
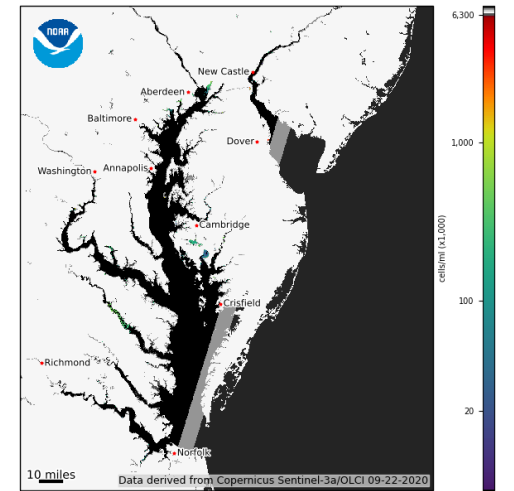
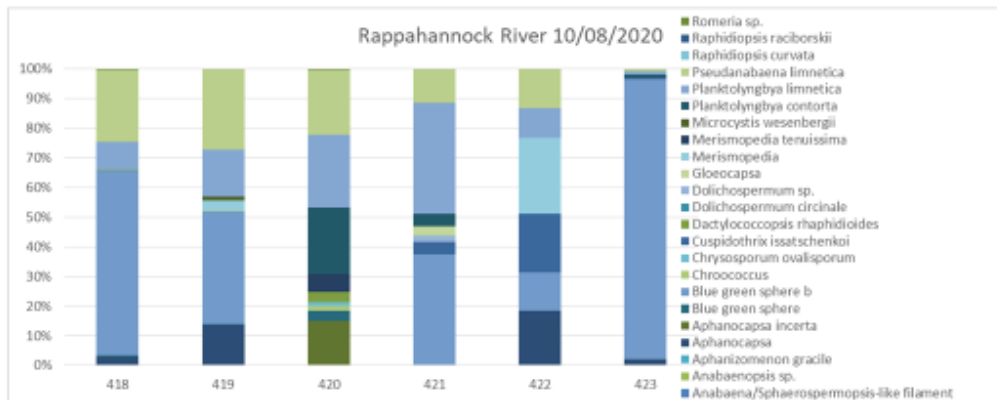
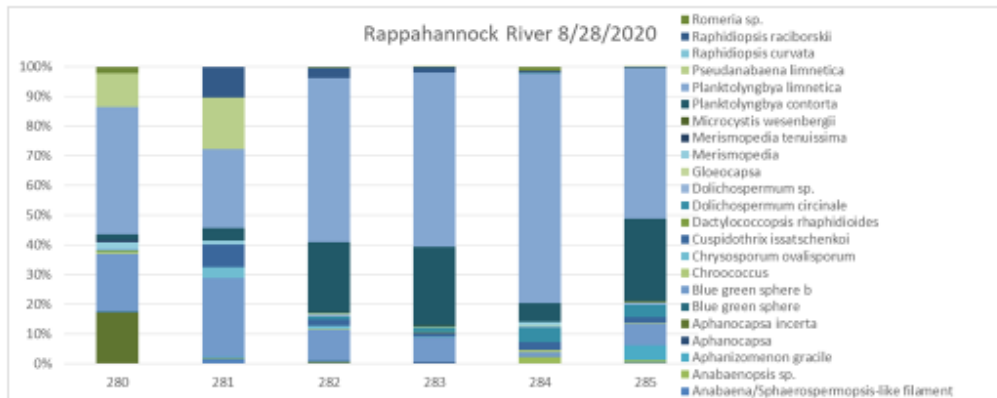


2022 Building Capacity for Biotoxins and Imaging Technologies

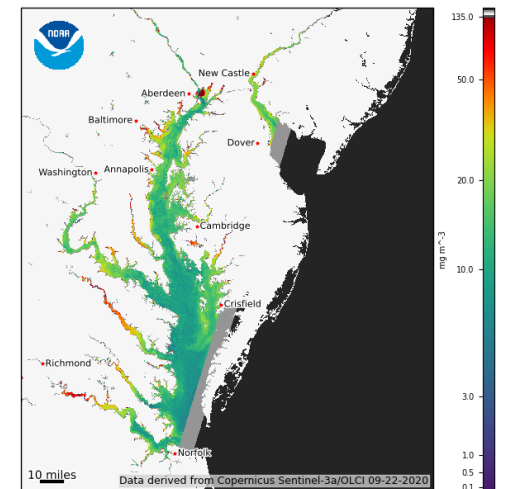


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Validation of NOAA hyperspectral satellite products



