CONTROLS ON CYANOBACTERIA GROWTH IN THE HUDSON RIVER ESTUARY

A Final Report of the Tibor T. Polgar Fellowship Program

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Rundquist, C. W. and S. E. G. Findlay. 2020. Controls on Cyanobacteria Growth in the Hudson River Estuary. Section I: 1-23 pp. *In* S.H. Fernald, D.J. Yozzo and H. Andreyko (eds.), Final Reports of the Tibor T. Polgar Fellowship Program, 2018. Hudson River Foundation.

ABSTRACT

In this study, the effects of light, nutrient and temperature on potential for cyanobacterial bloom formation were tested under simulated Hudson River conditions. The results from this experiment were then compared to actual Hudson River conditions in order to better predict the formation conditions for harmful cyanobacterial blooms. The first experiment measured the effects of temperature as well as nitrogen and phosphorus levels on the cyanobacteria concentration of small scale cultures with natural inoculants. The second experiment tested the variables of elevated nutrient levels and depth under field conditions at Norrie Point in Staatsburg, NY. After the cultures grew for three weeks, the results from the first experiment showed that increased temperature had a significant, positive effect on cyanobacterial concentration. Additionally, the results from the second experiment showed that elevated levels of nitrogen and phosphorus significantly increased cyanobacterial concentration. The results from this experiment convey that cyanobacteria can quickly grow up to harmful concentrations, in warm, nutrient-rich water. In the Hudson River, these conditions could be found in a relatively stagnant, open section of river, downstream of a sewage treatment plant, in late summer.

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INTRODUCTION

Cyanobacteria, or blue-green algae, are an integral part of aquatic ecosystems (WHO 1999a). Blue-green algae are photoautotrophic bacteria that can live in both salt and freshwater, often becoming a large part of the biomass in rivers and lakes. One key characteristic about cyanobacteria is that under certain conditions, they are able to grow at extremely rapid rates and reach very high abundances in what is known as a bloom. What is specifically dangerous about these blooms is that they sometimes contain certain types of cyanobacteria that produce toxins, known as cyanotoxins. These cyanotoxins pose a serious threat to bodies of water used for drinking water and recreational uses, causing serious damage to the surrounding ecosystem, citizens, and wild and domestic animals that live near the affected body of water (EPA 2017). Extended exposure can cause serious damage to the liver, including liver cancer, and can even be neurotoxic (Hitzfeld et al. 2000; Hudnell 2008; Carmichael 1994). These harmful effects are shown in a study of cyanobacteria that took place in Chesapeake Bay. According to the study, blue-green algae were responsible for a series of substantial bird and fish kills. The toxins also caused very real effects on humans, including skin rashes, nausea, and fevers (Tango and Butler 2008). Because of this harmful potential, cyanobacterial blooms are a primary issue in providing people with clean water. In the context of anthropogenic pollution and climate change, clean water is something that will only gain more importance in the coming decades (Hitzfeld et al. 2000). Furthermore, cyanobacterial prevalence has been increasing at a disproportionate rate to that of other phytoplankton, and are becoming more abundant in the U.S. and worldwide (Hudnell 2008; Taranu et al. 2015).

The lower Hudson River is a tidal, well-mixed, nutrient-rich estuary in eastern New York State (Fernald et al. 2007).The lower Hudson River has fluctuating values of turbidity, dissolved organic carbon, and nutrient levels, including nitrogen and phosphorus. Recently, the presence of cyanobacterial blooms has become an issue for the Hudson River Valley. The worst of these blooms mostly occur in the numerous slower-moving tributaries of the Hudson River (Fernald et al. 2007). An example of one of these blooms in the Hudson River valley occurred in the Wallkill River, in which cyanotoxins were reported up to 25 times the DEC's "High Toxin" threshold (Riverkeeper 2016). The cyanotoxins in this bloom posed a real threat to both the local ecosystem and community, harming aquatic organisms as well as domestic pets. If such a cyanobacterial bloom were to happen in the Hudson River, it would pose a real danger to the estuary ecosystem.

