2022 Building Capacity for Biotoxins and Imaging Technologies

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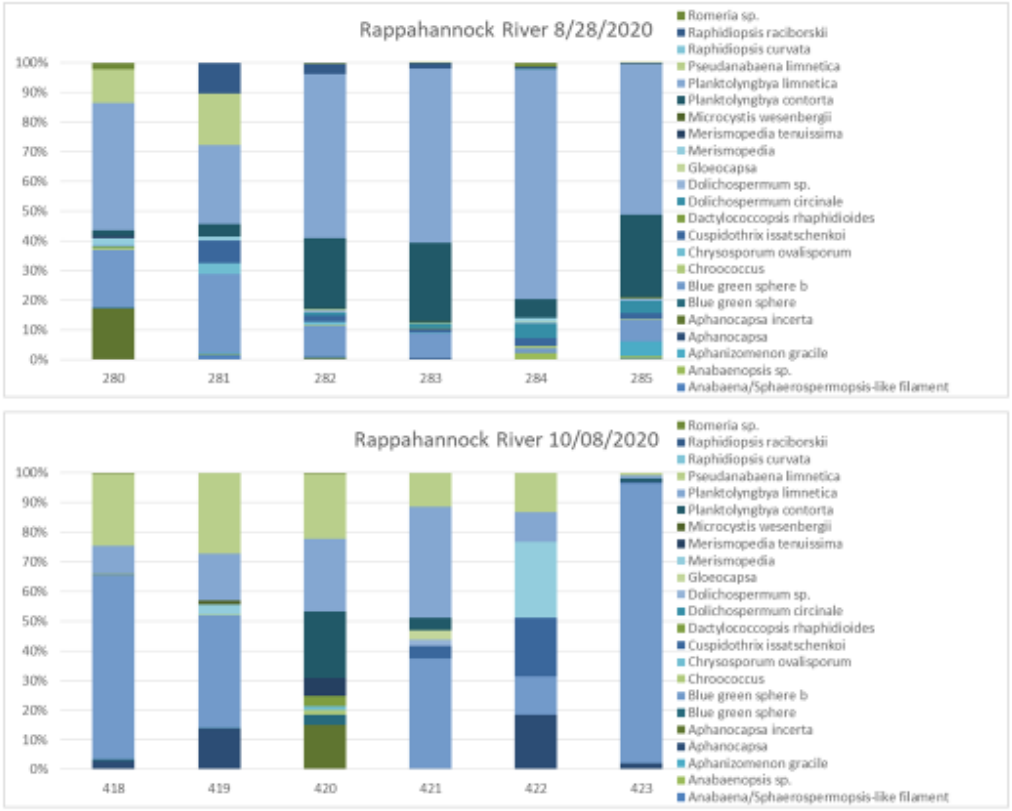
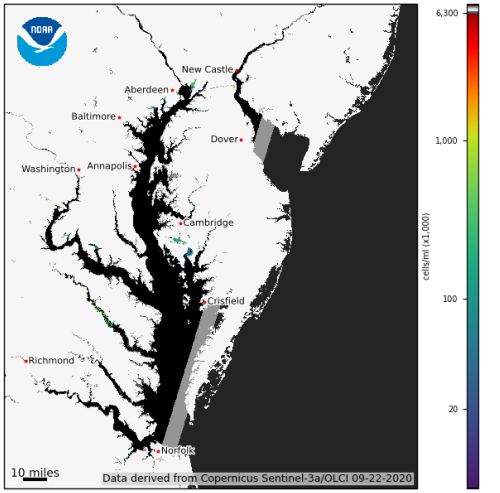
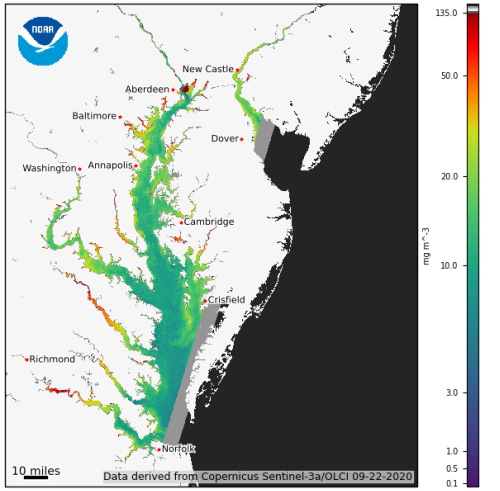
Validation of NOAA hyperspectral satellite products





