were also conducted at 14-day intervals to examine the potential role zooplankton may play in regulating phytoplankton biomass in SDPs. The purpose of this study was to determine the role that zooplankton play in the community of a typical SC coastal SDP. The primary hypotheses were as follows: (1) Zooplankton abundance is positively correlated with phytoplankton biomass (chl *a*) and negatively correlated with temperature. (2) Zooplankton diversity is negatively correlated with cyanobacteria concentrations. (3) Zooplankton grazing rates are negatively correlated with cyanobacteria concentrations.

CHAPTER 2

METHODS

Study Site

This study was conducted in a SDP (33°33'46.4"N and 79°01'47.7"W) near Murrells Inlet, SC, adjacent to townhomes located in Marina Colony (Fig. 2.1). Murrells Inlet is located in Georgetown county which receives 139.9 cm of rain per year on average (SC DNR). The main source of water for this pond is rain runoff. The pond is in an urbanized area with surrounding homes and vegetation and had high levels of nitrogen throughout the sampling period (Carruthers Master's Thesis, in prep.). The watershed sits at about 3.75×10^7 square meters and spans from Huntington Beach State Park to the southern end of Surfside beach (Williams et al. 2014). Of the 3.75×10^7 square meters of the watershed about 2.56×10^7 is covered with land, or just above 68% (Libes et al 2014). This pond empties via a drainage structure into a Murrells Inlet estuary.

Physical Parameters

A YSI 6820 multiparameter sonde was deployed just below the surface of the water directly in front of the outflow structure to determine dissolved oxygen (DO), temperature, specific conductivity, and pH. The YSI was calibrated via the instruction manual prior to each trip.

Field Sampling

The pond was sampled at 14-day intervals from June to October 2020 between 8 and 11am. One sampling trip, scheduled for September 3rd, was skipped due to COVID-19. A battery powered 12-volt DC diaphragm pump with a maximum flow rate of 6.84 L/s was used to collect water samples (Masson et al. 2004). Water was collected about 10 cm below the surface and filtered through a 200 μm sieve into a 1L container for collection of microzooplankton. The pump was also used to collect water samples for mesozooplankton. The pump hose was placed just below the water surface and water pumped into a 3.75L container. The water from the 3.75L container was then filtered through a 150 μm mesh cup. The cup was rinsed with a squirt bottle of DI water and emptied into 1L container. These samples were used for community composition and abundance analysis. For trips 7 and 8, which occurred on October 1st and 15th, a 150μm mesh net was also used for the collection of mesozooplankton to determine diversity (Harris et al. 2000). The net diameter was 0.3 m. Several net tows were conducted while standing on the outflow structure, tossing the net directly out in front of the structure and pulling it back in. The net was then placed within the outflow to collect the water flowing over the sides into the structure. Both microzooplankton and mesozooplankton samples were preserved using Lugol's solution. Whole water phytoplankton samples were obtained for phytoplankton abundance and community composition measurements (Carruthers Master's Thesis, in prep.).

For grazing experiments, nine 3.75 L bottles were filled with pond water using the diaphragm pump. Three bottles were filled with water filtered through a 200 μm sieve for the microzooplankton only treatment. Three additional bottles were filled with water

filtered through a 64 μm sieve to serve as a control which was not be small enough to remove all microzooplankton and therefore only acted as a control for mesozooplankton. Each sieve was rinsed with a squirt bottle filled with DI water to remove organisms from the sieve mesh. Three more bottles were not filtered and instead had whole water samples for a mesozooplankton and microzooplankton treatment. The bottles contained a small airspace to prevent oxygen depletion during the incubations. All bottles were placed in two opaque garbage bags, to ensure the incubations were done in the dark and to prevent phytoplankton photosynthesis during the incubation period. Bottles were washed and stored with 10% HCl between experiments. Before each sampling trip, the bottles were rinsed with pond water in triplicate.

Grazing Experiments

All nine bottles, while still in the opaque bags, were placed in a flowing seawater tank flushed with North Inlet estuary water for temperature control. The temperature was slightly lower but still comparable to pond temperature. Measurements of chl-*a*, to determine phytoplankton biomass, were taken every 2 h, for 12 h, starting with time point 0. A small subsample was removed from the bottles for filtering to keep the large bottles in the dark as much as possible. Samples for chl-*a* analysis were taken by filtering water through a glass fiber filter (Whatman GF/F) using a gentle vacuum. After filtration, the samples were stored in in a -80°C freezer until analysis. At the end of each experiment the remaining water from each bottle was filtered through a 200 μm sieve to collect the remaining mesozooplankton and preserved with Lugol's solution.

Lab Analysis

Community Composition Analysis

A dissecting microscope at 40x magnification was used to conduct qualitative and quantitative counts for the mesozooplankton samples. Johnson and Allen 2012 was used as an identification guide for mesozooplankton. Microzooplankton were counted and identified using an inverted microscope at 100x magnification. All individuals in the mesozooplankton and microzooplankton samples were counted and identified to the lowest taxonomic level possible. Stemberger 1979 was used as an identification guide for microzooplankton. Some copepod species are not able to be identified without looking at an adult male, therefore copepods were identified to family only. Microzooplankton community abundance was determined using the subsample sedimentation method described by Utermohl (1958). A 10 mL subsample was taken from a well-mixed 1L sample and placed in a settling chamber. The settling chamber was allowed to settle for 24 hours before analysis.

Phytoplankton Analyses

High performance liquid chromatography (HPLC) was used to determine chemosystematic phytoplankton photosynthetic pigments (Quiblier-Llobéras et al. 1996**)**. Samples were lyophilized for 24 h at -50̊ C, placed in 90% acetone (1.00 ml), sonicated, and extracted at -20̊ C for 18 - 20 h. Filtered extracts (250 µl) were injected into a Shimadzu HPLC equipped with a monomeric (Rainin Microsorb-MV, 0.46 x 10 cm, 3 µm) and a polymeric (Vydac 201TP54, 0.46 x 25 cm, 5 µm) reverse-phase C18 column in series. A nonlinear binary gradient consisting of the solvents 80% methanol:20% 0.50 M ammonium acetate and 80% methanol:20% acetone was used for pigment separations

(Pinckney et al. 1996). Absorption spectra and chromatograms (440 ± 4 nm) were acquired using a Shimadzu SPD-M10av photodiode array detector. Pigment peaks were identified by comparison of retention times and absorption spectra with pure standards (DHI, Denmark). The synthetic carotenoid β-apo-8'-carotenal (Sigma) was used as an internal standard.

Data Analysis

Data were analyzed using least-squares linear regressions to determine relationships between abundance and water quality parameters (DO, temperature, chl *a*) as well as zeaxanthin concentrations. Chl-*a* was used as a proxy for overall phytoplankton biomass, fucoxanthin was used for diatom biomass, and zeaxanthin for cyanobacteria biomass. The difference in pigment concentrations was taken per measurement, meaning the difference was taken between T_0 and T_2 then T_2 and T_4. These differences were graphed vs time to establish whether the grazing rate was relatively constant (Fig. 2.2). Once linear grazing responses were confirmed, the following formula was used to determine the 24-hour grazing rate:

$$R = ((T_{12}-T_0)/12) \times 24$$

With T_{12} as the concentration of pigment at hour 12 and T_0 was the pigment concentration at time point zero. To determine average grazing rates for each pigment, only values that showed a decrease in pigment were included. Similarly, when comparing the relationship between abundance and grazing only positive values, or decreases in pigment, were used.

