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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.

Induction of Proinflammatory Genes During Acute Infection with Wild Type or PdpA Knockouts of *Francisella noatunensis*

Po Jui (Patrick) Chen, Senior, Biochemistry, Microbiology
Rachel Faie Bessire, Senior, Microbiology
Mentor: John Hansen, Pathobiology

Francisella noatunensis is an emerging fish pathogen that causes disease and mortality for several economically important fish including tilapia and Atlantic cod. The Francisella Pathogenicity Island (FPI) is a region of the genome known to govern virulence for the genus. PdpA is one of the genes found in the FPI that has been implicated in the ability of *Francisella tularensis*, the human pathogen, to replicate in infected phagocytes. A recent study by our laboratory using zebrafish as a model host indicated that pdpA is important for the ability of *F. noatunensis* to cause mortality in infected zebrafish—thus the functional role of the pdpA protein appears to be conserved for the genus. Our goal is to compare the innate immune response of zebrafish during infection with either wild type or pdpA knockout strains of *F. noatunensis* to determine if the host immune response is altered. To accomplish this, we are monitoring the expression of key proinflammatory genes (IL1B and TNFA) using quantitative RTPCR during acute *Francisella* infection in zebrafish. The results from this study will provide additional information about the potential use of the *F. noatunensis* pdpA knockouts as live vaccine candidates for fish.

Culture Experiment: Elucidating the Roles of Sulfur Oxidizing Bacteria in Marine Nutrient Cycles

Yih En Lim, Senior, Biochemistry
Mary Gates Scholar
Mentor: Robert Morris, School of Oceanography

Bacteria play important roles in marine nutrient cycles. However, most marine bacteria are uncultured and little is known about their specific functions in marine nutrient cycles. Environmental members of the gamma proteobacterial sulfur oxidizer (GSO) clade have the genetic potential to use energy from sulfur oxidation to assimilate carbon, suggesting that they contribute to carbon and sulfur cycling in the oceans. Here we report the isolation and initial characterization of an open ocean representative from the GSO clade. Our isolate, GSO-NP4, was purified and its identity confirmed by 16S rRNA gene analyses. This isolate grows to relatively low cell densities (2 x E5 cells/ml) and has a doubling time of approximately 19 hours. Six 1L cultures were required to obtain 3.1g of DNA for genome sequencing. Six to twelve additional 1L cultures were established to obtain enough DNA to sequence the complete genome of GSO-NP4. Genomic information of this isolate is essential for future studies to determine the roles of GSOs in marine carbon and sulfur cycles.

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. mul-*

tiseries 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.

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 μm), would fall into Clade I, while smaller cells (≤ 3 μm) 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*.

Comparing Growth Rates of Common Estuarine Diatoms from Barkley Sound, British Columbia with Laboratory Cultures using *in situ* and Laboratory Growth Experiments

Moira (Meg) Regan, Senior, Oceanography
Mary Gates Scholar
Mentor: Virginia Armbrust, Oceanography
Mentor: Kathleen Newell, School of Oceanography
Mentor: Sara Bender, Oceanography

With the rise of atmospheric carbon dioxide, understanding the various facets of the carbon cycle is critical. In temperate coastal systems, the phytoplankton group of diatoms play a key role in the carbon cycle by sequestering carbon from the atmosphere into deep ocean sediments. Many scientists turn to laboratory culturing experiments to make predictions about the response of phytoplankton to changes in climate. But do these laboratory studies serve as realistic proxies for environmental responses? This study will compare growth rates of phytoplankton communities from Barkley Sound, British Columbia using field and laboratory experiments. Field experiments will comprise an *in situ* community growth rate measured using diffusion chambers in addition to on-ship incubations measuring the community growth rate in response to added nutrients. Field samples will be used to isolate the three dominant diatom species into a mixed-species culture for laboratory experiments. An analogous culture using laboratory isolates of the same species will be created, and the two cultures will be kept in a semi-continuous batch culture experiment tracking the growth rates in response to added nutrients. Comparisons of the growth rates *in situ*, shipboard, and in the laboratory will indicate how growth rates differ between natural phytoplankton assemblages and laboratory settings. Growth rates will be calculated using changes in chlorophyll *a* levels, a proxy for cell densities. If growth rates are much higher in the laboratory cultures than in the wild cultures, the implication is that there are limitations to growth in the natural environment that should be factored in when correlating laboratory experiments to the responses of natural phytoplankton assemblages. However, if there is no significant difference in growth rates between the experiments, then the laboratory conditions can provide similar conditions as in the field and laboratory culture experiments and can be easily used to predict environmental responses.

