Evaluating The Interactive Effects of Elevated pH and Hypoxia on Growth of The Diatom *Thalassiosira pseudonana*: Implications for Harmful Algal Blooms in The Laurentian Great Lakes

Jed Howrey as advised by Dr. Carlo Moreno

THE COLLEGE OF WOOSTER

Background

In the Laurentian Great Lakes, eutrophication, characterized by excessive nutrient inputs from fertilizer runoff, is a major issue. Harmful algal blooms (HABs) which result from eutrophication pose significant ecological and economic threats to aquatic ecosystems (Fig. 1). Hypoxia (i.e., dissolved oxygen < 2.0 mg O_2 L^{-1}) and elevated pH are adverse environmental conditions which commonly occur in ecosystems facing HABs, however their combined effects on phytoplankton growth are still not well understood. One group of phytoplankton species affected by HABs are diatoms, a critically important type of algae which produce around 40% of Earth's breathable oxygen. The aim of this study is to investigate the interactive effects of hypoxia and elevated pH on the growth of *Thalassiosira pseudonana*, a model diatom species.

Figure 1 - Harmful Algal Bloom
2011 Cyanobacteria bloom in the western basin of Lake Erie photographed by a NASA satellite.
Source: NOAA

Research Question

- **How do elevated pH and hypoxia affect the growth of the diatom *Thalassiosira pseudonana*?**
- **Are there significant interactive effects between elevated pH and hypoxia on the growth of the diatom *Thalassiosira pseudonana*?**

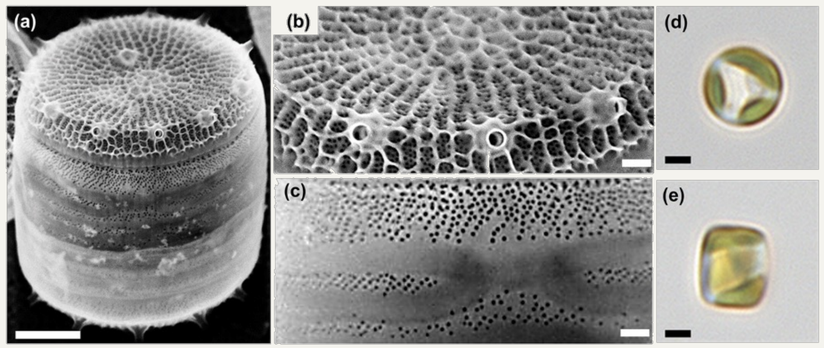


Figure 2 - *Thalassiosira pseudonana*
(a–c) Scanning electron microscopy of the *T. pseudonana* silica cell wall of a single whole cell (a) and details of the valve (b) and girdle band (c) regions. (d, e) Bright field microscopy image of single cells in valve (d) and girdle (e) view. Scale bars: (a–c) 1 µm, (d–e) 2 µm.
Source: Poulsen & Kröger, 2023

Experimental Setup

Stock cultures of *Thalassiosira pseudonana,* acquired from the University of Texas at Austin Culture Collection of Algae, were grown in F/2 medium. Cultures were maintained in a growth chamber with a temperature of 20°C, light intensity of ~900 lux, a 12:12 photoperiod, and orbital shaking set to 70 rpm. To kill contaminating bacteria and prevent future bacterial growth, penicillin and streptomycin (270µg/mL) were added to the stock culture.

For the experiment, 16 Erlenmeyer flasks were inoculated with *T. pseudonana* with each set of conditions; control, hypoxia, elevated pH, and combined hypoxia and elevated pH occupying 4 flasks (Fig. 3). To control the pH of the F/2 medium added to the flasks, NaOH was added until desired levels were achieved. To control the dissolved oxygen (DO), the relevant flasks were flushed with nitrogen gas and were sealed with gas-tight rubber septums once desired levels were reached.