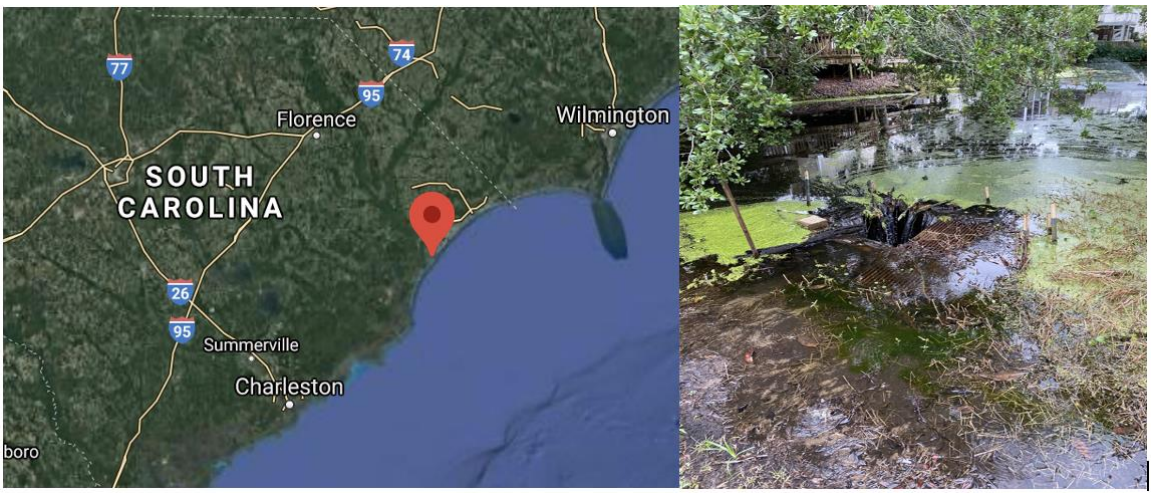

Figure 2.1: Study location. The picture on the left shows the approximate location within South Carolina that the pond is located. The picture on the right shows the outflow structure of the pond where samples were collected.

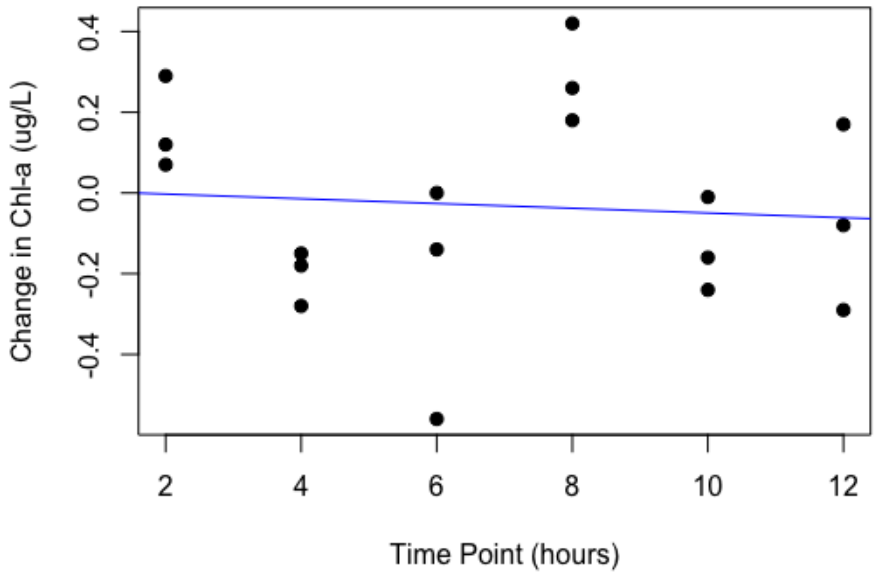

Figure 2.2: Change in chl a per time point from micro only #1 for July 9th. The line is not significantly different from zero indicating that the grazing rate is constant.

CHAPTER 3

RESULTS

3.1 Physical Parameters

Conductance in this pond indicates that this pond is freshwater (Table 3.1). The conductance and pH largely remain the same throughout the sampling period. Temperature continues to rise until August 20th, where a decline was first seen. The chl *a* concentration was much higher in the June and July sampling trips than the rest of the sampling period. Dissolved oxygen had a similar range for most trips outside of July 23rd, however this is only the range of dissolved oxygen for the morning.

3.1 Zooplankton Community Structure

The greatest mesozooplankton abundance was recorded on July 23rd, with 2.8 individuals per liter (indiv L^{-1}), while the lowest was recorded on September 17th, with 0.1 indiv L^{-1} (Fig. 3.1). Abundance peaked in July then continuously declined at the beginning of August through September, where abundance then increased slightly and leveled out in October. The highest abundance of microzooplankton was recorded on the same date as mesozooplankton, July 23rd, with 152,000 indiv L^{-1} (Fig. 3.2). Microzooplankton abundance followed a similar trend to mesozooplankton, with abundance increasing until July 23rd then decreasing afterward. However, microzooplankton abundance was at its lowest on October 1st, at 45,700 indiv L^{-1} in

contrast to the September 17th peak in mesozooplankton abundance. After October 1st the abundance slightly increases again on October 15th.

The most abundant mesozooplankton group was the copepods with an average abundance of 5.0 x 10^{-1} indiv L^{-1} averaged among all trips (Fig. 3.3). The second most abundant group was cladocerans with an average of 4.9 x10^{-2} indiv L^{-1}. Other crustaceans, decapods and ostracods, and isopods were the least abundant in this pond, with an average density of 8.9 x10^{-4} indiv L^{-1}. Amphipods had an average density of 2.7 x10^{-2} indiv L^{-1} and crustacean nauplii, likely copepod nauplii, were found to have an average density of 5.7 x10^{-2} indiv L^{-1}. Copepods were the dominant group among all trips, with cladocerans only coming close to that of copepods in the sample taken on October 15th. There was also a larger number of nauplii present in October compared to other months.

There were 5 unique families of copepods identified (Table 3.1). The most abundant family was Cyclopidae while the least abundant was Onceaidae. Amphipods and cladocerans both had 2 unique genera identified. The amphipod genera identified were *Gamarus* and *Calliopius* while *Bosmia* and *Daphnia* were the cladoceran genera. Isopods, decapods and ostracods had only one unique genus identified, *Eurydice*, *Pinnotheres* and *Euchonchoecia* respectively.

Sixteen unique microzooplankton genera were identified across all trips (Table 3.2). The most diverse sampling days were both in July, with 13 unique genera identified for both trips. The least diverse days were August 6th and October 1st having only 7 unique genera identified. The most abundant microzooplankton genus, averaged among all trips, was the testate amoeba *Netzelia*, at 222,300 indiv L^{-1}, followed by *Paramecium*, with 169,400

indiv L^{-1}, and then the testate amoeba *Arcella*, at 136,900 indiv L^{-1}. In the earlier months, such as June and July, *Netzelia* was the most abundant genus however, their prevalence decreased in August and they were no longer the most abundant by August 20th (Fig. 3.4). On August 20th there is almost even amounts of *Arcella*, *Netzelia* and *Paramecium*. From August 20th onwards *Paramecium* was the most abundant followed closely by *Arcella* while *Netzelia* abundance greatly declined.

3.2 Zooplankton Community Structure Relationship to Dissolved Oxygen

There was a positive relationship with DO concentrations for both mesozooplankton and microzooplankton abundance ($p < 0.05$) (Figs. 3.5 & 3.6). The highest abundance of mesozooplankton corresponded to the highest concentration of dissolved oxygen. However, there were similar abundances at the lowest and mid-range levels of dissolved oxygen. Without the high leverage data point there was no relationship for either mesozooplankton or microzooplankton.

3.3 Zooplankton Community Structure Relationship to Chl-a

Chl-*a* concentrations were positively correlated with both mesozooplankton and microzooplankton abundance ($p < 0.05$) (Figs. 3.7 & 3.8). However, without the high leverage points in each data set the relationships did not exist for either mesozooplankton or microzooplankton.

3.4 Zooplankton Community Structure Relationship to Temperature

Mesozooplankton abundance was not correlated with temperature with $p > 0.05$, while microzooplankton abundance was ($p < 0.05$) (Figs. 3.9 & 3.10). The highest pond water temperatures corresponded with the highest recorded average abundance for both

groups. However, the lowest temperature did not correspond with the lowest density for mesozooplankton while it did for microzooplankton.

3.5 Zooplankton Relationship to Zeaxanthin Concentrations

Mesozooplankton and microzooplankton abundance were correlated with the cyanobacterial photopigment zeaxanthin concentrations ($p<0.05$) (Figs. 3.11 & 3.12). Without the high leverage data points in both data there is no correlation for either group.

Microzooplankton diversity and zeaxanthin concentrations were positively correlated ($R^2 = 0.77$, $p < 0.05$) (Fig. 3.13). However, mesozooplankton abundance was not correlated with zeaxanthin concentrations ($R^2 = 0.06$, $p < 0.05$) (Fig. 3.14).