Cyanobacterial blooms are only able to form under certain ranges of water quality and atmospheric conditions (CDC 2017). Three main factors for the formation conditions of algal blooms identified by the World Health Organization are temperature, phosphorus and nitrogen levels, and light intensity (WHO 1999a). Cyanobacteria can withstand a wide range of temperatures from arctic lakes to hot springs; however, blooms only form in warmer conditions during the summer, and will usually last for a couple of weeks at a time (WHO 1999a). The trigger water temperature for cyanobacterial biomass dominance is about 25ºC, which means that blooms most frequently occur in the late summer months in the Hudson Valley (Fernald et. al 2007). Nitrogen and phosphorus are two of the primary nutrients that promote cyanobacterial growth and bloom formation (Davis et al. 2015). These nutrients are an important factor for cyanobacterial blooms for two reasons, the first being that cyanobacteria have a very high nutrient affinity and are able to

outcompete other phytoplankton for phosphorus and nitrogen. The second is that cyanobacteria are very efficient at nutrient storage, gathering enough at once to sustain up to four cell divisions (WHO 1999a). Sunlight conditions are important for the formation of blooms as most cyanobacteria prefer lower levels of light compared to other phytoplankton (Lopez et al. 2008). The optimal growth conditions of cyanobacteria are approximately half of direct sunlight level, and overexposure to intense light levels can be detrimental to cyanobacteria (WHO 1999b). Furthermore, transient microstratification in the Hudson River can lead to increased temperature, nutrient accumulation, and sunlight exposure. These conditions create a high overall phytoplankton biomass in the euphotic zone, and eventually can increase cyanobacterial dominance when resources become depleted (Fernald et al. 2007).

When trying to predict harmful algal blooms in the actual environment, it is very important to take these different factors of cyanobacterial growth into consideration. The purpose of this experiment was to analyze optimal conditions for the formation of harmful algal blooms in the Hudson River by testing different cyanobacterial growth factors in conditions similar to the Hudson River. The hypothesis that increased temperature, increased nitrogen and phosphorus levels, and higher sunlight exposure lead to increased cyanobacteria growth, was tested in this experiment The first experimental setup tested the effects of temperature, as well as elevated phosphorus and nitrogen levels on cyanobacterial growth in a controlled environment on land. The second experiment tested the variables of elevated nutrients and depth on isolated volumes of Hudson River water. This experiment examined cyanobacteria in conditions as close to the Hudson River as possible, without actually exposing the river to potentially toxic organisms. The

degree to which these different variables alter cyanobacterial growth will form a picture of what harmful algal bloom formation conditions look like in the Hudson River, helping to predict these blooms.

METHODS

Greenhouse Experiment

In the first experiment, the effects of temperature and increased nutrient levels on cyanobacterial growth were tested on small cultures in a controlled environment.

To begin culture growth, a natural inoculant was collected from a small pond with a history of algal blooms located in Tivoli, NY. This pond collection was then filtered to 35 µm to isolate the cyanobacteria for inoculation. In the experiment, a total of 16 cultures were prepared and subjected to four different treatments. First, sixteen 500 ml flasks were filled with deionized water. Half of these flasks were then fertilized using potassium nitrate and sodium phosphate. These flasks were raised to nitrogen and phosphorus levels twice that of natural levels documented from the Hudson River by Fernald et al.(2007), with concentrations of 70 µM nitrogen and 2.24 µM phosphorus. After, 20 ml of natural inoculant was added to all sixteen flasks and were ready for testing.

The experiment took place in a greenhouse on the Cary Institute of Ecosystem Studies campus, located in Millbrook, NY. In order to manipulate temperature, two clear 40”x20”x7” containers were placed adjacent to each other on a table to serve as water bath for the algal cultures. One container was subjected to an average ambient temperature of 25.34ºC, while the other was cooled to an average temperature of approximately 20.43ºC. This second container was cooled by a Fischer Scientific Isotemp

Water Bath, externally pumping cold water through a 2 ft long copper coil, placed in the water container. After this, shadecloth was draped over the experimental setup, controlling the cultures to receive an average sunlight level of 203.24 $\mu E/m^2/s$ during the day.

This experiment ran for three weeks, with the growth of the cultures being measured by a YSI Exo 2 sonde with chlorophyll-a and phycocyanin fluorometric probes in µg/L. The peak concentrations for all four treatments were analyzed by a repeated-measures Analysis of Variance (ANOVA), using the program JMP, version 13. In addition to this, laboratory chlorophyll measurements were taken on Day 11 and Day 14 of the experiment (Yéprémian et al. 2017). Temperature and light data were monitored throughout the experiment using HOBO Pendant water data loggers.

