Understanding and describing the growth of the coccolithophore *Emiliania huxleyi*, an important microalgae in the ocean

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INTRODUCTION

- *Emiliania huxleyi* (*E. hux*) is a unicellular microalgae (group of coccolithophores), found in most marine ecosystems around our planet
- Growth of this species is characterized by four growth phases: lag, exponential, stationary, and death phase
- Due to high abundance around the globe, this species is an integral part of the marine food-web
- *E. hux* is also a carbon sink, transporting much atmospheric CO_2 into the deep sea when it dies and sinks due to heavy coccoliths as a ballast material
- Once on the ocean floor, coccoliths build marine sediments and form chalk, such as the White Cliffs of Dover
- Coccoliths are also important as deterrent for being eaten by zooplankton (Strom et. al., 2017)
- This species plays a large role in the global carbon cycle with $CaCO_3$ coccolith production releasing CO_2 and photosynthesis capturing and eventually trapping it on the sea floor

Figure 1. Cliffs of Dover compared to *E. hux*

METHODS

- *E. hux* triplicate cultures were grown in artificial seawater made with environmentally relevant nutrient concentrations (low concentration of nitrogen)
- Flow cytometry and variable fluorescence measurements were taken daily over the duration of the experiment (four weeks)
- Nutrient samples were taken daily and frozen until analysis to track the nutrient content of the triplicates. Nutrients were measured using spectrometric analysis.
- Cell count, Median Chlorophyll-A (Chla-A), Median relative cell size, and photosynthetic quantum yield (indicator for cell health) were recorded

