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# Constraints on Phytoplankton Abundance and Cyanotoxin Production in the Tidal Freshwater James River

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

28-Apr-2020

by  
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# Abstract

## CONSTRAINTS ON PHYTOPLANKTON ABUNDANCE AND CYANOTOXIN PRODUCTION IN THE TIDAL FRESHWATER JAMES RIVER

By: Brittany A. Moretz

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

Virginia Commonwealth University, 2020.

Advisor: Dr. Paul A. Bukaveckas, Professor, VCU Department of Biology and Center for Environmental Studies

The tidal freshwater James River exceeds chlorophyll-a (CHL-a) water quality standards and the cyanotoxin microcystin is commonly present. Efforts to control harmful algal blooms in the James have brought about significant reductions in nitrogen and phosphorus inputs, but chlorophyll levels have not changed. In order to better understand the factors constraining harmful algal blooms in this system, we performed three factorial mesocosm experiments that tested how light (ambient, enhanced), mixing (weak mixing, well-mixed), and the availability of dissolved inorganic nitrogen (pre/post  $\text{NH}_4^+$  reductions) influence the abundance of phytoplankton (CHL<sub>a</sub>, particulate organic carbon [POC]) and cyanotoxin production (microcystin, anatoxin) in this system. Enhanced light treatments increased CHL<sub>a</sub> and POC in well-mixed mesocosms across all three experiments, while  $\text{NH}_4^+$  treatments had little effect on CHL<sub>a</sub> and POC. Light also had the greatest effect on toxin production, where microcystin production was favored under ambient light conditions and anatoxin production was favored under enhanced light conditions. CHL<sub>a</sub> and POC results suggest that light availability constrains phytoplankton production in the James and recent reductions in  $\text{NH}_4^+$  would not alone be expected to reduce CHL<sub>a</sub> concentrations in this system. Peak toxin levels observed in mesocosms were relatively low compared to other systems, suggesting that cyanobacteria blooms are unlikely to pose a significant threat in the James.

## Introduction

It is well-established that the frequency and severity of harmful algal blooms have been increasing worldwide, and many climate models predict a continued increase in bloom frequency and toxin production (Paerl & Calandrino 2011, Paerl & Otten 2013). Cyanobacteria dominate phytoplankton assemblages in many freshwater systems, and blooms have resulted in billions of dollars in annual economic losses from reductions in water quality and the degradation of aquatic ecosystems (De Figueiredo et al. 2004, Anderson et al. 2008, Dodds et al. 2009, Smith & Schindler 2009, Neilan et al. 2013). Several forms of cyanobacteria are capable of producing toxins that have deleterious effects on human health and aquatic resources (Paerl & Otten 2013). The hepatotoxin microcystin is a common cyanotoxin in freshwater systems for which the World Health Organization has established guidelines for consumption and exposure (WHO 2003, Wood et al. 2014). Understanding the factors constraining cyanobacteria abundance and the conditions favoring microcystin production has become a primary focus for water quality managers.

The tidal freshwater segment of the James River has been identified as the location of highest chlorophyll-a in the Chesapeake Bay and exceeds James River CHL-a water quality standards (Wood et al. 2014, Bukaveckas et al. 2011, Bukaveckas & Isenberg 2013). Contributions of cyanobacteria to total algal biomass are low (less than 10%) in comparison to some freshwater systems, particularly lakes, where cyanobacteria comprise upwards of 80% of biomass (Oliver 1994, Downing et al. 2001, Smith 2003, De Figueiredo et al. 2004, Marshall et al. 2009, Paerl & Otten 2013, Wood et al. 2014). Despite this, toxic cyanobacteria are commonly present, and microcystin is consistently found in water and tissues of higher trophic level consumers (Wood et al. 2014). New chlorophyll-based water quality standards for the James were designed in part to mitigate impacts from harmful algal blooms. In order to anticipate how the associated nutrient-based management plan will affect the abundance of harmful cyanobacteria, it is essential to understand which factors, or combination of factors, are constraining cyanobacteria abundance in this system.

Lentic (lake) systems have been the focus of a majority of studies on harmful cyanobacteria. In these systems, weak mixing and high residence time favor the formation of a prominent blue-green scum at the surface. Less attention has been paid to the occurrence of cyanobacteria blooms in rivers and estuaries, as light limitation in these turbid systems is thought to constrain bloom formation. However, blooms occur frequently in flowing waters (Soballe & Kimmel 1987, Reynolds & Descy 1996, Bukaveckas et al. 2011). An important but under-studied difference between lentic and lotic systems is the intensity of turbulent mixing. Mixing maintains particulate matter in suspension resulting in low light conditions. Diatoms tend to out compete cyanobacteria under these conditions due to their lower light requirements (Reynolds & Descy 1996, Huisman et al. 2004, Wells et al. 2016). Weak mixing favors cyanobacteria, which are able to regulate buoyancy with gas vesicles, thereby maximizing photosynthetic potential (Reynolds 1994, Wells et al. 2016). The presence of strong tidal and fluvial mixing forces in the James may account for the dominance of diatoms over cyanobacteria.

The quantity and form of anthropogenic nutrient inputs influence phytoplankton abundance, species composition, and toxin production (Anderson et al. 2008). Recently, studies have shown that targeting reductions in specific forms of nitrogen may be useful in reducing cyanobacteria abundance (Harris et al. 2016, Gasparini et al. 2017, Chaffin et al. 2018). Cyanobacteria are better competitors for chemically reduced forms of nitrogen, and high loads of ammonium ( $\text{NH}_4^+$ ) have been linked to the formation of harmful cyanobacteria blooms (Gasparini et al. 2017, Yang et al. 2017). In the James, point sources are a key factor influencing nutrient availability, especially during low discharge summer months (Wood and Bukaveckas 2014). Efforts to control algal blooms have brought about significant reductions in nitrogen and phosphorus inputs from point sources to the James. Corresponding reductions in estuarine nutrient concentrations have been observed, but chlorophyll levels have not changed (Wood & Bukaveckas 2014). Point sources account for nearly all ammonium inputs into the James, 76% of which are discharged in proximity to the region where peak chlorophyll and microcystin are observed (Bukaveckas and Isenberg 2013). Recent upgrades in wastewater treatment at this location have further reduced ammonium inputs by 56% at this location since late summer 2017. During the period when cyanobacteria blooms are most commonly observed (July-October), dissolved inorganic nitrogen (DIN) concentrations are now typically less than half of levels observed in the 1990's. It is unknown whether present levels are sufficiently low to limit cyanobacteria growth, or whether further reductions may be beneficial for controlling cyanobacteria blooms.

Mesocosm experiments were conducted in order to better understand the factors which constrain harmful algal blooms in the James Estuary. The use of mesocosms is advantageous in that each variable can be manipulated independently and interactions between parameters can be identified. Specifically, this work aims to improve our understanding of how light, mixing, and the availability of DIN influence the abundance of phytoplankton and cyanotoxin production in this system. Phytoplankton biomass was tracked using CHL<sub>a</sub> and POC as indicators of algal abundance. CHL<sub>a</sub> is commonly used as an indicator of algal abundance and is also the basis for water quality criteria. However, the amount of CHL<sub>a</sub> per unit biomass is known to vary in part on light conditions. POC is an alternative measure of algal abundance, however its concentration also reflects contributions from non-algal materials (e.g. terrestrial organic matter – allochthonous organic matter). Experiments were conducted in July, August, and September as these are the months where cyanobacteria abundance and toxin levels are typically highest (Wood et al. 2014). While microcystin is routinely monitored in this system, this was our first summer testing for anatoxin. Microcystin was tracked in all three months, while anatoxin was tracked in August, where we tend to observe peak microcystin concentrations.

A full factorial design was used to test the effects of three variables: solar irradiation (ambient, enhanced), N availability (pre/post  $\text{NH}_4^+$  reductions), and mixing (weak mixing, well-mixed). If light availability is the principal constraint on phytoplankton in the James, then we would predict that the enhanced light treatment would result in higher CHL<sub>a</sub> and POC. We further predict that the combination of enhanced light with stagnant conditions (weak mixing treatment) would specifically favor cyanobacteria that can regulate buoyancy, thereby resulting in higher microcystin and anatoxin production. If nitrogen availability constrains phytoplankton

production in the James, then we would predict that mesocosms with higher N levels (at pre- $\text{NH}_4^+$  reduction levels) would result in higher CHLa and POC than tanks receiving smaller dosages of  $\text{NH}_4^+$ . We further expect that reducing  $\text{NH}_4^+$  would have a larger effect on cyanobacteria, resulting in lower microcystin and anatoxin in the low  $\text{NH}_4^+$  treatments relative to ambient. There is also potential for interactive effects whereby enhanced light and ambient  $\text{NH}_4^+$  result in greater CHLa and POC than observed in response to either variable alone, and where enhanced light, ambient  $\text{NH}_4^+$ , and stagnant water result in greater microcystin and anatoxin production than any variable alone.

