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Fortnightly Effects of Urea Additions on Cyanobacteria in A Stormwater Detention Pond

Halley Carruthers

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FORTNIGHTLY EFFECTS OF UREA ADDITIONS ON CYANOBACTERIA IN A
STORMWATER DETENTION POND

by

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ABSTRACT

Increased urban and suburban population growth along the South Carolina (SC) coast has led to a rise in impervious surfaces, altering the course of stormwater runoff events. The construction of stormwater detention ponds (SDPs) is one of the many ways to best mitigate the flow of this water. In their function as natural pollutant traps, SDPs often contain increased levels of nutrients (nitrogen, N and phosphorus, P), which can lead to eutrophication. Under these high eutrophic conditions, primary production is overstimulated, and the formation of phytoplankton blooms, including harmful algal blooms (HABs) can occur. In recent decades, the forms of nitrogen (N) exported to coastal waters have changed, with more than half of all N fertilizers being urea-based. Research has also shown species-specific differences to various concentrations and forms of fixed N. More specifically, that cyanobacteria seem to thrive under higher concentrations of ammonium and urea. Furthermore, the photophysiology of freshwater phytoplankton has been studied to assess nutrient stress, lipid content, effects of UV radiation, and bloom dynamics, with less research on specific photo-physiological parameters in response to nutrients. However, there is a limited number of studies that has utilized Pulse Amplitude Modulated fluorometry to assess the photosynthetic efficiency and photophysiology of freshwater, phytoplankton communities in the North Inlet-Winyah Bay region. This proposed work aimed to examine the seasonal variability and photophysiology of phytoplankton communities in a one SDP over a short-term period in response to urea. These effects were tested using nutrient addition bioassays

under a 72 h incubation conducted every 14-days. Phytoplankton community composition varied throughout the season, coinciding with changes in nutrient concentration. Cyanobacteria made up a small percentage ($<30\%$) of the total phytoplankton community, contrary to our main hypothesis, only exhibited an insignificant, and weak response to urea additions. Urea additions also did not significantly increase the photophysiology of this phytoplankton community, except for the photosynthetic efficiency parameter.

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LIST OF SYMBOLS

F_v/F_m	Photosynthetic Quantum Efficiency
Φ_{PSII}	Effective Quantum Yield of Photosystem II
α	Initial slope of a rapid light curve
$rETR_m$	Maximum relative electron transport chain
E_k	Minimum saturation irradiance
NH_4^+	Ammonium
NO_{2+3}	Nitrite + Nitrate
$(CO(NH_2)_2)$	Urea
Chl a	Chlorophyll a

LIST OF ABBREVIATIONS

BMFL.....	Baruch Marine Field Lab
Ctrl	Control
HABs.....	Harmful Algal Blooms
HPLC	High Performance Liquid Chromatography
K-W ANOVA.....	Kruskal-Wallis Test
N.....	Nitrogen
NIWB.....	North Inlet-Winyah Bay
P	Phosphorus
PAM.....	Pulse Amplitude Modulated Fluorometry
P-I.....	Photosynthesis-Irradiance Curve
RLC.....	Rapid Light Curve
SC.....	South Carolina
Si	Silica
T ₀	Time Initial
TN	Total Nitrogen
TP	Total Phosphorus
U.....	Urea
UPSi.....	Urea + Phosphorus + Silica
WW	Whole Water

CHAPTER 1

INTRODUCTION

Increased urban and suburban population growth along the South Carolina (SC) coast has led to a rise in impervious surfaces, such as roads and parking lots, that can alter urban hydrology by increasing the volume and velocity of stormwater runoff events (Schroer et al., 2018). As a result, the development of various best management practices (BMP) have been implemented to mitigate water flow and improve water quality (Ellis et al., 2014; Chang et al., 2018; Beckingham et al., 2019). The construction of stormwater detention ponds (SDPs) is one of these BMPs. There are approximately 9,000 constructed SDPs along the SC coast (Schroer et al., 2018). In particular, about 100 ponds were constructed each year from 1994 to 2013, in the Charleston and Myrtle Beach areas (Smith et al., 2018; Beckingham et al., 2019).

From an engineering perspective, these ponds primarily act as reservoirs collecting water from nearby landscapes, allowing for the mitigation of pollutants and the control of the volume of runoff. Structurally, SDPs consist of a permanent and temporary pool. As contaminants are deposited into the pond, they undergo sedimentation and eventually collect in the permanent pool. During rain events, the temporary pool acts to manage flooding and lessen the severity of pollutants entering receiving waters. SDPs serve many functions such as, providing ecological habitat for birds, subsidizing ecosystem services, and raising aesthetic and property value, particularly in the southeastern US (Moore and Hunt, 2012; Ghermandi and Fichtman, 2015; Hassall and

Anderson, 2015). When SDPs are not properly maintained they can trap and transport environmental pollutants to nearby receiving waters (Van Metre et al., 2000; Thapalia et al., 2010).

A growing human population is correlated with increased nutrient inputs of nitrogen (N) and phosphorus (P). In their function as natural pollutant traps, SDPs often contain increased levels of these nutrients, leading to eutrophication. Under these high eutrophic conditions, primary production is overstimulated, and the formation of phytoplankton blooms, including harmful algal blooms (HABs) can occur (Paerl, 1997; Bricker et al., 1999; Anderson et al., 2002, 2008). For example, SDPs on Kiawah Island, SC, experience frequent algal blooms due to residential development and associated turf maintenance of surrounding golf courses (Lewitus et al., 2003). Studies have shown that a variety of phytoplankton species occupy the ponds including, raphidophytes, diatoms, and cyanobacteria depending on salinity (Lewitus et al., 2003; 2004; 2008). Seasonal changes in community composition have also been observed; Siegel et al. (2011) demonstrated that ponds on Kiawah Island showed shifts in community composition over the course of a summer season. One pond exhibited a shift in community throughout the summer with prasinophytes dominating in the beginning and end, and more diatoms in the middle of the summer season (Siegel et al., 2011). An adjacent pond, however, consistently exhibited a high density of diatoms throughout the summer (Siegel et al., 2011). Research has also shown species-specific differences in growth response to nutrient additions (Donald et al., 2011; 2013; Siegel et al., 2011; Reed et al., 2016; Sitta et al., 2018; Erratt et al., 2019). In recent decades, the forms of N exported to coastal waters have changed, with more than half of all N fertilizers being urea-based (Glibert et

al., 2006). This shift in the type of fertilizer usage has caused changes in species-specific phytoplankton dynamics with a significant increase in the prevalence of cyanobacterial blooms (Donald et al., 2011; 2013; Siegel et al., 2011; Reed et al., 2016; Sitta et al., 2018; Erratt et al., 2019). Moreover, research has shown that urea is more energetically favorable for various species of cyanobacteria, like *Microcystis aeruginosa* because it can serve as a N and C source (Finlay et al., 2010; Erratt et al., 2018; Krausfeldt et al., 2019). The reason for this is that cyanobacteria contain the enzyme urease, and associated transporter proteins, needed to assimilate and breakdown urea (Flores and Herrero, 2005; Veaudor et al., 2019).

Through genomic studies, it is reported that about 85% (264 out of 308) of cyanobacterial genomes possess the three structural genes that encode for urease, *ureA*, *ureB*, *ureC* (Flores and Herrero, 2005; Veaudor et al., 2019). The presence of the necessary transporter proteins in cyanobacteria, UrtABCDE, have also been discovered (Valladeres et al., 2002). The inactivation of some of these transporter proteins including, *urtA*, *urtB*, and *urtE*, have demonstrated a 97-98% decrease in urea uptake in a strain of *Dolichospermum* and *Synechococcus* (Valladeres et al., 2002).

Several studies have documented the impact of stormwater nutrients on phytoplankton communities, showing changes in community composition and growth (Lewitus et al., 2003; 2004; 2008; Siegel et al., 2011; Reed et al., 2016; Sitta et al., 2018). Specifically, high inputs of urea have caused significant blooms of cyanobacteria (Donald et al., 2011; 2013; Siegel et al., 2011; Reed et al., 2016; Sitta et al., 2018; Erratt et al., 2019). Cyanobacteria are able to further their expansion by utilizing urea (Valladeres et al., 2002; Flores and Herrero, 2005; Finlay et al., 2010; Erratt et al., 2018; Krausfeldt et

al., 2019; Veaudor et al., 2019). However, short-term variation in phytoplankton dynamics in response to urea additions, over the course of a season, have not been characterized in SDPs in the NIWB area.

The photophysiology of freshwater phytoplankton has often been measured using Pulse Amplitude Modulated Fluorometry (PAM) to assess nutrient stress, lipid content, effects of UV radiation, and bloom dynamics (Kromkamp et al., 2001, 2008; Masojidek et al., 2001; White et al., 2011; Zhang et al., 2011; Harrison, 2015). Some studies have also found increases in various photo-physiological parameters in response to nutrient additions (Harrison and Smith, 2013; Ramanna et al., 2014; Rattan et al., 2014). However, only one account of the utilization of PAM to assess the photophysiology of freshwater phytoplankton communities in the Southeastern US has been published in the scientific literature (Bergmann et al., 2002). Thus, more research is needed to assess the photophysiology of freshwater phytoplankton in the Southeastern US, specifically in the NIWB area.

The aim of this study was to analyze changes in community composition over a short-term time scale, and quantify photosynthetic efficiency (F_v/F_m), and other physiological characteristics of phytoplankton in an SDP from summer to fall in response to urea ($\text{CO}(\text{NH}_2)_2$) loading. Nutrient addition bioassays were conducted at 14-day intervals with four different treatments: time initial (T_0), control (Ctrl), urea (U), and U + phosphorus and silica (UPSi) to assess these changes. This research was focused on the primary research question of, how does phytoplankton community composition change over a short-term (14-day) period following urea, and other nutrient additions? The hypotheses were that: (i) U additions preferentially stimulate cyanobacterial growth and

the addition of UPSi (in excess) preferentially stimulates diatoms relative to cyanobacteria, and (ii) U and UPSi additions would result in a significant increase in F_v/F_m and RLC characteristics relative to the control group. The goal of this study was to gain insight on the ecology of natural SDP phytoplankton communities and provide information for stormwater management in order to better manage local SDPs in Georgetown and Horry county.