Figure 1: Picture of the experimental setup testing the effects of temperature and nutrients on cyanobacteria concentration. The higher temperature treatment is depicted on the left and the lower temperature is depicted on the right.

River Experiment

For the second experiment, the effects of elevated nutrient levels and depth on culture growth were tested under realistic Hudson River conditions.

The design of this experiment included a floating frame that was suspended in the Hudson River, with attached containers full of isolated river water and ambient cyanobacterial communities. To keep the rig afloat a 1m x 1m square was constructed out of 1-1/4" PVC piping. The square was held together by square elbow joints and PVC cement. Across the square, four lengths of nylon rope were strung both lengthwise and widthwise, forming four intersections in the middle of the square. Four 1.5 m long chains were then clipped to the four intersections, able to hang down into the water. On each of these chains, two LDPE 10 L clear cubitainers were fastened at the very top and at 1 m down the chain. In total, the rig had four cyanobacterial cultures growing at the surface level, and four growing at a depth of 1 m.

This experiment took place adjacent to the dock of the Norrie Point Environmental Center in Staatsburg, NY. The experimental rig was placed about 20 m offshore, upriver of the dock. One side of the PVC square was tied to the dock and the other side was tied to a submerged cinderblock, keeping the rig suspended in the same area during high and low tide. After this, all of the cubitainers were filled with unaltered Hudson River water. Half of the surface and submerged cultures had their nitrogen and phosphorus levels raised to twice the ambient level, as done in the first experiment. The cultures were sealed in the cubitainers and the whole rig was set to grow for two weeks. The growth of the cultures was monitored using the same phycocyanin and chlorophyll-a

fluorometric probes on the YSI Exo 2 sonde, measuring in µg/L. The measurements from these probes were analyzed by running the same repeated measures ANOVA test on the peak concentration for all four treatments. Water samples were also taken on Day 1 and Day 7 of the experiment, used for laboratory chlorophyll extraction measurements, as done in the first experiment.

Figure 2: A picture of the experimental rig testing the effects of nutrients and depth on cyanobacteria concentration, placed in the Hudson River at Norrie Point, Staatsburg, NY.

RESULTS

Greenhouse Experiment

At the outset of the greenhouse experiment, all four treatments of cultures started very slowly with little to no growth for the first week. After that point, the warmer temperature treatments started to grow abruptly, observed both quantitatively and qualitatively, turning green in color (Figures 1, 3, and 4). The high temperature treatment cultures then rose to a higher concentration than the cold treatments, as shown by the

error bars on both the chlorophyll and phycocyanin graphs. In addition to this, the higher temperature cultures reached their peak concentration at Day 13, which was slightly earlier than the colder temperature cultures on Day 14 (Figures 3 and 4). On Day 13, a repeated measures ANOVA test was run on the cyanobacteria concentration in relation to temperature, resulting in a p-value of less than .0001, indicating a significant effect of temperature on culture growth for both phycocyanin and chlorophyll-a measurements. All of the cultures exhibited growth for roughly the same amount of time, with no signs of further cyanobacterial accumulation at three weeks. The temperature variable was found to have a significant effect on cyanobacterial growth, determined through statistical analysis on both chlorophyll and phycocyanin parameters (ANOVA, $p<0.05$) (Figure 7).

The cultures enriched with nitrogen and phosphorus had greater overall measurements for both chlorophyll and phycocyanin. The cultures with elevated nutrients for both higher and lower temperature treatments had greater peak concentrations than their unaltered counterparts (Figures 3 and 4). This was not consistent among all four cultures within each treatment. The elevated levels of nitrogen and phosphorus did not have a significant effect on peak cyanobacterial culture growth (ANOVA, $p>0.05$). Overall, the cyanobacterial growth through all treatments was consistent and steady, verified through the similar measurements of both chlorophyll and phycocyanin pigments.

