Seasonal Variability of Nutrient Limitation and Impacts on Phytoplankton Community Composition in the Albemarle Sound

Maxfield Palmer

maxadair@live.unc.edu

THE UNIVERSITY of NORTH CAROLINA at CHAPEL HILL

UNC COLLEGE OF ARTS AND SCIENCES
Earth, Marine and Environmental Sciences

Introduction:

- Phytoplankton, small primary producers in water, produce a large portion of the oxygen on the globe and serve as the base for aquatic and marine food webs.
- Freshwater and brackish systems are under a growing water quality threat caused by an overgrowth of phytoplankton, commonly referred to as a harmful algal bloom.
- Harmful algal blooms occur when there is rapid growth of a phytoplankton species of that can cause fish kills, oxygen starvation, and release of toxic compounds into the water and air. An example of a bloom is shown in Figure 1.
- In brackish/freshwater areas like the Albemarle Sound, the culprit of a harmful algal bloom is cyanobacteria, also known as cyanophyta.
- The largest contributor to harmful algal bloom occurrence is nutrient input, which can come from industrial wastewater or agricultural runoff.
- Commonly, nitrogen and phosphorous can limit the growth of phytoplankton in estuarine systems (Smith, 2006).
- The experiment was conducted to determine whether nitrate or phosphate was most limiting to phytoplankton of if the nutrients were both required for growth, referred to as colimitation in 6 sites on the Albemarle Sound (Fig 2).
- Colimitation can occur when both nutrients are at low concentrations or when the addition of one nutrient causes the original nutrient in excess to become limiting (Andersen, 2007).
- The river basins that make up Albemarle Sound have industrial and agricultural companies with permits allowing them to dump wastewater into nearby waterways. These permits require limits for phosphorous and ammonium input into watersheds but don't require limits for nitrate input (EPA, 2021).
- Different sources may have different compositions nitrogen and phosphate, and knowing what nutrient is limiting could determine how to best reduce nutrients and improve water quality.

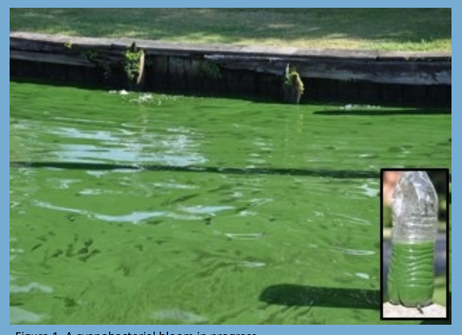

Figure 1. A cyanobacterial bloom in progress.
Photo credit: Chowan/Edenton Environmental Group

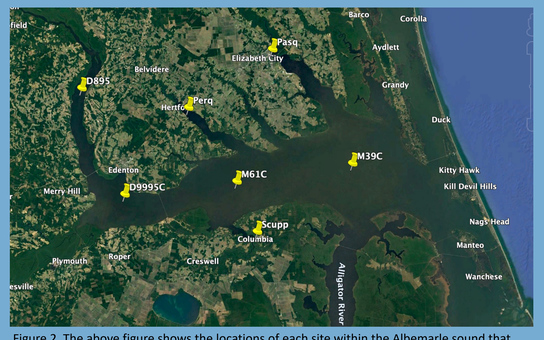

Figure 2. The above figure shows the locations of each site within the Albemarle sound that was sampled for the bioassays conducted during the experiment. Site "Scupp" was used for the April Bioassay and then replaced with Site "Perq" for the 2 remaining bioassays due to lower nutrient results from external experiments.

Methods:

- Water samples collected from 6 sites during 3 seasons (Spring/April, Summer/July, and Fall/October) (Fig 2).
- Initial samples were taken to determine pigment composition at start of bioassay, indicated "initial" in Figures 6-8.
- Each of the 6 sites had 12 cubitainers with 3L of site water 3 cubitainers going to each treatment group (Fig 4B). The treatment groups are: Added Nitrate (+N), Added Phosphate (+P), and Added Nitrate and Phosphate (+NP). Added chemicals are shown in Figure 3.
- Cubitainers were placed in incubation pool for 3 days (Fig 4A) and then samples taken from each were run through HPLC and processed through CHEMTAX to determine the percent of total chlorophyll a each taxa composed (Fig 5).
- Statistical analysis performed using a one-way ANOVA followed by a Tukey-HSD test for significant outputs from ANOVA

Experimental Treatments

Control
- 20 mg/L NaHCO3, 3.5 mL of 30 g/L NaHCO3

+N
- 20 mg/L NaHCO3, 3.5 mL of 30 g/L NaHCO3
- 50 uM sodium nitrate- 0.5 mL of 25 g/L NaNO3

+P
- 20 mg/L NaHCO3, 3.5 mL of 30 g/L NaHCO3
- 3 uM potassium dibasic phosphate 0.35 mL of 4.393 g/L K2PO4

+NP
- 20 mg/L NaHCO3, 3.5 mL of 30 g/L NaHCO3
- 3 uM potassium dibasic phosphate 0.35 mL of 4.393 g/L K2PO4
- 50 uM sodium nitrate- 0.5 mL of 25 g/L NaNO3

Figure 3. Shows the nutrients added to the water samples for each site for each treatment group. The control has sodium bicarbonate added so the phytoplankton do not become carbon limited during the bioassay.