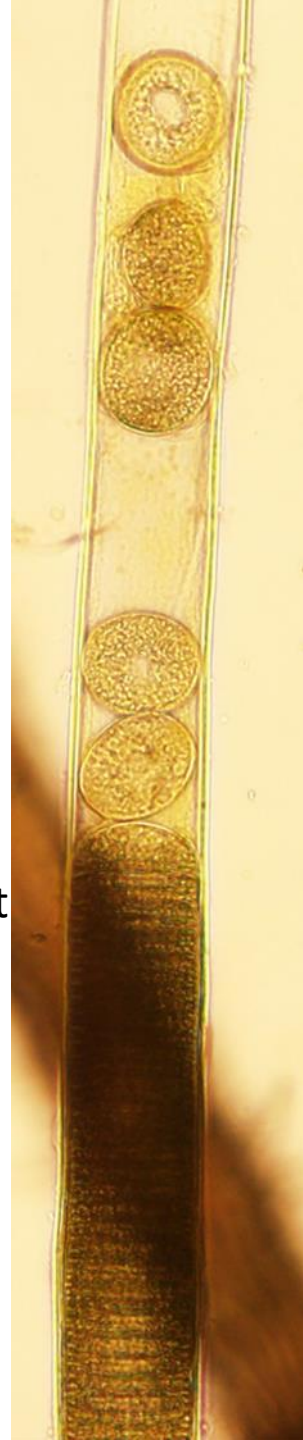
Cyanobacteria Index (Clyc) for Chesapeake Bay. Moderate and low concentrations may not be obvious to the eye.



Chlorophyll concentrations (mg m^{-3}) for Chesapeake Bay.

New Biotoxins Safety Protocol to allow for the continued development of a benthic protocol

- Collection method – grabs
- Prep method for taxonomy – identify dominant taxa
 - No cell counts
- Prep method for toxins –
 - Remove excess water
 - Weigh 5g of sample
 - Add 5 mL appropriate dilutant specific to each test
 - Lyse using freeze/thaw method with addition of mortar pedestal and t homogenizer
 - Separate vegetative material of liquid using centrifuge
 - Run using standard ELISA method
 - Reporting toxin ppb/g wet weight



Microcystis aeruginosa: Understanding the controls of toxin production during a bloom and potential impacts of climate change

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INTRODUCTION

- Harmful algal blooms (HABs) have increased in Virginia and worldwide over the last century due to and influx in anthropogenic activities.
- Microcystis aeruginosa*, a colonial cyanobacterium, is a common cyanobacteria forming species. It is known for production of the hepatotoxin microcystin, which can be harmful to aquatic and terrestrial organisms[1].
- As a result of climate change, *M. aeruginosa* is exposed to excess nutrients and increases in pCO₂. This has the potential to alter toxin production and proliferation of future blooms [2].
- This study can give insight on how policymakers and agencies can organize and how to implement restrictions in recreational lakes, drinking water reservoirs, and other waterways in current and future situations.

Objectives

- Is the growth of *M. aeruginosa* sensitive to pCO₂ and at what pCO₂ concentrations are growth rates optimal?
- Do increases in pCO₂ increase toxin production?
- From a resource management standpoint, when in their growth cycle are intracellular and extracellular toxins greatest for *M. aeruginosa*?

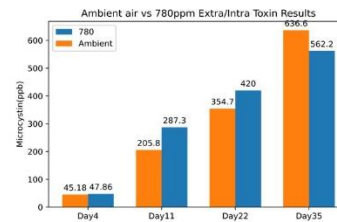
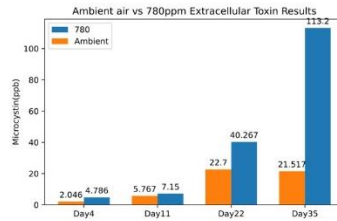
METHODS



