

The Role of Dissolved Organic Nitrogen in Bloom Formation of *Prorocentrum minimum*



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Prorocentrum minimum

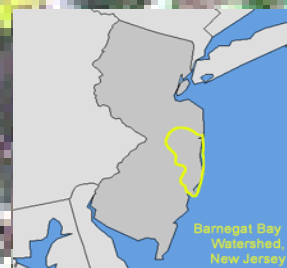
<http://www.upe.ac.za/botany/Dinos/dino.htm>



Introduction

Harmful Algal Blooms (HABs) have caused many problems on the eastern shore, including fish and shellfish kills that have been reported along the coast. These kills can be caused by phytoplankton producing various types of toxins and clogging the cilia of shellfish. It has been hypothesized that the types and amounts of nitrogen are factors in causing blooms (Fan et al. 2003). Determining causes to these blooms is an active field of research. *Prorocentrum minimum*, a dinoflagellate, secretes compounds that are toxic to molluscs, causing mortalities in their populations around the United States. *P. minimum*, along with other phytoplankton such as *Aureococcus anophagefferens*, is becoming an interest to researchers on the eastern shore of the United States. Barnegat Bay in New Jersey and the Chesapeake Bay in Maryland are of specific interest to us.

This research will help us to determine whether nitrogen types and ratios play a role in causing *P. minimum* blooms. We are comparing differences in effects of dissolved organic urea and lysine, DIN and dissolved inorganic nitrogen (nitrate, DIN) and different ratios of urea and nitrate on the growth of *P. minimum* (Berg et al. 1997).



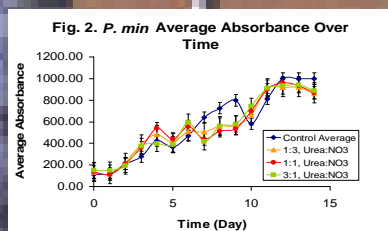
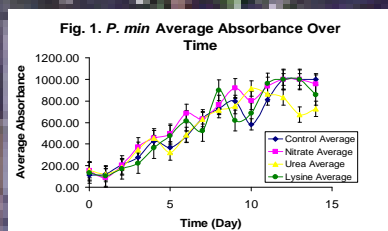
Picture courtesy of http://www.cse.noaa.gov/crs/lca/app_nbj.html



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Methods

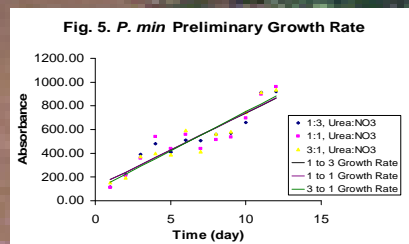
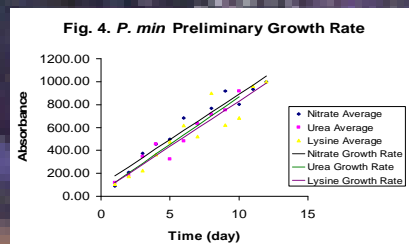
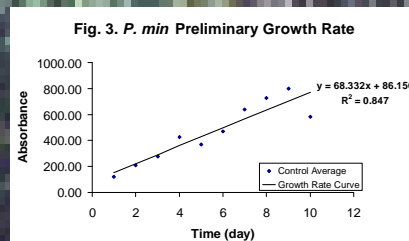
F/15 medium was made with 7 different types and ratios of nitrogen. The treatments included: control (no nitrogen), nitrate, urea, lysine, and ratios of urea to nitrate of 1:3, 1:1, and 3:1. Each treatment was set up in triplicate test tubes and inoculated with *P. minimum* that was grown on urea. Over a three-week period, absorbance measurements were taken for each of the triplicate test tubes using a Turner fluorometer. A fast repetition rate fluorometer (FRRF) was used on Day 9 to obtain values for Fv/Fm, which gives an indication of the photosynthetic efficiency of the cells.



Figures 1 and 2 (above). Average absorbances of *P. min* during the first 14 days of the experiment. Readings were taken every morning between 0900 and 1000.

Results

From average absorbance data (Figures 1 and 2), linear trend lines were applied to obtain growth rate curves (Figures 3-5). The data suggest that *P. minimum* grew faster with urea than with nitrate, lysine, or the urea:nitrate ratios. The Fv/Fm values (Figure 6) though show that the cells were more efficient with lysine, followed by nitrate, the ratios and then urea. It is possible that *P. minimum* grew faster with urea because the cells were grown in urea media before the inoculations were performed.



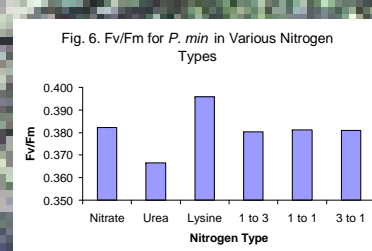
Figures 3-5 (above). Preliminary growth rates (first 12 days) of *P. min* based on absorbance values collected from a Turner Fluorometer.

Table 1 (below). Equations and R2 values for the growth rate curves.

Table 1. Growth Rate Equations (y=mx+b)	
Nitrate	y = 79.284x + 98.023, R2 = 0.947
Urea	y = 82.929x + 36.951, R2 = 0.9422
Lysine	y = 79.034x + 40.086, R2 = 0.8863
1:3	y = 62.414x + 115.54, R2 = 0.8949
1:1	y = 62.706x + 114.69, R2 = 0.8415
3:1	y = 66.124x + 90.53, R2 = 0.9028

References

- Berg, G.M., P.M. Glibert, M.W. Lomas, M.A. Burford. 1997. Organic nitrogen and growth by the chrysophyte *Aureococcus anophagefferens* during a brown tide event. *Mar. Biol.* 129:377-387.
- Fan, C., P.M. Glibert, J. Alexander, M.W. Lomas. 2003. Characterization of urease activity in three marine phytoplankton species, *Aureococcus anophagefferens*, *Prorocentrum minimum*, and *Thalassiosira weissflogii*. *Mar. Biol.* 142:949-958.



Discussion and Conclusions

A single experiment was performed and needs to be repeated. *P. minimum* appears to grow faster with urea than any of the other nitrogen types/ratios used in this experiment, but nutrients (nitrate/nitrite and phosphate) still need to be analyzed so that we can get some insight into the extent of their use by the phytoplankton. 3-6 chlorophyll a standards also need to be analyzed on a Perkin Elmer UV/VIS Spectrophotometer to obtain absorbance values at different wavelengths. These absorbances will then be placed on a regression curve to determine the chlorophyll concentrations of the standards. The standards will be analyzed on the Turner fluorometer to produce a standard curve so absorbance readings can be paired with chlorophyll concentrations from the Perkin Elmer analysis. This will allow us to associate our absorbance readings of the samples with chlorophyll concentrations. Future work will aim at determining the effect of DON, DIN and dissolved organic carbon (DOC) on HABs species like *Prorocentrum minimum* and *Aureococcus anophagefferens*.

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