

Bigelow | Laboratory for Ocean Sciences

Research Experience for Undergraduates The Gulf of Maine and the World Ocean

REU Symposium Program & Abstracts
Thursday, August 3, 2017



Oral Program

8:50 Introduction

- 9:00 Julia Park, Colby College, Waterville, ME
Effects of phosphate limitation on cell growth and toxin production in *Pseudo-Nitzschia* in the Gulf of Maine
Mentors: Dr. Peter Countway, Dr. Nicole Poulton, Dr. Stephen Archer, Katherine A. Hubbard
- 9:15 Theodore Bishop, Southern Maine Community College, South Portland, ME
qPCR-Based Diagnostic Survey of Protozoan Parasites in the Eastern Oyster (*Cassostrea virginica*) in Maine
Mentors: Dr. José A. Fernández Robledo, Nicholas D. Marquis, Dr. Nicholas R. Record, Dr. Peter D. Countway
- 9:30 Deja Best, North Carolina State University, Raleigh, NC
Observing the relationship between *Perkinsus marinus* and *Acartia tonsa*
Mentors: Dr. David Fields, Dr. Jose Anotonio and Jesica Waller
- 9:45 Matthew Weiser, Occidental College, Los Angeles, CA
Investigating Microbial Biofilm Formations on Crustal Rock Substrates
Mentors: Dr. Stephanie Carr, Tim D'Angelo, and Dr. Beth Orcutt
- 10:00 Michael Chen, Williams College, Williamstown, MA
Hiding in Plain Sight: the Globally Distributed Microbial Dark Matter Superphylum PAUC34f
Mentors: Dr. Eric Becraft, Dr. Maria Pachiadaki and Dr. Ramunas Stepanauskas
- 10:15 Lindsey Benedict, Pacific Lutheran University, Tacoma, WA
Low-oxygen respiration in microaerophilic iron-oxidizing Zetaproteobacteria
Mentors: Dr. David Emerson, Dr. Jake Beam

10:30 Break (30 minutes) *****

- 11:00 Ariel Petchel, Stockton University, Galloway, NJ
Exploration of Viral Diversity in the Ocean Using Single Virus Genomics – A Deep Sea Subsurface Case Study
Mentors: Dr. Joaquín Martínez Martínez, Dr. Ananda Bhattacharjee
- 11:15 Samantha Calderon, St. Peter's University, Jersey City, NJ
Effects of Viral Infection on phytoplankton species *Tetraselmis striata* under photoautotrophic conditions
Mentor: Dr. Joaquin Martínez Martínez
- 11:30 Janice DeStefano, Nova Southeastern University, Fort Lauderdale, FL
Identifying Sources of Haloperoxidase Activity in Coastal Seawater
Mentors: Dr. Stephen Archer, Kevin Posman
- 11:45 Clara Bird, Duke University, Durham, NC
Seasonal Gulf of Maine CDOM Variability as determined by satellites and ships
Mentors: Dr. Barney Balch and Dr. Catherine Mitchell
- 12:00 Dakota Young, Smith College, Northampton, MA
Finding Dominant Modes in Ocean Precipitation Phenology
Mentors: Dr. Karen Stamieszkin and Dr. Nicholas Record

12:15 Break (1:15 min) *****

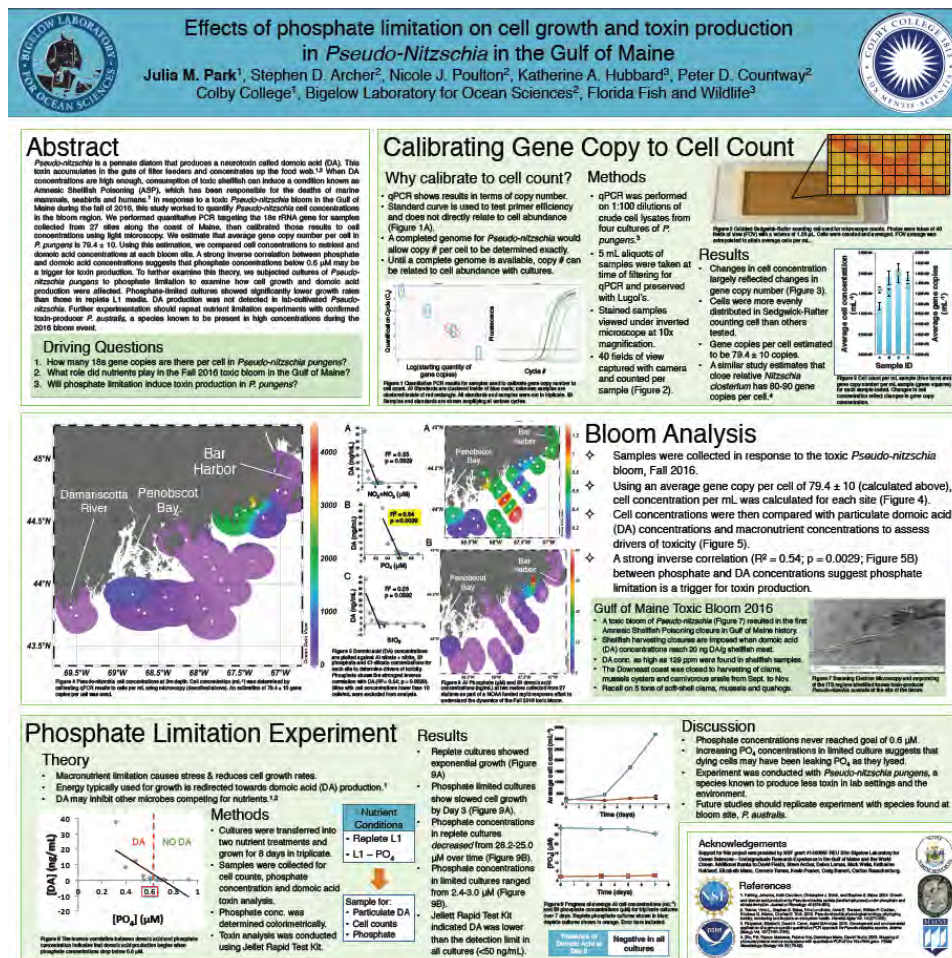
- 1:30 Anne Innes-Gold, Vassar College, Poughkeepsie, NY
Herbivore diversity and ecosystem function on coral reefs
Mentor: Dr. Doug Rasher
- 1:45 Emily Geske, Colby College, Waterville, ME
Ground shells as an antacid for buffering coastal acidification for shellfish aquaculture
Mentor: Dr. Nichole Price
- 2:00 Yoojin Cha, Colby College, Waterville, ME
Interactive effects of $p\text{CO}_2$ and temperature on the larval development of American lobster
Mentors: Dr. David Fields, Jesica Waller and Maura Niemisto
- 2:15 Nina Forziati, Simmons College, Boston, MA
Determining the lethal and sublethal toxicity of oxygenated hydrocarbons found in weathered oil
Mentors: Dr. Christoph Aeppli and Dr. Deedar Nabi
- 2:30 Devin Domeyer, Muhlenberg College, Allentown, PA
Shifting habitats in a warming Gulf: Compared growth and physiology of American lobsters to European green crabs under elevated temperatures.
Mentors: Dr. David Fields and Jesica Waller

Effects of phosphate limitation on cell growth and toxin production in *Pseudo-Nitzschia* in the Gulf of Maine

Julia M. Park¹, Peter D. Countway², Nicole J. Poulton², Stephen D. Archer², Katherine A. Hubbard³

¹Colby College, Waterville, ME ²Bigelow Laboratory for Ocean Sciences, East Boothbay, ME ³Florida Fish and Wildlife

Pseudo-nitzschia is a pennate diatom that produces a neurotoxin called domoic acid (DA). This toxin accumulates in the guts of filter feeders and concentrates up the food web. When DA concentrations are high enough, consumption of toxic shellfish can induce a condition known as Amnesic Shellfish Poisoning (ASP), which has been responsible for the deaths of marine mammals, seabirds and humans. In response to a toxic *Pseudo-nitzschia* bloom in the Gulf of Maine during the fall of 2016, this study worked to quantify *Pseudo-nitzschia* cell concentrations in the bloom region. We performed quantitative PCR targeting the 18s rRNA gene for samples collected from 27 sites along the coast of Maine, then calibrated those results to cell concentrations using light microscopy. We estimate that average gene copy number per cell in *P. pungens* is 79.4 ± 10 . Using this estimation, we compared cell concentrations to nutrient and domoic acid concentrations at each bloom site. A strong inverse correlation between phosphate and domoic acid concentrations suggests that phosphate concentrations below $0.6 \mu\text{M}$ may be a trigger for toxin production. To further examine this theory, we subjected cultures of *Pseudo-nitzschia pungens* to phosphate limitation to examine how cell growth and domoic acid production were affected. Phosphate-limited cultures showed significantly lower growth rates than those in replete L1 media. DA production was not detected in lab-cultivated *Pseudo-nitzschia*. Further experimentation should repeat nutrient limitation experiments with confirmed toxin-producer *P. australis*, a species known to be present in high concentrations during the 2016 bloom event.