3.6 Grazing Rates

There was no correlation between chl-*a* grazing rates and microzooplankton abundance ($R^2 = 0.71$), as relationship was not significant ($p>0.05$)(Fig. 3.15). However, there was a strong positive correlation between the combined abundance and chl-*a* grazing rate ($R^2 = 0.87$), which was significant ($p<0.05$)(Fig. 3.16). The lowest abundance in the microzooplankton only treatment correlated with the highest grazing rate for chl-*a* and the lowest grazing rate corresponded to the highest microzooplankton abundance (Fig. 3.15). The opposite trend was seen in the combined zooplankton treatment, with the highest grazing rate correlated to the highest abundance (Fig. 3.16). Although, the lowest abundance did not correlate with the lowest grazing rate and the lower abundance grazing rates did not differ much from the higher abundance grazing rates outside of the highest abundance point (Fig. 18). Zeaxanthin had the lowest rate of grazing in all three treatments while chl-*a* had the highest. The combined micro and meso treatment had similar grazing rates for fucoxanthin and chl-a, but a much lower rate for

zeaxanthin. These results suggest that mesozooplankton and microzooplankton contribute to overall chl *a* grazing nearly equally while mesozooplankton may have an edge in diatom grazing. This could be attributed to the size of diatoms as some are in the same size range of microzooplankton and therefore may be out of their grazing range.

Out of 24 samples, 10 exhibited an increase in chl *a* concentration, with 4 of those being the control treatments and 4 in the micro only treatment (Table 3.4). The highest increase in chl-*a* was in the July 23rd control treatment, with an increase of 12.01 $\mu g\ L^{-1}\ d^{-1}$, followed by the micro treatment from that same date, with an increase of 3.69 $\mu g\ L^{-1}\ d^{-1}$. By contrast, the largest decrease in chl *a* was in the mixed meso and micro treatment on July 23rd. The July 23rd trip had the highest chl *a* concentration of the sampling period. All of the treatments from the October 1st and 15th trips saw an increase in chl *a*. Outside of the October trips there were no mesozooplankton treatments that increased in chl *a,* which could be correlated with the low zooplankton abundance measured during those trips.

Nearly all treatments had an increase in zeaxanthin concentrations (Table 3.4). Out of 24 measurements there were only 6 instances where there was a decrease in zeaxanthin, 3 of which were recorded in control treatments. The largest increase in zeaxanthin concentration was recorded on July 23rd, similar to chl *a*, and was in the microzooplankton only treatment, with an increase of 0.89 $\mu g\ L^{-1}\ d^{-1}$. There were 2 measurements where there was a net zero change in zeaxanthin concentration, both of which occurred in the mixed meso and micro treatments on June 25th and October 1st. The largest decrease in zeaxanthin concentration was in the control treatment on September 17th, with 0.22 $\mu g\ L^{-1}\ d^{-1}$, followed by the mixed meso and micro treatment on

that same date, with 0.12 μg L^{-1} d^{-1}. The low rate of grazing, combined with the numerous treatments where growth was observed, suggest that cyanobacteria aren't the ideal food source for zooplankton.

Fucoxanthin concentrations decreased in all but 2 treatments, one of which was a control treatment on July 23rd (Table 3.4). The largest decrease was obtained for the mixed meso and microzooplankton treatment on July 23rd, with a decrease of 3.18 μg L^{-1} d^{-1}. The second largest decrease was also in a mixed meso and microzooplankton treatment on the August 6th trip, at 2.96 μg L^{-1} d^{-1}. The smallest decrease was recorded in the control treatment on August 6th, at 0.0160 μg L^{-1} d^{-1}. The only other increase in fucoxanthin concentration was seen in the microzooplankton only treatment on July 9th. While grazing was observed in nearly all samples the rate was low in most of them and likely not enough to control the diatom biomass based on the growth rates seen in an unaltered incubation from the same pond.

Table 3.1: Physical measurements of the pond. Measurements were taken between 9 and 11am, varying by trip.

Date	Dissolved Oxygen (mg/L)	Chl a (ug/L)	Temperature (C)	pH	Conductance (mS/cm)
June 25th	2.9	11.80	24.56	6.96	0.110
July 9th	2.25	7.13	25.54	6.90	0.196
July 23rd	10.08	17.14	28.07	7.56	0.312
August 6th	4.3	2.59	26.29	6.96	0.213
August 20th	4.3	2.35	24.67	7.52	0.164
September 17th	2.2	2.35	24.3	7.08	0.293
October 1st	4.3	1.20	20.81	8.21	0.198
October 15th	3.2	1.66	21.87	7.99	0.231

Table 3.2: Unique families of copepods. Each of the unique families of copepods identified and the month that they were present. There were two sampling techniques used in October, a pump and a net, which have been separated.

Family	July	August	September	October (pump)	October (net)
Pontillidae	x	x			x
Centropagidae		x	x	x	x
Cyclopidae	x	x		x	x
Onceaidae	x			x	x
Temoridae	x		x		x

Table 3.3: Unique genera of microzooplankton. Each of the unique genera of microzooplankton identified and the month that they were present.

Genus	June	July	August	September	October
Arcella	x	x	x	x	x
Gyrodinium	x	x	x	x	x
Stauroneis	x	x	x	x	x
Paramecium	x	x	x	x	x
Netzelia	x	x	x	x	x
Cylindrotheca	x	x	x	x	x
Anuraeopsis		x	x	x	x
Tintinnopsis		x	x		
Keratella		x			
Strombidium		x			
Colurella		x	x	x	
Lecane		x		x	
Microcodon		x			
Ptygura		x			
Monommata			x		
Lesquereusia				x	

Table 3.4: Average grazing rates and SD of triplicates from each treatment. A negative number indicates an increase in pigment while a positive number indicates a decrease in pigment (grazing). Zea stands for zeaxanthin and fuco stands for fucoxanthin. The meso treatment is the combined microzooplankton and mesozooplankton treatment.

Trip	Treatment	Rate (µg chl-*a* L^{-1} d^{-1})	Rate (µg zea L^{-1} d^{-1})	Rate (µg fuco L^{-1} d^{-1})
1	Control	0.42 (± 0.11)	0.06 (± 0.02)	0.28 (± 0.11)
1	Micro	1.04 (± 1.0)	-0.09 (± 0.28)	0.16 (± 0.10)
1	Meso	0.35 (± 1.5)	0.00 (± 0.02)	0.10 (± 0.21)
2	Control	-0.008 (± 1.0)	-0.08 (± 0.07)	0.13 (± 0.11)
2	Micro	0.37 (± 0.76)	-0.08 (± 0.05)	-0.03 (± 0.73)
2	Meso	0.59 (± 0.48)	-0.06 (± 0.04)	0.06 (± 0.10)
3	Control	-12.0 (± 4.2)	-0.53 (± 0.22)	-0.46 (± 0.18)
3	Micro	-3.69 (± 9.9)	-0.89 (± 0.55)	0.98 (± 2.7)
3	Meso	3.44 (± 11.6)	-0.14 (± 0.44)	3.18 (± 5.9)
4	Control	0.44 (± 0.27)	0.008 (± 0.05)	0.02 (± 0.16)
4	Micro	0.76 (± 9.1)	0.03 (± 0.05)	0.10 (± 0.20)
4	Meso	0.85 (± 0.51)	-0.02 (± 0.02)	2.96 (± 4.8)
5	Control	0.10 (± 0.58)	-0.06 (± 0.05)	0.03 (± 0.04)
5	Micro	0.48 (± 1.4)	0.008 (± 0.07)	0.08 (± 0.09)
5	Meso	0.16 (± 0.80)	-0.04 (± 0.09)	0.03 (± 0.08)
6	Control	0.42 (± 0.53)	0.22 (± 0.30)	0.17 (± 0.15)
6	Micro	-1.45 (±2.9)	-0.59 (± 2.9)	0.09 (± 0.16)
6	Meso	0.6 (± 2.1)	0.12 (± 0.12)	0.18 (± 0.11)
7	Control	-0.45 (± 0.31)	0.05 (± 0.21)	0.82 (± 1.3)
7	Micro	-1.67 (± 1.3)	-0.05 (± 0.06)	0.16 (± 0.04)
7	Meso	-1.04 (± 0.28)	0.00 (± 0.04)	0.30 (± 0.16)
8	Control	-1.56 (± 1.02)	-0.07 (± 0.14)	0.22 (± 0.06)
8	Micro	-2.45 (± 0.22)	-0.15 (± 0.04)	0.24 (± 0.08)
8	Meso	-2.27 (± 0.4)	-0.17 (± 0.05)	0.24 (± 0.03)