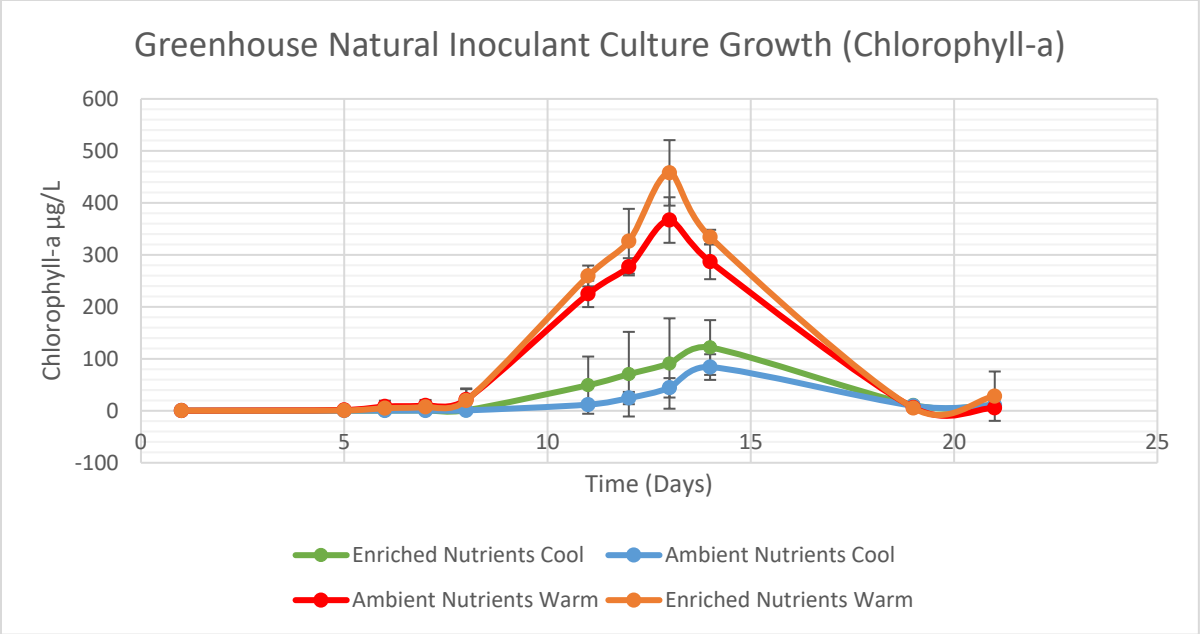

Figure 3: **Average chlorophyll (µg/L) measurements of four culture treatments grown over three weeks. Temperature was found to have a significant effect on culture growth ($p<0.05$), and enriched nutrient levels of nitrogen and phosphorus did not have a significant effect ($p>0.05$).**