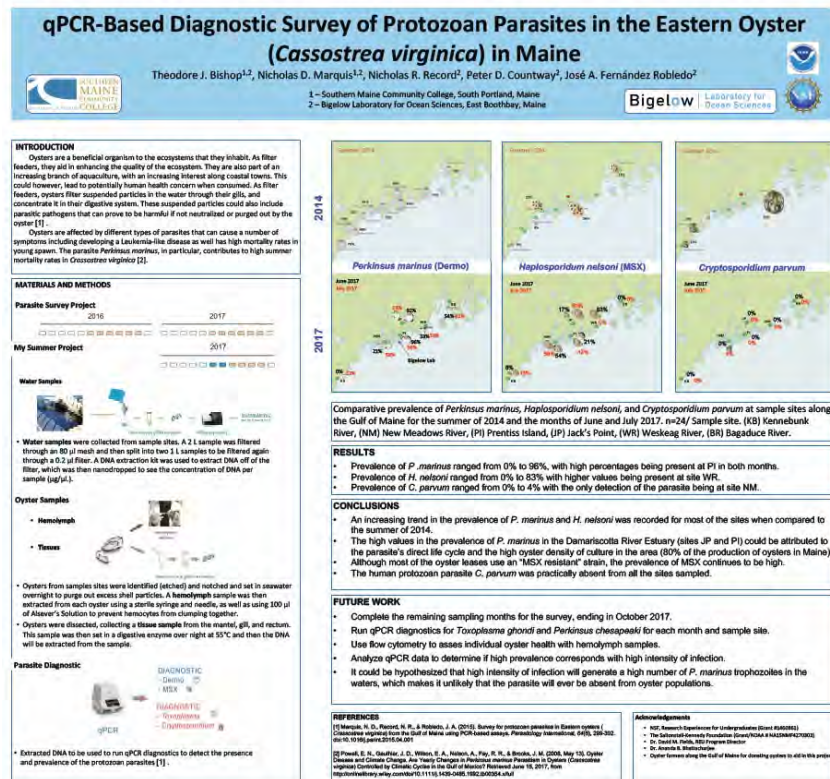


qPCR-Based Diagnostic Survey of Protozoan Parasites in the Eastern Oyster (*Cassostrea virginica*) in Maine

Theodore J Bishop^{1,2}, Nicholas D. Marquis^{1,2}, Dr. Nicholas R. Record², Dr. Peter D. Countway²,
Dr. José A. Fernández Robledo²

¹Southern Maine Community College, South Portland, ME ²Bigelow Laboratory for Ocean Sciences, East Boothbay, ME

Mollusc bivalves are key components of marine and estuarine environments because, as filter feeders, they play a critical role in maintaining water quality and ecosystem integrity. Bivalves also are an abundant resource for the coastal inhabitants, and in most places, traditional local harvesting of the natural populations is being substituted worldwide by semi-intensive aquaculture initiatives. Shellfish aquaculture -oysters in particular- has the potential to play a significant role in sustaining the coastal economy in the state of Maine. Concerns for developing oyster aquaculture include the presence of protozoan pathogens capable of producing large production losses and, through filter feeding, the ability to concentrate human pathogens that put the consumers of raw oysters at risk. Previously, we expand the Northeast range in the USA for the protozoans *Perkinsus marinus*, *Perkinsus chesapeaki* ("Dermo" disease), and *Haplosporidium nelsoni* (MSX), and report for the first time the detection of the human pathogens *Toxoplasma gondii* and *Cryptosporidium parvum*. We are now conducting a two-year survey to establish the base line of these parasites in Maine using qPCR-based assays. Analysis for *P. marinus*, *H. nelsoni*, and *C. parvum* for the months of June and July of 2017 reveals that the prevalence of *P. marinus* ranges from 0% to 96%, with high percentages being present at site PI in both months. The prevalence of *H. nelsoni* ranges from 0% to 83% with higher values being present at site WR, while *C. parvum* has the lowest prevalence ranging from 0% to 4% with the only detection of the parasite being at site NM. The oyster strain used in most cultures is labeled as "MSX resistant" however, our results indicate that the oysters get infected and with high intensities. Our preliminary results also show a persistence of *P. marinus* infections suggesting that there is a continuous release of infectious stages (*P. marinus* has a direct life cycle) and infections of new oyster batches.