## Methods

### *Study Site*

The James River is the third largest tributary of the Chesapeake Bay. The tidal freshwater segment extends about 115 km downstream from the fall line at Richmond, VA to the confluence with the Chickahominy River. Mesocosm experiments were conducted in July, August, and September 2018 at the Virginia Commonwealth University Rice Rivers Center, which is located in the lower portion of the tidal freshwater segment near Hopewell, VA at the location of the CHLa maximum (Avg.  $53.3 \text{ ug L}^{-1}$ ; Bukaveckas et al 2011). The facility consists of 24 2000-liter fiberglass tanks. The outdoor tanks are filled with water drawn directly from the James River containing a natural community of bacteria, phytoplankton and zooplankton.

### *Experimental Design*

Each experiment ran for 10 days, allowing time for algal growth and approximating the average freshwater replacement time (16 days) during this portion of the year (Wood & Bukaveckas 2014). Each treatment combination was replicated in three tanks and distributed in a randomized block design. Tanks used for ambient light treatments were fitted with covers that were designed to create light conditions representative of those in the James taking into account incident solar radiation, water clarity, and average depth (average underwater irradiance =  $4.8 \text{ E m}^{-2} \text{ d}^{-1}$ ; Wood & Bukaveckas 2014). Enhanced light treatments had the covers removed, resulting in 4-fold higher underwater irradiance relative to ambient ( $18 \text{ E m}^{-2} \text{ d}^{-1}$ ). Ambient nitrogen treatments were representative of the loading rate to this segment of the James prior to recent reductions in  $\text{NH}_4^+$  inputs ( $0.12 \text{ mg DIN L}^{-1} \text{ d}^{-1}$ ) with DIN added at a ratio of 65%  $\text{NH}_4^+$ , 35%  $\text{NO}_3$  (Bukaveckas and Isenberg 2013). Recent nutrient reductions were simulated by omitting the  $\text{NH}_4^+$  component of the N additions (DIN inputs =  $0.042 \text{ mg NO}_3 \text{ L}^{-1} \text{ d}^{-1}$ ). Dissolved inorganic phosphorus (DIP) was added at a rate consistent with the average loading rate into this segment of the James across all treatment combinations ( $0.03 \text{ mg DIP L}^{-1} \text{ d}^{-1}$ ).

Natural turbulence acts over a broad range of spatial and temporal scales, and it is challenging to generate suitable mixing conditions within experimental ecosystems (Sanford 1997). Particle mixing is a factor impacted by turbulence at all scales and has been shown to significantly impact structure and function within experimental ecosystems (Sanford 1997, Porter et al. 2010). Previous work at this facility has used aquarium pumps to simulate estuarine particle mixing, but this technique was unable to generate water column turbulence that is representative of a well-mixed system. The weak mixing treatment replicated this design, with each mesocosm



containing 4 submersible aquarium pumps (capacity per pump 1120 L h<sup>-1</sup>; Trache 2015). For the well-mixed treatment, we developed a mixing design whereby twelve mesocosms were fitted with variable pitch propellers, which previous work has shown can adequately simulate natural water column turbulence (Sanford 1997, Porter et al. 2010). We conducted pilot experiments to evaluate how effective the propellers were at maintaining the bulk of particulate matter in suspension and how long it takes for the mesocosms to become well-mixed over a range of RPM settings. Mesocosms were spiked with a sodium chloride solution, and a handheld YSI was used to track specific conductivity. Preliminary data showed that at a propeller speed of 30 RPM, conductivity measurements plateaued after ~90 sec, which provides a useful metric for assessing the mixing time (Porter et al. 2010).

### *Sample Collection & Analysis*

Data were collected to characterize physical and chemical conditions within the mesocosms and to assess treatment effects on the phytoplankton community. On day 0 and every 2 days following, a YSI 6600 multiparameter sonde was used to record spot measurements of water quality parameters (turbidity, dissolved oxygen, temperature, pH) and a Li-Cor photometer was used to measure light attenuation around mid-day. Water samples (2 L) were collected from each mesocosm on day 0 and every 2 days following and filtered for CHL-a and total suspended solids (TSS). On day 0 and the final day of the experiments, additional water samples were collected for microcystin, anatoxin (August), and nutrient (TN, TP, NH<sub>3</sub>, NO<sub>x</sub>, PO<sub>4</sub>) analyses. Samples collected for dissolved nutrients were syringe filtered in the field through Whatman glass filters (934-AH) and stored in 50 mL centrifuge tubes. Grazing by zooplankton has been shown to play a role in controlling phytoplankton abundance and toxin production in past mesocosm experiments, therefore zooplankton abundance was also monitored (Trache 2015). A 2 L sample was taken for zooplankton counts on day 0 and day 10, filtered through a 23 µm mesh sieve, and preserved in alcohol until counted by microscopy.

Samples for CHLa were filtered through Whatman glass filters (0.5 µm), extracted for 18 hours in buffered acetone, and analyzed by fluorometer (Arar and Collins 1997). TSS was determined gravimetrically using pre-weighed, pre-combusted filters (0.5 µm), a sub-sample of which was analyzed for particulate organic carbon on a Perkin-Elmer CHN analyzer. Nutrient concentrations were determined using a Skalar San<sup>++</sup> Automated Wet Chemistry Analyzer following standard methods (APHA 1992). Abraxis ELISA kits were used to measure microcystin (July, August, September) and anatoxin (August).

### *Statistics*

A three-way analysis of variance (ANOVA) was performed in order to test for significant influence of each treatment and their interaction terms. Statistical tests were performed on day 10 values for CHLa, POC, TSS, microcystin, and zooplankton. A Tukey HSD post-hoc test was performed where significant interactions occurred. All statistical analyses were performed in R.

## Results

### *Mesocosm Conditions*

Similar water temperatures were observed in the mesocosms and the estuary (Figure 1). Median water temperatures in the mesocosms during July, August, and September experiments were  $27.9 \pm 0.26$  °C,  $28.2 \pm 0.26$  °C, and  $25.2 \pm 0.25$  °C, (respectfully). Corresponding values for the estuary were ( $27.2 \pm 0.08$  °C,  $29.1 \pm 0.06$  °C,  $23.3 \pm 0.10$  °C, (respectfully). Median estuarine pH for the three experiments ( $9.0 \pm 0.03$ ,  $8.7 \pm 0.04$ ,  $7.1 \pm 0.01$ ) was lower than mesocosm pH ( $9.4 \pm 0.05$ ,  $9.0 \pm 0.04$ ,  $8.7 \pm 0.05$ ). During each 10-day experiment, pH was stable or declining in the estuary, whereas pH in the mesocosms tended to increase. Median dissolved oxygen in the July and August experiments was generally similar between the mesocosms ( $9.7 \pm 0.21$  mg L<sup>-1</sup>,  $8.8 \pm 0.09$  mg L<sup>-1</sup>, respectfully) and the estuary ( $10.1 \pm 0.08$  mg L<sup>-1</sup>,  $8.9 \pm 0.11$  mg L<sup>-1</sup>, respectfully). In the September experiment, average dissolved oxygen was greater in mesocosms ( $8.9 \pm 0.05$  mg L<sup>-1</sup>) than what was observed in the estuary ( $6.8 \pm 0.07$  mg L<sup>-1</sup>).

Initial TSS varied among months, increasing from July to August and September ( $20.75 \pm 1.60$  mg L<sup>-1</sup>,  $26.53 \pm 2.06$  mg L<sup>-1</sup>,  $31.42 \pm 3.17$  mg L<sup>-1</sup>, respectively; Figure 2). The propeller mixing system (well-mixed treatment) was more effective in maintaining particulate matter in suspension through the end of the experiments (median TSS day 6-10 =  $15.20 \pm 1.33$  mg L<sup>-1</sup>,  $15.88 \pm 1.00$  mg L<sup>-1</sup>,  $22.96 \pm 0.93$  mg L<sup>-1</sup>, respectively). The weakly mixed tanks resulted in TSS concentrations that were less than half that of the well-mixed tanks in the second half of the experiments ( $8.81 \pm 0.91$  mg L<sup>-1</sup>,  $2.18 \pm 0.46$  mg L<sup>-1</sup>,  $7.13 \pm 0.75$  mg L<sup>-1</sup>, respectively).