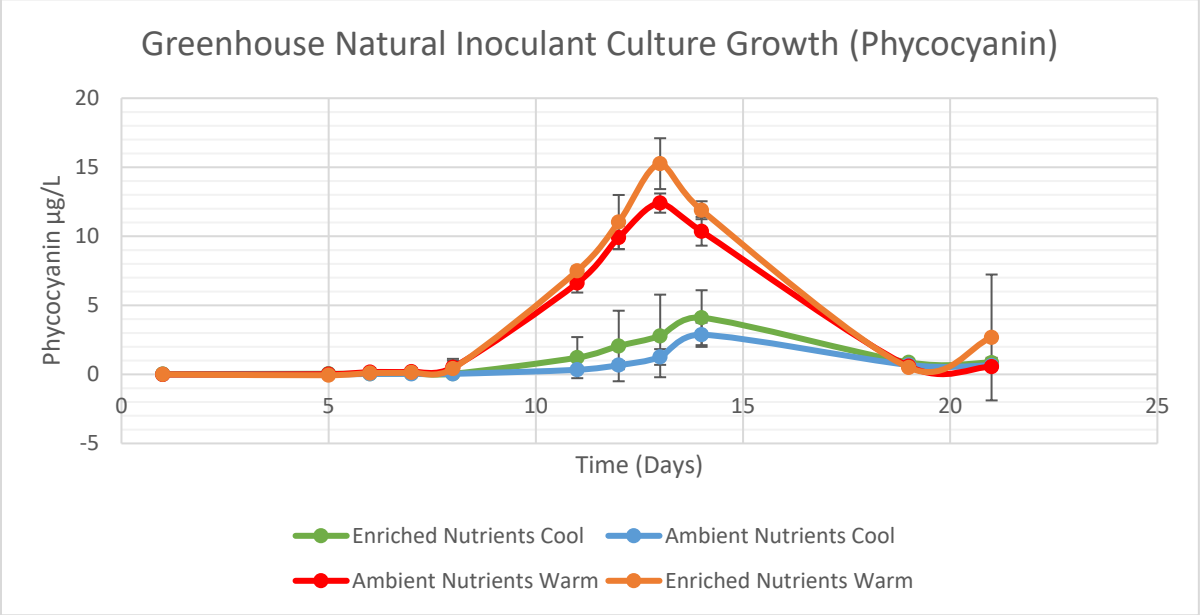

Figure 4: **Average phycocyanin (µg/L) measurements of four different treatments on cyanobacterial cultures, grown over three weeks. Temperature had a significant effect on culture growth ($p<0.05$), but elevated nitrogen and phosphorus levels did not have a significant effect ($p>0.05$).**

River Experiment

For the river experiment, the cultures grew rapidly overall, with noticeable growth on Day 3. In the beginning, the enriched surface cultures had the highest cyanobacterial concentration in comparison to other treatments, peaking on Day 5 for both chlorophyll and phycocyanin (Figure 5,6). From then on, the enriched surface treatment decreased in concentration, and the enriched bottom treatment exhibited the highest algal concentrations for the remainder of the experiment. In contrast, the two unaltered surface and bottom treatments had the lowest concentrations throughout the whole experiment. These cultures both peaked early, approximately one week into growth, and exhibited very small changes in concentration throughout the experiment. On Day 6, the enriched bottom culture was comparable to the unaltered bottom culture, but increased from that point on (Figure 5). By Day 8, the elevated levels of nitrogen and phosphorus had a significant effect on cyanobacterial concentration (ANOVA, $p<0.05$). This effect is illustrated through the comparison of peak concentrations between the enriched bottom and unaltered bottom treatments (Figure 7).

In terms of the growth depth variable, there was no clear trend in either the chlorophyll or phycocyanin data. For the enriched cultures, the surface culture treatments peaked first at Day 5, but then decreased and were surpassed by bottom culture concentrations (Figure 5,6). For phycocyanin, the higher concentration of the enriched bottom treatment was more apparent, as shown by the error bars at the end of the experiment (Figure 6). In contrast, the chlorophyll data had more variance in the measurements. Furthermore, the enriched surface treatment exhibited the highest peak chlorophyll concentration among all other treatments (Figure 5). For the unaltered

cultures, both the surface and bottom treatments had very similar readings for both probes. Overall, the bottom unaltered treatment had greater values, but these data points often overlapped with the surface unaltered cultures. Depth did not have an effect on the peak concentration of cyanobacterial culture growth (ANOVA, $p>0.05$).