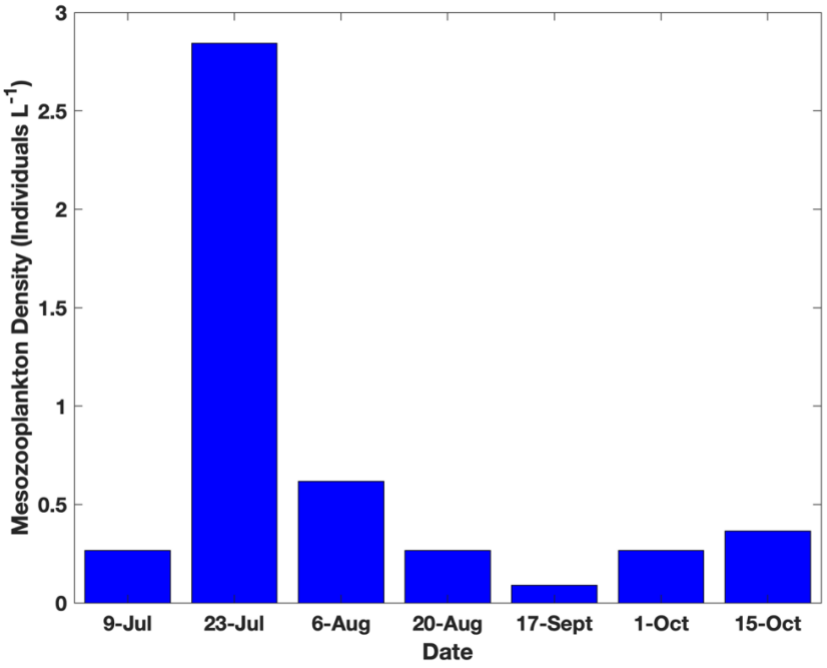

Figure 3.1: Average mesozooplankton density (Individuals/L^{-1}). Taken from a Murrell's Inlet stormwater detention pond from July 9th to October 15th.

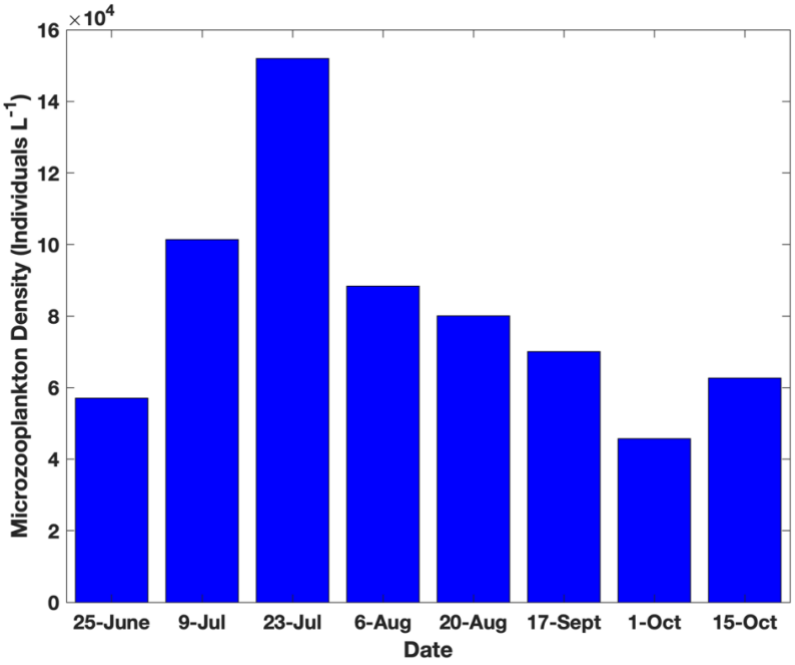

Figure 3.2: Average microzooplankton density (Individuals/L^{-1}). Taken from a Murrell's Inlet stormwater detention pond from July 9th to October 15th.

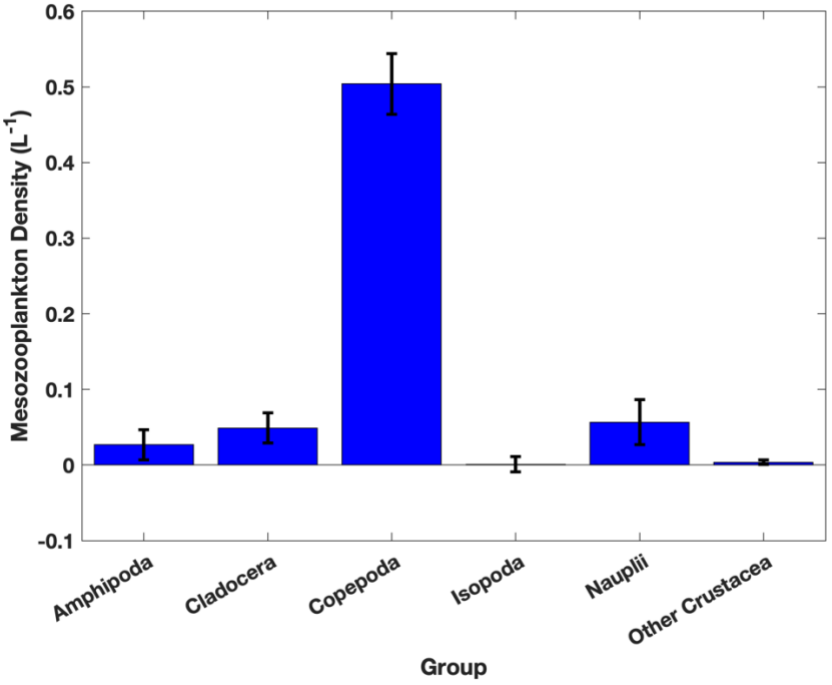

Figure 3.3: Density of mesozooplankton group (Individuals/L^{-1}). This is averaged among all trips.

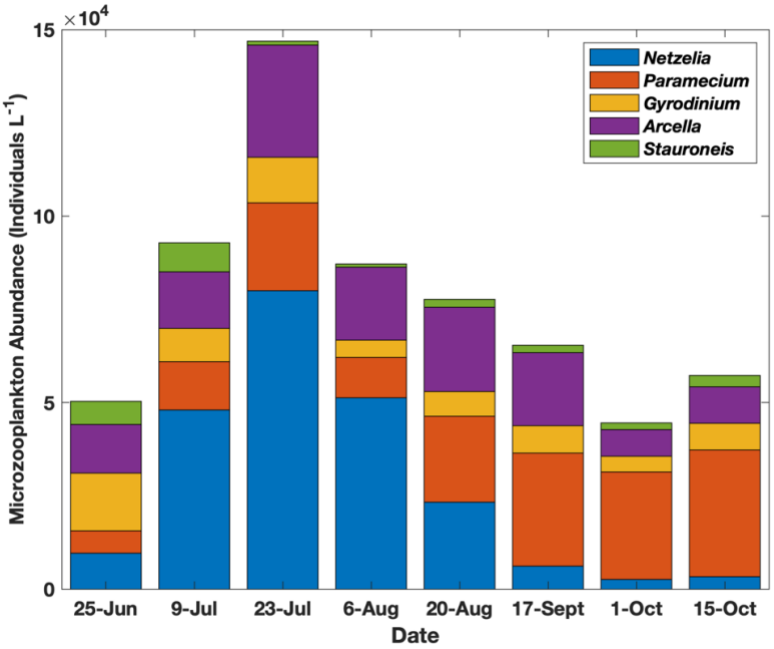

Figure 3.4: Density of microzooplankton genera each trip (Individuals/L^{-1}). Only the top 5 genera were included.

