UNIVERSITY of WASHINGTON

Undergraduate Research Symposium May 17, 2013 Mary Gates Hall

Online Proceedings

SESSION 2I

AQUATIC MICROBIOLOGY

Session Moderator: Frieda B. Taub, Aquatic & Fishery Science

251 MGH

3:45 PM to 5:15 PM

* Note: Titles in order of presentation.

Uncovering the Evolutionary Relationships of Pseudo-nitzschia

Terence Sebastian Leach, Freshman, Pre-Sciences
Mentor: Gabrielle Rocap, Oceanography
Mentor: Michael Carlson, Oceanography

Pseudo-nitzschia is a genus of phytoplankton widely dispersed throughout the world known for its production of a neurotoxin called domoic acid. As there is not a lot known about which species of *Pseudo-nitzschia* are associated with the neurotoxin, research on domoic acid is very limited. Little is known about the evolutionary relationships within the genus, but in 2002, the genus was broken into two clades (Lundholm, et al. 2002). Recently, the validity of these clades was tested by the sequencing of the Ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) gene in 14 species of *Pseudo-nitzschia* (Guannel, unpublished data). As there are currently 37 known species, the goal of this project is to sequence DNA, using the RuBisCO gene, from more species in order to fill out more of the phylogenetic tree. Cultures of 6 strains of species lacking known RuBisCO sequences were grown to mid-exponential phase and then had their DNA extracted. After the DNA was isolated, polymerase chain reaction (PCR) was conducted in order to amplify the gene. Before the DNA was sent for sequencing, the PCR products were run on an agarose gel confirming that the RuBisCO gene had in fact been successfully isolated and amplified. After the DNA is sequenced and placed on the phylogenetic tree, the physical characteristics (cell size, photopigments, etc.) of species making up each clade are then compared. This provides additional support as closely related organisms are likely to have similar traits. For example, we predicted that species with a larger cell size ($> 3\ \mu$m), would fall into Clade I, while smaller cells ($\leq 3\ \mu$m) would be placed in Clade II. Through the addition of these species to the phylogenetic tree, further research can be conducted to pinpoint where the production of domoic acid lies in the evolutionary history of *Pseudo-nitzschia*.

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Correlation between Fluorometer and Flow Cytometer Readings as a Proxy for Photosynthetic Cell Abundance

Jennefer Annet Lopez, Junior, Aquatic & Fishery Sciences
Mary Gates Scholar
Mentor: Gabrielle Rocap, Oceanography
Mentor: Jaclyn Saunders, Oceanography

The use of a fluorometer as a proxy for cell counting is a common method to track phytoplankton culture growth. A fluorometer works by emitting a beam of light, which excites the electrons in chlorophyll a, causing it to emit residual light. The fluorometer then measures the intensity and wavelength of that light. This information helps identify the relative abundance of cells present without giving a direct cell count. When conducting experiments involving the growth rate of photosynthetic cells it would be more beneficial to know the direct cell count as opposed to relative chlorophyll abundance because it is possible they may not correlate exactly as cells have the ability to adjust the ratio of chlorophyll in response to differences in light used in culturing. The marine picocyanobacteria, comprised of the genera Prochlorococcus and Synechococcus, are the most abundant photosynthetic organisms on Earth. I will culture five strains of picocyanobacteria in batch cultures using artificial seawater media in varying light and temperature conditions. I will measure the relative fluorescence of the chlorophyll a in the cultures at regular intervals throughout the growth cycle of the batch cultures. At the same time I will also measure the cell abundance using the flow cytometer. The flow cytometer will individually count and characterize the cells by chlorophyll fluorescence, accessory pigments, and cell size. My hypothesis is that the fluorescence based proxy of cell abundance measured by the fluorometer will highly correlate with the cell count from the

flow cytometer, but that exact cell counts will vary under different culturing conditions. My results will be able to provide scientists who use the fluorometer as a proxy for growth a better understanding of the actual number of photosynthetic cells growing in each tube.

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The Ecology of Marine Diatom Viruses: Characterizing Viruses that Infect *Pseudo-nitzschia*

Nicolette Danielle (Nicolette) Donohue, Senior, Oceanography, Biology (General)
Mary Gates Scholar
Mentor: Gabrielle Rocap, Oceanography
Mentor: Michael Carlson, Oceanography

Diatoms are unicellular photosynthetic algae, or phytoplankton, and account for approximately 20% of global primary production. The pennate diatom *Pseudo-nitzschia* can produce a neurotoxin called domoic acid (DA) that bioaccumulates in the tissues of shellfish when this diatom blooms. DA poisoning causes life-threatening conditions in mammals and humans when these shellfish are ingested. Parameters such as shellfish exposure length and bloom toxicity can be found by understanding what regulates these diatom communities. One mechanism of bloom regulation that we know little about is that of viral infection, despite viruses being the most abundant predator in the ocean. After a toxic *Pseudo-nitzschia* bloom at Sunset Beach, Oregon was sampled in 2009, the first *Pseudo-nitzschia* infecting virus (PmDNAV) was isolated by infecting the host *P. multiseries* Clnn-16. I hypothesize that there are many different viruses that can infect the genus *Pseudo-nitzschia* in addition to the PmDNAV. Over the past year, I worked to isolate additional *Pseudo-nitzschia* viruses from three blooms collected in the Pacific Northwest: Penn Cove, Friday Harbor, and Sunset Beach, Oregon. I have identified potential viruses in 12 of 48 infection experiments using these bloom samples. Three strains of *Pseudo-nitzschia*: GGA2, GGA3, and GGB1, which represent the species *P. multiseries* and *P. cingulata*, were infected by putative viruses from two blooms, while many others were only infected by one or none. The data suggests that independent of species, certain strains of *Pseudo-nitzschia* are more permissive to viral infection than others. As well, in June 2012 I isolated seven new strains of *Pseudo-nitzschia* from Golden Gardens, WA. I genetically identified these strains by extracting DNA, running PCR, and sequencing the Internal Transcribed Spacer region. I will test these strains to determine how permissive they are, and how effective they will be to use as hosts when working to isolate new viruses.