CHAPTER 2

MATERIALS AND METHODS

2.1 STUDY SITE

This study was conducted in a SDP located in Murrells Inlet, SC (33°33'46.2"N 79°01'47.3"W), behind the Marina Colony Condominium community (Fig. 1). The pond is in a highly urbanized area surrounded by vegetation and drains directly into Murrells Inlet. The main sources of catchment into this pond come from rainfall and runoff from the adjacent townhomes.

2.2 EXPERIMENTAL DESIGN

Bioassays were conducted in 14-day intervals between June to October 2020 from this one pond, with a total of 8 sampling dates. Water was collected at the surface using a battery- operated diaphragm pump (Pentair ShurFlo). Water for time 0 measurements were collected in 10% HCl acid washed 1000 mL flasks (2) and incubation water was collected in 10% HCl acid washed 750 mL flasks (15 replicates). The flasks were rinsed in triplicate with pond water prior to collection. Nutrients were added to the flasks to create the following treatments: (1) Control (no nutrients added), (2) Urea (U), and (3) U + Phosphate + Silica (UPSi). U was added in equimolar concentrations of 50 $\mu\text{mol/L}$ as the final experimental concentration. Phosphate as KH_2PO_4 and silica as $\text{Na}_2\text{O}_3\text{Si}_5\text{H}_2\text{O}$ were added in excess concentrations of 6.25 $\mu\text{mol/L}$ and 65.625 $\mu\text{mol/L}$ respectively, to ensure that they were not limiting. The flasks were placed into two water tables in front of the Baruch Marine Field Lab (BMFL) in Georgetown, SC to maintain ambient water

temperature while allowing for exposure to natural light. Flasks were gently mixed three times a day during the 72 h incubation period. One to two sheets of fiberglass neutral density screens were used to reduce ambient irradiance based on weather patterns. Subsamples for nutrient measurements, microscopy, photopigment, and photosynthesis analysis were taken at the start of experimentation and at 72 h. Subsamples for photopigment analysis were filtered into two size fractions, whole water (WW) and $<20\mu\text{m}$ water ($<20\mu\text{m}$) to discern the composition of different sized phytoplankton.

2.3 WATER QUALITY

Water quality parameters (temperature, conductivity, dissolved oxygen, pH, turbidity, and Chlorophyll *a* (Chl *a*)) were measured using a YSI 6820 sonde probe every 14 days. Daily water temperatures for the water tables were based on measurements taken from the Oyster Landing environmental monitoring station located at BMFL.

2.5 NUTRIENT ANALYSES

Filtered ($0.45\ \mu\text{m}$) samples were analyzed for total nitrogen (TN), total phosphorus (TP), orthophosphate (PO_4^{3-}), nitrate + nitrite (NO_{2+3}^-), and ammonium (NH_4^+) at time 0 using a Technicon nutrient AutoAnalyzer3. A total of three 20 mL scintillation vials were collected for analysis. Urea ($\text{CO}(\text{NH}_2)_2$) concentrations were analyzed at time 0 for the last three sampling dates (9/17/20; 10/1/20; 10/15/20) using a V-1200 Spectrophotometer VWR following the Revilla et al. method (2005). A total of three 20 mL scintillation vials were collected for analysis.

2.5 MICROSCOPY

Subsamples (20 ml) were collected from each of the bioassay containers for microscopic analysis. Samples were immediately preserved with 2 mL of Lugol's

solution and stored in the dark at room temperature until ready for analysis. An inverted microscope was used to assess each sample qualitatively and identify phytoplankton down to the genus level.

2.6 PHOTOPIGMENT MEASUREMENTS

Phytoplankton photopigment concentrations were measured using High Performance Liquid Chromatography (HPLC) (Pinckney et al., 2001a; Roy et al. 2011). Aliquots (50-100 mL) of the incubation water were filtered under a gentle vacuum (<50 KPa) through a glass fiber filter (25 mm dia. Whatman GF/F), immediately frozen, and stored at -80°C. Filters were lyophilized for 18-24 hours at -50 °C. Photopigments were extracted by adding 750 µL of 90% aqueous acetone solvent followed by storage for 12-20 hours at -20°C. Filtered extracts (250 µL) were injected into a Shimadzu HPLC with a single monomeric column (Rainin Microsorb, 0.46 × 1.5 cm, 3 µm packing) and a polymeric (Vydac 201TP54, 0.46×25 cm, 5 µm packing) reverse-phase C18 column in series. A non-linear binary gradient consisting of solvent A (80% methanol: 20% 0.5 M ammonium acetate) and solvent B (80% methanol: 20% acetone) was used for the mobile phase (Pinckney et al. 2001a). Absorption spectra and chromatograms (440 ± 4 nm) were obtained using a Shimadzu SPD-M10av photodiode array detector and pigment peaks were identified by comparing retention times and absorption spectra with pure standards (DHI, Denmark). The synthetic carotenoid β-apo-8'-carotenal (Sigma) was used as an internal standard. The software ChemTax (v. 1.95) was used to determine the relative abundances of major phytoplankton groups (Pinckney et al. 2001, Lewitus et al. 2005, Higgins et al. 2011). ChemTax provided estimates of the

relative abundances of major algal groups (e.g., chlorophytes, cryptophytes, cyanobacteria, diatoms, dinoflagellates, etc.) based on total chl *a* in units of $\mu\text{g chl } a \text{ l}^{-1}$.

2.7 PHOTOSYNTHETIC EFFICIENCY MEASUREMENTS

A Walz Water-Pulse-Amplitude-Modulated Chl *a* fluorometer (PAM) was used to measure photosynthetic performance using rapid light curves (RLCs) (Schreiber et al., 1986). RLCs are different from traditional photosynthesis-irradiance (P-I) curves because each step of illumination does not provide sufficient time for photosynthesis to reach steady state (Schreiber, 1997, 2004). These curves provide information on the present state of photosynthesis for photoautotrophs by measuring effective quantum yields (Φ_{PSII}) and relative electron transport rates (rETR); thereby indicating the physiological state of the cells (Ihnken et al., 2011). The Φ_{PSII} describes whether absorbed light photons are used to transport electrons through photosystem II and is calculated by the difference in maximal chlorophyll fluorescence and steady-state fluorescence, divided by the maximal chlorophyll fluorescence (Genty et al., 1989). rETRs provide a relative rate of electrons passing through photosystem II and are calculated by multiplying Φ_{PSII} by the photosynthetically active radiation (Beer et al., 2001).

Subsamples (5 ml) were collected at 14-day intervals and at the end of each bioassay for measurements of RLCs using the Walz Water-PAM. Cells were dark-adapted for 30 minutes and then exposed to a saturation pulse at the end of eight different and increasing actinic photosynthetic active radiation intensities, with a 30 s duration between each increment (Ralph and Gademann, 2005). The RLCs were fitted to a curve using a Marquardt-Levenberg regression function (Platt et al., 1981). From this function, the photosynthetic efficiency (F_v/F_m), initial slope (α), maximum relative electron

transport rate ($rETR_m$), and minimum saturation irradiance (E_k) were obtained and evaluated. F_v/F_m is a ratio that measures the efficiency of photosynthesis through the indication of the maximum quantum yield of PSII (Genty et al., 1989). The initial slope or α indicates the light adaptation state and is proportional to the Φ_{PSII} (Schreiber et al., 2004). The $rETR_m$ is the maximum value of $rETR$, and E_k is the irradiance at which this maximum value is achieved (Beer et al., 2001; Schreiber et al., 2004).

2.8 STATISTICS

Statistical analyses were performed using R version 1.3.1073. The data were not normally distributed based on a Shapiro-Wilks test, so the nonparametric Kruskal-Wallis test (K-W ANOVA) and Dunn's *post-hoc* tests were used. These tests were used to assess the differences between urea addition response with algal group. They were also used to analyze the differences between treatment and RLC parameters.



Figure 2.1 (a) Google maps image of Marina Colony Condominiums in Murrells Inlet, SC. (b) Image of Marina Colony SDP.

CHAPTER 3

RESULTS

3.1 POND CONDITIONS

The Marina Colony pond ranged in temperature between 21.9°C - 28.1°C, with the lowest temperature in October and the highest temperature in July (Table 3.1). The average temperature was $24.5^{\circ}\text{C} \pm 2.31^{\circ}\text{C}$. This pond was fresh with an average pH of 7.4 ± 0.504 and a mean conductivity of $0.21 \text{ mS/cm} \pm 0.065$. Concentrations of NH_4^+ were higher than $\text{NO}_3^- + \text{NO}_2^-$ throughout the sampling season, with especially high concentrations on 7/23/20 (NH_4^+ 29.40 ± 0.76 ; $\text{NO}_3^- + \text{NO}_2^-$ 1.24 ± 0.48 ; Table 3.2). The average NH_4^+ concentration was $13.0 \pm 8.9 \text{ } \mu\text{mol/L}$, while the average $\text{NO}_3^- + \text{NO}_2^-$ was $3.28 \pm 1.65 \text{ } \mu\text{mol/L}$. This pond had an average PO_4^{3-} concentration of $1.09 \pm 0.234 \text{ } \mu\text{mol/L}$ and an average total P concentration of $1.65 \pm 0.303 \text{ } \mu\text{mol/L}$. The average TN concentration of this pond was $50.1 \pm 13.02 \text{ } \mu\text{mol/L}$, with the highest concentration of TN at $72.8 \text{ } \mu\text{mol/L}$ on 7/23/20. Relative to the Redfield ratio of 16 N:1 P, ambient N:P ratios were higher at about 25:1 (Redfield, 1958). Based on TN concentrations, urea was added in excess on 6/25/20, 7/9/20, 8/20/20, and 10/1/20 based on the experimental concentration of $50 \text{ } \mu\text{mol/L}$ used. For the urea samples collected from this pond (9/17/20; 10/1/20; 10/15/20), concentrations were low, with an average concentration of $1.10 \pm 0.559 \text{ } \mu\text{mol/L}$ (Table 3.3).