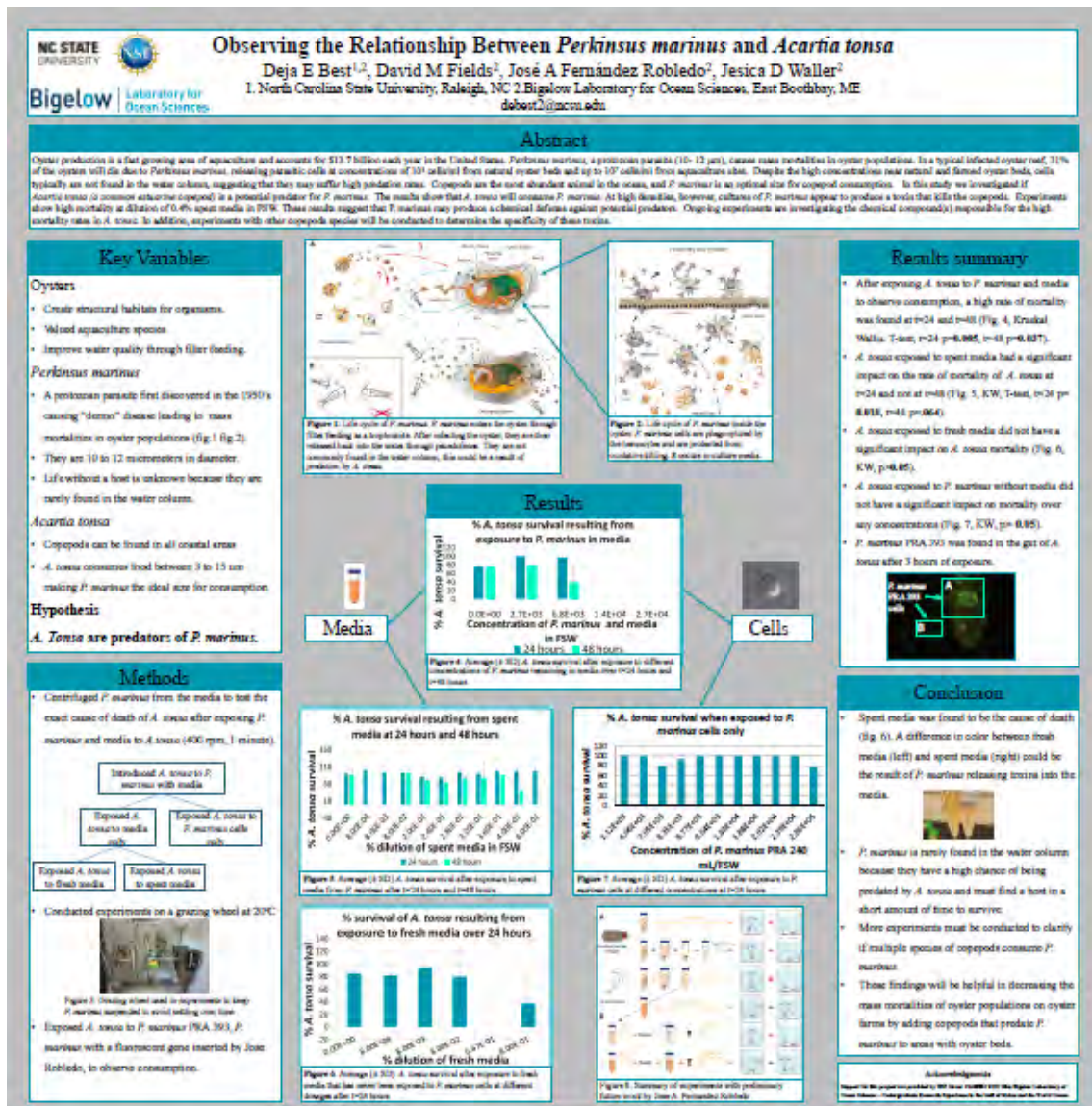


Observing the relationship between *Perkinsus marinus* and *Acartia tonsa*

Deja E Best^{1,2}, David M Fields², José A Fernández Robledo², Jessica D Waller²

¹North Carolina State University, Raleigh, NC ²Bigelow Laboratory for Ocean Sciences, East Boothbay, ME

Oyster production is a fast growing area of aquaculture and accounts for \$13.7 billion each year in the United States. *Perkinsus marinus*, a protozoan parasite (10- 12 µm), causes mass mortalities in oyster populations. In a typical infected oyster reef, 31% of the oysters will die due to *Perkinsus marinus*, releasing parasitic cells at concentrations of 10³ cells/ml from natural oyster beds and up to 10⁷ cells/ml from aquaculture sites. Despite the high concentrations near natural and farmed oyster beds, cells typically are not found in the water column, suggesting that they may suffer high predation rates. Copepods are the most abundant animal in the ocean, and *P. marinus* is an optimal size for copepod consumption. In this study we investigated if *Acartia tonsa* (a common estuarine copepod) is a potential predator for *P. marinus*. The results show that *A. tonsa* will consume *P. marinus*. At high densities, however, cultures of *P. marinus* appear to produce a toxin that kills the copepods. Experiments show high mortality at dilution of 0.4% spent media in FSW. These results suggest that *P. marinus* may produce a chemical defense against potential predators. Ongoing experiments are investigating the chemical compound(s) responsible for the high mortality rates in *A. tonsa*. In addition, experiments with other copepods species will be conducted to determine the specificity of these toxins.



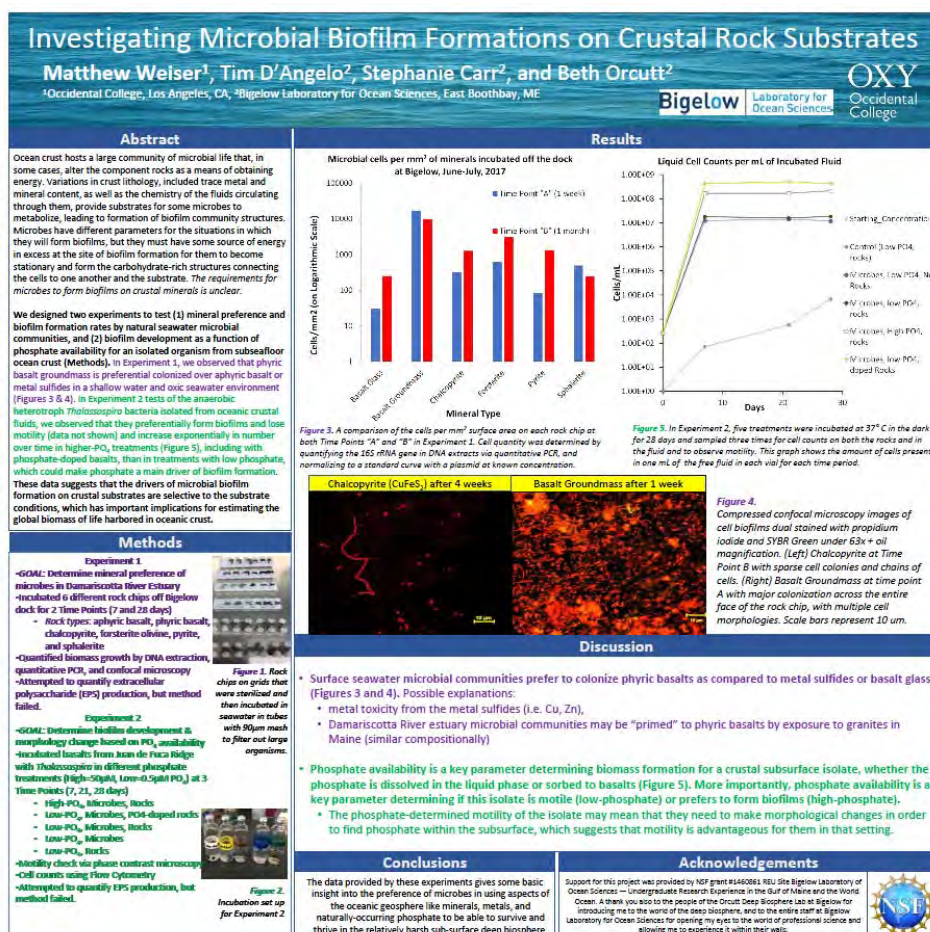
Investigating Microbial Biofilm Formations on Crustal Rock Substrates

Matthew Weiser¹, Dr. Stephanie Carr², Tim D'Angelo², and Dr. Beth Orcutt²

¹Occidental College, Los Angeles, CA ²Bigelow Laboratory for Ocean Sciences, East Boothbay, ME

Ocean crust hosts a large community of microbial life that, in some cases, alter the component rocks as a means of obtaining energy. Variations in crust lithology, included trace metal and mineral content, as well as the chemistry of the fluids circulating through them, provide substrates for some microbes to metabolize, leading to formation of biofilm community structures. Microbes have different parameters for the situations in which they will form biofilms, but they must have some source of energy in excess at the site of biofilm formation for them to become stationary and form the carbohydrate-rich structures connecting the cells to one another and the substrate. *The requirements for microbes to form biofilms on crustal minerals is unclear.*

We designed two experiments to test (1) mineral preference and biofilm formation rates by natural seawater microbial communities, and (2) biofilm development as a function of phosphate availability for an isolated organism from subseafloor ocean crust (Methods). In Experiment 1, we observed that phytic basalt groundmass is preferential colonized over aphyric basalt or metal sulfides in a shallow water and oxic seawater environment (Figures 3 & 4). In Experiment 2 tests of the anaerobic heterotroph *Thalassospira* bacteria isolated from oceanic crustal fluids, we observed that they preferentially form biofilms and lose motility (data not shown) and increase exponentially in number over time in higher-PO₄ treatments (Figure 5), including with phosphate-doped basalts, than in treatments with low phosphate, which could make phosphate a main driver of biofilm formation. These data suggests that the drivers of microbial biofilm formation on crustal substrates are selective to the substrate conditions, which has important implications for estimating the global biomass of life harbored in oceanic crust.