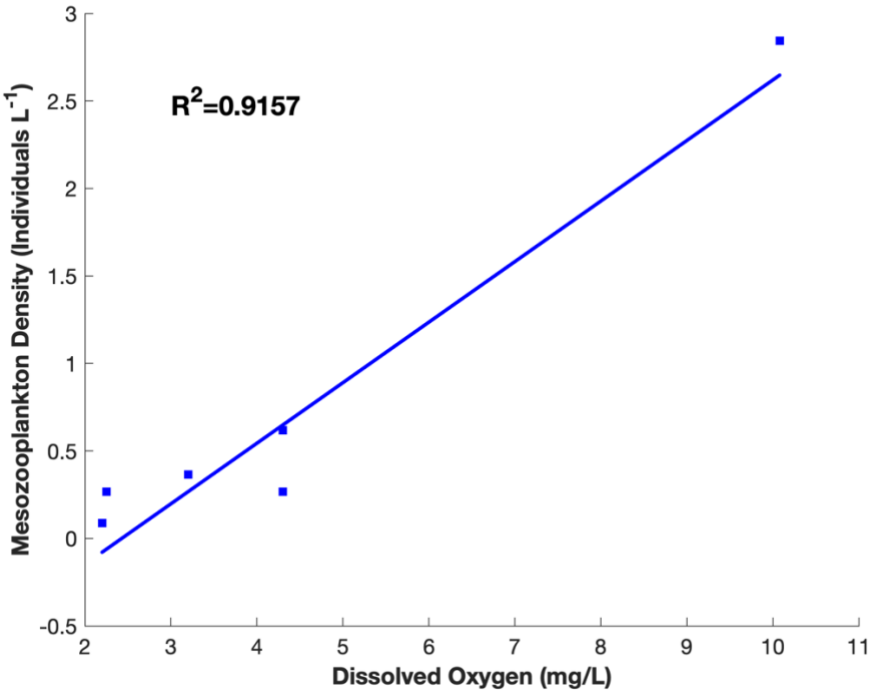

Figure 3.5: Mesozooplankton density (Individuals/L^{-1}) vs DO (mg/L). The slope of the line was not significantly different from zero when the high leverage point was removed ($p>0.05$).

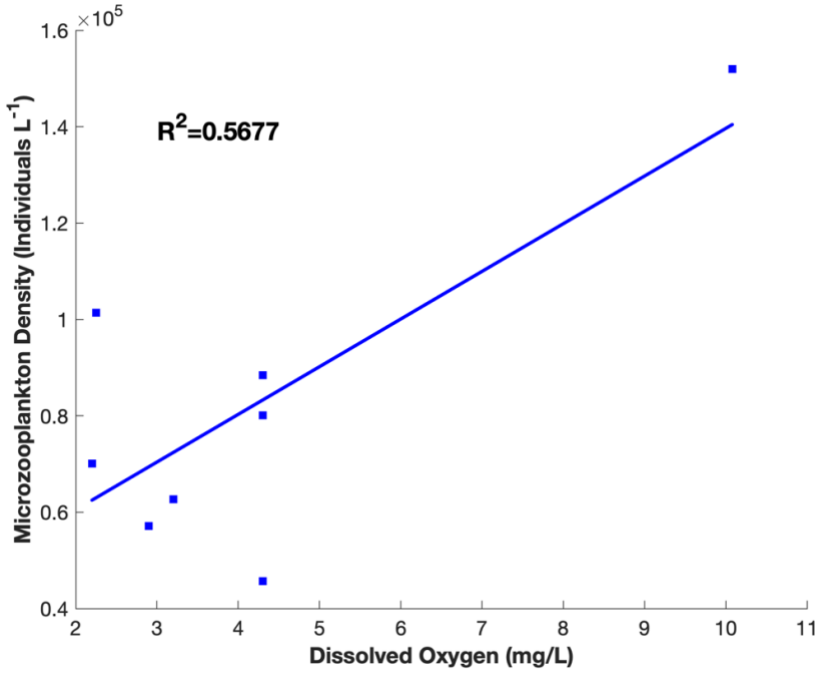

Figure 3.6: Microzooplankton density (Individuals/L^{-1}) vs DO (mg/L). The slope of the line was not significantly different from zero when the high leverage point was removed ($p>0.05$).

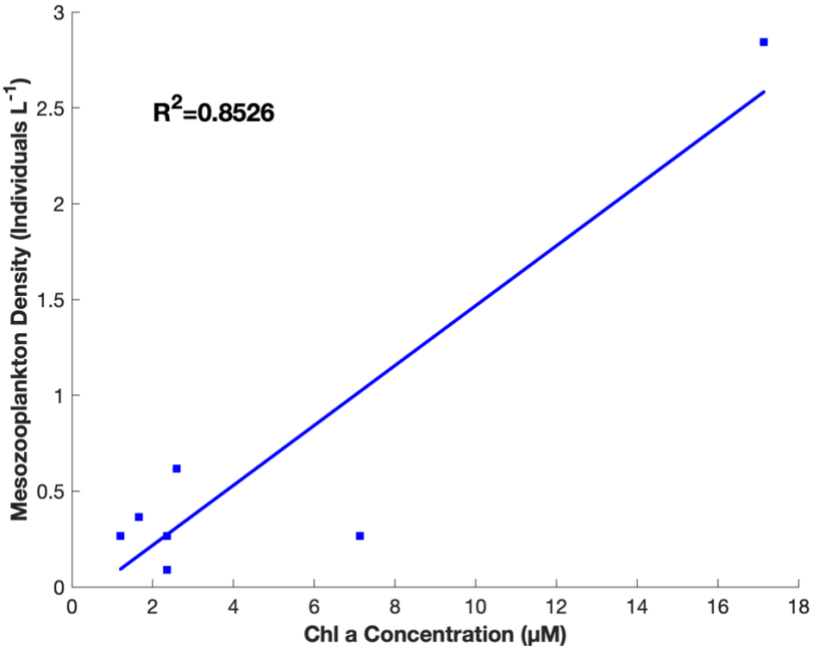

Figure 3.7: Mesozooplankton density (Individuals/L^{-1}) vs chl a (µg L^{-1}). The slope of the line was not significantly different from zero when the high leverage point was removed ($p>0.05$).

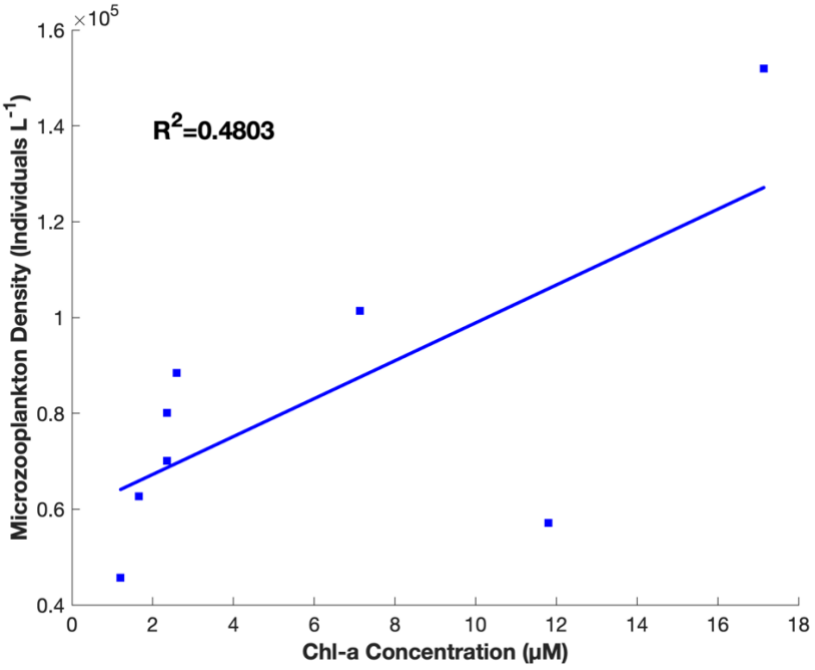

Figure 3.8: Microzooplankton density (Individuals/L^{-1}) vs chl a (µg L^{-1}). The slope of the line was not significantly different from zero when the high leverage point was removed ($p>0.05$).

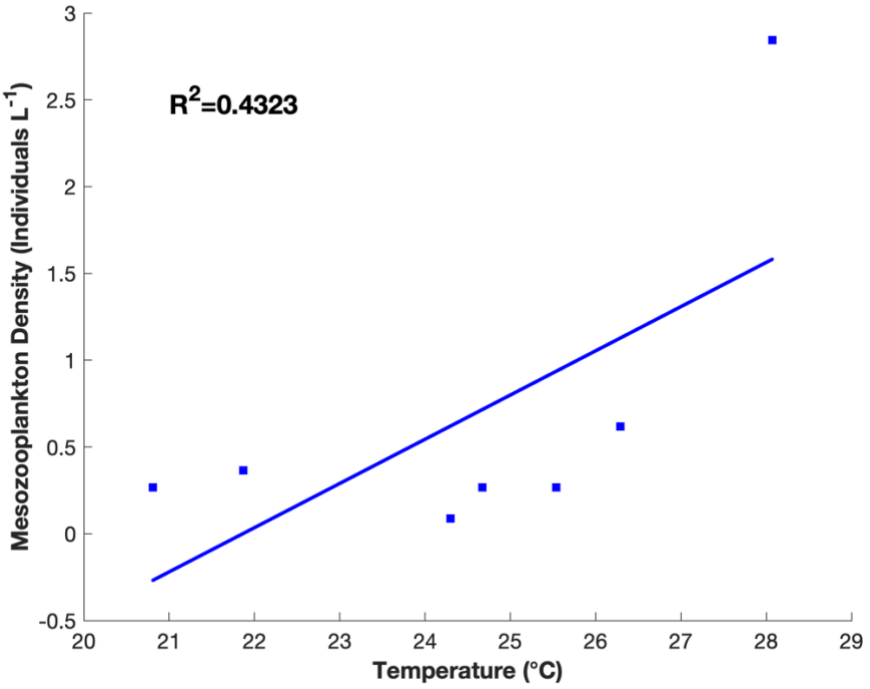

Figure 3.9: Mesozooplankton density (Individuals/L^{-1}) vs temperature (°C). The slope of the line was not significantly different from zero ($p>0.05$).

