Role of Phosphorus on Phytoplankton Concentrations

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INTRODUCTION

Phytoplankton are the primary producers of the marine ecosystem and are consequently often the subject of environmental and scientific research. As species of phytoplankton are extremely diverse, it is difficult to completely elucidate the mechanisms by which their productivity is regulated. However it has been shown that the most dominant factor involved in limiting productivity is nutrient availability. There is still debate over the specific nutrient that is the most limiting, the best candidates being Phosphorus and Nitrogen or possibly Silicon in the case of diatoms (Roelke et al. 1999). All of these nutrients are necessary for basic biological processes, however certain species flourish at their optimal nutrient ratios. Charleston Harbor is a estuarine environment with a temperate climate that supports a biannual phytoplankton bloom. Nutrients flood in from rivers, such as the Ashley and Cooper, and mix with the more saline and nutrient-diluted ocean waters. In the similar ecosystem of Chesapeake Bay, phytoplankton growth rates were limited by dissolved inorganic phosphorus (DIP) during the spring (Malone et al. 1996). Spring was the time of year with the most nutrient input from rivers, and as dissolved inorganic nitrogen (DIN) and dissolved silicate (DSi) were available at much higher concentrations than DIP, it was Phosphorus that the organisms ran out of first. After the spring bloom died, summer phytoplankton were limited by DIN (Malone et al. 1996). In another seasonal study, Nitrogen alone was shown to always stimulate phytoplankton growth, Phosphorus alone stimulated growth in May and July, and their combination stimulated growth in July and August (Vrede et al. 1999). It is also important to note that phytoplankton productivity due to nutrient availability cannot be independently observed in natural habitats. A large reason for this is their predation by zooplankton. Zooplankton species play a large role in selecting for phytoplankton size. While small phytoplankton flourish in low nutrient environments, high pressure from zooplankton grazing selects for larger phytoplankton (Cottingham 1999). Some noxious phytoplankton blooms are detrimental to coastal environments and management techniques are actively being pursued in order to select for more beneficial species. It has been found that edible phytoplankton bloom quickly in nutrient-sensitive communities, followed by slower growing, inedible blooms which are less desirable. One possible management scheme has been proposed that involves pulsing nutrients from a point input so that edible phytoplankton have multiple blooms and the inedible blooms are inhibited (Roelke et al. 1999). Our study investigates the fluctuations in phosphate levels during Spring in Charleston Harbor and attempts to correlate these fluctuations with phytoplankton blooms by measuring corresponding chlorophyll concentrations over four weeks. We also take a look at the effect of temporally spaced pulses of a limiting amount of Phosphorus in a specific phytoplankton diatom species, *Phaedactylum* from the Sargasso Sea.

MATERIALS AND METHODS

Field Experiment

We collected 150ml water samples at low tide at the North end of the Grice Marine Laboratory. We collected the first sample Wednesday February 28th and continued to take samples each consecutive Wednesday following until March 21st. We filtered 50ml for each of the four sample dates and stored them at -80˚C for chlorophyll analyses. We then cut the chlorophyll covered filter paper into tiny pieces each in a tube filled with 6ml of acetone for forty-eight hours. The 4 tubes were centrifuged and the final chlorophyll concentration in the supernatant was measured using a fluorometer. The other 100ml of each of the 4 collections was stored at
-20˚C used for phosphate readings. A standard phosphate curve ($y=1.532x + 0.2351$) was derived with y representing (Absorbance-Turbidity) and x representing phosphate concentration ([PO4-]). A spectrophotomer was used to obtain an initial turbidity reading of the untreated samples. After adding 10ml of Mixed Reagent, a second absorbance reading was recorded. The final phosphate reading *(x)* was extracted from the standard curve.

Image 1. (left) Charleston Harbor Map with arrow indicating sample location.

Image 2. (right) North shore of Grice Marine Facility.