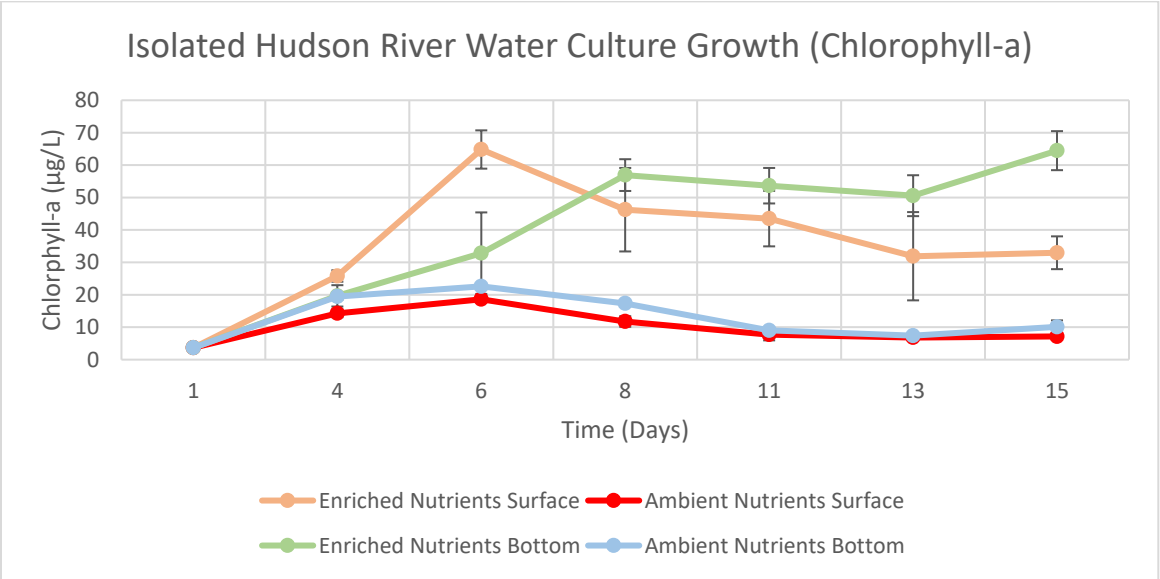

Figure 5: **The average chlorophyll (µg/L) measurements for cyanobacterial culture growth with nutrient level and depth variables. Elevated levels of nitrogen and phosphorus had a significant effect on culture growth over two weeks. Depth did not have a significant effect.**

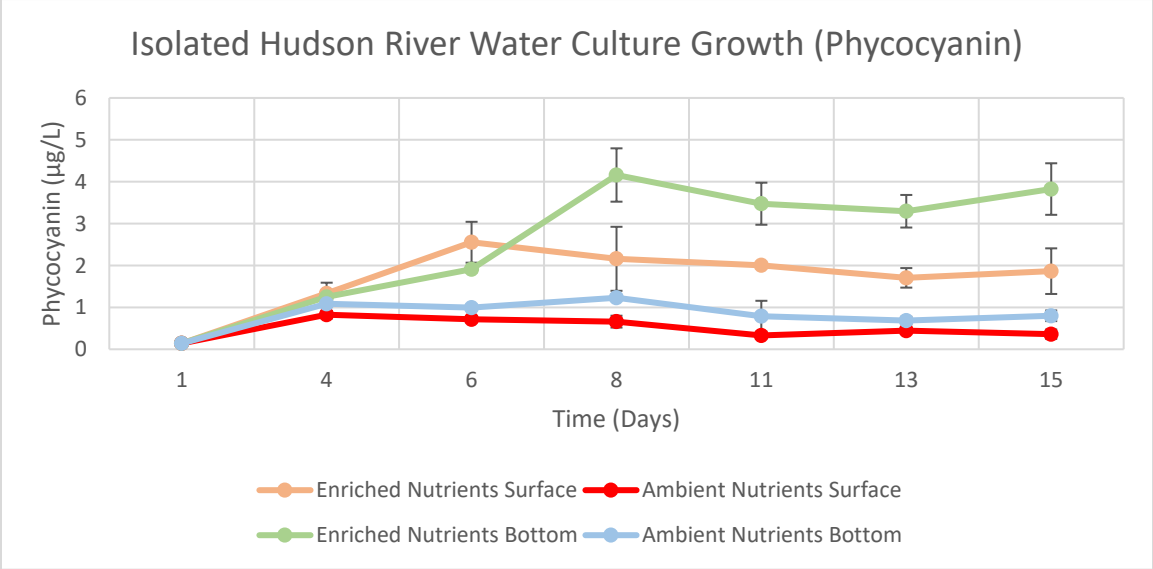

Figure 6: **The phycocyanin (µg/L) measurements for four treatments with nutrient levels and depth on cyanobacterial culture growth over two weeks. Elevated levels of nitrogen and phosphorus had a significant effect on culture growth, and the growth depth variable did not have a significant effect.**