3.2 COMMUNITY COMPOSITION

Total Chlorophyll a

Overall, WW and <20µm samples resembled a similar pattern to one another in T_0 total chl *a* concentration with the lowest concentrations on 10/1/20 and the highest concentrations on 7/23/20. At the start of the experiment (6/25/20), concentrations were 4.68 ± 0.88 µg/L (WW; Fig. 3.1) and 3.45 ± 0.172 µg/L (<20µm; Fig. 3.2). On 7/23/20, concentrations greatly increased to 17.0 ± 1.61 µg/L for WW and 15.6 ± 0.60 µg/L for <20µm, thereby reflecting the highest levels for the sampling season. Total chl *a* concentrations drastically decreased and remained steady through the end of the experiment (10/15/20). The lowest concentrations were observed on 10/1/20 at 1.21 ± 0.08 µg/L for WW and 1.27 ± 0.45 µg/L for <20µm.

Cyanobacteria

WW and <20µm cyanobacteria samples displayed similar trends in their abundance throughout the sampling season (Figs. 3.3 & 3.4). There was a general increasing trend in percent abundance with values fluctuating from 6/25/20 to 9/17/20, followed by a notable increase in abundance until 10/15/20. In both size fractions, cyanobacteria contributed the most to chl *a* concentrations on the last sampling date, 10/15/20, with <20µm cyanobacteria contributing more (WW: $25.3 \pm 8.97\%$; <20µm: $28.6 \pm 7.73\%$). Cyanobacteria contributed the least to chl *a* concentrations on the first sampling date, 6/25/20, with WW cyanobacteria contributing more (WW: $1.97 \pm 0.76\%$; <20µm: $1.32 \pm 0.72\%$).

3.3 NUTRIENT ADDITIONS

Data for all bioassays were combined and normalized to the control group to determine the overall response of each algal group for each nutrient treatment. This was done by calculating a ratio relative to control for each algal group by dividing the concentration in response to U additions by the average of the control group.

Urea

Overall, U additions did stimulate a response in phytoplankton communities. For WW samples, chlorophytes, diatoms, and euglenophytes exhibited the strongest response to U additions, while haptophytes, cryptophytes, and cyanobacteria exhibited the weakest response (Fig. 3.5). Chrysophytes and dinoflagellates exhibited moderate responses to U additions. For <20 μ m samples, Euglenophytes and chrysophytes exhibited the strongest response to U additions, while all other groups exhibited minimal responses to U additions (Fig. 6). A K-W ANOVA test revealed a significant difference in WW group responses to U additions ($p \leq 0.001$). A Dunn's *post-hoc* test ($p \leq 0.05$) revealed that haptophytes exhibited a statistically significant response to urea additions from all other algal groups, except chrysophytes and dinoflagellates. In addition, there were significant differences between diatoms-chrysophytes ($p = 0.0022$) and euglenophytes-chrysophytes ($p = 0.0088$). Similarly, for the <20 μ m samples, there was also a significant difference in group response to U additions (K-W ANOVA Test, $p \leq 0.001$). A Dunn's *post-hoc* test revealed ($p \leq 0.05$) that haptophytes were statistically significant from chrysophytes and cyanobacteria in their response to urea additions. Another significant difference was found between chrysophytes and chlorophytes ($p = 0.0418$). Likewise, diatoms-

chrysophytes ($p = 0.0050$) and euglenophytes-chrysophytes ($p = 0.0011$) were also significantly different in their response.

Urea + Phosphorus + Silica (UPSi)

For WW and $<20\mu\text{m}$ samples, diatoms exhibited a stronger response to U (Fig. 3.7 & 3.8). No significant difference was observed between the U and UPSi treatments based on a K-W ANOVA test ($p \geq 0.001$; Table 3.4). Response ratios for U and UPSi were also compared among all algal groups. Based on a K-W ANOVA test, a significant difference was only observed for dinoflagellates at a p of 0.024 for WW and 0.000 for $<20\mu\text{m}$. Dinoflagellates exhibited a stronger response to U than UPSi for both size fractions (Fig. 3.9 & 3.10).

3.4 MICROSCOPY

Microscopy samples were consistent with HPLC samples and revealed chlorophytes and diatoms as the dominant algal groups. Common genera found in the samples were the chlorophytes, *Scenedsmus*, *Desmodesmus*, *Selenastrum*, and *Monoraphidium*, along with the diatoms, *Amphipleura*, *Gyrosigma*, and *Aulacoseira*.

3.5 PAM MEASUREMENTS

Photosynthetic Efficiency (F_v/F_m)

All measurements for F_v/F_m were combined for each bioassay (Fig. 3.11). There was an increasing trend with decreasing variability in F_v/F_m response to the different treatments with U and UPSi showing similar distribution. The lowest F_v/F_m values were observed in the T_0 treatment, while the highest values were observed in the U and UPSi treatments. A K-W ANOVA test revealed a significant difference between treatments ($p \leq 0.001$). A Dunn's *post-hoc* test revealed that all groups were significantly different from

each other, except for U-UPSi ($p = 0.659$, $p \leq 0.05$). Though the increase was small, this indicates that U and UPSi significantly increased the photosynthetic efficiency relative to the control group and T₀.

Initial slope (α)

All measurements for α were combined for each bioassay (Fig. 3.12). Data showed a slight increase in α , followed by a slight decrease in UPSi. Overall variability of α for the different treatments remained similar. However, based on a K-W ANOVA test and Dunn's *post-hoc* test, there was a significant difference between T₀-Ctrl ($p = 0.0134$) and T₀-U ($p = 0.00016$).

Relative Maximum Electron Transport Rate ($rETR_m$)

All measurements for $rETR_m$ were combined for each bioassay (Fig. 3.13). A similar trend to α can be observed with minimal changes in $rETR_m$ between treatments. Following a K-W ANOVA test ($p \leq 0.001$), a Dunn's *post-hoc* test showed that there was only one significant difference between U-T₀ ($p = 0.0036$; $p \leq 0.05$). Relative to the Ctrl group, nutrient additions of U and UPSi did not significantly change the $rETR_m$.

Minimum Saturation Irradiance (E_k)

All measurements for E_k were combined for each bioassay (Fig. 3.14). Data for all treatments showed similar distributions with no significant differences based on a K-W ANOVA test ($p \geq 0.001$).

Table 3.1 Temperature, pH and conductivity of the Marina Colony pond from 6/25/20 to 10/15/20. Average (Avg.); standard deviation (S.D.).

Date	Temperature (°C)	pH	Conductivity (mS/cm)
6/25/20	24.56	6.96	0.11
7/9/20	25.54	6.9	0.196
7/23/20	28.07	7.56	0.312
8/6/20	26.29	6.96	0.213
8/20/20	24.67	7.52	0.164
9/17/20	24.3	7.08	0.293
10/1/20	20.81	8.21	0.198
10/15/20	21.87	7.99	0.231
Avg.	24.51	7.40	0.07
S.D.	2.32	0.50	0.21
Median	24.62	7.30	0.21

Table 3.2 Initial nutrient concentrations of the Marina Colony pond from 6/25/20 to 10/15/20. TN (Total Nitrogen); TP (total phosphorus); Average (Avg.); Standard Deviation (S.D.).

Date	NO₃⁻ + NO₂⁻ (μmol/L)	NH₄⁺ (μmol/L)	PO₄³⁻ (μmol/L)	TN (μmol/L)	TP (μmol/L)
6/25/20	3.51	6.35	0.84	34.4	1.36
6/25/20	3.46	6.54	0.85	33.1	1.23
6/25/20	3.27	6.17	0.82	33.8	1.26
7/9/20	2.26	8.18	0.83	43.4	1.32
7/9/20	2.22	8.46	0.82	43.9	1.45
7/9/20	2.17	8.43	0.83	44.1	1.32
7/23/20	0.83	30.2	1.38	72.8	2.03
7/23/20	0.82	29.1	1.39	70.2	2.07
7/23/20	1.65	28.8	1.35	70.8	1.99
8/6/20	3.92	13.5	1.29	55.7	1.91
8/6/20	3.83	13.5	1.29	53.3	1.84
8/6/20	3.86	13.5	1.28	54.4	1.84
8/20/20	5.92	7.91	1.13	43.7	1.79
8/20/20	5.73	6.02	1.11	48.2	1.75
8/20/20	5.73	8.10	1.13	50.1	1.65
10/1/20	5.23	8.52	0.94	44.7	1.61
10/1/20	5.19	8.27	0.93	41.6	1.43
10/1/20	5.22	8.40	0.93	43.7	1.50
10/15/20	4.21	11.9	1.90	60.2	2.59
10/15/20	4.45	11.8	1.92	58.6	2.59
10/15/20	4.22	12.5	1.88	64.2	2.72
Avg.	3.70	12.2	1.18	50.7	1.77
S.D.	1.56	7.60	0.36	12.0	0.44
Median	3.86	8.46	1.13	48.2	1.75

Table 3.3 Initial urea concentrations of the Marina Colony pond from 9/17/20 to 10/15/20. Average (Avg.); Standard Deviation (S.D.).