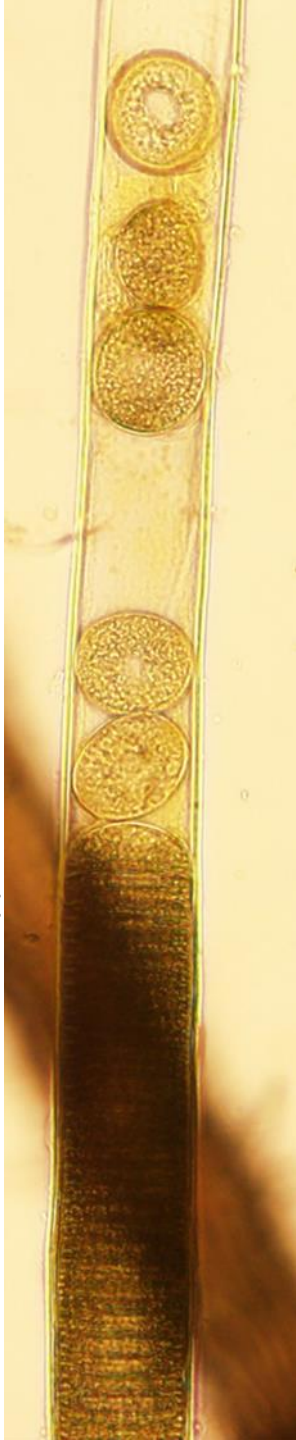
Cyanobacteria Index (CIcyano) for Chesapeake Bay. Moderate and low concentrations may not be obvious to the eye.



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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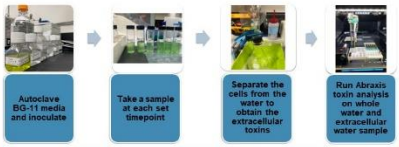
INTRODUCTION

- 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 cyanoHAB 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 pCO_2. 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*?

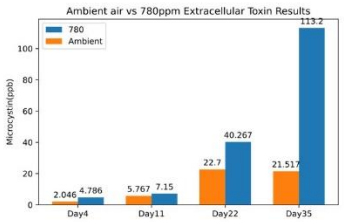
METHODS

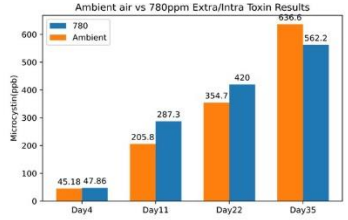


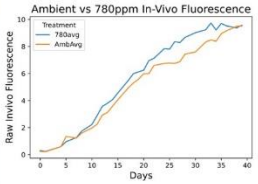
Experimental set up

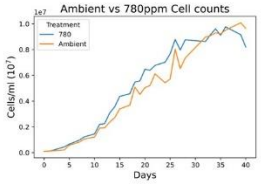
- Experiments employed batch culturing techniques (in triplicates) that were maintained in a walk-in temperature controlled environmental chamber. Set at 25°C.
- CO_2 concentrations were maintained by gentle and constant bubbling with AirGas-filtered air mixtures enriched with ambient air, 780ppm and 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 CO_2 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 (ADDA) (EPA ETV) (EPA Method 546) ELISA kit [PN: 520011] and CAAS cube.

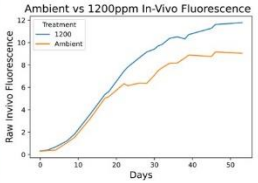
RESULTS

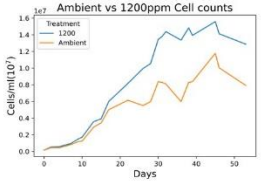










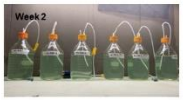


DISCUSSION

- 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.



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1Anderson, D.M., Glibert, P.M., Burkholder, J.M., 2002. Harmful algal blooms and eutrophication: Nutrient sources, composition, and consequences. Estuaries 25, 704–726. https://doi.org/10.1007/BF02804901

2Wagner, N.D., Osburn, F.S., Wang, J., Taylor, R.B., Boedecker, 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/toxins11100601