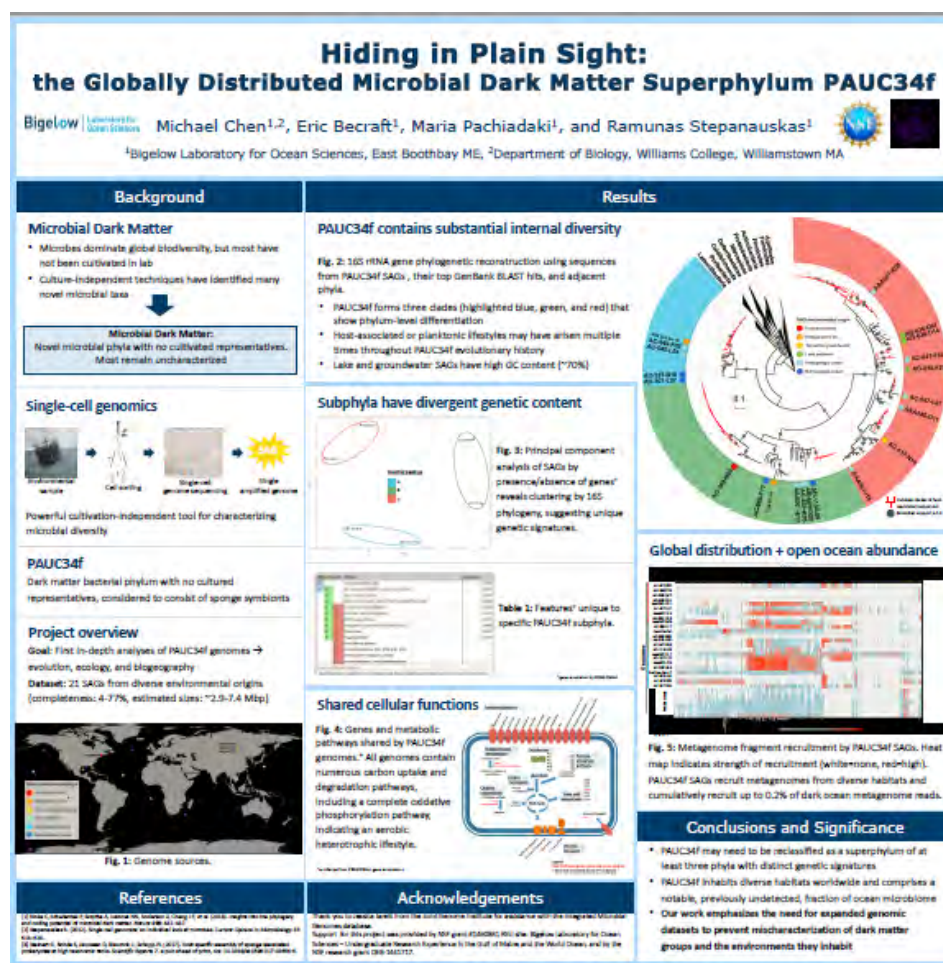


Hiding in Plain Sight: the Globally Distributed Microbial Dark Matter Superphylum PAUC34f

Michael Chen^{1,2}, Eric Becraft¹, Maria Pachiadaki¹, and Ramunas Stepanauskas¹

¹Bigelow Laboratory for Ocean Sciences, East Boothbay, ME ²Department of Biology, Williams College, Williamstown, MA

Microorganisms comprise most of the world's known biodiversity, although most have yet to be cultivated in the laboratory and remain uncharacterized. These novel, uncultivated microbial phyla have been referred to as "microbial dark matter." Single-cell genomics has proven to be a powerful tool for characterizing these groups by providing genetic blueprints for microorganisms without the need for cultivation. Here, we apply single-cell genomics to the dark matter bacterial phylum "PAUC34f," which has been identified in many sponge microbiome surveys and is widely considered to consist of sponge-specific symbionts. We used 21 single-cell amplified genomes identified as PAUC34f, originating from a variety of environments including the open ocean, lake sediments and terrestrial subsurface groundwater, to provide the first in-depth characterization of this group's evolution, ecology, and biogeography. We show that bacteria assigned to PAUC34f contain substantial evolutionary diversity, spanning three phylum-level clades with divergent genetic compositions, possibly warranting redesignation of PAUC34f as a superphylum. These heterotrophic organisms contain numerous carbon uptake and degradation pathways, and host-association seems to have evolved independently multiple times throughout the evolutionary history of this group. We also show that PAUC34f recruits metagenome fragments from diverse environments worldwide and constitutes an unexpectedly large fraction of the dark ocean microbiome that has previously gone undetected. This work highlights the power of single-cell genomics to characterize microbial diversity, and emphasizes the need for expanded genomic datasets to prevent mischaracterization of dark matter groups and the environments they inhabit.

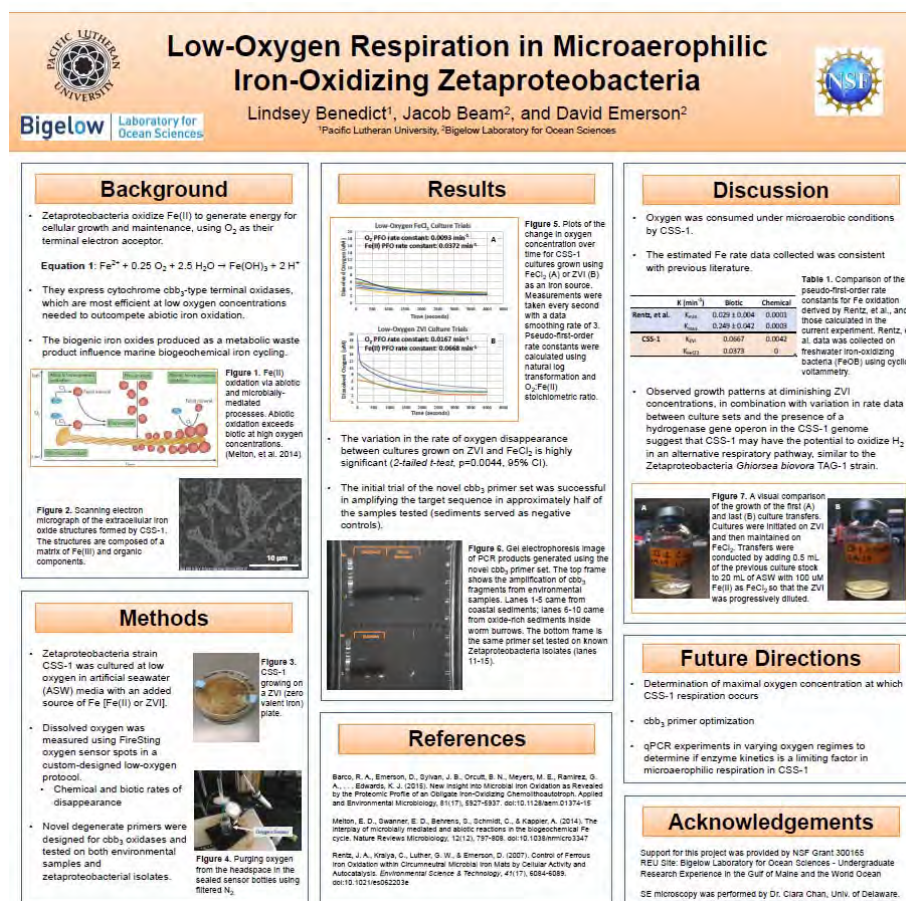


Low-oxygen respiration in microaerophilic iron-oxidizing Zetaproteobacteria

Lindsey Benedict¹, Jacob Beam², David Emerson²

¹Pacific Lutheran University, Tacoma, WA ²Bigelow Laboratory for Ocean Sciences, East Boothbay, ME

Marine biogeochemical cycles are influenced by the metabolic activity of lithoautotrophic microorganisms. Zetaproteobacteria are a class of marine, microaerophilic iron oxidizing bacteria that thrive in zones of high Fe(II) and low oxygen—such as bioturbated sediments—where the biological rate of iron oxidation outcompetes the rapid chemical oxidation of iron. Zetaproteobacteria encode for high-affinity terminal oxidase genes (e.g., *cbb₃* cytochrome oxidase), which are more efficient at low oxygen concentrations. Zetaproteobacteria strain CSS-1 was isolated from a shallow, micro-oxic, bioturbated surface sediment from the coast of Maine. In this study, we monitored oxygen consumption in strain CSS-1 under low O₂ conditions (<10 μmol L⁻¹), and estimated net O₂ consumption rates assuming pseudo-first order kinetics. Here we show that CSS-1 estimated O₂ consumption rates were statistically different when the organism was grown on zero valent iron (ZVI) as a source of Fe(II) than when grown on ferrous chloride—0.0167 min⁻¹ and 0.0093 min⁻¹, respectively (p=0.0044). The increased rate of oxygen consumption on ZVI—a source of both Fe(II) and H₂—was reflected by the presence of genes in strain CSS-1 for both Fe(II) and H₂ utilization as electron donors. Degenerate primers targeting the cytochrome *cbb₃* oxidase gene amplified the target gene fragment in known Zetaproteobacteria isolates and bioturbated sediment samples where Zetaproteobacteria were previously identified. These results represent a new tool for assessing the presence of Zetaproteobacteria in environmental samples. The course of this research generated novel protocols for studying Zetaproteobacteria under controlled oxygen regimes and added new data to the small but growing catalog of Zetaproteobacterial isolates. Continued respiration experiments and qPCR assays under varying physicochemical conditions will further contextualize strain CSS-1's position in the class.