Total nutrient concentrations (TN, TP) generally increased during the 10-day experiment, with highest increases observed in the high light tanks (Figure 3). Dissolved nutrient concentrations increased for tanks receiving nutrient amendments, but exhibited little change in tanks without nutrient additions.

### *Chlorophyll-a*

Initial CHLa concentrations in the September experiment ( $14.48 \pm 2.94$  µg L<sup>-1</sup>) were less than half that observed at the start of the July and August experiments ( $28.39 \pm 1.35$  µg L<sup>-1</sup> and  $39.49 \pm 2.66$  µg L<sup>-1</sup>, respectively; Figure 4). CHLa generally increased over the 10 days for each of the three experiments, with the greatest final concentrations observed in July ( $73.61 \pm 6.76$  µg L<sup>-1</sup>), followed by August ( $51.93 \pm 7.77$  µg L<sup>-1</sup>) and September ( $30.99 \pm 3.37$  µg L<sup>-1</sup>). It is important to note that in July and August, CHLa peaked on day 10 of the experiment. In the September experiment, a peak was observed on day 4, followed by a decline and a second smaller peak on day 10.

There were no significant main effects on CHLa in the July experiment (Table 1), though there was a significant interactive effect observed between Mixing and Light factors. When the system was well-mixed, enhanced light treatments resulted in significantly greater CHLa than ambient light treatments ( $106.24 \pm 12.55$  µg L<sup>-1</sup> and  $63.49 \pm 9.33$  µg L<sup>-1</sup>, respectively; Figure 5). There was no significant difference among enhanced light and ambient light treatment groups in the weakly mixed tanks ( $64.42 \pm 8.55$  µg L<sup>-1</sup> and  $74.06 \pm 18.00$  µg L<sup>-1</sup>, respectively). A significant interactive effect was also observed between Mixing and Ammonia factors. When the system

was weakly mixed, tanks with no  $\text{NH}_4^+$  additions resulted in significantly less CHLa than tanks that received  $\text{NH}_4^+$  additions ( $40.34 \pm 7.04 \mu\text{g L}^{-1}$ ,  $99.95 \pm 9.69 \mu\text{g L}^{-1}$ , respectively). There was no significant difference among CHLa in well-mixed tanks that received  $\text{NH}_4^+$  additions and those that did not. In the August experiment, only the main effects of Mixing and Light resulted in significant differences in CHLa on day 10 of the experiment. Tanks that were well-mixed and those receiving enhanced light had greater CHLa than the weakly-mixed and ambient light treatments, respectively. In the September experiment, there was a significant 3-way interaction between mixing, light, and  $\text{NH}_4^+$  factors. The combination of enhanced light and  $\text{NH}_4^+$  additions in the well-mixed mesocosms resulted in significantly greater CHLa than all other treatment combinations except for the low-mix, ambient light, with  $\text{NH}_4^+$  additions. All other treatment combinations with enhanced light resulted in lower CHLa concentrations than those receiving ambient light, and the combination of the low-mix treatment with enhanced light resulted in the lowest CHLa concentrations overall.

#### *Particulate Organic Carbon*

POC concentrations generally increased during the experiments, particularly in the High Light tanks (Figure 5). In the July experiment, there was a significant interactive effect observed between Mixing and  $\text{NH}_4^+$  factors. The combination of weak mixing and no  $\text{NH}_4^+$  additions resulted in significantly lower POC than any other treatment combination. There was also a significant difference observed among light treatments, with significantly greater POC observed in mesocosms receiving enhanced light than in those receiving ambient light treatments. In August, there was a significant interactive effect observed between Mixing and Light factors, whereby the well-mixed with enhanced light treatment combination resulted in significantly greater POC than any other treatment combination, and the weakly-mixed tanks with ambient light resulted in significantly lower POC than any other treatment combination. There was also an interactive effect observed between mixing and  $\text{NH}_4^+$ . The well-mixed tanks with  $\text{NH}_4^+$  additions resulted in significantly greater POC than any other treatment combination. The well-mixed tanks with no  $\text{NH}_4^+$  additions resulted in significantly greater POC than either weakly-mixed treatment combination. In September, the well-mixed with enhanced light treatment combination had significantly greater POC than any other treatment combination. Additionally, well mixed ambient light had significantly greater POC than either of the low mix treatment combos. POC measurements generally yielded a greater number of significant treatment effects than for CHLa, except for the September experiment (Table 1). Across all three experiments, statistical models accounted for a greater proportion of the variation in POC (adj.  $R^2=0.74$ ,  $0.95$ ,  $0.94$ , respectively) than for CHLa (adj.  $R^2=0.51$ ,  $0.61$ ,  $0.82$ , respectively).

POC and CHLa tracked each other most closely in the July and August experiments ( $R^2=0.51$ ,  $0.61$ , respectively; Figure 6). In the August experiment, CHLa and POC most closely tracked each other when CHLa and POC values were low (CHLa  $< 60 \mu\text{g L}^{-1}$ , POC  $< 6 \text{ mg L}^{-1}$ ). All treatment combinations fell within this range except for those that were well-mixed with enhanced light, which had the greatest observed POC and CHLa concentrations. At elevated POC concentrations, corresponding CHLa observations were more variable. In the September experiment, there was a notable difference in POC concentration among weakly mixed tanks receiving ambient light (POC  $< 3 \text{ mg L}^{-1}$ ), and well-mixed tanks receiving enhanced light (POC

> 3 mg L<sup>-1</sup>), while the range of CHLa concentrations observed was similar among treatments. TN tracks POC in July and August mesocosm experiments (Figure 6). Elevated concentrations of nitrate were associated with low CHLa and POC across all three experiments, and lower concentrations were associated with higher CHLa and POC (Figure 3; Figure 5).

### *Microcystin*

Initial microcystin concentrations were greatest in the July experiment, and less than 0.1 µg L<sup>-1</sup> in both August and September experiments (Figure 5). In both July and September experiments, day 10 microcystin was greater than day 0 microcystin across all treatment combinations. In the July experiment, light was the only factor that had a significant impact on microcystin. During the July experiment, the largest increases in CHLa and POC were observed in the well-mixed tanks with enhanced light, but these showed relatively little change in microcystin. In contrast, the largest increases in microcystin were observed among the well-mixed tanks with ambient light, which exhibited smaller increases in POC and CHLa. In the August experiment, there was an interactive effect observed between NH<sub>4</sub><sup>+</sup> and Light treatments. Tanks with NH<sub>4</sub><sup>+</sup> additions and ambient light resulted in more than double the amount of microcystin than any other treatment combination. For September, the largest increases in microcystin were observed in the tanks with greatest increase in POC and CHLa (+Mix+Light+ NH<sub>4</sub><sup>+</sup>). High mixing and high light tanks that did not receive NH<sub>4</sub><sup>+</sup> addition exhibited a similar increase in CHLa and POC, but not microcystin. In the September experiment, a 3-way interactive effect was observed whereby microcystin in tanks receiving the well-mixed, enhanced light, +NH<sub>4</sub><sup>+</sup> treatment combination was significantly greater than in any other treatment combination.

### *Anatoxin*

Initial anatoxin concentration was double that of microcystin (Figure 5). Day 10 anatoxin concentrations were 2-3 times greater in tanks that were well-mixed with enhanced light than any other treatment group. Anatoxin and microcystin concentrations were approximately equivalent in well-mixed tanks with ambient light. Anatoxin concentrations were greater than microcystin concentrations in all treatment groups with enhanced light. The greatest anatoxin concentrations occurred in the well mixed, enhanced light tanks with no external NH<sub>4</sub><sup>+</sup> additions, while the greatest microcystin concentrations were observed in the low mix, low light tanks with external NH<sub>4</sub><sup>+</sup> additions. Anatoxin concentrations were greater than microcystin concentrations in all treatment groups with enhanced light.

### *Zooplankton*

Mesocosms which exhibited the largest increase in CHLa and POC generally exhibited the highest zooplankton densities at the end of the experiment (Figure 7). In the July experiment, day 10 rotifer concentrations were less than initial densities across all treatment combinations. There was a Mixing x Light interactive effect observed on both rotifers and mesozooplankton, whereby the combination of well-mixed and enhanced light treatments resulted in significantly greater rotifers and mesozooplankton than any other treatment combination. The factors that had a significant effect on mesozooplankton concentrations were consistent with those that significantly affected CHLa concentrations. On day 10 of the August experiment, mesozooplankton concentrations were greater than initial across all treatment combinations,

while rotifers concentrations were only greater than initial tanks receiving the well-mixed treatment, with the exception of the weakly mixed, enhanced light,  $\text{NH}_4^+$  addition combination. There was no significant difference in rotifers among treatment combinations, while the well-mixed tanks had significantly greater mesozooplankton than the low-mix tanks. In September, day 10 rotifer and mesozooplankton concentrations were greater on day 10 than on day 0 across all treatment combinations. There was no significant difference in mesozooplankton among treatment combinations, while tanks with  $\text{NH}_4^+$  additions resulted in significantly greater rotifers than tanks with no  $\text{NH}_4^+$  additions.