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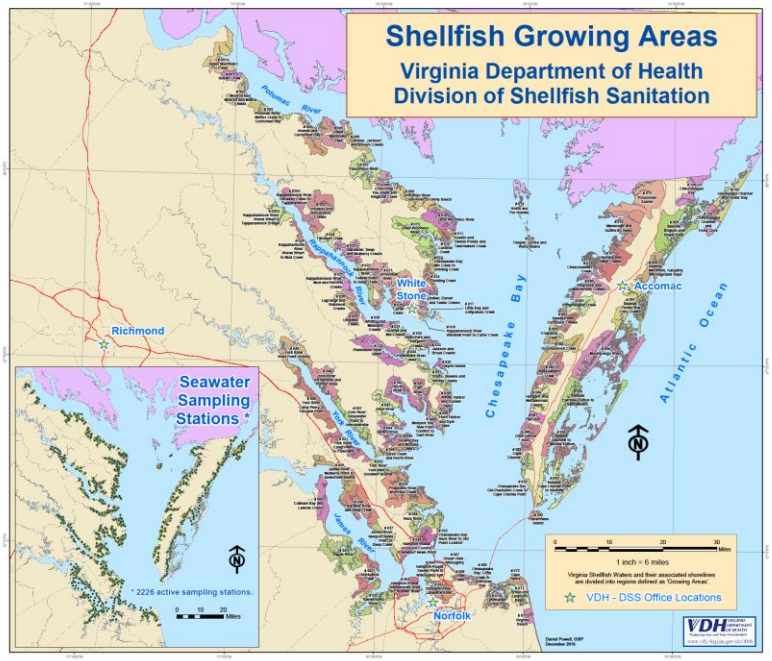
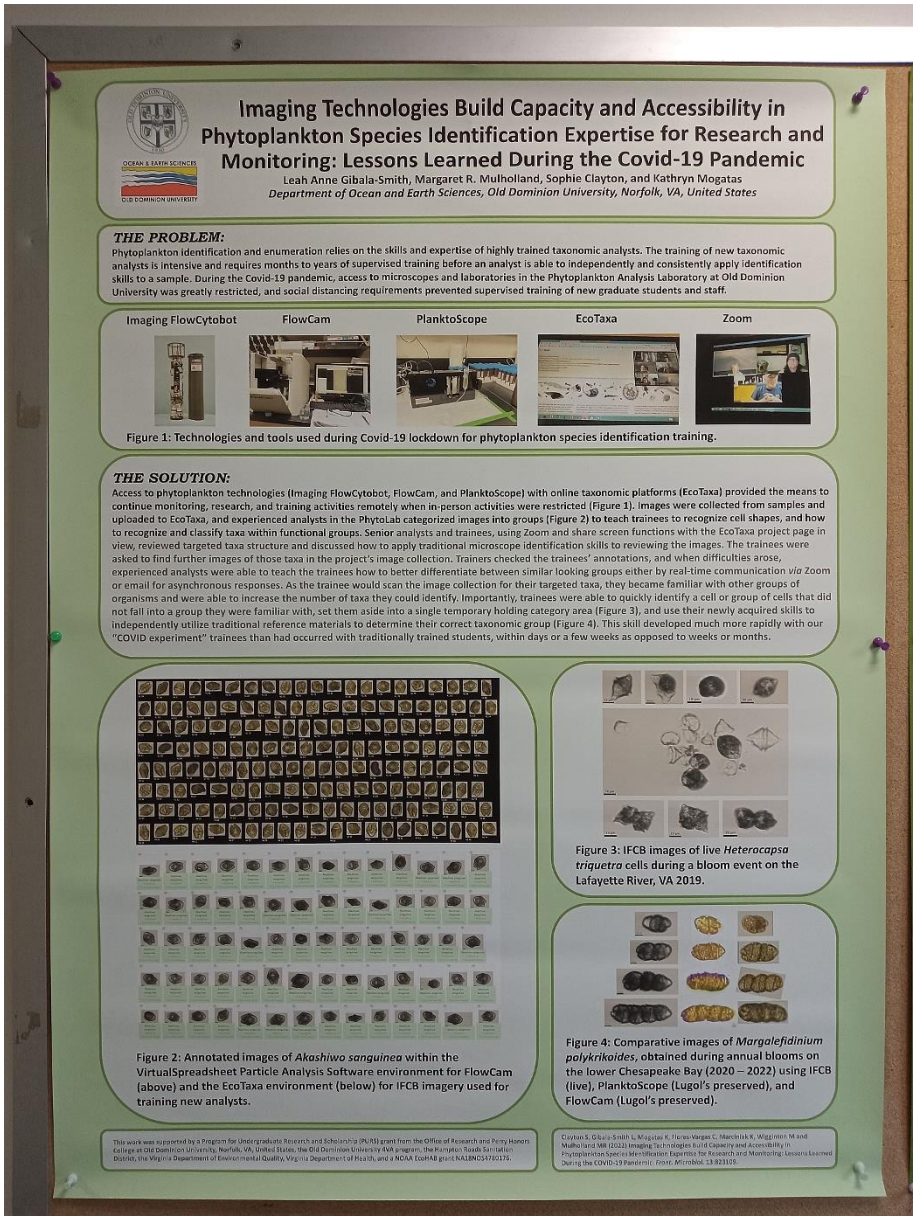
Building image libraries using FlowCam, IFCB, and Planktoscope





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 with in bloom dynamics missed by infrequent sampling

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