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.

Foraminifera Assemblages in Semiahmoo Bay, WA from 2001 to 2012

Adrienne M. (Adrienne) Sorenson, Senior, Earth and Space Sciences: Geology
Mentor: Elizabeth Nesbitt, Earth And Space Sciences
Mentor: Ruth A. Martin

The purpose of this study was to do a temporal investigation of the benthic forminifera in northern Puget Sound, specifically Semiahmoo Bay north of Bellingham Bay, to correlate any fluctuations in species diversity or abundance with environmental parameters. Semiahmoo Bay is influenced by an influx of both sediment and water from the Fraser River as well as mixing with ocean waters that travel in through the Strait of Juan de Fuca. The area is of interest because it is less impacted by anthropogenic activity than the rest of the Puget Sound and this allows for comparisons with other areas that are more heavily industrialized. Foraminifera are single celled, marine protists with specific habitat requirements. The sensitivity of these animals to their ecosystem allows us to use them for environmental monitoring. This study examines the foraminiferal genera and species present in sediments from this region spanning twelve years; from 2001 to 2012. The samples used were collected by the Washington Department of Ecology annually in April. Results of this investigation show an average of 15 species of foraminifera were present each year with three species dominating the samples. These species are *Bucella frigida*, *Elphidium frigidum* and *Elphidium hannai*, which are present in every sample. There are 10 to 12 other species that occur in small numbers present in most samples. One species, *Cassidulina limbata*, which is intolerant to pollution, indicates that this water is clean. There are only a few agglutinated species found in Semiahmoo Bay which is in contrast to the high abundance found in Bellingham Bay. This is significant because the dominant agglutinated species in Bellingham Bay are known to be opportunistic colonizers of polluted waterways. Thus, the foraminiferal assemblage in Semiahmoo Bay corroborates the hypothesis that the area is not subject to the degree of environmental stress as other embayments of Puget Sound.

Comparison of Dinoflagellate Cyst Assemblages in the Basins of Effingham Inlet, Uchucklesit Inlet, and Imperial Eagle Channel, Vancouver Island, British Columbia, Canada

Lyndsey Marie (Lyndsey) Sandwick, Senior, Oceanography
Mentor: Evelyn Lessard, School of Oceanography
Mentor: Kathleen Newell, School of Oceanography
Mentor: Kirsten Feifel, Oceanography

Some species of dinoflagellates develop into dormant, spore-like structures known as 'cysts' that sink to the sediment as a part of their lifecycle. Causes of the production of cysts include changes in water temperature and nutrient concentrations as well as sexual reproduction. Studying the role of cysts as a means of proliferation and expansion of dinoflagellate populations is an important part of understanding their place within the ecosystem. The study of cysts can strengthen our understanding of how dinoflagellate blooms can affect other populations within an ecosystem through their functions as producers and consumers as well as their roles in the global carbon cycle. If the dominant species in these blooms is capable of producing toxins that are dangerous to humans and other organisms, predicting the location and density of the blooms is vital. This study investigates the differences in concentrations and diversity of dinoflagellate cysts within the shallow sediments among Effingham Inlet, Uchucklesit Inlet, and the head of Imperial Eagle Channel in Vancouver Island, British Columbia. Sampling took place between January 22nd and February 3rd, 2013 aboard the University

of Washington's R/V Thomas G. Thompson. A comparison of the dinoflagellate cyst assemblages among the basins will be performed through the use of sediment cores, stains, and epifluorescence microscopy. The dinoflagellate cysts will be enumerated using a modified version of the method described by Yamaguchi et al. (1995). Previous studies have found variations in the composition of assemblages of dinoflagellate cysts within these basins that could be attributed to differing circulation patterns affecting the distributions and diversity of the dinoflagellate cyst assemblages. It is reasonable to hypothesize that these differences will be reflected in variations in the relative abundance and variety of cysts found in these locations.