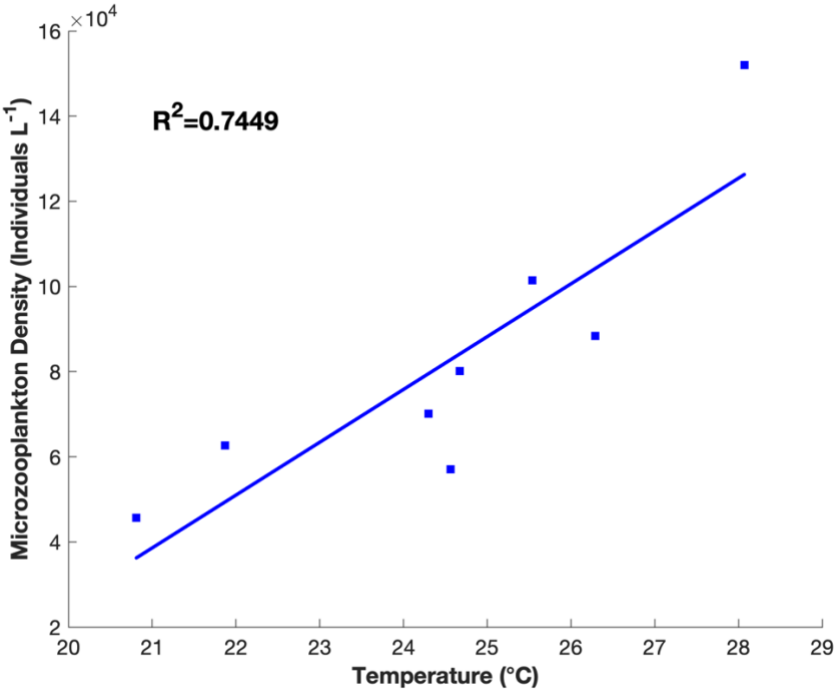

Figure 3.10: Microzooplankton density (Individuals/L^{-1}) vs temperature (°C). The slope of the line was significantly different from zero even without the high leverage point ($p<0.05$).

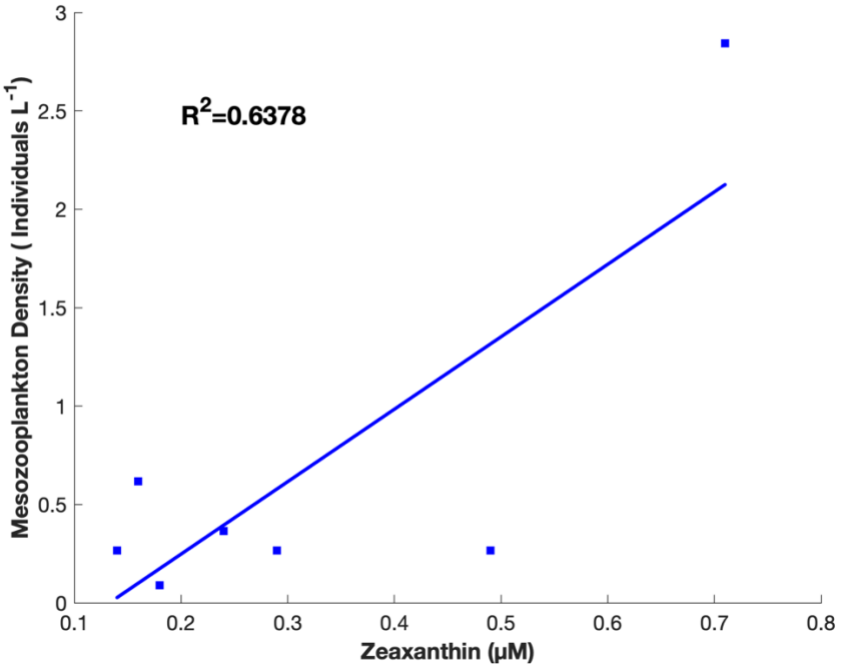

Figure 3.11: Mesozooplankton density vs zeaxanthin (µg L^{-1}). The slope of the line was not significantly different from zero when the high leverage point was removed ($p>0.05$).

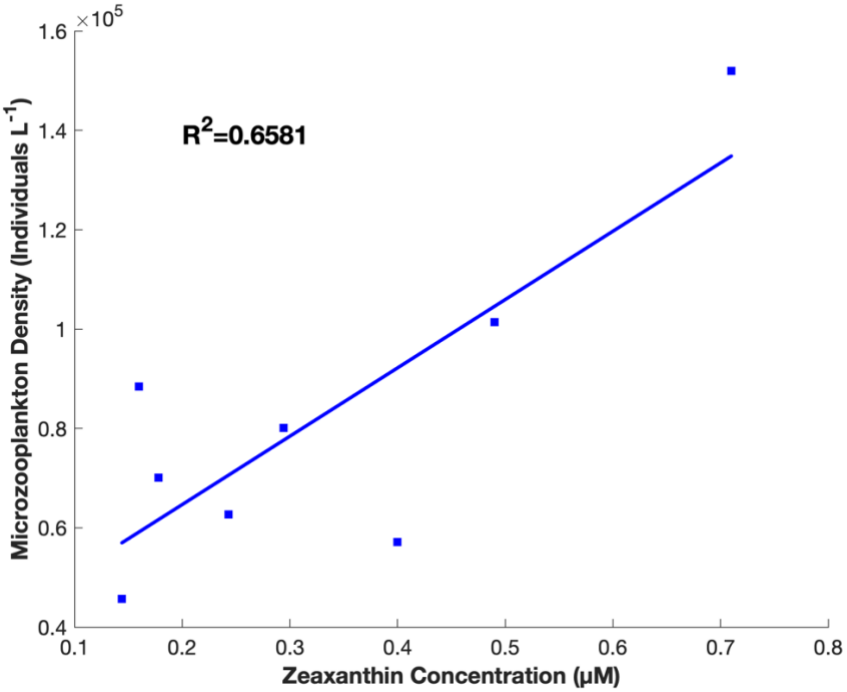

Figure 3.12: Microzooplankton density vs zeaxanthin (µg L^{-1}). The slope of the line was not significantly different from zero when the high leverage point was removed ($p>0.05$).

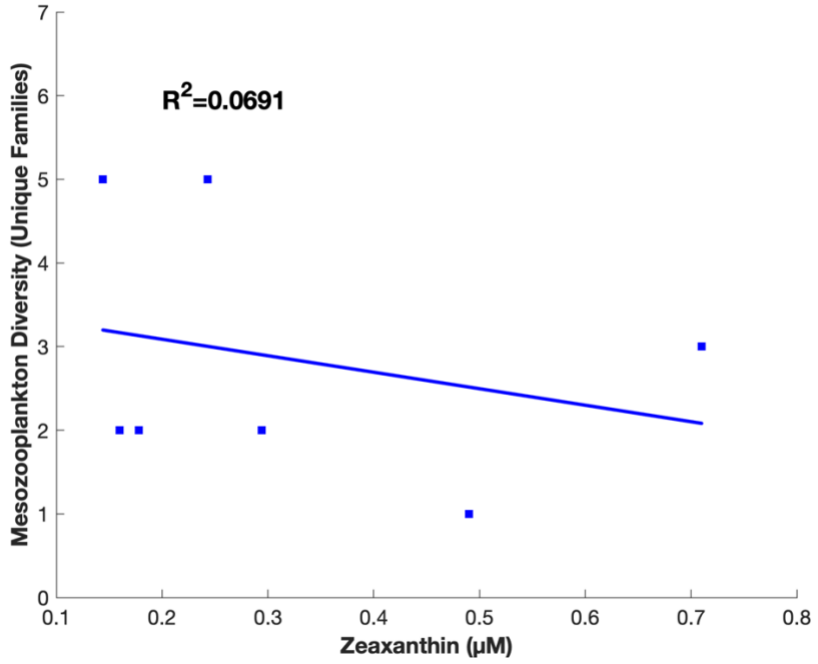

Figure 3.13 Mesozooplankton diversity vs zeaxanthin (µg L^{-1}). Mesozooplankton diversity is in terms of unique families. The slope of the line was not significantly different from zero ($p>0.05$).