## Discussion

In order to better understand the factors constraining harmful algal blooms in the James, we tested the effects of mixing, light, and DIN availability on phytoplankton abundance and toxin production in three mesocosm experiments. Overall, CHLa and POC results support the hypothesis that light is the principal constraint on phytoplankton production in the James. A clear treatment effect was observed in the well-mixed mesocosms whereby enhanced light increased both CHLa and POC across all three experiments. We also found that despite reducing total DIN inputs by 65%, the removal of external  $\text{NH}_4^+$  additions had little effect on CHLa and POC. Overall, these results suggest that current DIN loads are sufficient to support algal growth, and that recent reductions in  $\text{NH}_4^+$  would not alone be expected to bring down CHLa concentrations in the James.

Although we expected toxin concentrations to be greatest in the weakly-mixed tanks with enhanced light, tanks that received this treatment combination observed among the lowest microcystin and anatoxin concentrations in each of our experiments (Figure 5). Light had the greatest treatment effect on toxin production, although the effects differed for microcystin vs. anatoxin. Anatoxin concentrations generally tracked phytoplankton abundance, with greatest concentrations in the well-mixed tanks with enhanced light, while microcystin concentrations were greatest in tanks with ambient light. Our results suggest that different conditions may favor the production of one toxin over the other, and although metrics of algal abundance generally lack utility in predicting microcystin concentrations in the James (Bukaveckas et al. 2018), it may be useful to explore their capacity to predict anatoxin concentrations in this system.

A key difference between the tidal James and lentic systems that maintain high cyanobacteria biomass is the intensity of turbulent mixing, which influences both light and nutrient availability. Enhanced light had the clearest impact on algal abundance across each of the experiments, but the effect was enhanced in the well-mixed tanks. Unexpectedly, the weakly mixed tanks observed among the lowest CHLa and POC across the three experiments. The phytoplankton community in the James is dominated by diatoms, which, because of their silica-based cell walls, have a higher sedimentation rate (Marshall et al. 2009). In the absence of strong mixing, it may be that a greater proportion of the phytoplankton community was lost through settling to the bottom of the mesocosms.

The utility of any experiment depends in part on the degree to which it mimics in situ conditions. During the September experiment, CHLa peaked on day 4 rather than on day 10 as in the two

preceding months (Figure 4). Since toxin response variables were measured on day 10, the September discussion surrounding toxin concentrations will be considered separately from the July and August experiments. Tanks that received the +Mix+Light+NH<sub>4</sub><sup>+</sup> treatment combination observed the greatest increase in microcystin, CHLa, and POC in the September experiment. Interestingly, in the July and August experiments the response of microcystin to the light treatment was generally opposite that of CHLa and POC (Figure 5). Elevated microcystin concentrations were clearly favored under ambient light conditions, with greatest concentrations occurring in control mesocosms. This is supported by previous work showing that microcystin-producing cyanobacteria may have an ecological advantage under light limited conditions (Chaffin et al 2018). Although NH<sub>4</sub><sup>+</sup> addition alone did not have a significant impact on microcystin production, it is important to note that well-mixed tanks with ambient light had greater microcystin concentrations in tanks receiving the NH<sub>4</sub><sup>+</sup> treatment than those that did not. This pattern was also present in the weakly mixed tanks in both July and August experiments. The relationship between microcystin-producing cyanobacteria and increased dissolved nitrogen supply has been reported in other systems (Gobler et al 2016), and it may be useful in future work to investigate a potential interactive effect present where the combination of ambient light and reductions in nutrient inputs may lower microcystin concentrations in this system.

Our results suggest that the experimental conditions within the mesocosms may have shifted the dominance of the cyanobacteria assemblage, thereby affecting which toxins are produced. The conditions favoring anatoxin production were consistent with those that favored overall phytoplankton abundance. Tanks with the lowest microcystin concentrations had the greatest anatoxin concentrations, while tanks with the greatest microcystin concentration had the lowest anatoxin concentrations. Microcystin has generally been used as an indicator of harmful algal blooms in this system, but our results suggest that it may be useful to continue to track anatoxin as the conditions that favor its production are those present in the James.

Traditionally, algal biomass reduction strategies have focused on establishing targets for nutrient load reductions, but additional abiotic and biotic factors in natural environments introduce uncertainty in our understanding of algal community dynamics (Heisler et al 2008). It was notable that removing NH<sub>4</sub><sup>+</sup> additions reduced DIN inputs by 65%, yet had little observable impact on CHLa in well-mixed tanks across all three mesocosm experiments. Additionally, our toxin results indicate that different conditions may favor the production of one cyanotoxin over another, and it may be useful to screen for multiple toxins to better assess harmful algal bloom risks. This work highlights a need for more community level multifactor experiments in order to strengthen our understanding of harmful algal dynamics and to better predict and mitigate blooms (Kudela et al. 2016).

Our mesocosm experiments were designed in part to create more favorable conditions for cyanobacteria than are generally observed in the James (e.g. high light, weak mixing). Despite this, peak toxin levels were relatively low compared to those observed in other systems. Although we should be cautious about interpreting short-term experiments, these findings suggest that despite the eutrophic nature of the James, cyanobacteria blooms are unlikely to pose

a significant threat. Tidal forcing, which generates strong mixing and high suspended sediment loads, appears to strongly favor diatom over cyanobacteria dominance.

## Tables

Table 1: Statistical analysis (ANOVA) testing the effects of mixing (M), light (L), and ammonia additions (A) and their interactions on dependent variables measured on day 10 of each experiment.

Month	Variable	M	L	A	M*L	M*A	L*A	M*L*A	Adj. R2
July	Microcystin	NS	0.001	NS	NS	NS	NS	NS	0.37
	CHLa	NS	NS	NS	0.020	0.004	NS	NS	0.51
	POC	0.03	<0.001	0.001	NS	0.006	NS	NS	0.74
	TSS	<0.001	<0.001	0.046	0.026	0.022	NS	NS	0.78
	Rotifers	<0.001	0.018	NS	0.039	NS	NS	NS	0.56
	Mesozooplankton	<0.001	<0.001	NS	0.002	0.028	NS	NS	0.75
August	Microcystin	0.043	<0.001	0.026	NS	NS	0.023	NS	0.66
	CHLa	<0.001	0.001	NS	NS	NS	NS	NS	0.61
	POC	<0.001	<0.001	NS	0.003	<0.001	NS	NS	0.95
	TSS	<0.001	<0.001	NS	NS	NS	NS	NS	0.76
	Rotifers	NS	NS	NS	NS	NS	NS	NS	0.00
	Mesozooplankton	NS	0.03	NS	NS	NS	NS	NS	0.04
September	Microcystin	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	0.005	0.89
	CHLa	0.001	<0.001	<0.001	<0.001	NS	0.008	0.014	0.82
	POC	<0.001	<0.001	0.002	<0.001	NS	NS	NS	0.94
	TSS	<0.001	0.006	NS	NS	0.041	0.037	NS	0.83
	Rotifers	NS	NS	0.047	NS	NS	NS	NS	0.25
	Mesozooplankton	NS	NS	NS	NS	NS	NS	NS	0.00