Culture Pulsing Experiment

We added 2ml of a Phaedactylum culture to each of fourteen flasks containing 250ml of sterile 35‰ sea water (diH2O and Crystal Sea Salt Mix). The flasks were provided with an abundant level of essential nutrients excluding Phosphorus (0.25ml $NaNO_3$, 0.50ml $Na_2SiO_3 \cdot 9H_2O$, 0.25ml trace metals, and 0.25ml vitamins). Each experimental group had 3 replicates. There were 4 experimental groups, 3 of which received the same total amount DIP (150µl) over 3 weeks. Set one received the entire amount the first week. Set two received two 75µl treatments five days apart, and Set 3 received three 50µl treatments each five days apart. Set four did not receive any phosphate additions. All of these flasks were kept in an 22˚C incubator on a 12:12 light dark schedule. Chlorophyll readings were taken with a fluorometer from subsamples of each flask on March 23, 28 and April 4th.

Image 3. (Left) Cultures of *Phaedactylum* in incubator.

Image 4. (Right) Microscopic view of *Phaeodactylum tricornutum*.

RESULTS

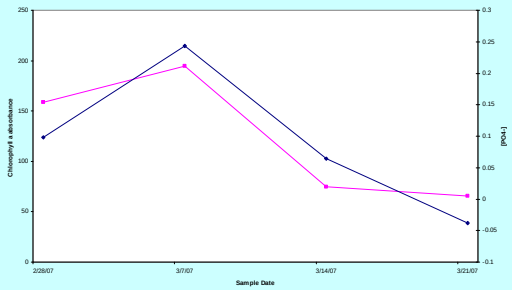

Figure 1. The temporal distribution of observed phosphate (blue line) and chlorophyll a (pink line) concentrations of shore samples. A peak in chlorophlyll a concentration occurs at the beginning of March mirroring the peak of phosphate concentration.

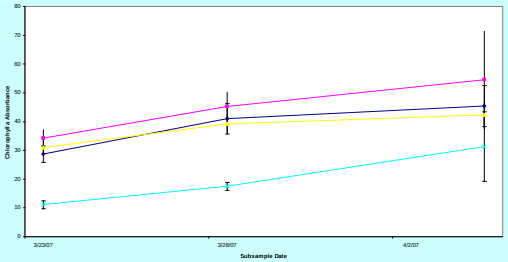

Figure 2. The chlorophyll absorbance of Phaedactylum culture subsamples over 3 weeks. Group 1 (blue) received 150 µl DIP at the first treatment. Group 2 (pink) received two 75 µl treatments 5 days apart. Group 3 (yellow) received three 50 µl treatments with 5 days between each treatment. Group 4 (teal) received no DIP at all. There is no statistically significant difference amongst Groups 1-3. Group 4 shows a significant reduction in productivity.

CONCLUSIONS

- Phosphate concentrations at the North shore of Grice Marine Facility were high in early March and correspond with an increase in phytoplankton concentrations. This is in accordance with a study on Chesapeake Bay that reported high phytoplankton blooms in early Spring that were limited by DIP (Malone et al. 1996).

- *Phaedactylum* did not reflect different growth rates as a result of phosphate pulsing. However, lack of DIP resulted in significantly lower chlorophyll concentrations.
- Possibly, our phosphate treatments of 150µl were non-limiting as *Phaedactylum* is a diatom species that grows exceptionally well in most conditions.

- Preliminary observations at the cellular level revealed a clumping of the diatom individuals in the pulsed experimental groups. The untreated Set four did not show clumping but rather cells remained isolated. While it appeared Set 3 had the most clumping activity this was not monitored regularly throughout the experiment and data is purely subjective. However, colonization of phytoplankton in response to temporally spaced Phosphate availability is a possible topic for further investigation.

- One study reveals a necessary 7 day time period for phytoplankton to uptake at least 50% of DIP (Reinhardt et al. 2005). This would suggest our experimental design was limited by time and perhaps pulses would have significant effects on chlorophyll concentrations if the time between each pulse was longer.

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