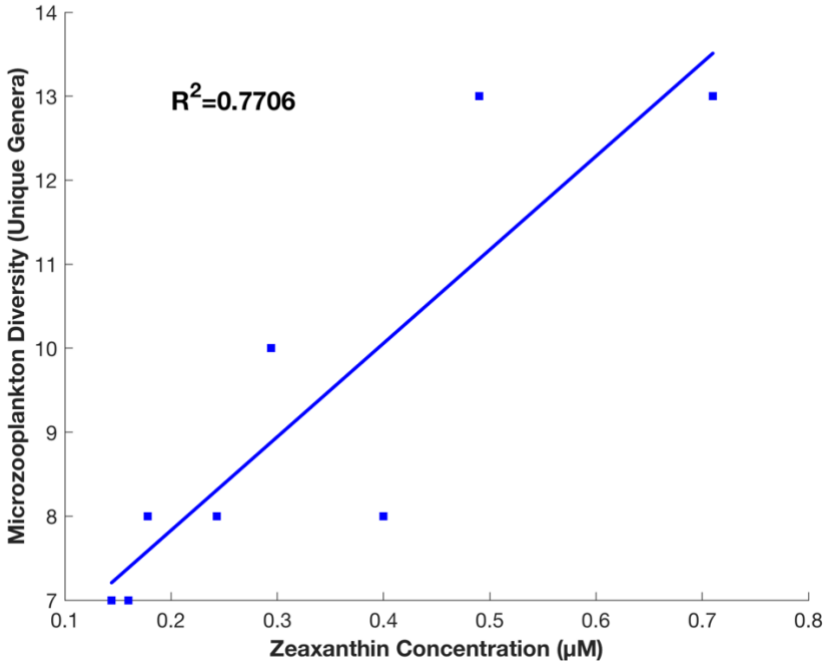

Figure 3.14: Microzooplankton diversity (Individuals/L^{-1}) vs zeaxanthin (µg L^{-1}). Microzooplankton diversity is in terms of unique genera. The slope of the line was significantly different from zero ($p<0.05$).

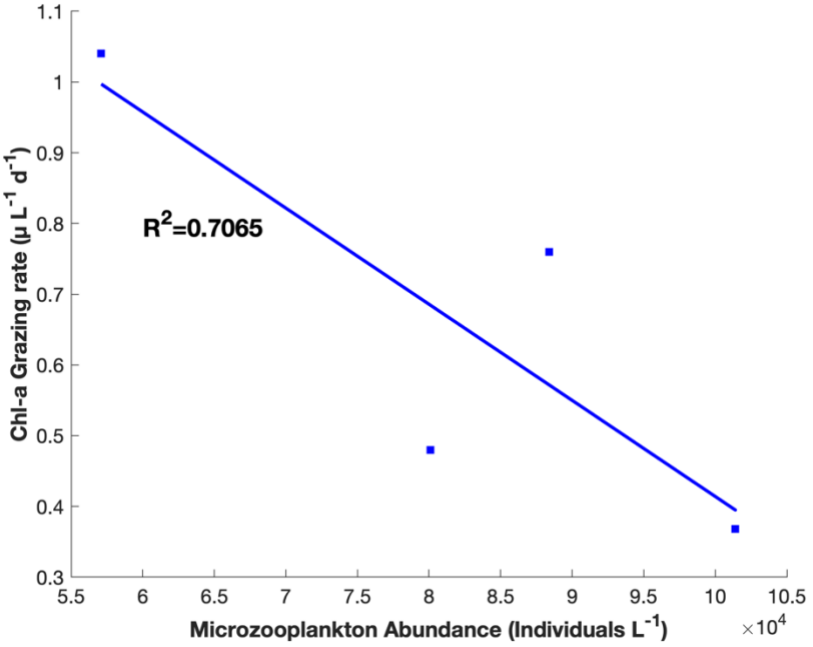

Figure 3.15: Microzooplankton density vs chl a grazing rate (μg L^{-1} d^{-1}). The slope of the line was not significantly different from zero (p>0.05).

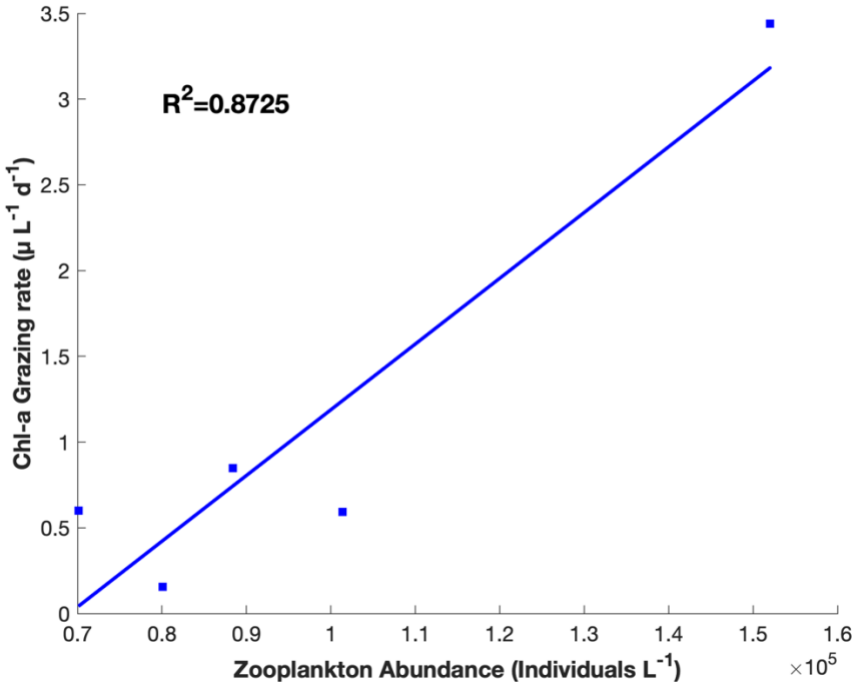

Figure 3.16: Combined density vs chl a grazing rate (μg L^{-1} d^{-1}). The slope of the line was not significantly different from zero (p>0.05).

CHAPTER 4

DISCUSSION

While there were many species of mesozooplankton identified, the mesozooplankton community in this pond was clearly dominated by copepods and cladocerans. The abundance of copepods and cladocerans is not surprising as these species dominate in other ponds and freshwater ecosystems as well (Frisch and Green 2007). There was a large increase in nauplii abundance in the month of October, particularly in the pump samples. The high prevalence in pump samples indicates that this likely not due to the change in sampling techniques. The high nauplii abundance measurements correlate with the lowest chl-*a* levels rather than the highest levels. Zooplankton, such as copepods, tend to release eggs when chl-*a* levels are high to ensure there is enough food for them to survive and grow (Seebens et al. 2009). However, this does not seem to be the case in this pond.

While there was a large amount of nauplii seen on October 1st, the adult mesozooplankton abundance showed a small decrease while nauplii increased even more. The low chl-*a* concentrations and low adult population could indicate that there was a high mortality rate during this sampling period, possibly due to low food availability. Another possibility of declining adult population could be that copepod adults are entering a diapause phase in the sediment in anticipation of unfavorable conditions, with water quality variables possibly acting as a cue (Seebens et al. 2009). Similar to diapause

phase, the nauplii could be leaving the pond with the flow of water heading towards the outflow structure, given the organisms were collected from the outflow structure. Lower temperatures lead to larger adults, for both cladocerans and copepods, and therefore larger clutches (Allan 1976). The observed increase in nauplii could be related to lower temperatures documented during this time, leading to larger clutches. Although this is less likely because there was no relationship between mesozooplankton abundance and temperature.