# Figures

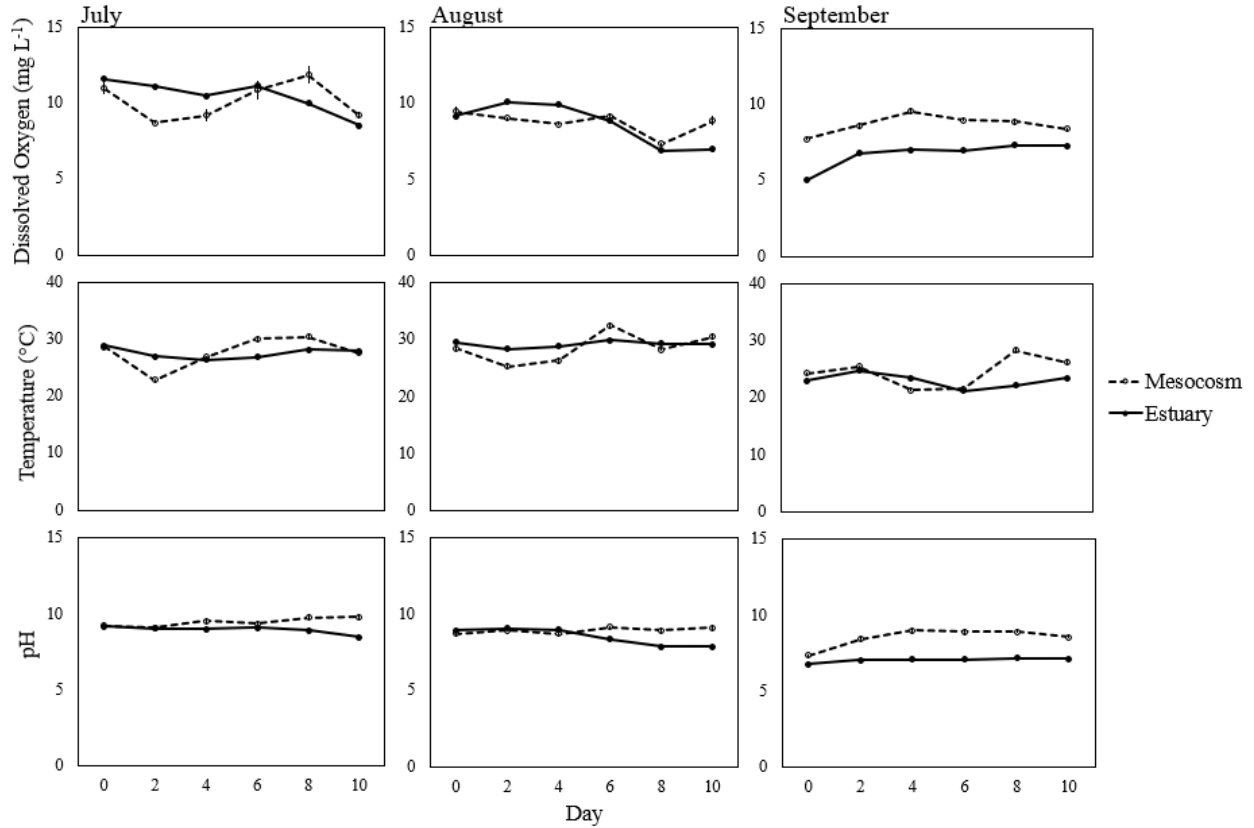


Figure 1: Mesocosm and in-situ estuarine water quality conditions during July, August, and September experiments. Mesocosm temperature, pH, and DO values represent median  $\pm$  SE of a single daily measurement collected from each tank. Estuarine values represent averages of water quality data gathered during mesocosm sample collection from a continuous monitoring station at the VCU Rice Rivers Center Pier.

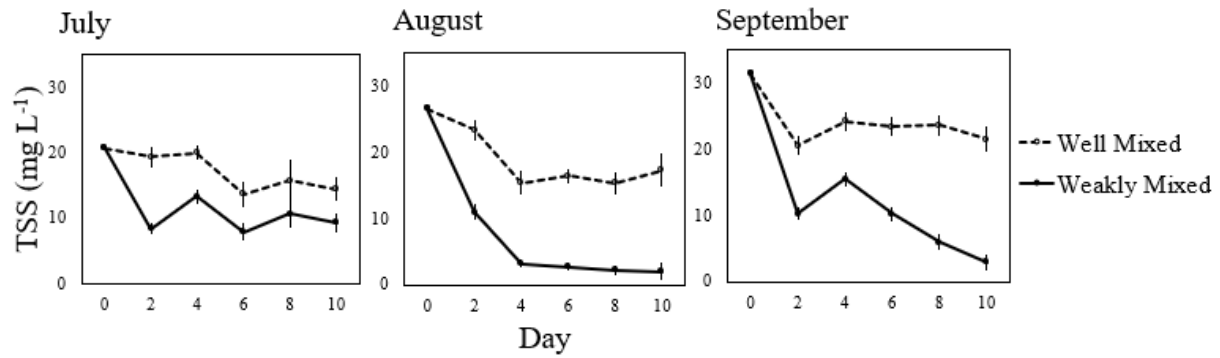


Figure 2: TSS time series of mixing treatment groups in July, August, and September mesocosm experiments. TSS values represent median  $\pm$  SE of daily measurements collected from each tank receiving weakly-mixed or well-mixed treatments.

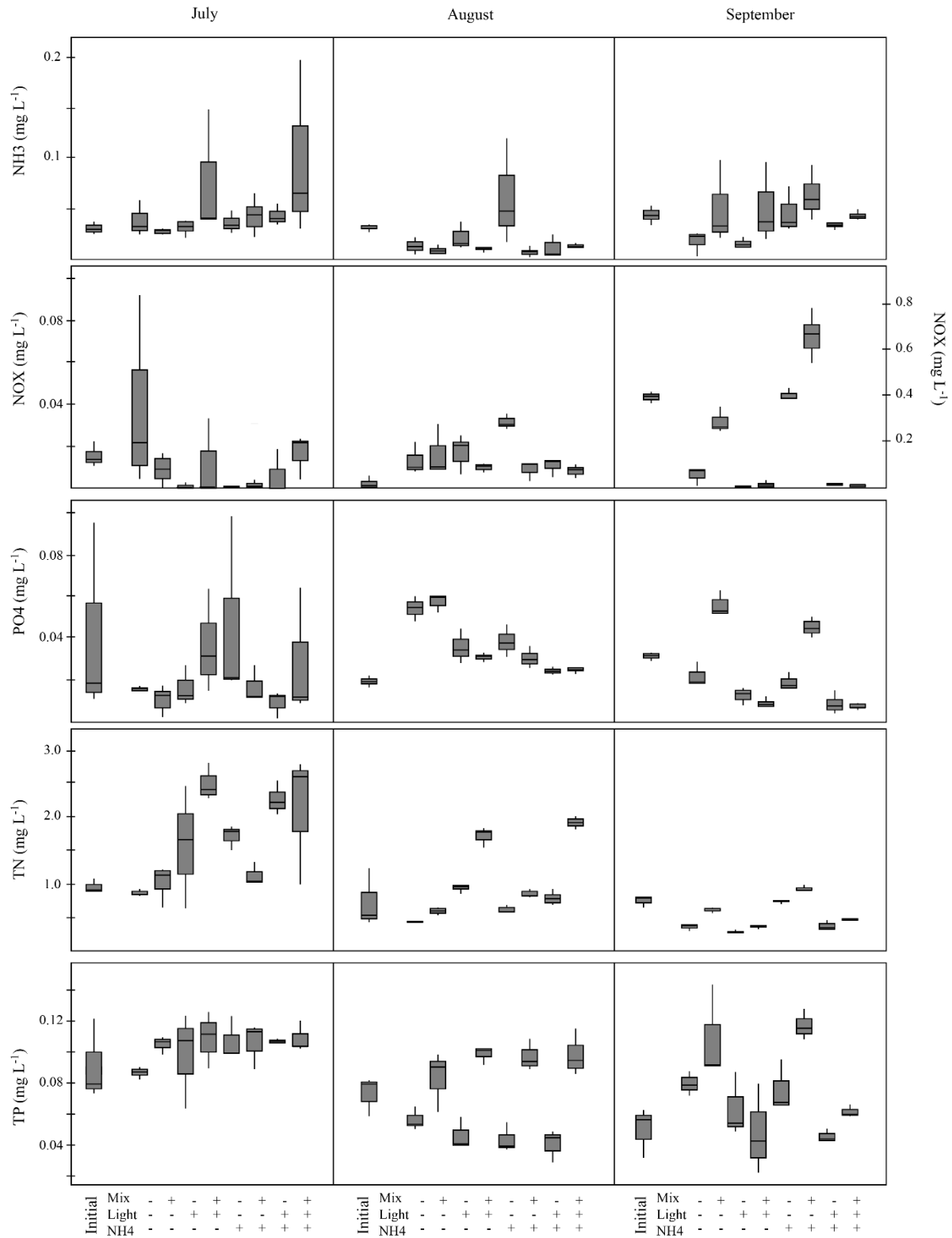


Figure 3: Initial and final concentrations of NH<sub>3</sub>, NO<sub>x</sub>, PO<sub>4</sub>, TN, and TP for July, August, and September mesocosm experiments. NO<sub>x</sub> concentrations in September were an order of magnitude greater than in the July and August and are plotted on a secondary axis.