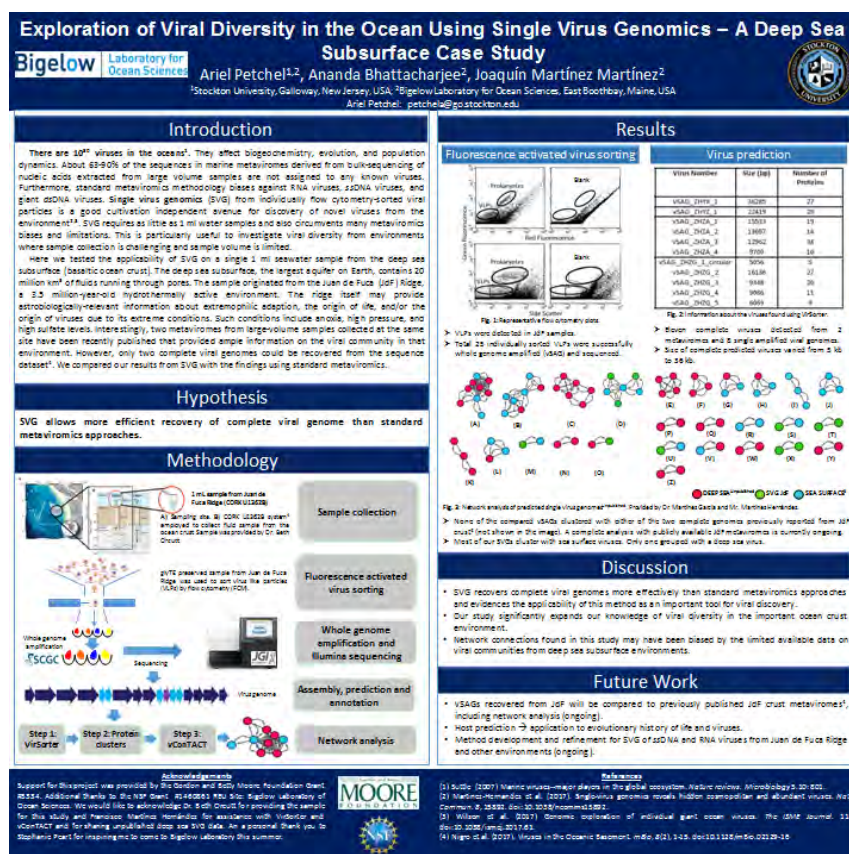


Exploration of Viral Diversity in the Ocean Using Single Virus Genomics – A Deep Sea Subsurface Case Study

Ariel Petchel¹, Dr. Ananda Bhattacharjee², Dr. Joaquín Martínez Martínez²

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There are 10^{30} viruses in the oceans affecting biogeochemistry, evolution, and population dynamics. About 63-90% of the sequences in marine metaviromes that are derived from bulk-sequencing of nucleic acids and extracted from large volume samples are novel. Furthermore, standard metaviromics methodology is biased against RNA, ssDNA, and giant dsDNA viruses. The use of single virus genomics (SVG) on individually flow cytometry-sorted viral particles is a good cultivation-independent avenue for the discovery of novel viruses from the environment. SVG requires as little as 1 ml water samples and also circumvents many metaviromics biases and limitations. This is particularly useful to investigate viral diversity from environments with limited sample volume. We tested the applicability of SVG on a 1 ml seawater sample from the deep sea subsurface or basaltic ocean crust. The deep sea subsurface, the largest aquifer on Earth, contains 20 million km³ of fluids running through pores. The sample originated from the Juan de Fuca (JdF) Ridge, a 3.5 million-year-old hydrothermally active environment. The ridge itself may provide information about extremophilic adaption, the origin of life, and the origin of viruses due to its extreme conditions. We predicted eleven complete dsDNA virus genomes using an SVG bioinformatics pipeline, which is significant compared to the recovery of only two complete virus genomes by metaviromics from 70 L of seawater collected from the same sampling site in a previous study. A network analysis of the genomes revealed similarities at the protein level to both sea surface and deep sea viruses. However, network connections found in this study may have been biased by the limited available data on viral communities from deep sea subsurface environments. Overall, this study significantly expands our current knowledge of viral diversity in the important ocean crust environment and highlight the power of SVG as a tool for viral diversity discovery.

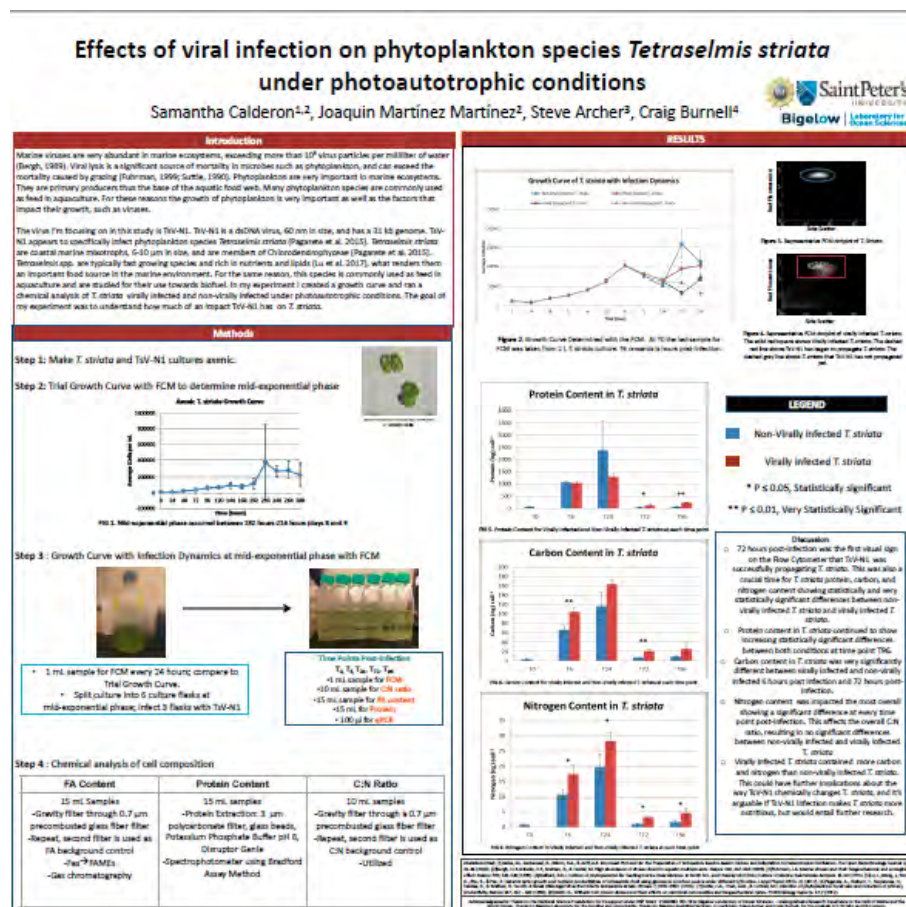


Effects of viral infection on phytoplankton species *Tetraselmis striata* under photoautotrophic conditions

Samantha Calderon¹, Dr. Joaquin Martínez Martínez²

¹ St. Peter's University, Jersey City, NJ ² Bigelow Laboratory for Ocean Sciences, East Boothbay, ME