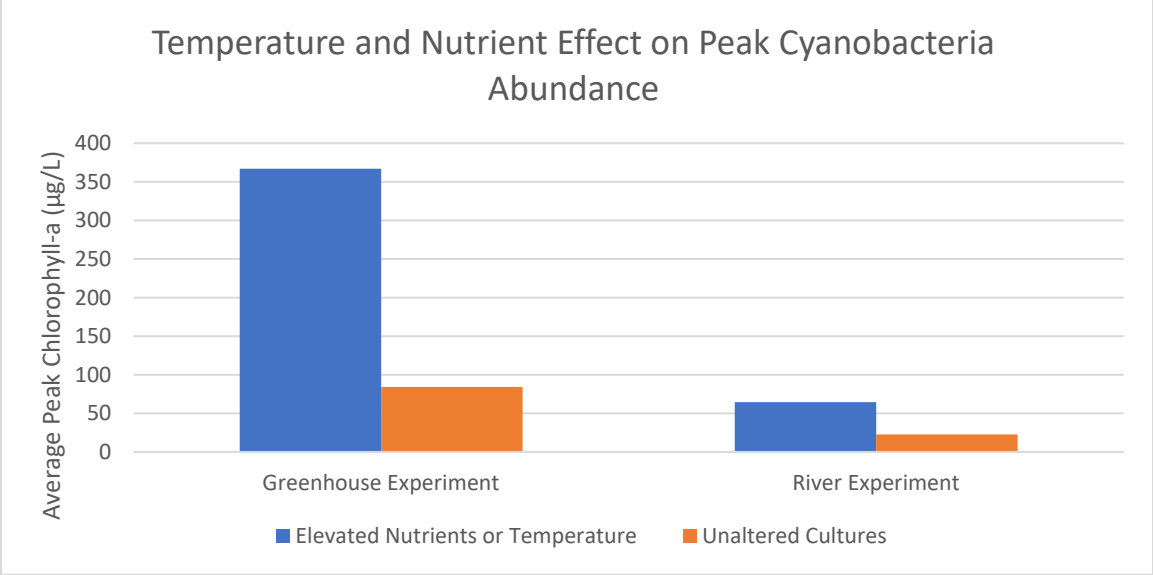

Figure 7: **The comparison of average peak growth for unaltered hot and unaltered cold treatments in the greenhouse experiment, and enriched submerged and unaltered submerged treatments in the river experiment. Temperature had a significant effect on cyanobacterial growth in the greenhouse experiment and enriched nutrient levels had a significant effect on culture growth in the river experiment.**

DISCUSSION

Overall, in these two experiments, both increased temperature and elevated nitrogen and phosphorus levels had a positive, significant effect on cyanobacterial growth. The effect of the temperature variable was shown in the greenhouse experiment, testing cultures with a natural inoculant in unaltered and enriched nutrient water. At the outset of culture growth, the higher temperature treatments distinguished themselves as having a greater cyanobacterial concentration. This was not only shown in the great increase of chlorophyll and phycocyanin levels, but also noticeable through a visible green hue. In the greenhouse experiment, temperatures above the critical 25ºC point (Fernald et. al. 2007) made the cyanobacterial cultures grow faster and reach significantly higher concentrations. This was a clear relationship, as the greenhouse experiment used regular deionized water as the base for the cultures, with only a small amount of other

phytoplankton and zooplankton to interact with the cyanobacteria. In the river experiment, the average water temperature measured at the Hudson River Environmental Conditions Observing System (HRECOS) Norrie Point hydrological measurement station was 26.84ºC. This value is clearly above the 25ºC point, and while not tested as a variable, could definitely have helped all of the cultures to produce growth over the two week period. This late summer hot temperature is also the cause of more frequent natural cyanobacterial blooms during this time, and could provide an explanation for the substantial amount of cyanobacteria in the river which allowed the cultures to grow without an external inoculant. In summary, this experiment reinforced the findings by Fernald et al. (2007); the cultures grown in temperatures above 25ºC had significantly higher concentrations than the cultures grown at approximately 20ºC.

In the experiment that took place in the river, the doubled levels of nitrogen and phosphorus had a significant, increasing effect on cyanobacterial concentration. For cultures growing both a meter under the water and at the surface, the enriched cultures were able to better reach and maintain higher cyanobacterial concentrations. Similar to the greenhouse experiment, the enriched cultures in the river experiment showed qualitative and quantitative signs of higher concentrations, early on in the experiment. The enriched cultures had a more intense green hue and ultimately reached significantly higher peak concentrations than their unaltered counterparts. In the river experiment, it is important to take into consideration that the cultures contained large portions of river water, capturing ambient communities of bacteria, algae, and zooplankton. These other organisms provided competition for the cyanobacteria in terms of gathering nitrogen and phosphorus to grow. An explanation then, for the success of cyanobacterial cultures in

enriched conditions, could be the high nutrient affinity of cyanobacteria, and their ability to outcompete other organisms and take full advantage of excess nutrients. The positive effect of nitrogen and phosphorus on cultures was also slightly conveyed in the greenhouse experiment. While the elevated nutrient levels were shown to not have a significant effect on culture concentration, almost all of the enriched treatment concentrations were slightly higher than the unaltered treatments. Furthermore, both of the enriched culture treatments had higher peak concentrations, with the enriched hot culture treatment being greater than the hot unaltered culture treatment. In conclusion, when nitrogen and phosphorus were added to Hudson River water, cyanobacteria were able to take advantage of the environment and grow to significantly higher concentrations than unaltered cultures.