Date	Urea ($\mu\text{mol/L}$)
9/17/20	2.26
9/17/20	1.02
9/17/20	0.113
10/1/20	0.978
10/1/20	0.978
10/1/20	0.813
10/14/20	1.31
10/14/20	1.20
10/14/20	1.20
Avg.	1.10
S.D.	0.559
Median	1.02

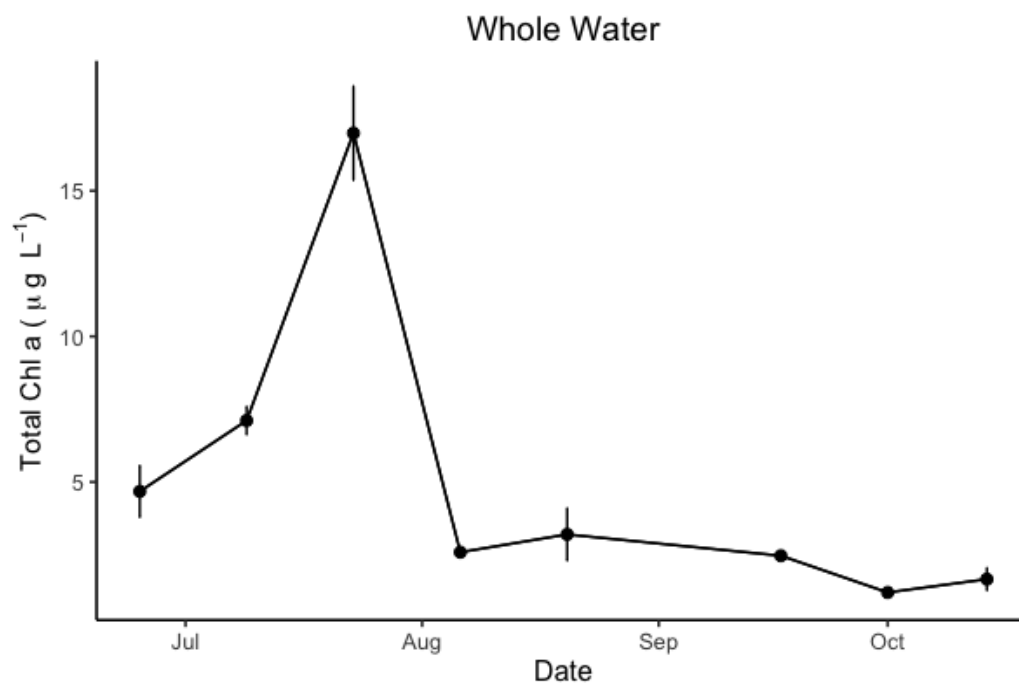


Figure. 3.1 Total Chl *a* concentration (µg/L) of WW phytoplankton (mean \pm SD; $n = 5$) in the Marina Colony pond at the start of the bioassays from 6/25/20 to 10/15/20.

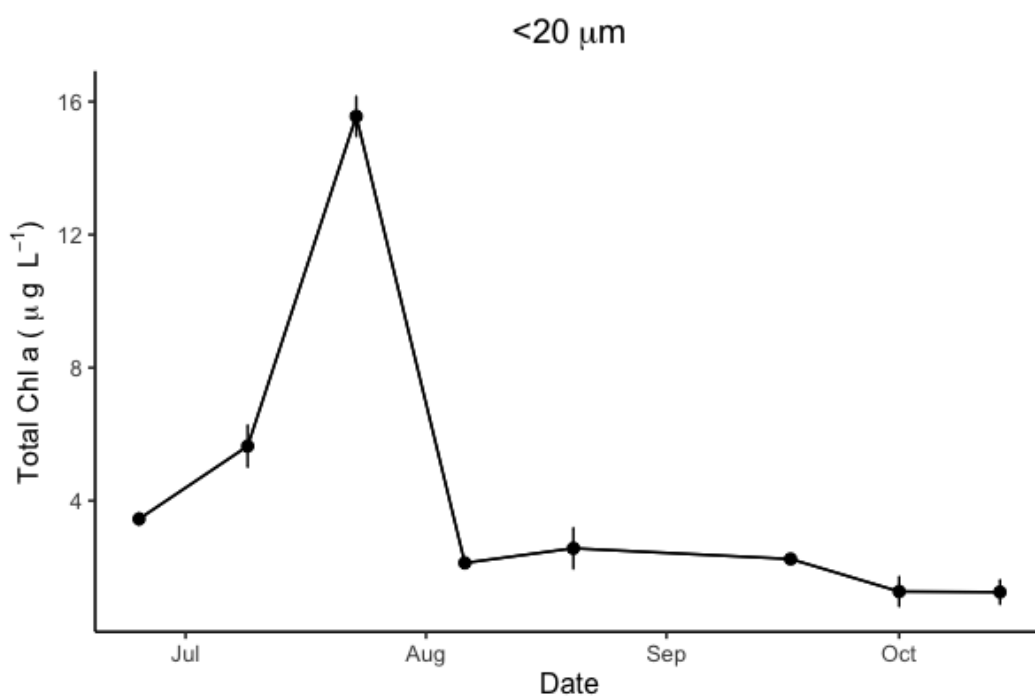


Figure 3.2 Total Chl *a* concentration ($\mu\text{g/L}$) of <20 μm phytoplankton (mean \pm SD; $n = 5$) in the Marina Colony pond at the start of the bioassays from 6/25/20 to 10/15/20.

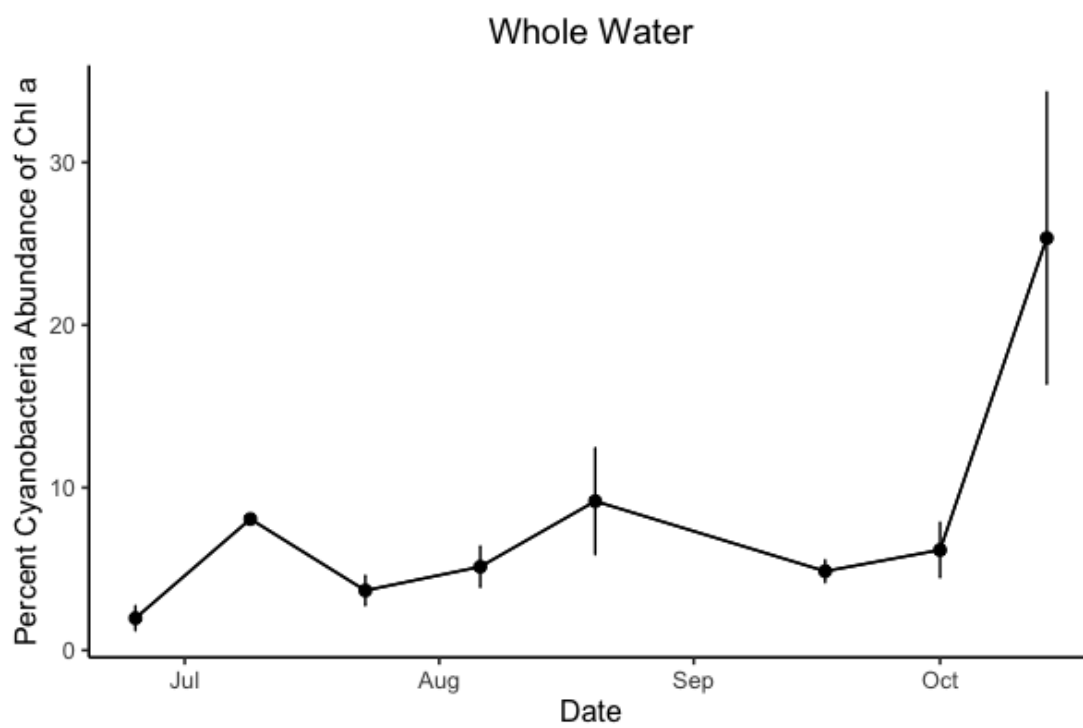


Figure 3.3 Percent cyanobacteria abundance of chl *a* for WW phytoplankton (mean \pm SD; $n = 5$) in the Marina Colony pond at the start of the bioassays from 6/25/20 to 10/15/20.

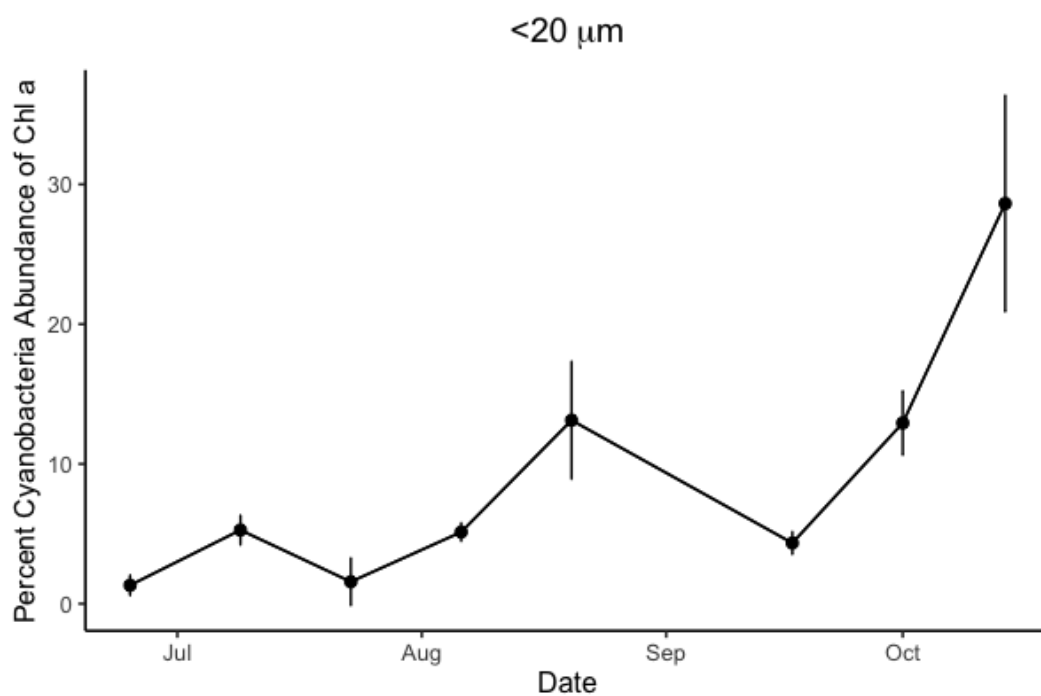


Figure 3.4 Percent cyanobacteria abundance of chl *a* concentration for <20 μm phytoplankton (mean \pm SD; $n = 5$) in the Marina Colony pond at the start of the bioassays from 6/25/20 to 10/15/20.