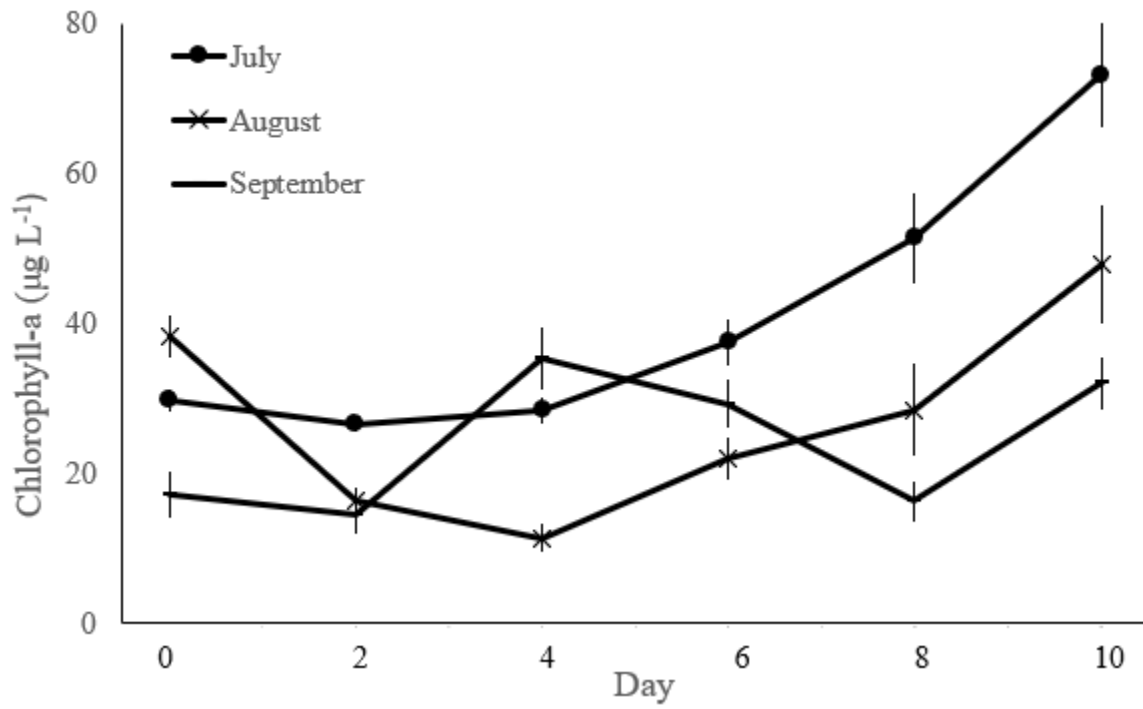


Figure 4: Median CHLa  $\pm$  SE across all mesocosms in July, August, and September mesocosm experiments.

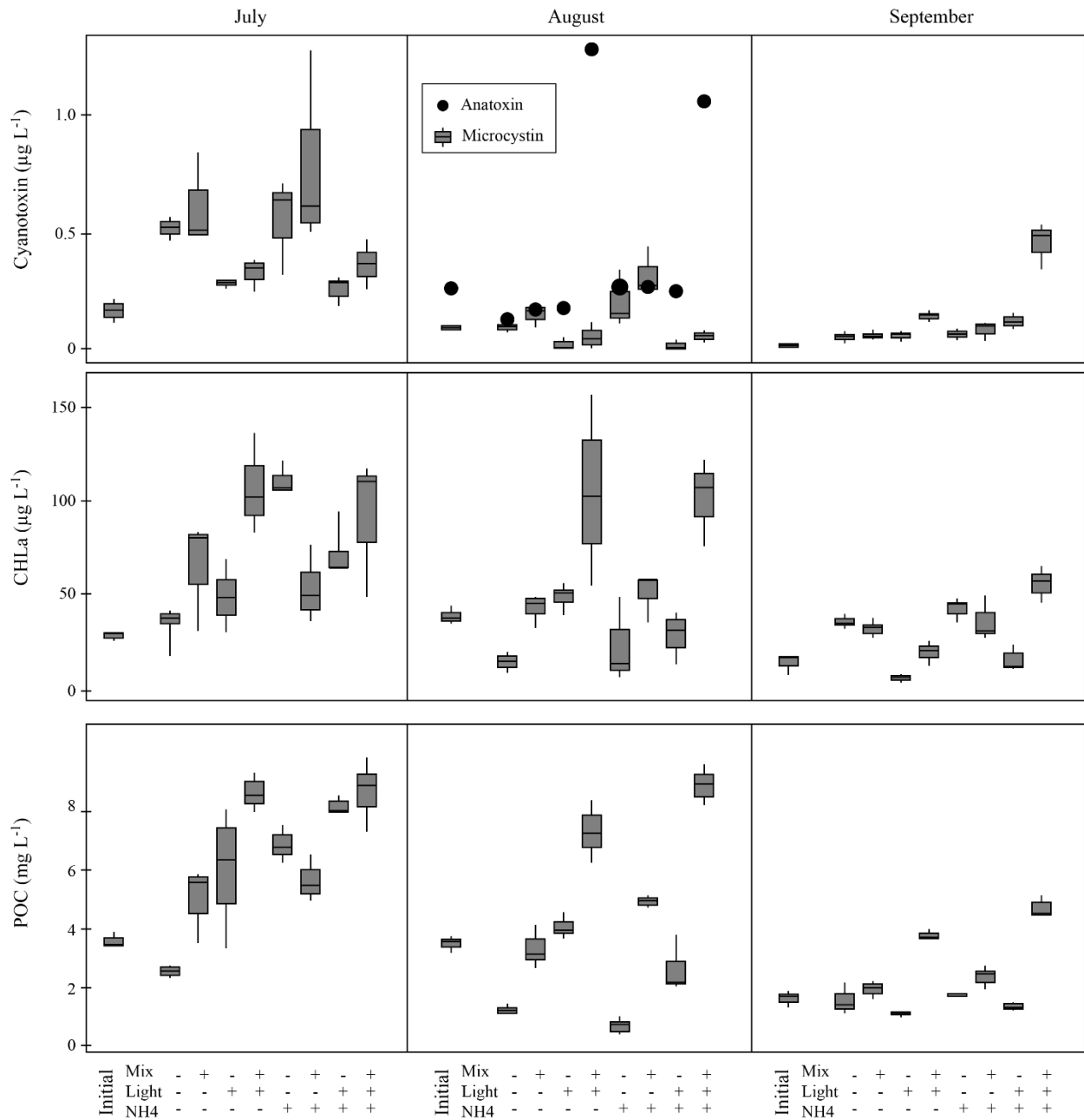


Figure 5: Median values for cyanotoxins, CHLa and POC on Day 0 (Initial) and Day 10 for July, August, and September mesocosm experiments. Median values for individual treatments are shown as ( - - - ) Low Mix, Low Light, No  $\text{NH}_4^+$  ( + - - ) Well-mixed, Low Light, No  $\text{NH}_4^+$  ( - + - ) Low Mix, Enhanced Light, No  $\text{NH}_4^+$  ( + + - ) Well mixed, Enhanced Light, No  $\text{NH}_4^+$  ( - - + ) Low Mix, Low Light,  $\text{NH}_4^+$  Addition ( + - + ) Well mixed, Low Light,  $\text{NH}_4^+$  Addition ( - + + ) Low Mix, Enhanced Light,  $\text{NH}_4^+$  Addition ( + + + ) Well mixed, Enhanced Light,  $\text{NH}_4^+$  Addition.