The zooplankton abundance trend for microzooplankton and mesozooplankton was similar, with increases until the peak on July 23rd then declining from there. The large increases and decreases seen throughout the mesozooplankton sampling is likely due to the imperfect sampling method leading to artificially low or high numbers. Microzooplankton abundances match well with what Pace and Orcutt 1981 recorded in a Georgia lake in August, with their total abundance ranging from 35,000 to over 100,000 individuals per liter. The mesozooplankton abundances they measured were higher overall, but their surface abundance matched closely with the abundance seen in this pond, both sitting around 0.1 individuals per liter. Pace and Orcutt also observed an increase in nauplii in the fall, similar to the trend observed in this pond. Sitta et al. 2018 observed higher mesozooplankton abundances than seen in this pond with a study that also took place in coastal South Carolina, with most of their abundances measuring greater than 1 individual per liter. The big peak in mesozooplankton abundance in July also correlated with the peak in phytoplankton abundance. Due to the positive correlation with chl-a concentrations, it is likely that this factor is influenced the initial increases in zooplankton abundance due to the increase in food availability. While the July 23rd data

are a high leverage point the relationship with chl-a and microzooplankton and mesozooplankton is still present and is weakly positive. Mesozooplankton abundance showed a strong positive relationship with grazing rates so this weak positive relationship could be related to the initial undersampling that occurred during earlier trips. Chl-*a* concentrations are likely the biggest factor in predicting zooplankton abundance trends for this pond as this relationship has been well documented in freshwater environments (Kagami et al. 2002, Liu and Dagg 2003).

While the relationship with DO was significant and positive for both zooplankton size groups there is a noticeably weaker relationship for microzooplankton. However, within both data sets there is a high leverage point. When this high leverage point is removed the relationship with DO is nearly non-existent although, mesozooplankton abundance still has a weak positive relationship. Both micro and mesozooplankton species seem relatively tolerant to DO levels below 5 mg/L, with a range of abundances at these concentrations, which has been noted in previous studies (Karpowicz et al. 2020). The drive behind that high leverage point is likely due to other environmental factors such as chl-*a* and temperature. Therefore, DO concentrations are not a great predictor for zooplankton abundance nor is it likely that low DO concentrations that are above hypoxic levels, or 2 mg/L, would hinder their growth. However, it is important to note that DO levels vary greatly throughout the day and therefore this measurement only gives insight into levels is during the morning period in the pond.

Temperature is one variable where there is some divergence in the relationship for mesozooplankton and microzooplankton. While both have a positive relationship, it is only significant and strong for microzooplankton. Temperature has been observed as a

driving factor in microzooplankton community composition, leading to significant changes in structure (Rose et al. 2009). This trend is observed when looking at the specific genera abundances. When temperature was noticeably lower, on August 20th, there was a shift in the dominant genera, with *Paramecium* overtaking *Netzelia* as the dominant microzooplankton. The temperature decline and community shift continue through October and the population looks very different from how it started, *Paramecium* is the dominant genera while *Netzelia* isn't even top 3 in abundance. Therefore, temperature could be a good predictor of micrzooplankton community structure in this pond.

While there was a positive correlation between both micro and mesozooplankton abundance and zeaxanthin concentration it is due to a high leverage data point. The high leverage point is also likely influenced by total chl-*a*, as zeaxanthin usually only constituted a small percentage of total chl-*a* of 10% or less. Without that data point the relationship falls apart for both groups. The abundance diversity relationships for the two groups are opposite of each other. Mesozooplankton see a negative relationship which was expected, although the sampling in earlier trips was likely under representative of the true diversity in the pond and therefore cannot be given much weight. The positive relationship for microzooplankton diversity is unusual, although it can likely also be attributed to the correlation between chl-*a* because of the low concentrations of zeaxanthin. Cyanobacteria likely did not greatly affect the zooplankton community structure or abundance, however there was an observed negative trend with grazing rates.

In freshwater ecosystems mesozooplankton are considered to be the main contributors to grazing of phytoplankton, contrary to marine systems where it is

microzooplankton (Sommer and Sommer 2006). Although, contrary to Gobler et al. 2007, the microzooplankton grazing rates were higher in June and lower in September. The combined grazing treatment had a higher average grazing rate as well as less instances where there was an increase in chl-*a* than the microzooplankton only treatment. The treatments that experienced an increase in pigment could be attributed to phytoplankton synthesizing more chl-*a* per cell in order to adapt to the dark, rather than phytoplankton growth as shown in experiments using these phytoplankton communities (Alvarez-Fernandez and Reigman 2014). In experiments using PAM fluorimetry the phytoplankton in this pond exhibited a strong adaptive response to reduced light intensities, supporting this theory (Carruther's Master's Thesis, in prep). However other pigments, such as fucoxanthin, are able to give us more insight because diatoms are a popular food source for zooplankton (Liu et. al 2016). In almost all treatments we see a decline in fucoxanthin, indicating that grazing did occur. Fucoxanthin grazing rates also point to mesozooplankton as the main driver of phytoplankton grazing, with an average rate more than three times that of the microzooplankton and control treatments. Certain zooplankton grazing rates can affected by the size of phytoplankton available, so the cause of mesozooplankton dominance could be due to the diatoms in the pond being larger and therefore not available to microzooplankton grazers, while mesozooplankton might not have the same limitations for smaller particles microzooplankton can feed on (Bogdan and Gilbert 1984). The general chl-*a* grazing trend could also be influenced by the size of other phytoplankton in the pond. The phytoplankton growth rate in the pond exceeded grazing rates nearly every trip, therefore it is more likely that phytoplankton are

controlled by bottom-up controls rather than zooplankton grazing, a top-down control (Carruthers Master's Thesis, in prep).

Unlike chl-*a* and fucoxanthin microzooplankton and mesozooplankton had similar grazing rates and trends for zeaxanthin. In nearly all microzooplankton only and combined treatments, zeaxanthin concentrations increased. These results mirror other studies that have linked cyanobacteria to reduce grazing. Several hypotheses for this reduced grazing include mechanisms such as toxin release, interfering with grazing assemblages and preference. It is unlikely toxin release is the cause, due to positive correlations with density and diversity, and more likely to be due to zooplankton cyanobacteria being less palatable or an interference with their grazing assemblages. Zooplankton have demonstrated food preferences based not only on size but also taste (DeMott 1986). It is clear that zooplankton in this pond do not find cyanobacteria to be an ideal food source and would rather feed on other phytoplankton. Given that zeaxanthin made up a relatively small percentage of total chlorophyll zooplankton were able to choose their food based on taste and quality preference, which seems to align well with diatoms. If cyanobacteria were to bloom in this pond it is unlikely that the present zooplankton community would be able to control the population.

Mesozooplankton were likely under sampled from trip 1 through 6 due to minimal water being processed as well as the pump hose limiting zooplankton capture due to its small opening. The net samples likely gave a more accurate measure of diversity and abundance, with the last two trips having the most diverse samples. Therefore, relationships between abundance and diversity and water quality parameters may not be accurate or hold much weight. The control in the grazing experiments were filtered

through a 64 μm sieve, which would not remove all of the microzooplankton grazers. Therefore, the control is only a true control for mesozooplankton while it cannot be compared as a control to the microzooplankton only treatment. Nearly all of the data sets presented had a high leverage point affecting the relationships seen. Therefore, the conclusions drawn here are made with limited confidence and more research is needed to improve that confidence level.

Zooplankton have demonstrated the ability to respond to varying water chemistry parameters quickly. Temperature and chl *a* both had strong positive correlations with zooplankton abundance in this pond, while DO is less of a concern. Microzooplankton had a clear community shift that lined up well with temperature, indicating that anthropogenic warming could permanently alter the community in this pond, disrupting the natural ecosystem balance. Management practices should focus on water quality variables that can be controlled, such as nutrient pollution, to promote biodiversity in this pond and minimize the effects of anthropogenic warming. Zeaxanthin concentrations did not have a strong effect on abundance or diversity however, concentrations were low compared to total chl *a*. Mesozooplankton generally exerted stronger grazing pressure than microzooplankton, although grazing pressure was greatly reduced for both groups when looking at zeaxanthin pigments, or cyanobacteria. As both zooplankton groups showed reduced grazing, the use of herbivory to contain cyanobacteria blooms appears limited. Therefore, it is important that new BMP strategies are introduced to prevent excess nutrients from entering the pond and possibly causing a bloom. Zooplankton play many important ecological roles such as acting as a water quality indicator or a trophic

transfer link in the food web. Research on zooplankton communities in SDPs are lacking and therefore should continue to be explored.

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