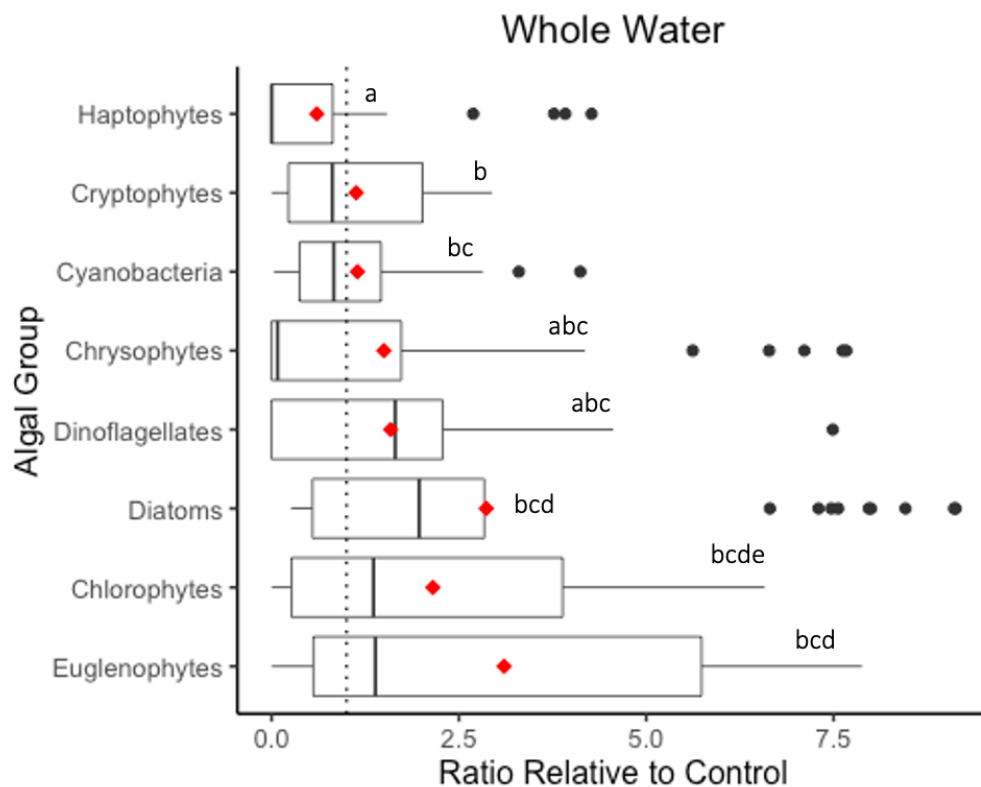


Fig. 3.5 WW ratio of urea addition effects relative to the control group for all algal groups in the Marina Colony pond across bioassays from 6/25/20 to 10/15/20. K-W ANOVA and Dunn's *post-hoc* tests were performed and indicated a significant difference between algal groups and their response to urea additions. Different lower-case letters indicate significant groups, the same lower-case letter indicate not significant groups. The vertical dashed line indicates no difference in response from the control and the red diamond indicates the mean.

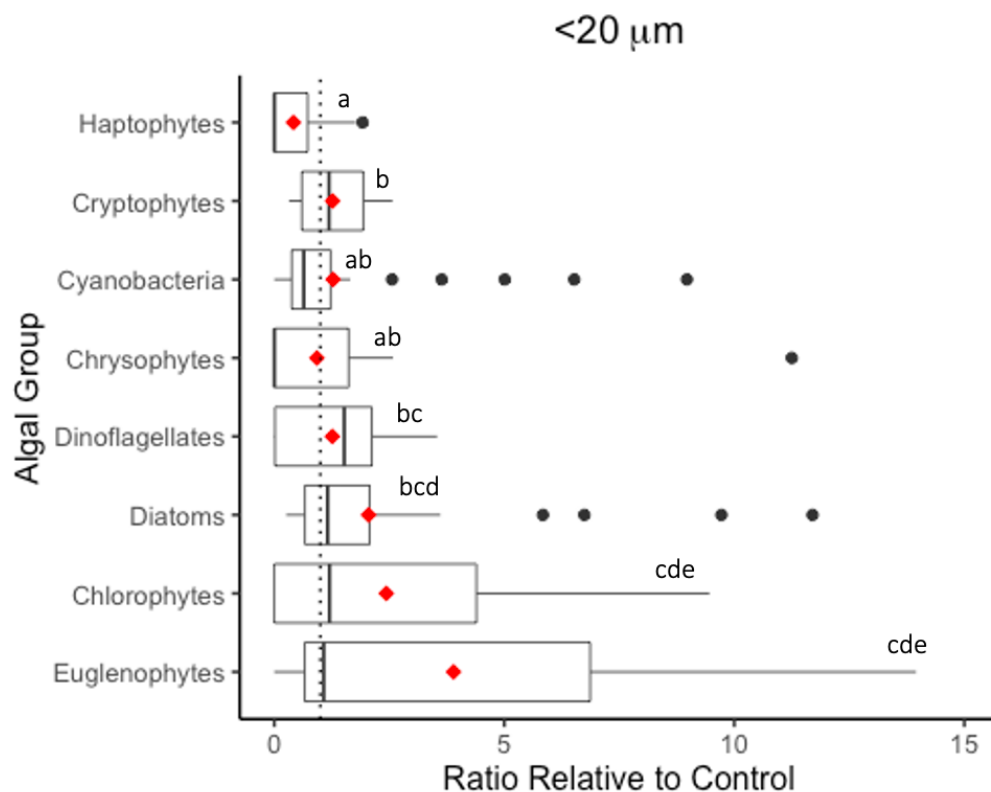


Fig. 3.6 $<20\mu\text{m}$ ratio of urea addition effects relative to the control group for all algal groups in the Marina Colony pond across bioassays from 6/25/20 to 10/15/20. K-W ANOVA and Dunn's *post-hoc* tests were performed and indicated a significant difference between algal groups and their response to urea additions. Different lower-case letters indicate significant groups, the same lower-case letter indicate not significant groups. The vertical dashed line indicates no difference in response from the control and the red diamond indicates the mean.

Table 3.4 K-W ANOVA results of UPSi addition effects for all algal groups, for each size fraction. Only dinoflagellates showed a statistically significant difference between U and UPSi treatments (WW p-value = 0.024; <20 μ m p-value = 0.000).

Algal Group	Whole Water		< 20 μm	
	x²	p	x²	p
Chlorophytes	0.080	0.777	3.114	0.078
Cryptophytes	0.047	0.829	1.576	0.209
Chrysophytes	2.203	0.138	0.567	0.452
Cyanobacteria	0.176	0.675	0.433	0.511
Diatoms	0.236	0.627	0.445	0.505
Dinoflagellates	5.091	0.024	12.249	0.000
Euglenophytes	0.775	0.379	1.125	0.289
Haptophytes	2.967	0.085	3.779	0.052

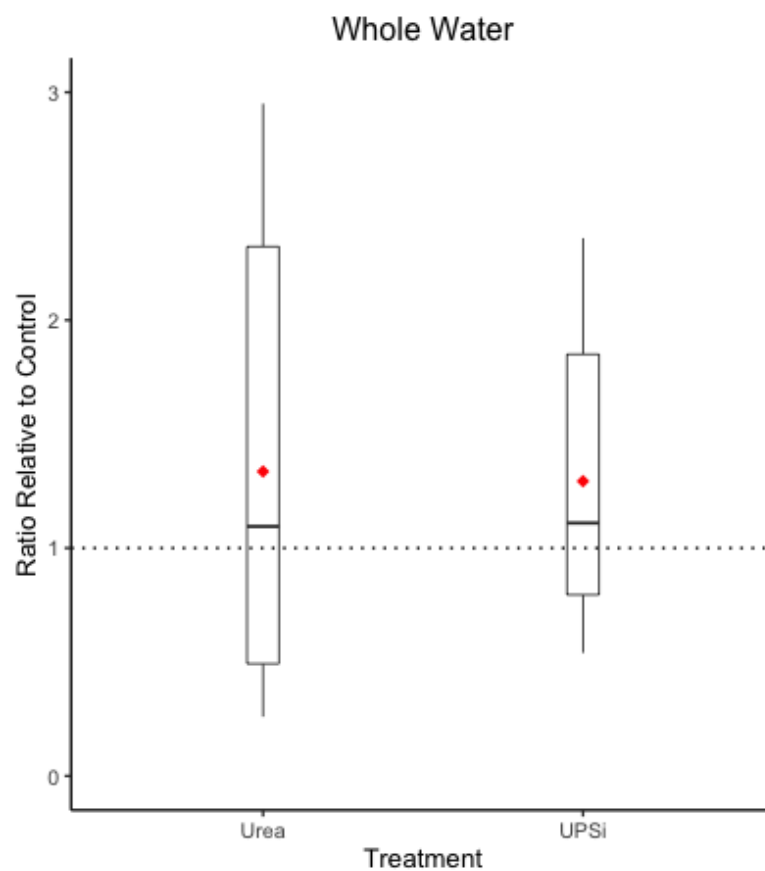


Figure 3.7 Ratio of U and UPSi addition effects relative to control for WW diatoms. Data was combined for all bioassays. No significant difference was observed between treatments (K-W ANOVA test, $p \geq 0.001$). The vertical dashed line indicates no difference in response from the control and the red diamond indicates the mean.

