IMPACT OF NUTRIENT AND LIGHT LIMITATION ON THE TOXIC DINOFLAGELLATE *KARENIA BREVIS*

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BACKGROUND

- Harmful algal blooms (**HABs**) pose significant ecological and economic challenges globally, with **Florida** serving as a hotspot. These blooms, driven by nutrient inputs and complex environmental factors, particularly affect coastal regions.
- ***Karenia brevis***, a toxic mixotrophic (photoautotrophy and heterotrophy) dinoflagellate, contributes notably to HAB occurrences in Florida.
- Understanding the dynamics of HABs formation, including the role of **nutrients** and **prey** availability, is critical. This study investigates the effects of nutrient and light limitation on ***Karenia brevis*** growth, alongside its grazing behavior on ***Synechococcus* sp.**

OBJECTIVES

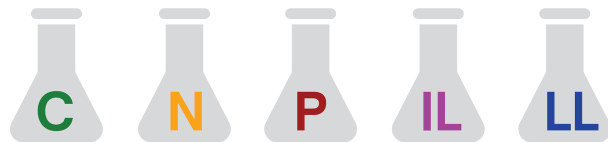
- What are the effects of **reduced nutrient** concentrations and diminished light intensities on the growth and population densities of ***Karenia brevis*** in laboratory cultures?
- Additionally, how does the introduction of ***Synechococcus* sp.** as prey influence the behaviour and growth of ***Karenia brevis*** under the same conditions of environmental limitation?

METHODOLOGY

5 experimental conditions: Control (**C**), Nitrogen limited (**N**), Phosphate limited (**P**), Intermediate light (**IL**) and Low light (**LL**).

Nutrient ratios:	**Light conditions:**
C,IL,LL: N:P=**16:1**	C,N,P: **120** µmol m-2s -1 photons
N: N:P=**3:1**	IL: **60** µmol m-2s -1 photons
P: N:P=**85:1**	LL: **30** µmol m-2s -1 photons

- **Experiment 1**: Light microscopy, Nutrient analysis, Pigment analysis;
- **Experiment 2**: Grazing assay, Fluorescence microscopy.



FINDINGS

- **EXPERIMENT 1 - LIMITING CONDITIONS**

Statistically significant differences were observed in cell yield between control and **nutrient-limited treatments**. **Light intensity** did not significantly affect cell yield, although low light conditions delayed exponential phase growth. There results are reflected in the **chlorophyll-a** analysis.

- **EXPERIMENT 2 - GRAZING ASSAY**

Unexpected lower yields observed in the presence of ***Synechoccus* sp.** Fluorescence microscopy indicated no active feeding of ***Karenia brevis***.

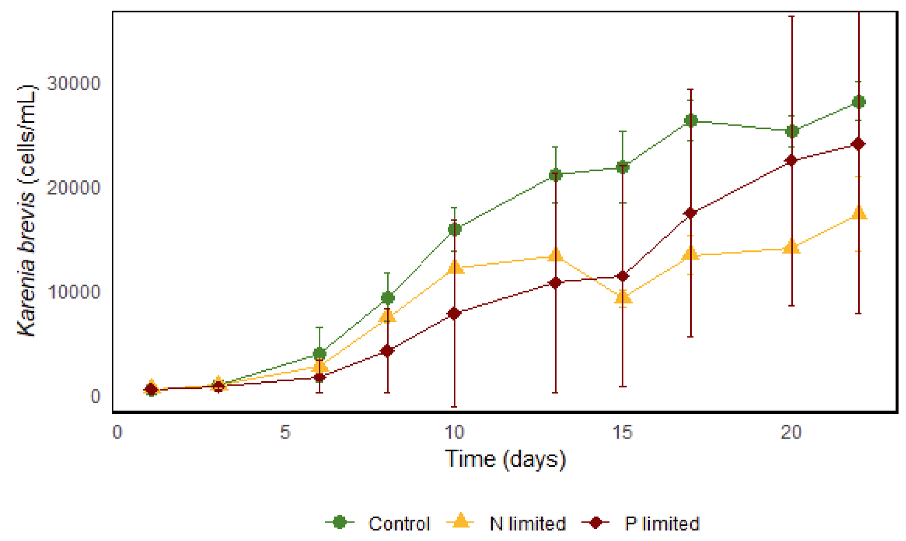


Figure 1. Cell counts of the experimental groups (C, N, P) plotted against time.Error bars are representative for the value range (standard deviation) of the replicates within each group.