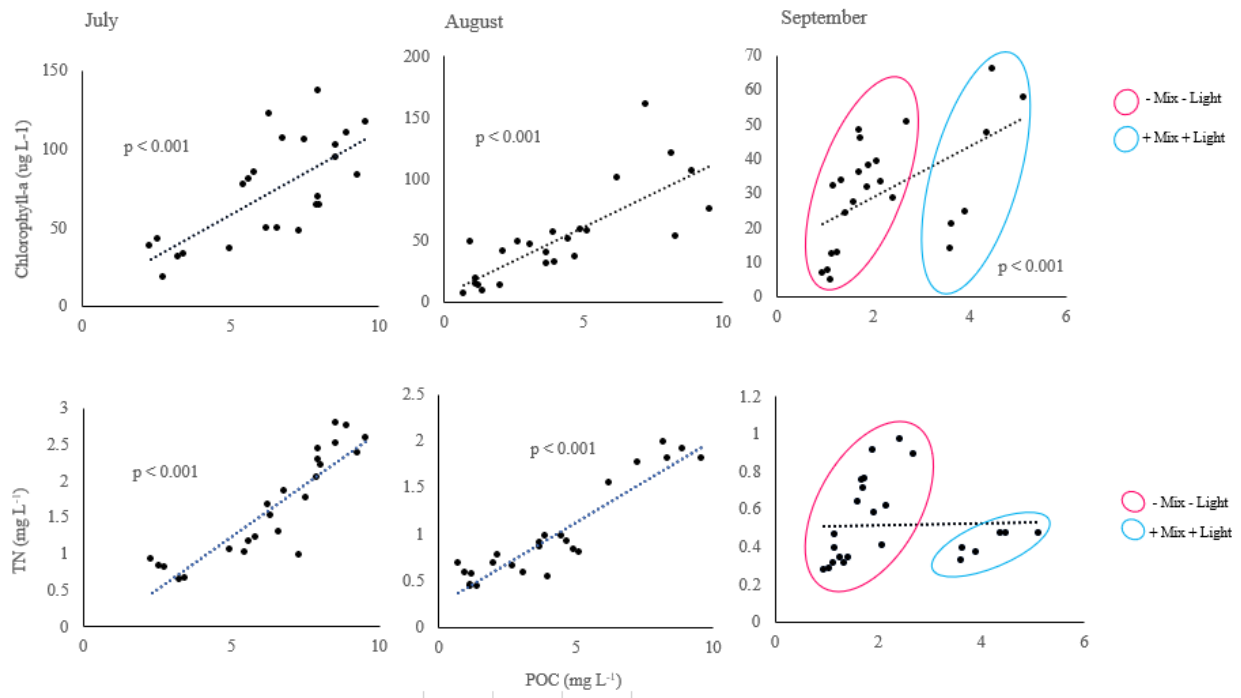


Figure 6: Relationships between CHLa and POC, and between TN and POC in July, August, and September mesocosm experiments. Points represent measurements from individual mesocosms on Day 10 of each experiment. P-values from linear regression are shown where relationships were significant. Responses of +Mix+Light and -Mix-Light treatment combinations are circled in the September figures.

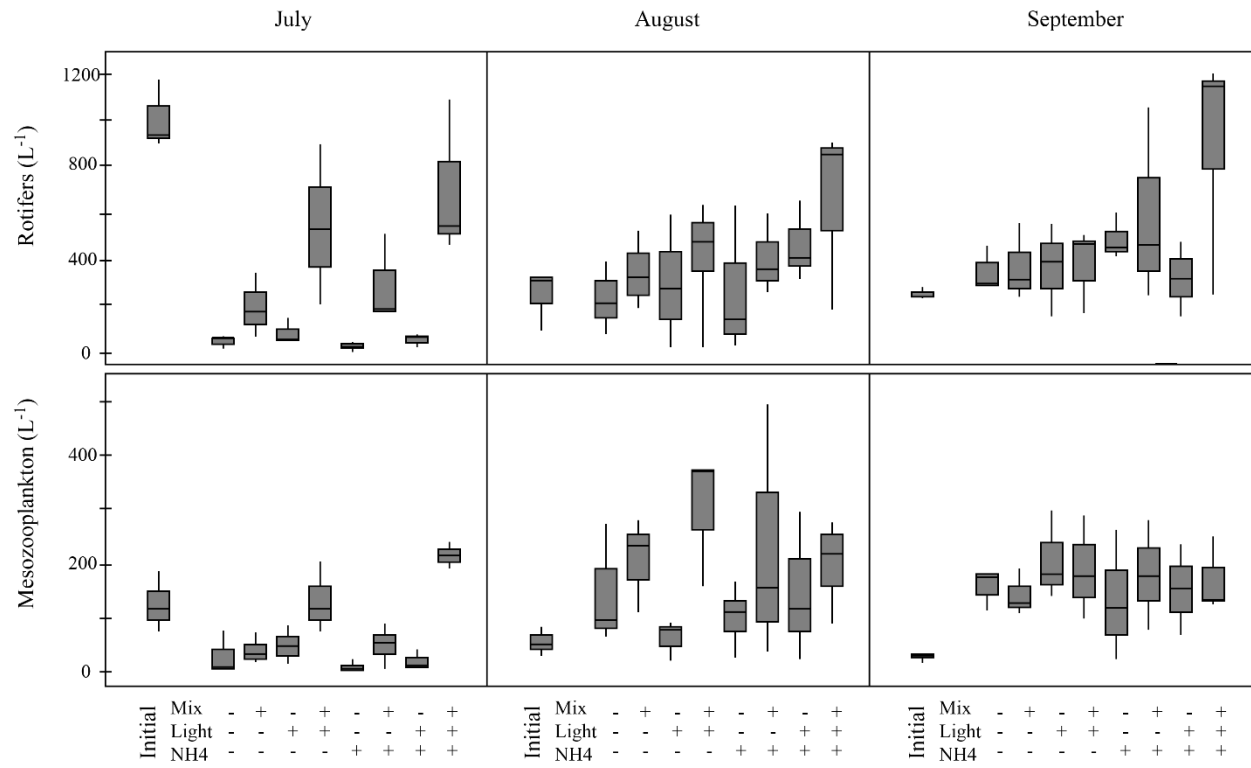


Figure 7: Initial and final concentrations of rotifers and mesozooplankton (Cladocerans, Copepods, Nauplii) for July, August, and September mesocosm experiments.