RESULTS

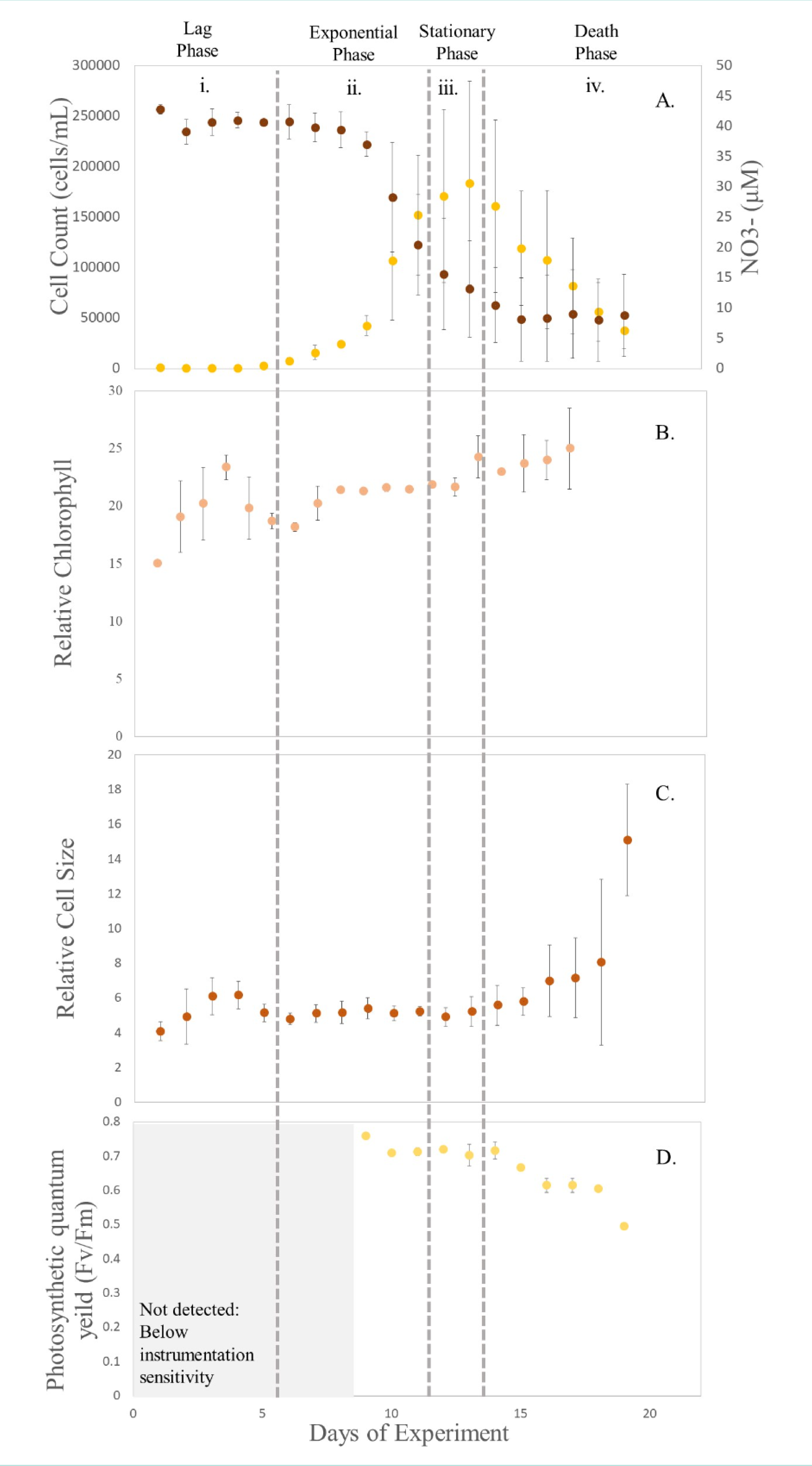

Figure 2. Cell Responses
A. Cell Counts per day compared to NO3 per day
B. Relative Chlorophyll levels per day
C. Relative Cell Size per day
C. Photosynthetic quantum yield per day

- lag phase: $\mu = 0.20$ d^{-1}
- exponential phase: $\mu = 0.60$ d^{-1}
- stagnant phase: $\mu = 0.10$ d^{-1}
- death phase: $\mu = -0.29$ d^{-1}
- *E. hux* grew at a growth rate of 0.6 until NO_3 was drawn down to 47.8% of its initial concentration. The cells slowed down growth to a rate of 0.1 and 30.7% residual NO_3.
- Once NO_3 was nearly drawn down, the cells stopped replicating but changed some of their cellular properties (grew larger and increased cellular chlorophyll). Cells did not draw down NO3 to completion.
- The photosynthetic quantum yield started to drop at the end of the exponential phase and more significantly during the death phase.

$$\mu = \frac{\ln(D) - \ln(D_o)}{t}$$

Figure 3. Dense *E. hux* culture before mixing
Most cells settled on bottom

Figure 4. Dense *E. hux* culture after mixing
Cells uniformly in media

CONCLUSION

- While our initial experimental design did not work (grow the red tide *Karenia brevis* and feed with *E. hux* and *Pro* cultures) I was able to characterize the growth of an important phytoplankton species, *E. hux*. Using the dataset, we will now be able to time *E. hux* harvesting for this experiment much better.
- It took the cells about five days to acclimate to their new conditions before starting to grow exponentially.
- Biovolume and Chla-A per cell increased, hinting to phosphorus limitation of the cells (not measured) rather than nitrogen - (McKew et al 2015).
- *E. hux* appears to be PO4 limited, strongly reduced its capability to efficiently photosynthesize. This experiment showed that PO_4 needs to be enhanced in the medium in order to deplete the cells in N.
- Peak harvesting time is between days 11 and 13, after which cell health quickly declines.
- During death phase, *E. hux* increased in size, making it harder for certain grazers to consume but the enlargement likely leads to faster sinking and thus increases the role this organism plays in the global carbon cycle.

FUTURE DIRECTIONS

- *E. hux* will be harvested at its peak growth and fed to *Karenia brevis*, the algae that makes up the red tide here in Florida. This will be used to understand if *Karenia brevis* can utilize organic matter produced by *E. hux* as a food source
- Due to *E. hux* not utilizing all NO_3, it is important to wash all nutrients off when harvesting it to feed to *Karenia brevis*, and/or to enhance PO_4 in the medium
- Cells need to be harvested at around 150,000-200,000 cells/mL for the nutrient ratio applied in this experiment, which was the maximum density measured in the triplicates

REFERENCES

Assmy, P., & Smetacek, V. (2009). Algal Blooms. *Elsevier EBooks*, 27–41. https://doi.org/10.1016/b978-012373944-5.00001-8

Li, J., Nedwell, D. B., Beddow, J., Dumbrell, A. J., McKew, B. A., Thorpe, E. L., & Whitby, C. (2015). *amoA* Gene Abundances and Nitrification Potential Rates Suggest that Benthic Ammonia-Oxidizing Bacteria and Not Archaea Dominate N Cycling in the Colne Estuary, United Kingdom. *Applied and Environmental Microbiology*, *81*(1), 159–165. https://doi.org/10.1128/aem.02654-14

Maxwell, K., & Johnson, G. N. (2000). Chlorophyll fluorescence—a practical guide. *Journal of Experimental Botany*, *51*(345), 659–668. https://doi.org/10.1093/jexbot/51.345.659

Strom, S. L., Bright, K. J., Fredrickson, K. A., & Cooney, E. (2018). Phytoplankton defenses: Do Emiliania huxleyi coccoliths protect against microzooplankton predators? *Limnology and Oceanography*. https://doi.org/10.1002/lno.10655