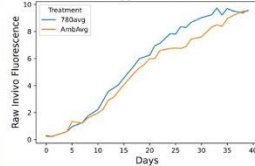
Experimental set up

- Experiments employed batch culturing techniques (in triplicates) that were maintained in a walk-in temperature controlled environmental chamber. Set at 25°C.
- CO₂ concentrations were maintained by gentle and constant bubbling with AirGas-filtered air mixtures enriched with ambient air, 780ppm and 1200ppm.
- Six 2 Liter bottles of BG-11 media were autoclaved and cooled.
- Each bottle was inoculated to have a final concentration of 99,583 cells/ml.
- Saturating irradiance was provided by a cool white fluorescent light banks at a 12-12 hour light: dark cycle.
- In-vivo fluorescence was measured with a fluorometer using the non-acidified method to calculate biomass along with cell counts with a hemocytometer.
- pH was measured weekly to monitor CO₂ concentrations.
- Toxins measurements were taken at 5 time points during the growth cycle
 - Early
 - Mid exponential
 - Late exponential
 - Peak
 - Crash (not shown)
- Each sample was filtered on a GF/F filter to separate cells from water to obtain extracellular toxins.
- Whole water (Intracellular and extracellular together), extracellular toxins, and GF/F filter were stored in -80 Freezer until analysis.
- Toxin analysis were conducted using Eurofins Abraxis Microcystins/Nodularins (ADA) (EPA ETV) (EPA Method 546) ELISA kit [PN: 520011] and CAAS cube.

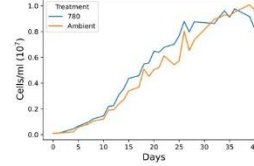
RESULTS



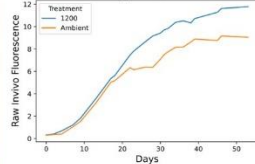
Ambient vs 780ppm In-Vivo Fluorescence



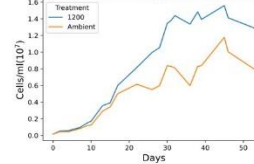
Ambient vs 780ppm Cell counts



Ambient vs 1200ppm In-Vivo Fluorescence



Ambient vs 1200ppm Cell counts



REFERENCES

1 Anderson, D.M., Gilbert, P.M., Burkholder, J.M. 2002. Harmful algal blooms and eutrophication: Nutrient sources, co-occurrence, and consequences. *Estuaries* 25: 704-726. <https://doi.org/10.1007/s12237-002-0050-0>

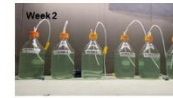
2 Wagner, N.D., Osburn, F.S., Wang, J., Taylor, R.B., Boedeker, A.R., Chambliss, C.K., Brooks, B.W., Scott, J.T. 2019. Biological Stoichiometry Regulates Toxin Production in *Microcystis aeruginosa* (UTEX 2385). *Toxins* 11, 601. <https://doi.org/10.3390/tox11090601>

DISCUSSION

- Cell count maximum concentrations of the 780 ppm treatment had a mean of 8,791,667 cells/ml compared to the ambient concentration mean of 8,041,667 cells/ml. Growth rates did not show any significant change.
- Toxin production of the 780 ppm treatment showed a difference between the filtered extracellular toxins as the density of the cultures increase.
- Further analysis is needed for toxins production and growth rate for the 1200 ppm treatment to confirm the difference between treatments.

Future Plans

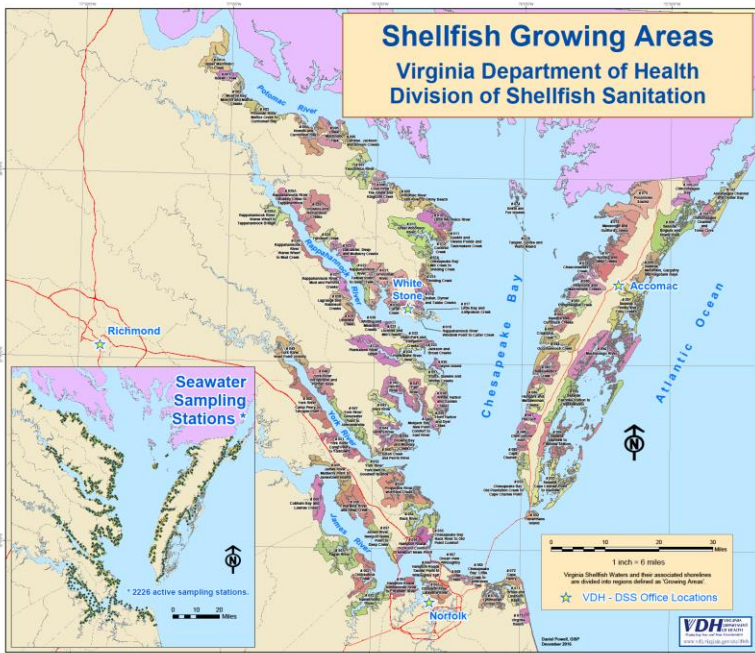
- Scaling the project down, add more replicates for each treatment, and implementing semi-continuous culturing techniques.
- Conduct toxin analysis for 1200ppm treatment.
- Conducting robust statistical analysis across treatments.