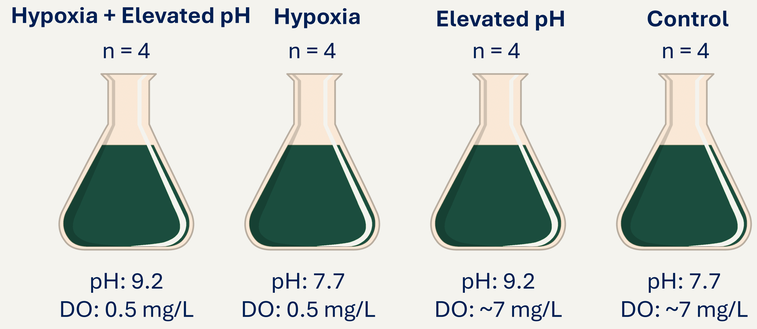


Figure 3 - Setup of Experimental Groups
Setup of treatment groups in the study.

Data Collection

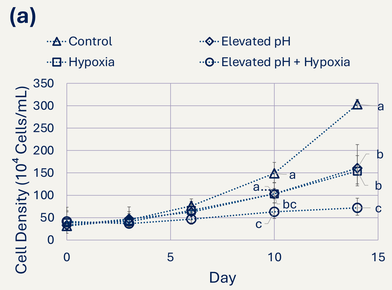
Subsamples were collected on days 0, 3, 6, 10, and 14 after inoculation. To determine cell density, a hemocytometer was used. For each flask, measurements were taken twice and averaged. For cell size, two cells from each flask were selected randomly and photographed. The subsequent photographs were uploaded to Infinity Analyze and size measurements were taken and averaged for each flask. Specific growth rate was calculated using the cell density measurements from days 0 and 14 and the following equation:

$$\mu = \frac{\ln(CD_{14}) - \ln(CD_0)}{t_{14} - t_0}$$

Statistical Analysis

To test for differences in cell density and cell size between treatment groups over time, a two-way repeated measures ANOVA was used. To account for multiple comparisons, the Bonferroni adjusted significance level was set at α=0.025. Because cell density did not meet assumptions of normality, a log transformation was used. For post-hoc analysis, Sidak-corrected pairwise comparisons were conducted. To test for differences in specific growth rate between treatment groups, a one-way ANOVA was conducted. For post-hoc analysis, Tukey's HSD was used.

Results



- For cell density, the interaction between treatment and time was significant (df=12, F=11.502, p<0.001) (Fig. 4a).
- For cell size the interaction between treatment and time was not significant (df=12, F=0.855, p=0.597) (Fig. 4b)
- For specific growth rate, treatment had a significant effect (df=3, F=17.364, p<0.001) (Fig. 4c)

Figure 4 - Experimental Results
Cell density (a), cell size (b), and specific growth rate (c) response in cultures of the diatom *Thalassiosira pseudonana* maintained for 14 days under four treatment groups (control, hypoxia, elevated pH, combined elevated pH and hypoxia). For cell density, each data point represents the back-transformed mean (± back-transformed 95% CI) of four replicate cultures. For cell size each data point represents the mean (± SE) of four replicate cultures. For specific growth rate each bar represents the mean (± SE) of four replicate cultures. Cell density means that are significantly different in Sidak-corrected pairwise comparisons and specific growth rate means that are significantly different in Tukey's test are indicated by different letters (p<0.05).

Discussion

The experimental findings have a few key implications. First, the significant interactive effects between elevated pH and hypoxia on cell density and specific growth rate show that previous studies, which have only measured the effect of a single variable, likely understate the true effect that HABs have on diatoms. Additionally, since such differences only appeared after around 10 days, the duration of HABs is important to the magnitude of the effects. Second, the lack of significant differences in cell size implies that *T. pseudonana* may not face increased predation pressure resulting from HABs. Given the importance of diatoms to humans and the fact that, in many places, nutrient loading targets are still not being met, the relative inability of the government to reduce eutrophication in the Great Lakes should be extremely alarming.

Limitations and Future Aims

A major limitation of the study was that only two abiotic factors; dissolved oxygen and pH, were tested. Going forward it would we interesting to look at how additional factors such as turbidity and the presence of microcystin toxins affect diatoms. Additionally, it would be interesting to conduct a study on diatoms co-cultured with *Microcystis aeruginosa*, the predominant cyanobacterial species in Great Lakes HABs, to determine if these conditions confer a competitive advantage.