Phytoplankton are the base of the aquatic food web thus feed a range of organisms from microscopic to large. Many phytoplankton species are commonly used as feed in aquaculture and are studied for their use in biofuel. For these reasons it is crucial to understand the growth of phytoplankton as well as factors that impact their growth, such as viruses. Viral lysis is a significant source of mortality in microbes such as phytoplankton, and can exceed the mortality caused by grazing. However, viral propagation and lysis and the effects of these processes can be quite unique to its host. In this experiment I studied the effects that virus TsV-N1 has on the cellular composition of phytoplankton species *Tetraselmis striata* under photoautotrophic conditions. Specifically, I quantified relative changes in cellular carbon, nitrogen, total protein and fatty acids content during the progression of viral infection, compared to non-infected control cultures. 72 hours post-infection was a crucial time for *T. striata* protein, carbon, and nitrogen content showing statistically significant ($P < 0.05$) and very statistically significant ($P < 0.01$) differences between non-virally infected *T. striata* and virally infected *T. striata*. However, Carbon and Nitrogen content significantly ($P < 0.05$) and very significantly ($P < 0.01$) increased from at least 6 hours after inoculating with TsV-N1. Protein content showed increasing significant differences between virally infected and non-virally infected *T. striata* from time point T72 ($P < 0.05$) to time point T96 ($P < 0.01$). These results suggest that *T. striata* infected with TsV-N1 may become more nutritional than non-infected *T. striata*. This experiment significantly increases our relatively limited understanding so far of TsV-N1 infection dynamics and adds to the overall understanding of the consequences of viral infection of important members of the marine phytoplankton community, from the individual cell to the ecosystem level.

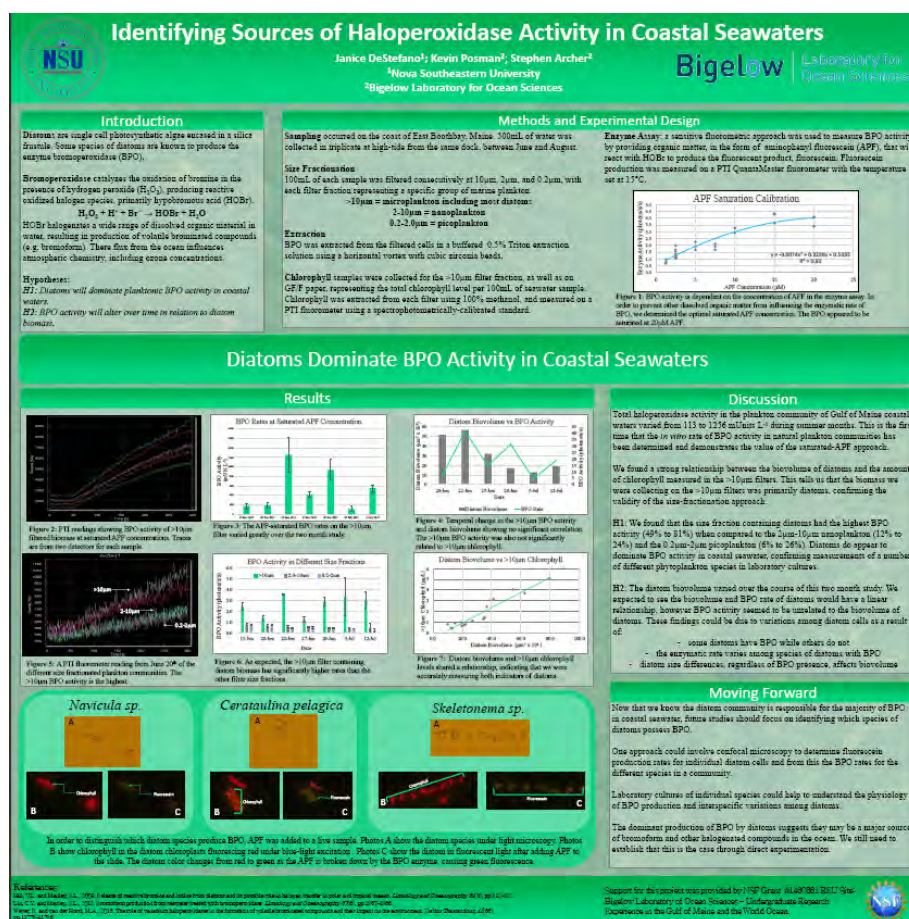


Identifying Sources of Haloperoxidase Activity in Coastal Seawater

Janice DeStefano¹; Kevin Posman²; Dr. Stephen Archer²

¹Nova Southeastern University, Fort Lauderdale, FL ²Bigelow Laboratory for Ocean Sciences, East Boothbay, ME

Haloperoxidase enzymes have been linked to the production of reactive halogen gases that influence atmospheric ozone concentrations. Very little is known about oceanic sources of these volatile halogenated compounds. Seaweeds and some phytoplankton are known to contain bromoperoxidase (BPO) enzymes, which catalyze the oxidation of bromine in the presence of hydrogen peroxide (H_2O_2) producing reactive volatile oxidized compounds, mainly hypobromous acid (HOBr). HOBr halogenates a wide range of dissolved organic material in water to produce volatile halogenated compounds, including bromoform ($CHBr_3$). Previous laboratory cultures have shown that some diatoms have high BPO activity. Using a size fractionation approach, we expected to see that diatoms dominated BPO activity in coastal seawater in comparison to other phytoplankton fractions containing primarily picoplankton and nanoplankton. A novel fluorescent assay was used to determine BPO activity in natural phytoplankton communities. Here we show that the $>10\mu m$ size fraction containing primarily diatom biomass did produce the highest BPO activity in coastal seawater from the Gulf of Maine; this activity comprised 50-80% of the total BPO activity. There was a clear relationship between the biomass of diatoms and the amount of chlorophyll in the $>10\mu m$ diatom fraction. We also found that over a 2 month period the BPO rates had no clear relation to the total biovolume of diatoms, possibly due to variation in BPO activity in individual diatom species. Some of the diatoms accounted for microscopically may not contain BPO, while others containing BPO may vary in the individual enzymatic rate and cell size. Marine diatoms may be significant producers of ozone-depleting $CHBr_3$, considering their global distribution and population size in comparison to other organisms containing bromoperoxidase.