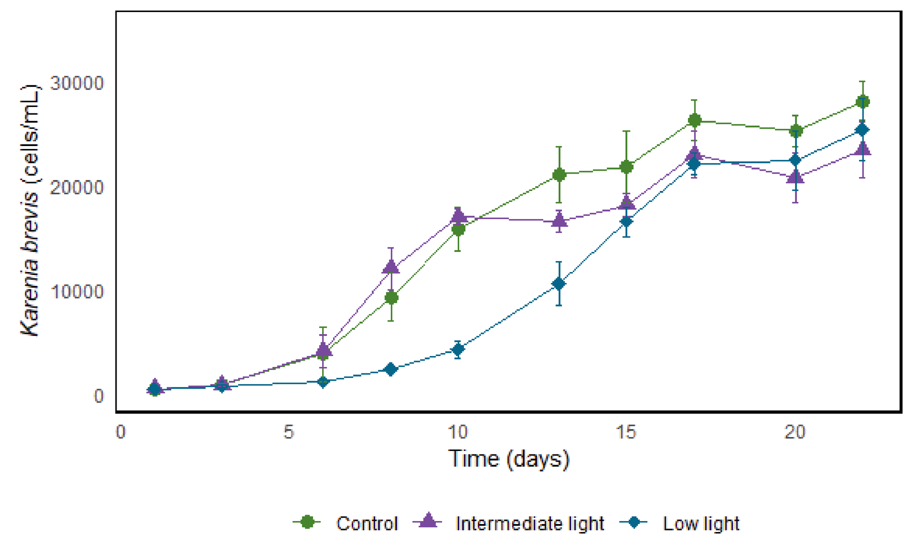


Figure 2. Cell counts of the experimental groups (C, IL, LL) plotted against time.Error bars are representative for the value range (standard deviation) of the replicates within each group.

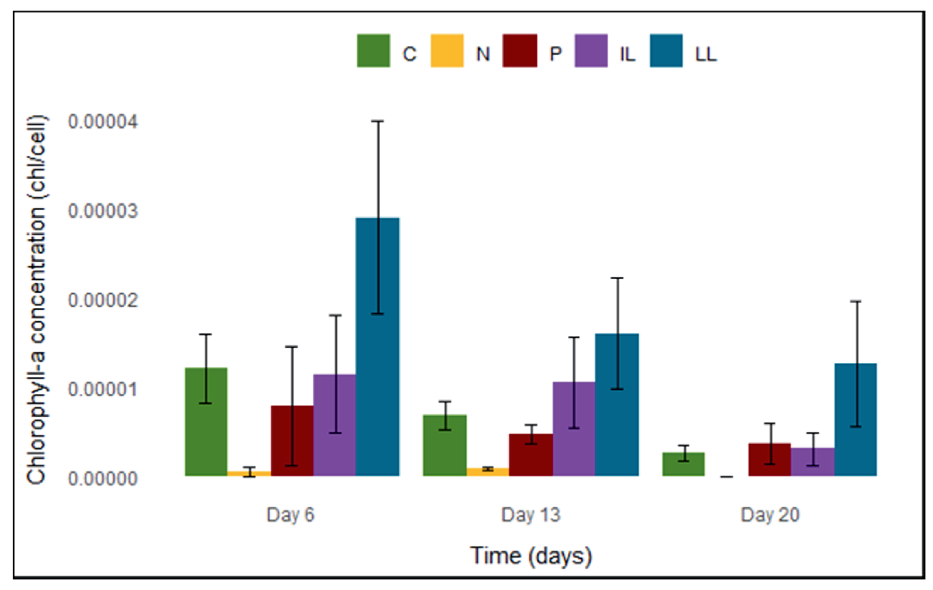


Figure 3. Concentration of chlorophyll-a pigments during the experiment. The values were averaged between the replicates in each group, with the error bars representing the standard deviation.

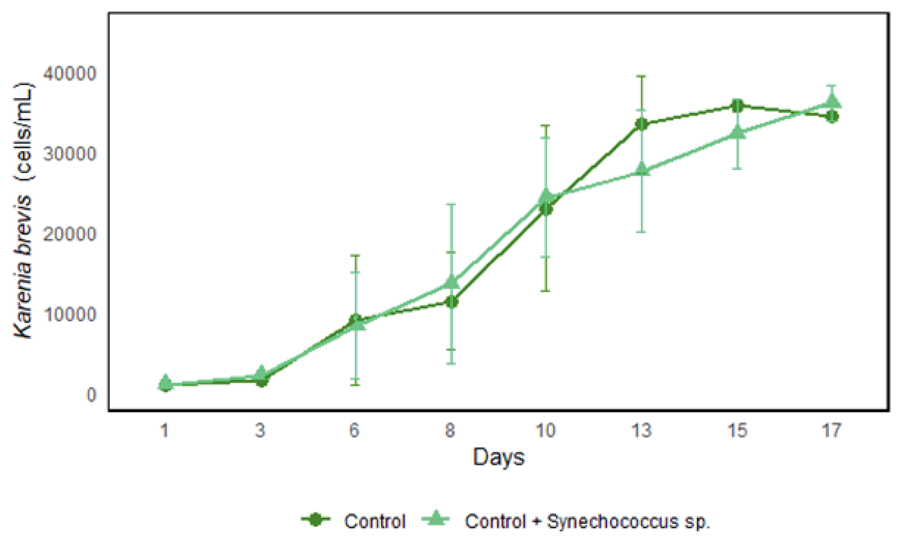


Figure 5. Cell counts for the experimental groups: Control (n=2), Control+*Synechococcus sp.* (n=3). Error bars are representative for the value range (standard deviation) of the replicates within each group.