Figure 4. (A). Incubation process of the bioassay. The use of the incubation pool allows for the most similar environmental conditions (temperature and light availability) while experimenting. (B). Cubitainers used for the bioassays.
Photo credit: Madison Sholes

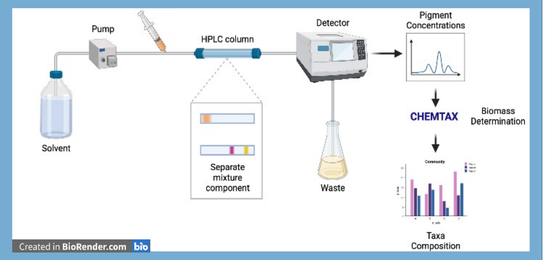

Figure 5. The process for how the total chlorophyll a composition for each taxa was determined using HPLC and CHEMTAX, a program for finding taxa abundances using pigment inputs.

Results:

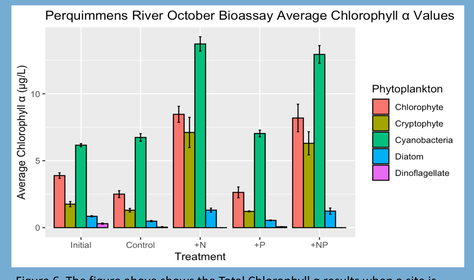

Figure 6. The figure above shows the Total Chlorophyll a results when a site is nitrate limited. You will see an increase in both the +N and +NP treatment due to the presence of nitrate in both treatment groups.

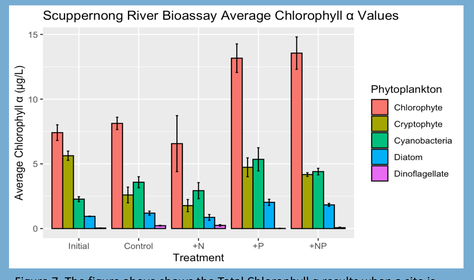

Figure 7. The figure above shows the Total Chlorophyll a results when a site is limited by phosphate. You will see an increase in both the +P and +NP treatment due to the presence of phosphate in both treatment groups.

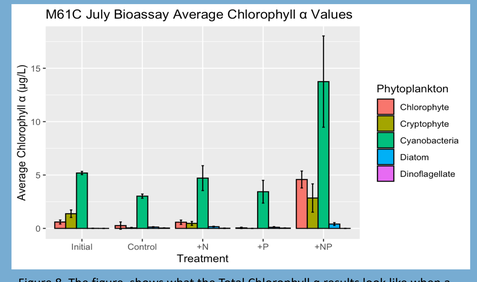

Figure 8. The figure shows what the Total Chlorophyll a results look like when a site is limited by both nitrate and phosphate.

Limiting Nutrients:

Taxa	Site	Spring	Summer	Fall
Cyanophyta	698	P		
Cyanophyta	500		N	N
Cyanophyta	39C	-	NP	-
Cyanophyta	61C	N	NP	NP
Cyanophyta	9995C	NP	NP	N
Cyanophyta	275	-	NP	-
Cyanophyta	895	-	N	-

Figure 9. The figure above shows the limiting nutrients for Cyanobacteria at each station during their respective seasons. Blanks were stations unsampled during the season they fall beneath. Squares with "-" were not nutrient limited.

Taxa	Site	Spring	Summer	Fall
Chlorophyta	698	P		
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Chlorophyta	39C	N	NP	-
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Chlorophyta	9995C	N	NP	N
Chlorophyta	275	P	NP	-
Chlorophyta	895	-	NP	NP

Figure 10. The figure above shows the limiting nutrients for Chlorophyta, also known as Chlorophytes, at each station during their respective seasons. Blanks were stations unsampled during the season they fall beneath. Squares with "-" were not nutrient limited.

Conclusions:

- During the Summer Bioassay, many station's Cyanobacteria populations saw a shift from not being nutrient limited, to being limited by nitrate or both nitrate and phosphate (Fig 9).
- Cyanobacteria growth isn't the only group limited by nitrate, to show another example, the second largest taxa, Chlorophyta, experiences growth limited by nitrate or colimited by a combination of nitrate and phosphate (Fig 10).
- Given the component of nitrate composing cyanobacterial growth inducing treatments (+N and +NP) in every site in warm summer months, prime time for harmful algal blooms, nitrate inputs into water systems need to be limited to improve water quality.
- Phosphorous remains very important given many results showing colimitation.

Broader Impacts:

- Waste permits, while already requiring limits on ammonium, need to also require limits on total nitrogen that is dumped into water systems.
- As systems become more nutrient rich with nitrogen and phosphorous, known as eutrophication, in fresh/brackish water systems, cyanobacterial harmful algal blooms will continue with nitrate input into water, drastically reducing water quality and ecosystem health while increasing public health risk in surrounding areas.
- Knowing what nutrient is limiting allows for best management practices for reducing nutrients from factory wastewater and agriculture runoff that can be used to target nitrate and make waters safer.

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- Photography: Chowan / Edenton Environmental Group

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