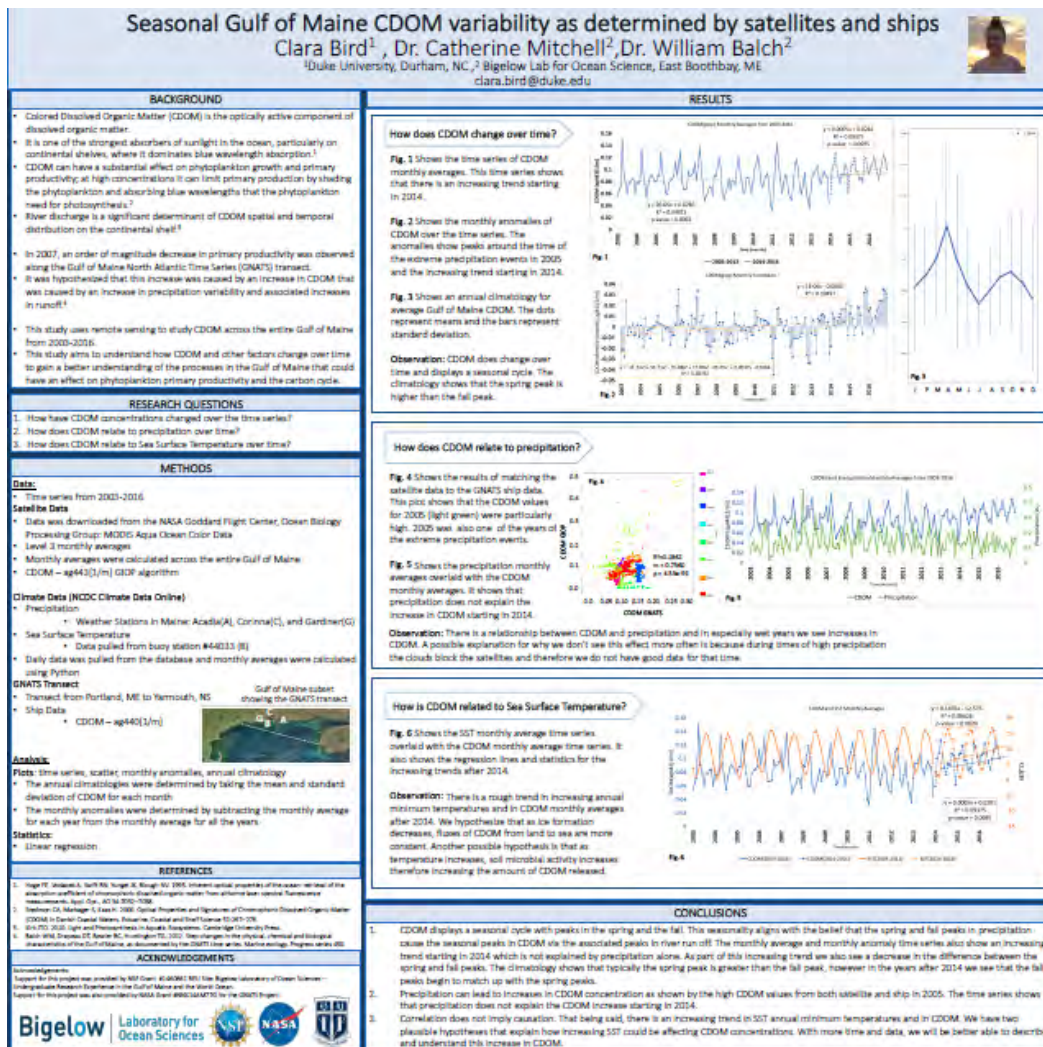


Seasonal Gulf of Maine CDOM Variability as determined by satellites and ships

Clara Bird¹, Dr. Catherine Mitchell² and Dr. Barney Balch²

¹Duke University, Durham, NC ²Bigelow Laboratory for Ocean Sciences, East Boothbay, ME

Colored Dissolved Organic Matter (CDOM) is the optically active component of dissolved organic matter, it is one the strongest absorbers of light in the ocean, particularly on continental shelves where it can dominate absorption in the blue region of the spectrum. CDOM can limit primary production by shading the phytoplankton and absorbing blue wavelengths needed for photosynthesis. In 2007, a significant decrease in primary productivity was observed along the Gulf of Maine North Atlantic Time Series (GNATS) transect which has never completely recovered. It was hypothesized that this decrease was in part due to an increase in CDOM caused by an increase in precipitation variability and associated increases in runoff. This study uses remote sensing to study CDOM across the Gulf of Maine from 2003-2016. Here we show that there is a seasonal CDOM cycle across the Gulf as well as an increasing trend starting in 2014. This trend was associated with a decrease in precipitation and an increase in temperature. We present hypotheses pertaining to why this recent upwards trend in gulf-wide CDOM has occurred. These results are relevant to the dissolved organic carbon budget across the entire Gulf and help elucidate the factors that regulate the land-to-sea carbon transfer in this coastal system.



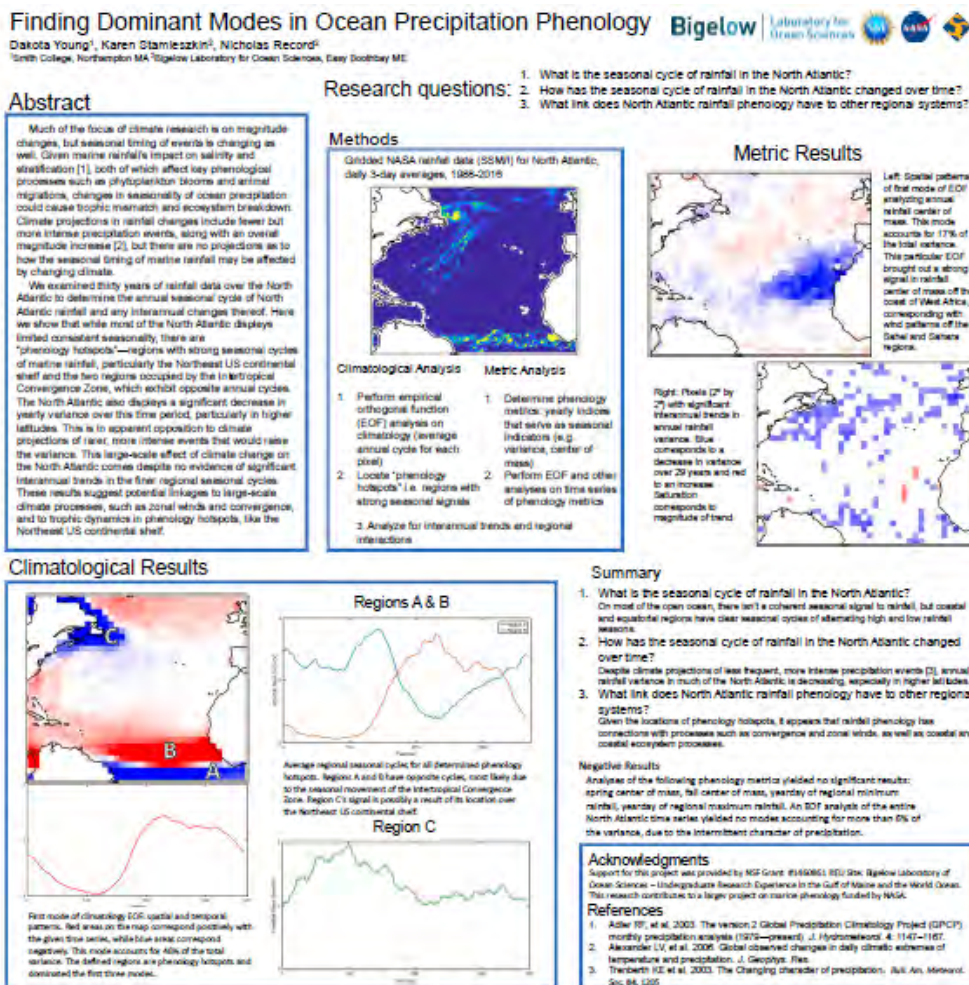
Finding Dominant Modes in Ocean Precipitation Phenology

Dakota Young¹, Dr. Karen Stamieszkin² and Dr. Nicholas Record²

¹Smith College, Northampton, MA ²Bigelow Laboratory for Ocean Sciences, East Boothbay, ME

Much of the focus of climate research is on magnitude changes, but seasonal timing of events is changing as well. Given marine rainfall's impact on salinity and stratification, both of which affect key phenological processes such as phytoplankton blooms and animal migrations, changes in seasonality of ocean precipitation could cause trophic mismatch and ecosystem breakdown. Climate projections in rainfall changes include fewer but more intense precipitation events, along with an overall magnitude increase, but there are no projections as to how the seasonal timing of marine rainfall may be affected by changing climate.

We examined thirty years of rainfall data over the North Atlantic to determine the annual seasonal cycle of North Atlantic rainfall and any interannual changes thereof. Here we show that while most of the North Atlantic displays limited consistent seasonality, there are “phenology hotspots”—regions with strong seasonal cycles of marine rainfall, particularly the Northeast US continental shelf and the two regions occupied by the Intertropical Convergence Zone, which exhibit opposite annual cycles. The North Atlantic also displays a significant decrease in yearly variance over this time period, particularly in higher latitudes. This is in apparent opposition to climate projections of rarer, more intense events that would raise the variance. This large-scale effect of climate change on the North Atlantic comes despite no evidence of significant interannual trends in the finer regional seasonal cycles. These results suggest potential linkages to large-scale climate processes, such as zonal winds and convergence, and to trophic dynamics in phenology hotspots, like the Northeast US continental shelf.