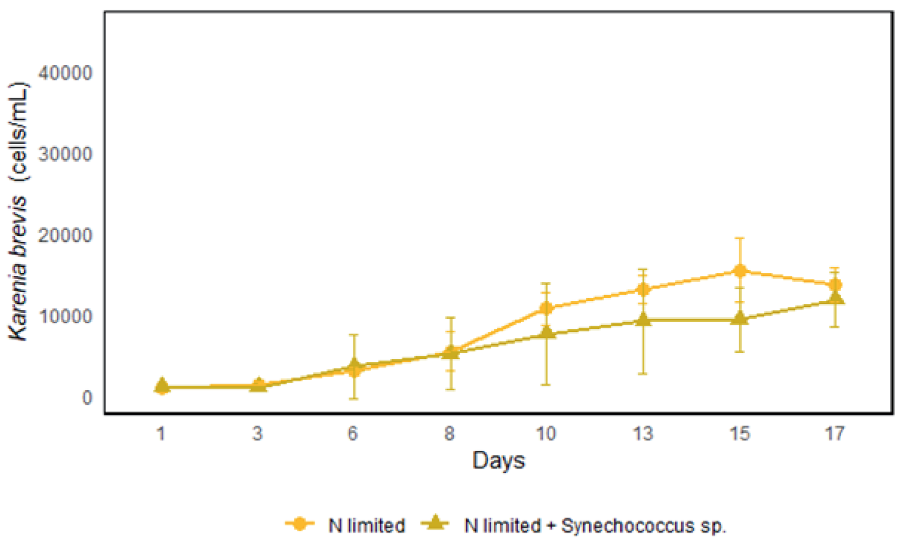


Figure 6. Cell counts for the experimental groups: N-limited (n=2), N-limited+*Synechococcus sp.* (n=3). Error bars are representative for the value range (standard deviation) of the replicates within each group.

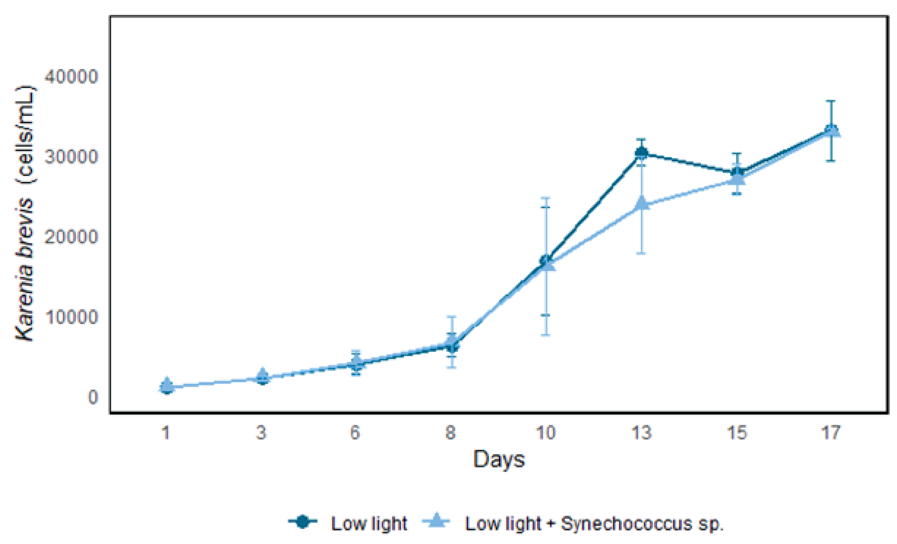


Figure 7.Cell counts for the experimental groups: Low light (n=2), Low light+*Synechococcus sp.* (n=3). Error bars are representative for the value range (standard deviation) of the replicates within each group.