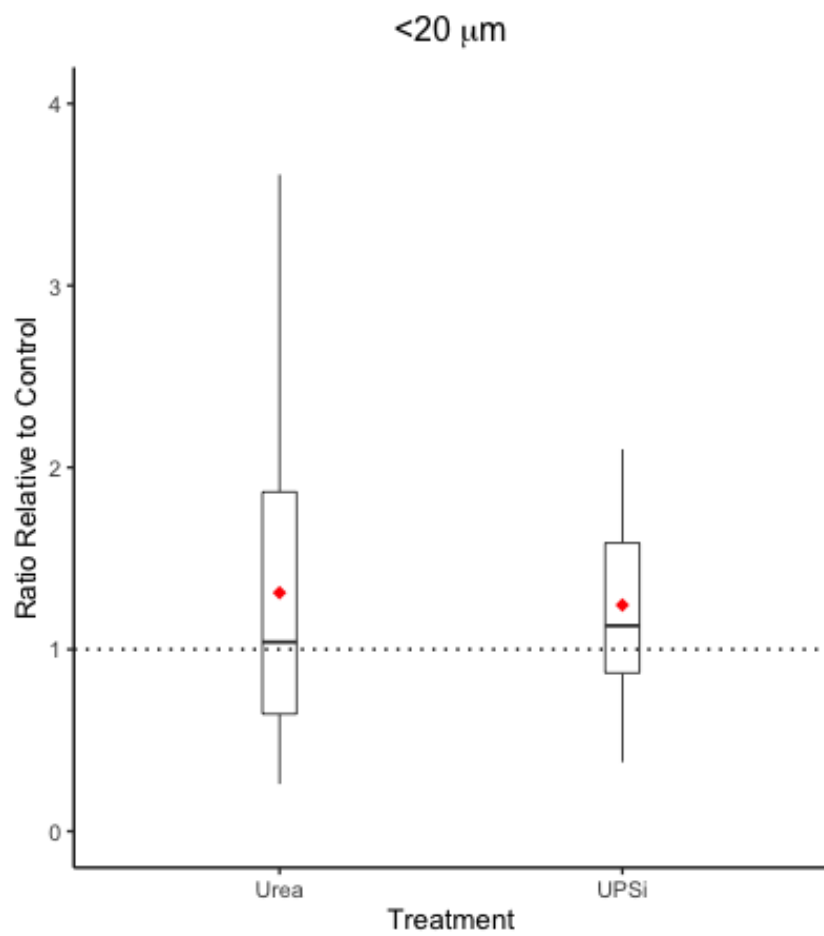


Figure 3.8 Ratio of U and UPSi addition effects relative to control for <20μm diatoms. Data was combined for all bioassays. No significant difference was observed between treatments (K-W ANOVA test, $p \geq 0.001$). The vertical dashed line indicates no difference in response from the control and the red diamond indicates the mean.

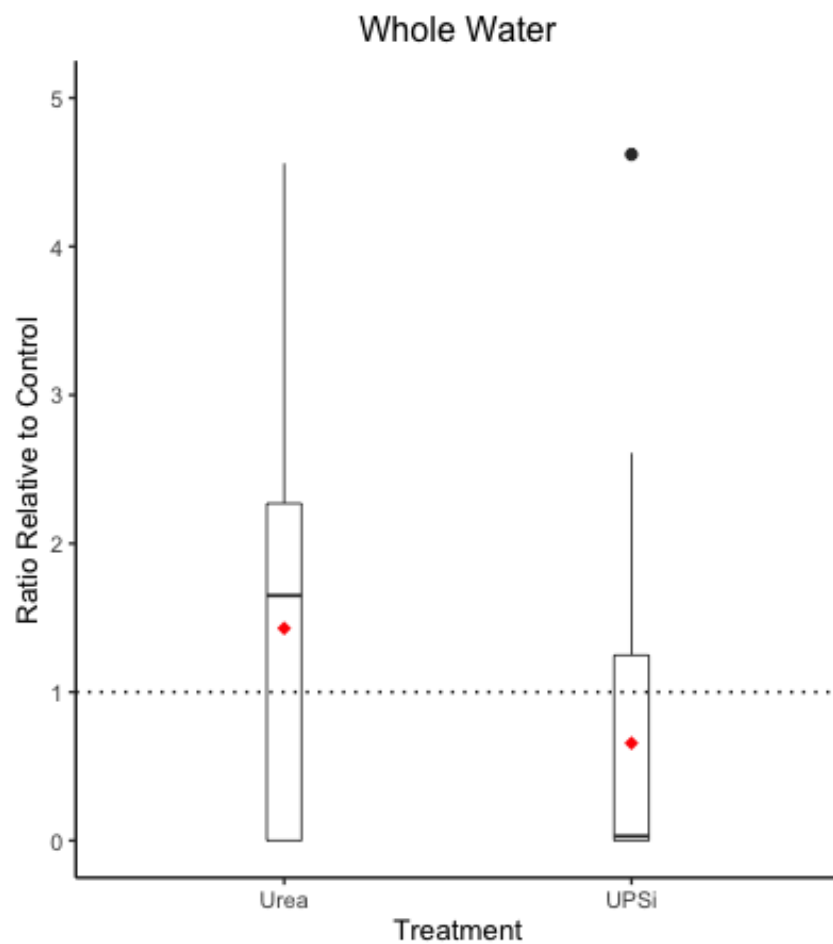


Figure 3.9 Ratio of U and UPSi addition effects relative to control for WW dinoflagellates. Data was combined for all bioassays. A significant difference was observed between treatments (K-W ANOVA test, $p \leq 0.001$). The vertical dashed line indicates no difference in response from the control and the red diamond indicates the mean.

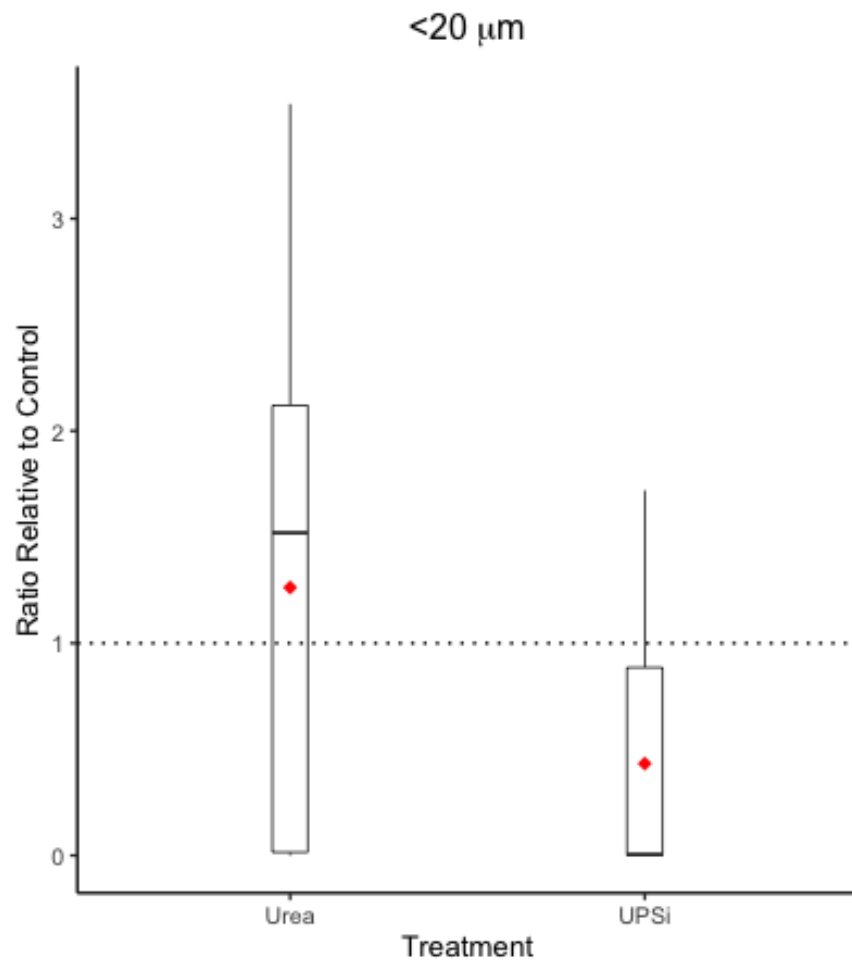


Figure 3.10 Ratio of U and UPSi addition effects relative to control for <20 μm dinoflagellates. Data was combined for all bioassays. A significant difference was observed between treatments (K-W ANOVA test, $p \leq 0.001$). The vertical dashed line indicates no difference in response from the control and the red diamond indicates the mean.

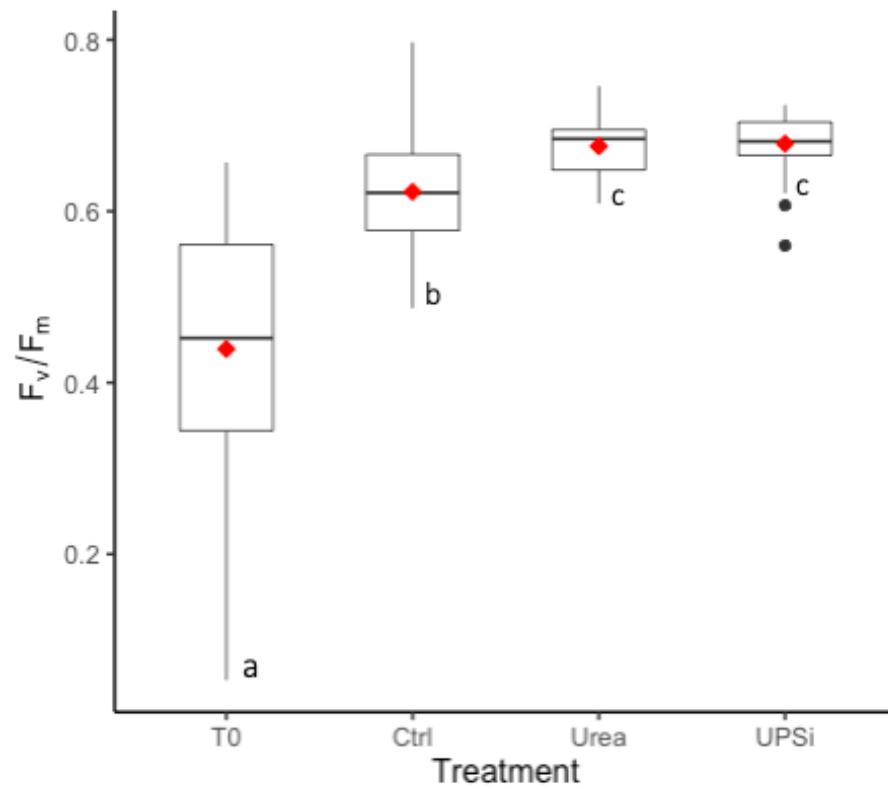


Figure 3.11 Photosynthetic efficiency (F_v/F_m) of phytoplankton across bioassays for each treatment. Different letters indicate significant differences between groups, the same letter indicates no significant difference between groups. Statistical significance was tested to $p \leq 0.001$ (K-W ANOVA) and $p \leq 0.05$ (Dunn's *post-hoc* test).

