Grace Davenport
James Madison University

Biotic drivers of brown tide formation from co-competing phytoplankton

1) Problem Harmful algal blooms (HABs) are a global threat, affecting virtually every coastal country in the world **[1]**. Toxic HABs can lead to illness and death in fish, birds, and marine mammals, as well as humans. Even non-toxic HABs can degrade ecosystem function and damage fisheries through sheer accumulation of biomass, costing local economies millions of dollars annually. According to the National Oceanic and Atmospheric Administration, HABs cost the United States at least $50 million each year **[2]**. Climate change is a potential catalyst to the growth of these blooms, as noted by the United Nations' Intergovernmental Panel on Climate Change. Higher global temperatures provide HABs a competitive edge over other phytoplankton, such as diatoms or green algae. Additionally, longer periods of surface warming throughout the year encourages lakes to stratify earlier in the spring and destratify later in the fall. Lake stratification reduces vertical mixing, in turn creating an ideal environment for HAB growth **[3].**

One specific pelagophyte phytoplankton, *Aureococcus anophagefferens*, causes a type of HAB known as an annual “brown tide” along the East Coast, from New York to Virginia **[4]**. In recent years, *A. anophagefferens* has even been detected off the coasts of South Africa and China. These blooms pose a threat to ecosystems and humans by causing water column hypoxia/anoxia, recruitment failures in commercially important shellfish, and destruction of seagrass communities **[4].** When “brown tides” form, *A. anophagefferens* dominates the ecosystem, often to the complete exclusion of all other phytoplankton **[4]**. *A. anophagefferens* blooms are a model system for studying HAB ecology. Previous research **[1] [4] [6]** has largely focused on abiotic drivers of brown tides (e.g. nutrient input), but relatively little is known about biotic factors from co-competing phytoplankton. My research is focused here. **Specifically, I am utilizing an RNA-seq approach to characterize the global gene expression patterns of *A. anophagefferens* in response to co-competing phytoplankton.** Identifying specific allopathic genes can help us determine strategies that *A. anophagefferens* utilizes to outcompete co-occurring phytoplankton.

2) Research Methodology and Results Before I could extract RNA, I first had to inoculate common co-occurring phytoplankton, *A. anophagefferens* (strain 3368), *Synechococcus baccilarus* (1333)*, Karenia brevis* (2281) and *Phaeodactylum tricornutum* (2561) and measure their initial growth. I prepared a batch of L1 media minus silica. I poured eight flasks with 100mL of media each. For the two flasks that would contain *P. tricornutum,* I added silica (1.06×10^{-4} M) because diatoms require silica for proper cell wall growth. I then autoclaved all the flasks and added sterile-filtered vitamins (thiamine - 2.96×10^{-7} M ; biotin - 2.05×10^{-9} M; cyanocobalamin 3.69×10^{-10} M). Vitamins must be added after autoclaving because they are heat sensitive. I inoculated 1 mL of each phytoplankton strain into two flasks. Strains 3368 and 2561 were initially incubated at 16.0 degrees Celsius; strains 2281 and 1333 were incubated at 24.4 degrees Celsius. These temperatures mimic the phytoplankton’s natural habitats.

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I quantitatively measured phytoplankton health with two mechanisms. To track growth, I measured fluorescence at five wavelengths (440, 480, 540, 590, 625 nm) over the course of one week, collecting time points every 24 hours. These wavelengths measure chlorophyll and accessory pigment fluorescence and serve as a proxy for growth (**Figure 1**). When first inoculated into a new flask, phytoplankton numbers commonly remain stagnant due to an adjustment period known as the lag phase. Next, phytoplankton rapidly reproduce (exponential growth phase) until they deplete the environment's nutrients and space (stationary phase). Both 3368 and 2561 strains followed this growth pattern, unlike strains 2281 and 2561 (**Figure 1**). To improve growth, I transferred strains 2281 and 2561 to a 28 degree Celsius incubator. Increasing the incubation temperature returned strains 2281 and 2561 to their expected growth curves.

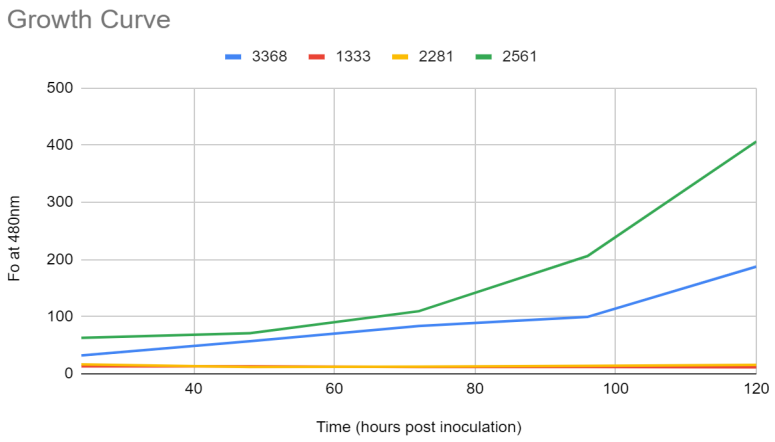

Figure 1: Linear graph displaying phytoplankton growth at initial incubation temperatures.