ACKNOWLEDGMENTS

This project was funded by the Chesapeake Bay Program. Thank you to the Ocean and Earth Graduate Student Organization for the travel award. Very grateful to the Mulholland Lab and Barshis Lab for allowing me to use their equipment. Lastly, a special thanks to Imani Booth for assistance in processing samples, Kathryn Mogatas and Leah Gibala-Smith for mentoring and support.

Building image libraries using FlowCam, IFCB, and Planktoscope



Imaging Technologies Build Capacity and Accessibility in Phytoplankton Species Identification Expertise for Research and Monitoring: Lessons Learned During the Covid-19 Pandemic

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THE PROBLEM:
Phytoplankton identification and enumeration relies on the skills and expertise of highly trained taxonomic analysts. The training of new taxonomic analysts is intensive and requires months to years of supervised training before an analyst is able to independently and consistently apply identification skills to a sample. During the Covid-19 pandemic, access to microscopes and laboratories in the Phytoplankton Analysis Laboratory at Old Dominion University was greatly restricted, and social distancing requirements prevented supervised training of new graduate students and staff.



Figure 1: Technologies and tools used during Covid-19 lockdown for phytoplankton species identification training.

THE SOLUTION:
Access to phytoplankton technologies (Imaging FlowCytobot, FlowCam, and PlanktoScope) with online taxonomic platforms (EcoTaxa) provided the means to continue monitoring, research, and training activities remotely when in-person activities were restricted (Figure 1). Images were collected from samples and uploaded to EcoTaxa, and experienced analysts in the PhytoLab categorized images into groups (Figure 2) to teach trainees to recognize cell shapes, and how to recognize and classify taxa within functional groups. Senior analysts and trainees, using Zoom and share screen functions with the EcoTaxa project page in view, reviewed targeted taxa structure and discussed how to apply traditional microscope identification skills to reviewing the images. The trainees were asked to find further images of those taxa in the project's image collection. Trainers checked the trainees' annotations, and when difficulties arose, experienced analysts were able to teach the trainees how to better differentiate between similar looking groups either by real-time communication via Zoom or email for asynchronous responses. As the trainee would scan the image collection for their targeted taxa, they became familiar with other groups of organisms and were able to increase the number of taxa they could identify. Importantly, trainees were able to quickly identify a cell or group of cells that did not fall into a group they were familiar with, set them aside into a single temporary holding category area (Figure 3), and use their newly acquired skills to independently utilize traditional reference materials to determine their correct taxonomic group (Figure 4). This skill developed much more rapidly with our "COVID experiment" trainees than had occurred with traditionally trained students, within days or a few weeks as opposed to weeks or months.



Figure 2: Annotated images of *Akashiwo sanguinea* within the VirtualSpreadsheet Particle Analysis Software environment for FlowCam (above) and the EcoTaxa environment (below) for IFCB imagery used for training new analysts.

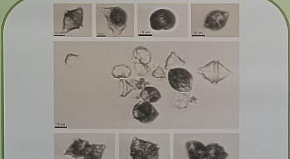


Figure 3: IFCB images of live *Heterocapsa triquetra* cells during a bloom event on the Lafayette River, VA 2019.



Figure 4: Comparative images of *Margalefidinium polykrikoides*, obtained during annual blooms on the lower Chesapeake Bay (2020 - 2022) using IFCB (live), PlanktoScope (Lugo's preserved), and FlowCam (Lugo's preserved).

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Clayton S., Gibala-Smith L., Mogatas K., FlowCam: C., Mulholland R., Mogatas K., Mulholland M. and Mulholland M. (2022) Imaging Technologies Build Capacity and Accessibility in Phytoplankton Species Identification Expertise for Research and Monitoring: Lessons Learned During the COVID-19 Pandemic. *Front. Microbiol.* 13:821195.

2023 Additional HAB projects

- Continued collaboration with VIMS to determine relationship between cells counts and the presence of shellfish toxins in tidal waters
- Assessment of automated image instrumentation for the detection of rare/uncommon HAB species in monitoring programs, and well as informing us as to the within bloom dynamics missed by infrequent sampling

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