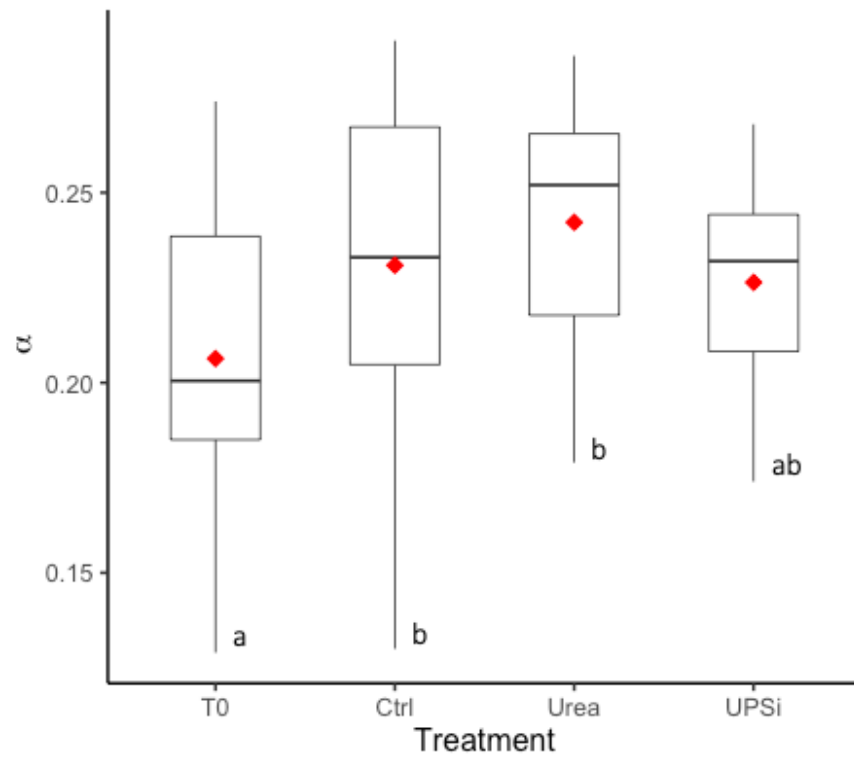


Figure 3.12 Initial slope (α) of phytoplankton across bioassays for each treatment. Different letters indicate significant differences between groups, the same letter indicates no significant difference between groups. Statistical significance was tested to $p \leq 0.001$ (K-W ANOVA) and $p \leq 0.05$ (Dunn's *post-hoc* test).

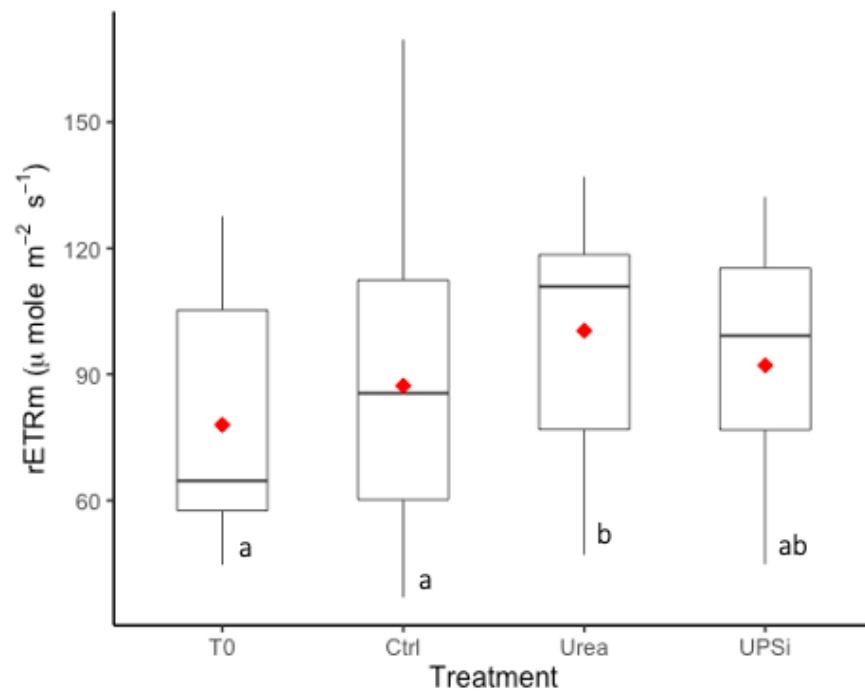


Figure 3.13 Maximum relative electron transport rate (rETRm) of phytoplankton across bioassays for each treatment. Different letters indicate significant differences between groups, the same letter indicates no significant difference between groups. Statistical significance was tested to $p \leq 0.001$ (K-W ANOVA) and $p \leq 0.05$ (Dunn's *post-hoc* test).

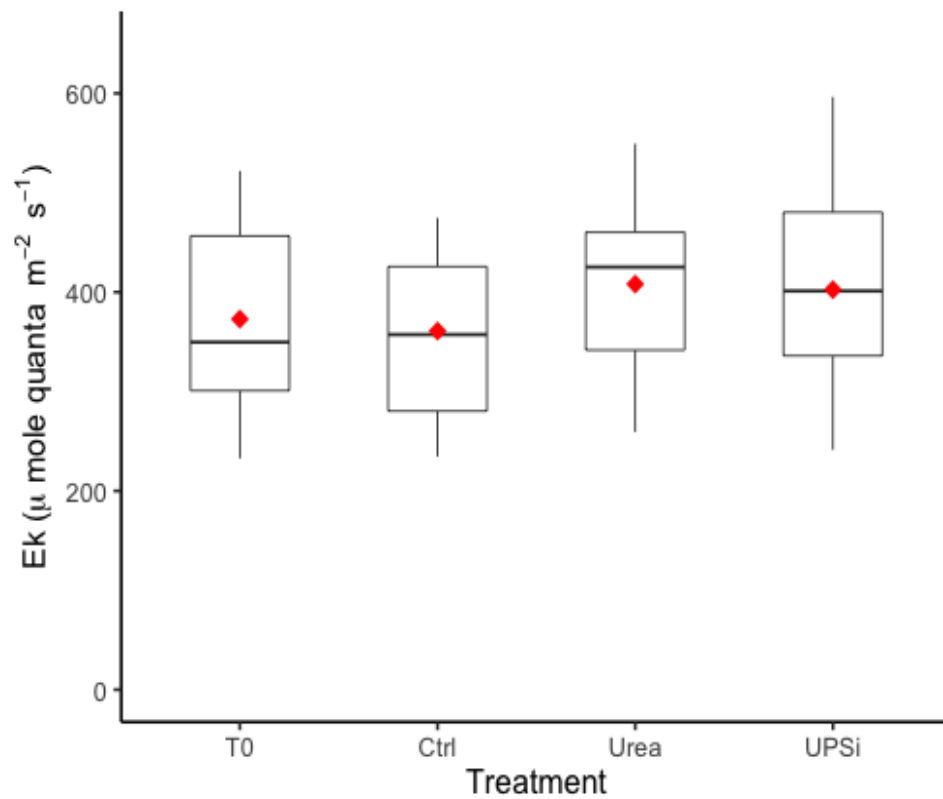


Figure 3.14 Minimum saturation irradiance (E_k) of phytoplankton across bioassays for each treatment. K-W ANOVA results indicated that there were no significant differences between groups. Statistical significance was tested to $p \leq 0.001$ (K-W ANOVA) and $p \leq 0.05$ (Dunn's *post-hoc* test)

CHAPTER 4

DISCUSSION

The aim of this study was to assess the stimulatory effects of urea additions on cyanobacteria and quantify the photophysiology of phytoplankton in a SDP located in Murrells Inlet, SC. Several studies have been conducted on phytoplankton communities in SDPs in the state of South Carolina on Kiawah Island (Lewitus 2003, 2004, 2008; Siegel et al., 2011; Reed et al., 2016; Sitta et al., 2018). In addition, the increased availability of urea has often associated with cyanobacteria growth in these ponds (Siegel et al., 2011; Reed et al., 2016; Sitta et al., 2018). However, these ponds in Kiawah Island are known to be tidally influenced, and of a higher salinity than the pond studied in Murrells Inlet (Lewitus et al., 2003; 2004; 2008). Additionally, they are commercially influenced due to the number of golf courses present (Lewitus et al., 2003), whereas the pond in Murrells Inlet is surrounded by residential areas. Regardless, no studies have been conducted on ponds in the NIWB region that assess natural phytoplankton dynamics on a more frequent scale of every two weeks. The photophysiology of natural, freshwater phytoplankton communities has also been frequently studied (Kromkamp et al., 2001, 2008; Masjidek et al., 2001; Zhang et al., 2011; Harrison, 2015), with fewer studies assessing this response to changes in nutrient concentration (Harrison and Smith, 2013; Ramanna et al., 2014; Rattan et al., 2014). Moreover, there is only one study that has

analyzed effects of urea additions on the photophysiology of a natural, freshwater phytoplankton community in the Southeastern US (Bergmann et al., 2002).