Utilizing a pulse amplitude modulated (PAM) fluorometer, I quantified “stress” by measuring photosystem II efficiency (as described in **[5]**). For thirty minutes, I dark adapted all flasks, effectively opening all phytoplankton reaction centers. While maintaining aseptic technique under the fume hood, I transferred two mL of each phytoplankton from their original flask to a cuvette and placed the cuvette into the PAM fluorometer. I recorded the initial fluorescence (Fo) at five wavelengths (440, 480, 540, 590, 625 nm). This value indicates the minimum fluorescence when all reaction centers are open. Next, I added six microliters of DCMU to the cuvette and recorded the max fluorescence (Fm). DCMU forces all photoreceptors to accept photons, effectively closing all reaction centers. To calculate the Fv/Fm value, I used the following formula: (Fm-Fo)/Fm. If the Fv/Fm value is above 0.5, photosystem II is running

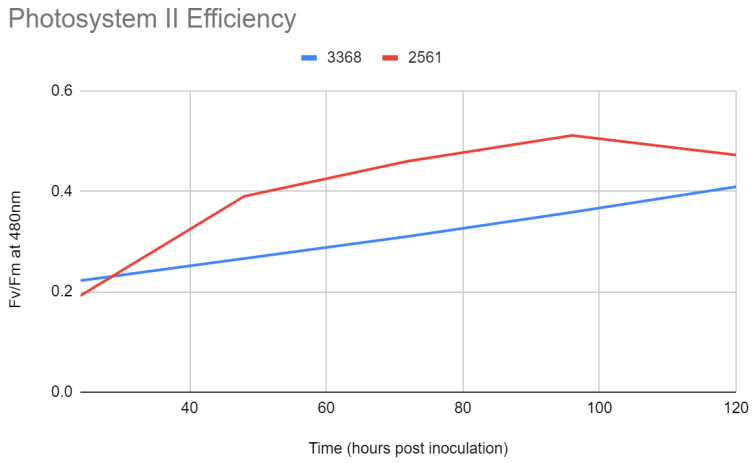

Figure 2: Linear graph quantifying phytoplankton stress levels. Over time, strains 3368 and 2561 eventually adapted to their new environment.

efficiently, indicating low stress **[5]**.
When first incubated, strains 3368 and 2561 photosystems II operated at 20% efficiency, indicating stress (**Figure 2**). This is a normal response to a new environment as the phytoplankton are adjusting to an influx of space and nutrients. Eventually, both strains reached greater than 40% efficiency, which is considered healthy. Strains 2281 and 2561 are not included in **Figure 2** due to their minimal initial growth.

3) Future Work Each strain is healthy according to their fluorescence and Fv/Fm values. These initial measurements were necessary to prevent the confounding variable—phytoplankton health—from entering the second part of the experiment. Over the next month, I will introduce *A. anophagefferens* and one co-occurring phytoplankton competitor into opposite sides of a custom-built membraned flask (**Image 1**). This type of flask was first utilized by a research team studying bacteria and diatom interactions. For their experiment, a commercially available DuranÒ flask was modified to contain a 29 mm neck and side opening. A clamp and membrane filter held the two flasks together, along with a silicon O-ring to prevent leaks **[6]**. I will recreate this double-sided, membraned flask for my experiment. At mid-log and early stationary phases of growth, I will extract *A. anophagefferens'* RNA using the RNeasy Power Water kit (QIAGEN). Then, I will send these samples to Azenta Life Sciences for sequencing. This will elucidate the underlying cellular responses that makes *A. anophagefferens* a dominant competitor. By examining every gene and its expression level, I am likely to discover multiple changes to the *A. anophagefferens* genome that will require additional experiments.

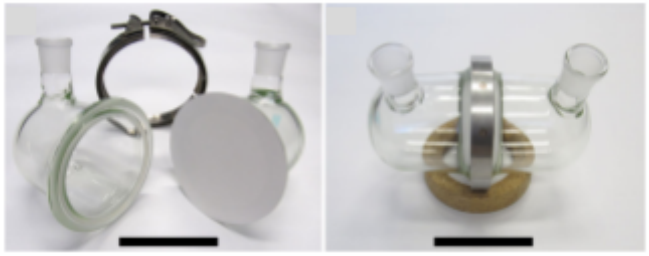

Image 1: Membraned flask modified from **[6]**. The black bar represents 10 cm.

Utilizing the skills I have learned, I intend to continue in this field at the WHOI-MIT Joint Program graduate school, eventually extrapolating data to produce HAB predictive models. I will employ proper culturing techniques for toxic microorganisms, micro pipetting and aseptic technique that I learned from my current research. Ultimately, my goal is to mitigate these blooms, working toward better protection of our coastal zones and its users.

Literature Cited:

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