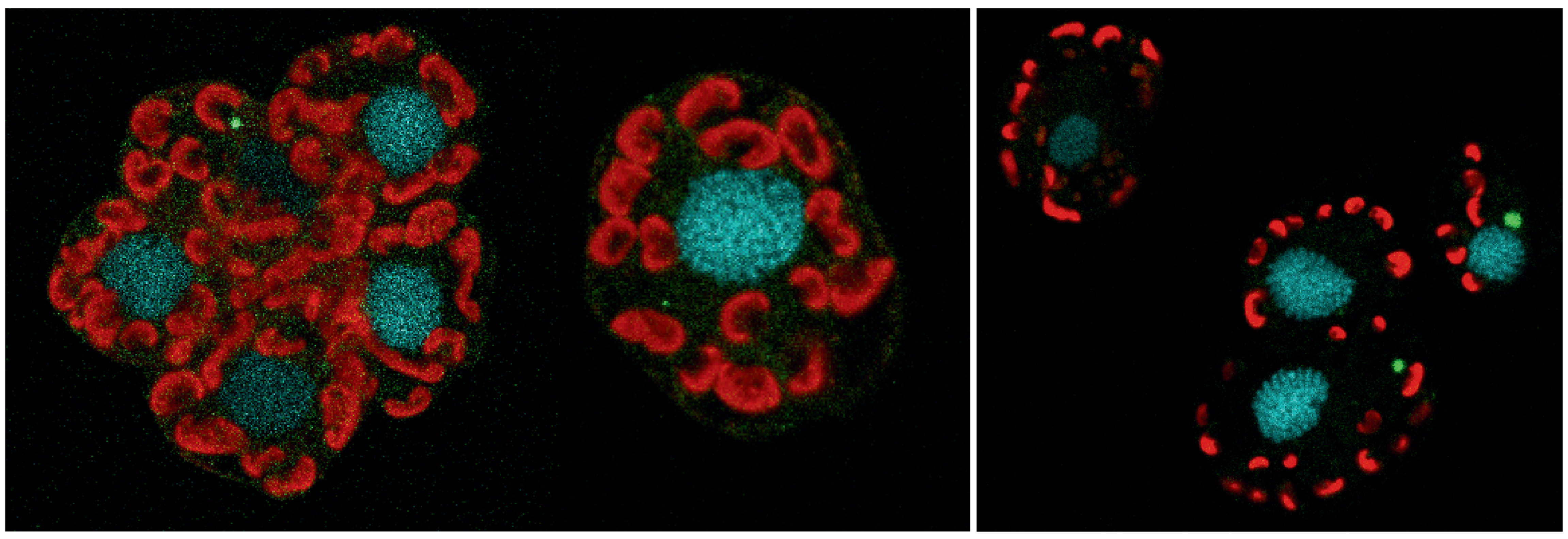


Figure 4. Confocal fluorescence microscopy images of *Karenia brevis*. ***Left:*** Low light + *Synechococcus* sp. treatment; ***Right:*** N-limited + *Synechococcus* sp. treatment. In blue emission are shown the nucleus, in red emission the chloroplasts and in green emission an unidentified organelle.

TAKE-HOME MESSAGE

- **Nitrogen** scarcity significantly reduced cell densities, but unclear response to **phosphate** limitation.
- Contrary to initial hypotheses, the addition of ***Synechococcus* sp.** resulted in slightly lower maximum cell yields, challenging the anticipated benefits of mixotrophic feeding.
- Fluorescence microscopy analysis further details the absence of mixotrophic interactions.

CONCLUSIONS

- The current study is a first step, leading to a more in-depth analysis aimed at the production of **toxins** and **lipid biomarkers** by ***Karenia brevis***.
- The observed effects of nutrient and light limitations offer implications for understanding the ecological factors influencing the proliferation of ***Karenia brevis*** and the potential formation of HABs in natural environments.

Anderson, D. M., Cembella, A. D., & Hallegraeff, G. M. (2012). Progress in understanding harmful algal blooms: paradigm shifts and new technologies for research, monitoring, and management. Annual review of marine science, 4, 143-176;Glibert, P. M., & Burkholder, J. M. (2018). Causes of harmful algal blooms. Harmful algal blooms: A compendium desk reference, 1-38; Guillard, R. R. (1975). Culture of phytoplankton for feeding marine invertebrates. In Culture of marine invertebrate animals: proceedings—1st conference on culture of marine invertebrate animals greenport (pp. 29-60). Boston, MA: Springer USHardison, D. R., Sunda, W. G., Shea, D., & Litaker, R. W. (2013). Increased toxicity of Karenia brevis during phosphate limited growth: ecological and evolutionary implications. PLoS One, 8(3), e58545; Jeong, H. J., Yoo, Y. D., Kim, J. S., Seong, K. A., Kang, N. S., & Kim, T. H. (2010). Growth, feeding and ecological roles of the mixotrophic and heterotrophic dinoflagellates in marine planktonic food webs. Ocean science journal, 45, 65-91.; Landsberg, J. H., Flewelling, L. J., & Naar, J. (2009). Karenia brevis red tides, brevetoxins in the food web, and impacts on natural resources: Decadal advancements. Harmful Algae, 8(4), 598-607.; https://www.vox.com/2021/8/4/22606025/florida-red-tide-dead-fish-beach-algae-bloom