4.1 POND CONDITIONS

The TN pool was mainly comprised of NH_4^+ , at concentrations ranging from 30.2 $\mu\text{mol/L}$ to 6.02 $\mu\text{mol/L}$ throughout the season. The samples taken for urea measurements (9/17/20–10/15/20) were comparatively low, ranging from 2.26 $\mu\text{mol/L}$ to 0.113 $\mu\text{mol/L}$. This could be due to the hydrolysis of urea as it is readily hydrolyzed into ammonium carbonate by urease (Glibert, 2006). Studies have shown the time it takes urea to speciate depends on various factors such as, timing of application, weather, soil temperature, and pH (Khakural and Alva, 1995; Wali et al., 2003).

This pond had a ratio of about 25 TN:1 TP; relative to the Redfield ratio of 16 N:1, this observed ratio is higher, and indicates the pond to be P limited (Redfield, 1958). However, the bioassays indicate that P was not limiting, but rather N was limiting. Similar studies done on freshwater lake systems, have also shown N limitation to occur in mesocosm experiments (Lewis et al., 2011).

4.2 COMMUNITY COMPOSITION

WW and $<20\mu\text{m}$ size fractions had the highest concentrations of initial total chl *a* on 7/23/20 at $17.0 \pm 1.61 \mu\text{g/L}$ and $15.6 \pm 0.60 \mu\text{g/L}$ for WW and $<20\mu\text{m}$ respectively. These results coincided with the highest initial TN concentrations for the season at 72.8 $\mu\text{mol/L}$, 70.2 $\mu\text{mol/L}$, and 70.8 $\mu\text{mol/L}$. These results are consistent with established literature as it is commonly known that high amounts of nutrients promote phytoplankton growth (Bricker et al., 1999; Anderson et al., 2002, 2008; Siegel et al., 2011; Reed et al., 2015, 2016; Sitta et al., 2018). By 8/6/20, both total chl *a* and TN concentrations

decreased and remained low through the end of the experiment. In a similar study, Reed et al. (2015) also found higher initial abundances of chl *a* in the summer (~25.0 µg/L) compared to the fall (~14.0 µg/L) in pond K075 on Kiawah Island, SC.

Overall, cyanobacteria made up a small percentage (<30%) of the total chl *a* composition throughout the season. Cyanobacteria abundance accounted for a maximum of 25.3% (WW) and 28.6% (<20µm) in mid-October and a minimum of 1.92% (WW) and 1.32% (<20µm) in late-June. Similar values were observed in another pond studied on Kiawah Island with cyanobacteria making up a maximum of 36% and a minimum of 6%; these values occurred in July and June, respectively (Siegel et al., 2011). However, another pond on Kiawah Island showed much lower contributions of cyanobacteria to total chl *a*, with ~9% in August and September, and ~6% in June and July (Siegel et al., 2011).

4.2 NUTRIENT ADDITIONS

Urea

Current literature shows that cyanobacteria grow best under dissolved organic nitrogen sources, specifically urea (Donald et al., 2011; 2013; Siegel et al., 2011; Reed et al., 2016; Sitta et al., 2018; Erratt et al., 2019). Nonetheless, cyanobacteria showed a weak response to U additions relative to the control group in this study, and a Dunn's *post-hoc* test showed that they were not significant in their response compared to all phytoplankton groups, except haptophytes (WW). Therefore, these results did not support the hypothesis that urea would preferentially stimulate cyanobacteria relative to other phytoplankton groups. Rather urea stimulated chlorophytes, diatoms (WW), and euglenophytes the most. A laboratory study shows contrasting results to the current study

that indicate a stronger response both in terms of highest nutrient drawdown and consumption rate from cyanobacteria under urea conditions compared to chlorophytes (Erratt et al., 2019). Conversely, natural cyanobacteria in Lake Erie had the highest growth rates in response to NH_4^+ and an intermediate response to urea (Chaffin and Bridgeman, 2014). Siegel et al., (2011) found that cyanobacteria produced significantly higher biomass in response to both sources of N, $\text{NH}_4^+ + \text{U}$ (K67), as well as individually, U (K61) and NH_4^+ (K67), in two SDPs on Kiawah Island. Also on Kiawah Island, during summer 2012, diatoms exhibited the highest growth rates under urea additions in pond K075 relative to cyanobacteria, while in fall 2011, cyanobacteria had slightly higher growth rates (Reed et al., 2016).

Urea + Phosphorus + Silica (UPSi)

In the presence of excess P and Si, diatoms showed a stronger response to urea, along with no significant difference in nutrient response between U and UPSi. This indicates that silica likely was not a limiting nutrient for this algal group in this SDP. Therefore, Si did not preferentially stimulate diatoms as anticipated. The only group that showed a significant difference between U and UPSi treatments were dinoflagellates with a stronger response to U, possibly indicating that P or Si may have inhibited their growth response.

4.3 PAM MEASUREMENTS

Photosynthetic Quantum Efficiency (F_v/F_m)

As expected, the U and UPSi treatments showed a significant increase in F_v/F_m relative to the Ctrl group, and the T_0 group. This small increase (~ 0.10) could be due to issues with the incubation experiments. For these treatments, the average F_v/F_m ratio was

the highest, and closest to the optimal literature value, 0.83, indicating that the nutrient amended cells were more efficient relative to the control and time initial treatments (Björkman and Demmig, 1987; Johnson et al., 1993). One study done on Neuse River Estuary did not find a significant difference between the control and other nutrient treatments in F_v/F_m response, and ratios were lower than in this study across all treatments (Bergmann et al., 2002). In nutrient amended conditions, F_v/F_m responses on phytoplankton in Lake Erie and Lake Ontario also increased relative to the control group and nutrient replete condition, respectively (Rattan et al., 2014; Harrison and Smith, 2013). Nonetheless, Harrison and Smith (2013) did not observe a significant difference in measurement. Similar F_v/F_m ratios for the nutrient amended treatments were observed for these studies that ranged from ~0.6-0.7 (Harrison and Smith, 2013).

Initial slope (α)

No significant difference was observed for U and UPSi relative to the Ctrl group, however there was a significant increase in α between U and T₀. There was also a significant difference between T₀ and Ctrl. This indicates that through experimentation and thus, relative to the T₀ treatment, the phytoplankton were acclimated to lower irradiances with a higher average of α for U. Similarly, it was observed in Lake Erie that phytoplankton exhibited an increase in α compared to nutrient limited treatments, with the highest α seen in the N-amended treatment (Rattan et al., 2014). One study however, did not find a significant increase in α between treatments with added N and P (Harrison and Smith, 2013).

Relative Maximum Electron Transport Rate ($rETR_m$)

Similarly to α , there was a significant difference between U- T_0 , and so the U and UPSi treatments did not significantly change $rETR_m$ values relative to the Ctrl group as expected. This might imply that P or Si had an inhibitory effect on $rETR_m$. Rattan et al., (2014) found the same increasing trend as α with the N-amended treatment showing the highest $rETR_m$ value, along with a significant difference relative to T_0 , but not the control group. $rETR_m$ was also shown to increase in N-amended treatment relative to an unamended treatment (Harrison and Smith, 2013).

Minimum Saturation Irradiance (E_k)

No significant differences were observed between treatments for E_k , possibly indicating that $rETR_m$ was achieved at around the same time. E_k values for a freshwater phytoplankton community in Lake Ontario exhibited a significant increase relative to unamended treatments (Harrison and Smith, 2013). Despite these results herein, E_k fluctuates continuously and rarely is an instantaneous measurement, and so should be analyzed with caution (Sakshaug et al., 1997).

4.4 CYANOBACTERIA CONTRIBUTION

Cyanobacteria may not have bloomed in this pond because certain conditions were not optimal, such as temperature and nutrients. Research has shown that the optimal temperature range for cyanobacterial growth is 20-25°C or >30°C (Coles and Jones, 2000; Domis et al., 2007). The average temperature in this pond was 24.5°C \pm 2.31°C. Although this is within the range of reported values, the hydrolysis of urea could have occurred before the cyanobacteria were able to utilize it and respond accordingly. It is

also possible that the cyanobacteria were outcompeted by the most dominant groups, diatoms and chlorophytes.

4.5 STUDY LIMITATIONS

Experimental error in this study could have come from the pump under sampling leading to low phytoplankton densities. In addition, the incubation bottles may not have received an adequate amount of light throughout the incubation period due to the control of the use of one to two pieces of fiber density screens based on the weather forecast. This could alter the phytoplankton community because they were only receiving 34% (2 screens) to 64% (1 screen) of natural irradiance. Another limitation to this study could be that when filtering, all phytoplankton may not have been properly accounted for when rinsing the funnels, especially when rinsing the $<20\mu\text{m}$ funnels.

4.6 FUTURE STUDIES

Future research should focus on assessing urea concentrations of this pond prior to experimentation, in order to ascertain absolute nutrient concentrations. It also would be interesting to consider a fifth treatment to look at the effects of U+P on its own to further discern nutrient limitations in this pond. To further expand on this project, it would be of interest to incorporate a temperature regime to examine the synergistic effects of temperature and nutrients on phytoplankton dynamics in this pond.

4.7 CONCLUSION

Rapid change in agriculture practices has led to the rise of urea-based fertilizers, with compounding effects on freshwater and receiving water systems, as a result of eutrophication (Glibert et al., 2006). Despite the fact, it was evident that eutrophication likely did not occur in this pond and more specifically, cyanobacteria did not respond as

expected to urea additions. Cyanobacterial blooms are not known to occur in this pond, and it is likely that the urea was already hydrolyzed into ammonium, thus leading to a weak response in U additions. In addition, the water temperature may not have been warm enough to stimulate cyanobacterial growth. It was also evident that urea additions did not necessarily increase the photophysiology of this phytoplankton community significantly, except in F_v/F_m . This could be because the incubation bottles were not subjected to enough irradiance through the incubation period. Regardless, this study provides valuable insights into the photophysiology of phytoplankton communities in SDPs in the NIWB region. This study suggests that more research needs to be done on natural, freshwater phytoplankton communities on a more frequent scale that assess nutrient addition effects and photophysiology, especially in the NIWB region.

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