## References

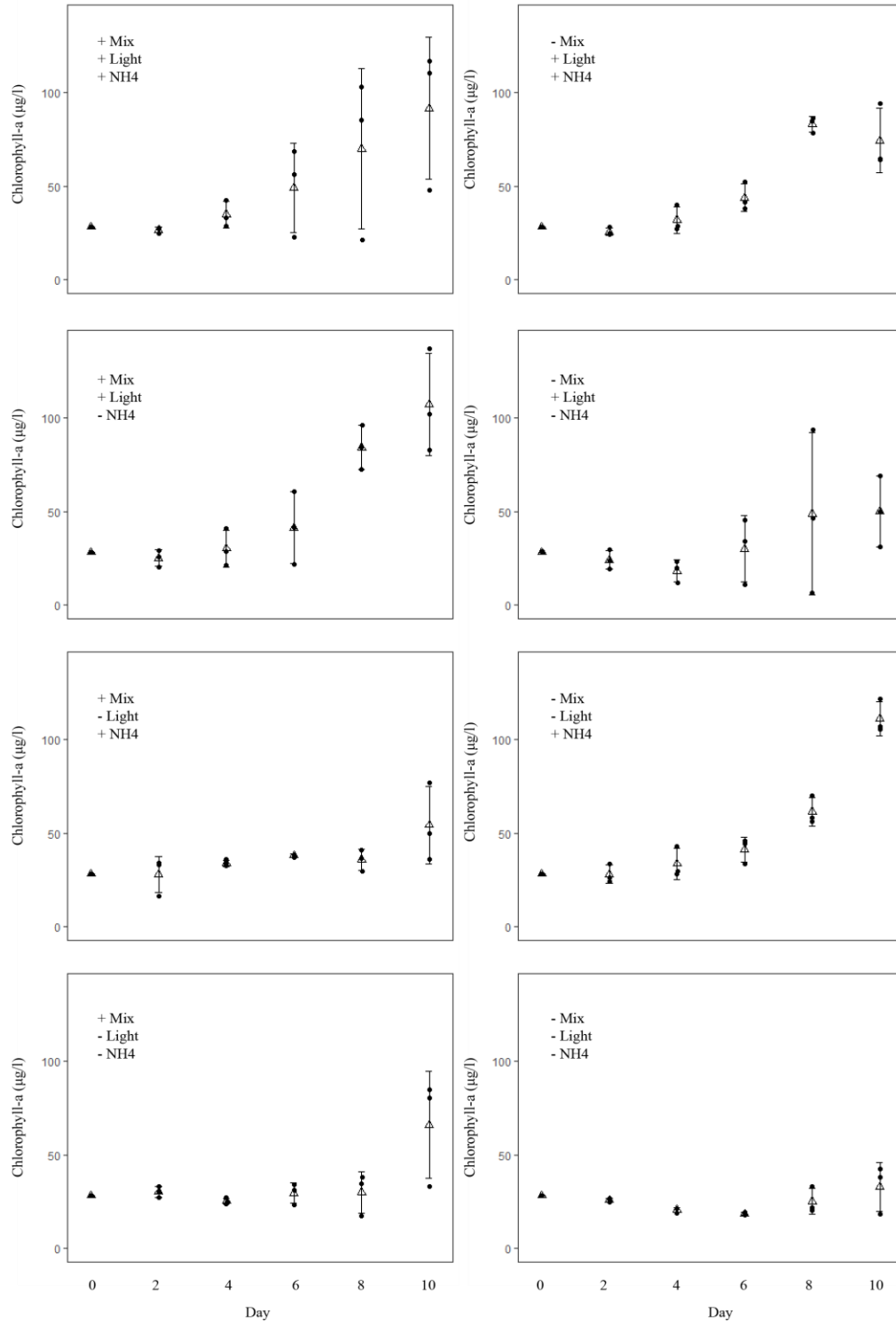
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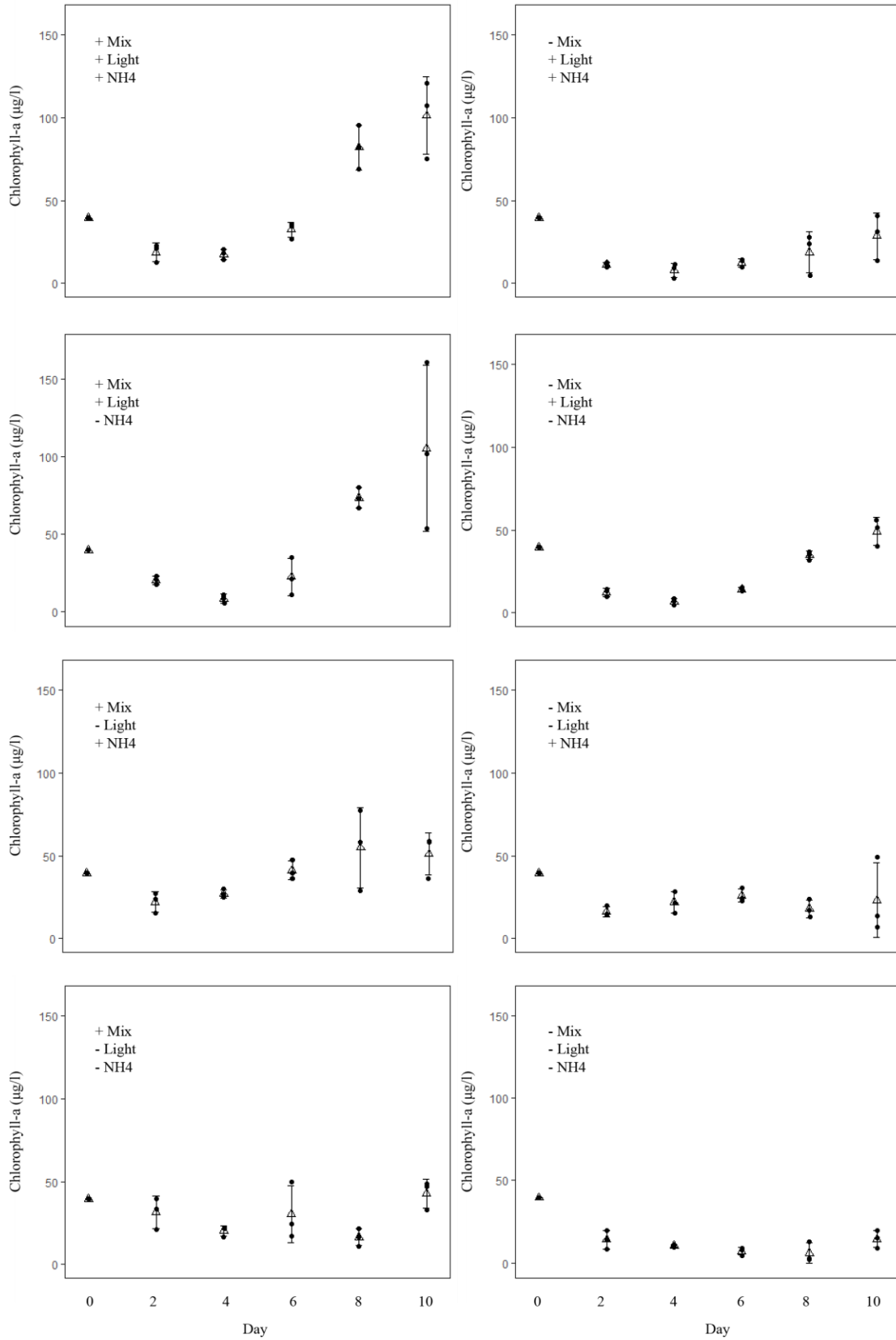
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# Supplemental Figures

July



# August



# September

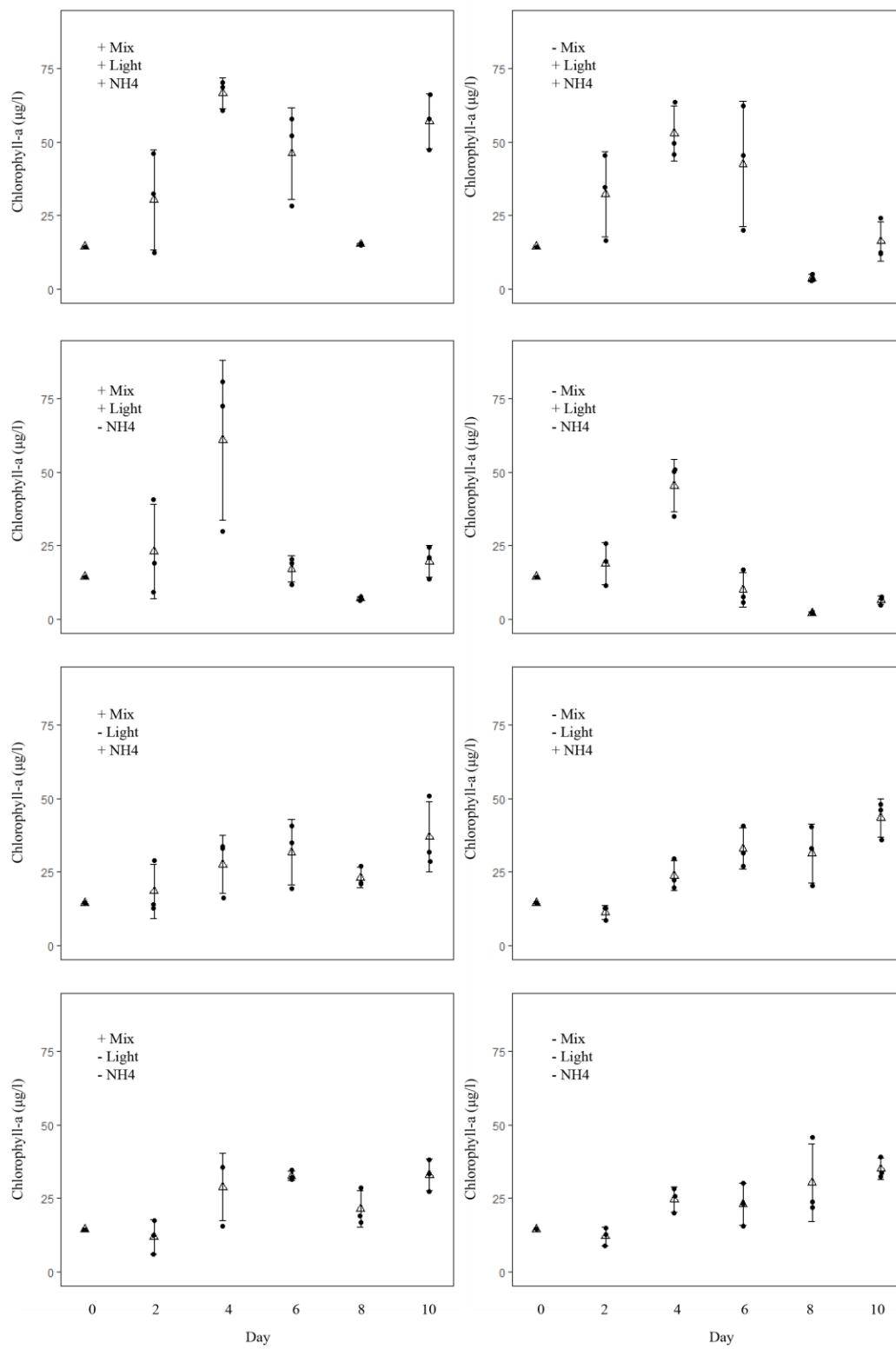


Figure S.1: Time series of average daily CHLa  $\pm$  SD for each treatment combination in July, August, and September mesocosm experiments. Points represent measurements from individual mesocosms on each day of each experiment.