In the river experiment, the depth variable modified various growth conditions of cyanobacteria, most notably light level. Sunlight level changes from approximately 3265.89 $\mu E/m^2/s$ at the surface the water, to approximately 914.45 $\mu E/m^2/s$ at one meter below the surface. Overall, depth did not have a significant effect on culture concentration in the river experiment; however, the bottom cultures exhibited high variance, but generally were able to reach and maintain slightly higher cyanobacterial concentrations than the surface cultures. This is especially conveyed in the relationship between the surface and bottom elevated nutrient cultures. The surface culture quickly peaked before the bottom, but then decreased in concentration. In contrast, the bottom culture, showed more steady growth and reached higher concentrations towards the end of the experiment. When analyzing this relationship, the sunlight level is one of the most important to take into consideration, with the surface cultures receiving 75%-50% of

maximum sunlight and submerged cultures receiving 25%-10% of sunlight. Over the course of the two week experiment, the average sunlight level measured by the adjacent Norrie Point meteorological HRECOS station during the peak of the day was 5225.429 $\mu E/m^2/s$. A possible explanation for the relationship of the surface and bottom nutrient treatments could be that the surface treatment grew very quickly due to the large amount of sunlight, but then started to die off because the sunlight was too intense. This would fit the characteristic of cyanobacteria as being sensitive to intense levels of sunlight. In contrast, the bottom culture would receive a lower but sufficient amount of sunlight, allowing the cultures to grow at a steadier rate.

The results of elevated temperature and nutrients increasing cyanobacterial growth become significant when put into the context of the actual conditions of the Hudson River. From the greenhouse experiment, it was determined that temperatures above 25ºC significantly increased cyanobacterial concentration. This immediately identifies a time window in the Hudson River in which the temperature is above 25ºC, and therefore more prone to cyanobacterial blooms. The average river temperature during the experiment at Norrie Point was approximately 27ºC, corroborating the theory that the late summer has ideal temperature formation conditions for cyanobacterial blooms. Furthermore, the water temperature at Norrie Point exceeded 25ºC from July 2 through September 10, showing that there is a large time window for optimal cyanobacterial bloom formation conditions. This temperature threshold also identifies possible areas that are more susceptible to blooms. These would be slow moving, open sections of the river that can be easily heated by the sun for longer periods of time, allowing the temperature to rise. In addition, this experiment conveyed the importance of the effect that

temperature has on cyanobacterial growth; increased temperature can cause just a small amount of pond water grow to a high level status bloom concentration as classified by the New York State DEC. At a concentration this high, harmful cyanobacteria would produce enough toxins for the culture to be harmful to human health (NYSDEC 2018). In conclusion, temperature is a vital formation condition for cyanobacteria, with optimal temperature conditions occurring during the late summer in the Hudson River.

The experiment that took place within the Hudson River attempted to replicate, as closely as possible, conditions for growing cyanobacteria without actually releasing cyanobacterial blooms into the river. Thus, the effects of the variables in the river experiment on cyanobacterial growth would be very similar to the conditions of the actual Hudson River. In terms of the elevated nitrogen and phosphorus levels, this means that the addition of nutrients into the Hudson River could easily contribute to cyanobacterial growth.

In conclusion, the formation conditions of increased temperature as well as increased levels of nitrogen and phosphorus were shown to have a significant positive effect on cyanobacterial concentration. Under these conditions, cyanobacterial cultures are able to reach concentrations high enough to be considered dangerous to human health, within just a week of growth. In the Hudson River, these conditions would take the form of a slow moving, open section of river, downstream of a nutrient polluting source. In the warm waters of the late summer, an area like this would be susceptible to cyanobacterial blooms.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank the Hudson River National Estuarine Research Reserve, the Hudson River Foundation, and the Polgar Fellowship program for giving me this opportunity to conduct research. I would also like to thank Stuart Findlay for advising my research project. In addition, David Fischer, Heather Malcolm, and the Cary Institute of Ecosystem Studies were very helpful in providing me assistance and resources with my research. Lastly, I would like to thank Sarah Fernald and the staff at the Norrie Point Environmental Center for their guidance and for allowing me use of their facilities.

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