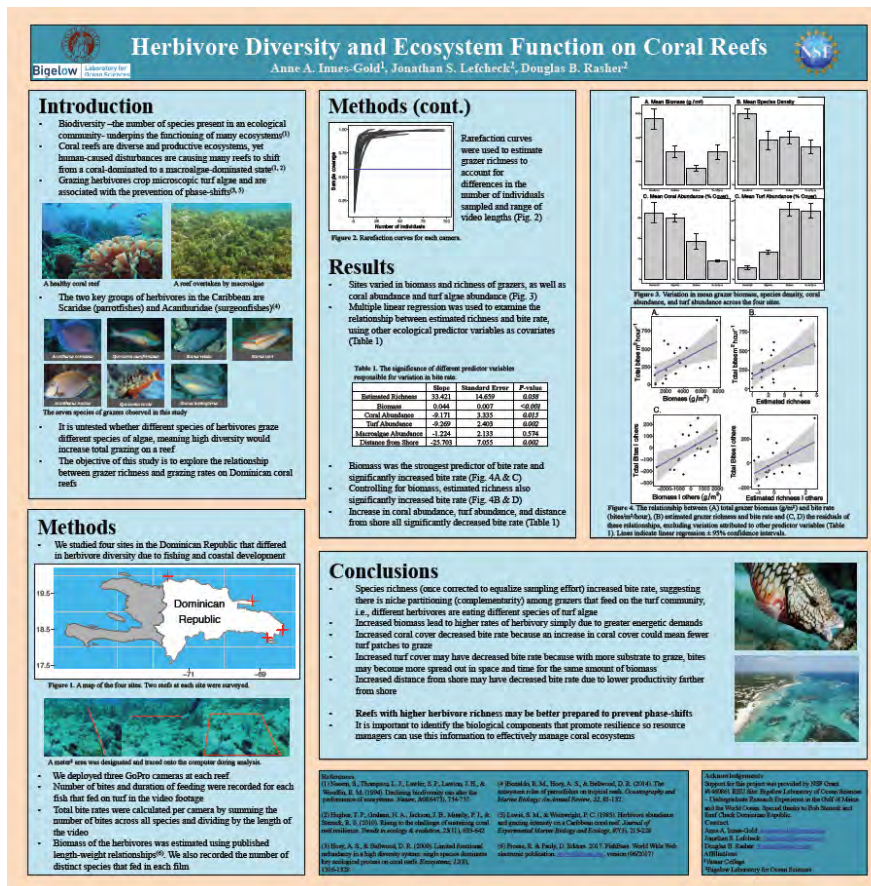


Herbivore diversity and ecosystem function on coral reefs

Anne A. Innes-Gold¹, Dr. Jonathan S. Lefcheck² and Dr. Douglas B. Rasher²

¹Vassar College, Poughkeepsie, NY ²Bigelow Laboratory for Ocean Sciences, East Boothbay, ME

Coral reefs are diverse and productive ecosystems that provide benefits to millions of people. Due to human-caused disturbances coral reefs, especially those in the Caribbean, are undergoing phase-shifts to a macroalgae-dominated ecosystem. Herbivorous fish species are associated with the prevention and reversal of these phase-shifts. Grazing herbivores crop microscopic turf algae, while browsing herbivores take bites of large, upright macroalgae. Different browser species have been shown to target various species of macroalgae, however it is unknown if grazers show similar complementarity, meaning they would target different species of microscopic algae within the turf. If this were true, then as grazer diversity increases, grazing rates per unit area would increase as well. The objective of this study was to determine a relationship between grazer diversity and grazing rates. Data were collected from eight reefs around the Dominican Republic; these reefs differ in herbivore diversity due to fishing and coastal development. At each reef, cameras were deployed to gather data on species richness and grazing rates, and benthic surveys were conducted to characterize reef composition. A linear model was used to explore the relationship between richness and bite rate, including covariates such as herbivore biomass, coral abundance, turf abundance, and distance from shore. Biomass was the strongest predictor of herbivory, and showed a significant positive relationship with bite rate. However, when controlling for biomass, richness significantly increased bite rate. Distance from shore, coral abundance, and algal abundance all had significant negative relationships with bite rate. Since reefs with higher richness showed higher bite rates, this suggests that grazers may target different turf algae species. As bite rate increases, a greater biomass of algae is being consumed, so reefs with higher herbivore diversity may be better prepared to prevent phase-shifts.

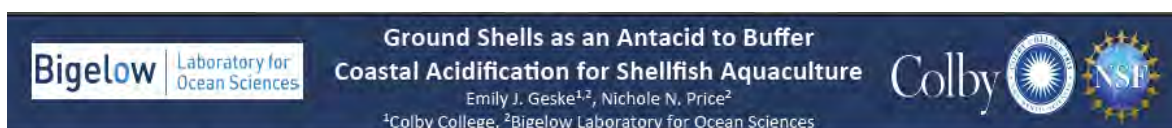


Ground shells as an antacid for buffering coastal acidification for shellfish aquaculture

Emily Geske¹, Dr. Nichole Price²

¹Colby College, Waterville, ME ²Bigelow Laboratory for Ocean Sciences, East Boothbay, ME

Anthropogenic CO₂ emissions are a driver of ocean acidification. Decreased pH within the ocean makes it more difficult for calcifying organisms to form calcium carbonate structures, impacting growth and development. This research project investigated localized 'liming' as a potential remediation strategy to buffer acidification in a coastal environment for application on commercial oyster farms. Bench-top dissolution experiments were conducted in the laboratory to test the impact of three different crushed oyster shell size fractions (250µm, 500µm, >1000µm) at six different pCO₂ treatments (250, 500, 1000, 1500, 2000, 4500µatm) on water chemistry. The data show that smaller fractions were found to have greater dissolution rates and dissolve at a higher saturation state than larger size fractions. When compared to carbonate chemistry parameters surrounding a commercial oyster aquaculture facility (Mook Sea Farm) on the Damariscotta River in Maine (USA), these findings suggest farmed oysters are on the brink of needing remediation today, but will be more vulnerable in the near future to increasing acidification.

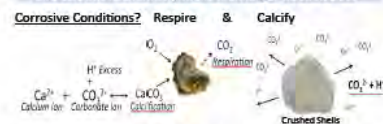


Abstract
CO₂ emissions are a driver of ocean acidification. Decreased pH makes it more difficult for calcifying organisms to form calcium carbonate structures, impacting growth and development. This research project investigated localized 'liming' as a potential remediation strategy to buffer acidification in a coastal environment for application on commercial oyster farms. Bench-top dissolution experiments were conducted in the laboratory to test the impact of three different ground shell (GS) size fractions (250µm, 500µm, >1000µm) at six different pCO₂ treatments (250, 500, 1000, 1500, 2000, 4500µatm) on water chemistry. The data show that smaller fractions were found to have greater dissolution rates and dissolve at a higher saturation state than larger size fractions. When compared to carbonate chemistry parameters in a oyster farm (Mook Sea Farm) on the Damariscotta River in Maine (USA), these findings suggest farmed oysters are on the brink of needing remediation today, however, will be more vulnerable in the near future to increasing acidification.

- Background**
- Acidification in the open ocean occurs more slowly than in coastal waters
 - Increasing atmospheric CO₂ leads to more aqueous CO₂; carbonic acid, which dissociates into bicarbonate and H⁺ ions; Excess hydrogen ions contribute to increased acidification
 - Calcifying organisms experience abnormalities in growth and development in more acidic conditions compared to less acidic conditions
 - Two forms of calcium carbonate: Calcite and aragonite
 - Saturation states [Ca²⁺][CO₃²⁻]/[CaCO₃]
 - Ω > 1 precipitation = dissolution; Ω < 1 net precipitation; Ω < 1, net dissolution; Ω < 1.5 calcifying organisms expend more energy to maintain and build calcium carbonate structures

- Research Questions**
1. Are the present-day oyster farm conditions corrosive?
 2. Can localized 'liming' be a feasible solution for buffering ocean acidification for shellfish aquaculture?

- Hypotheses**
1. Through concentrated respiration and calcification, oyster farms create conditions counter productive to oyster growth
 2. The smallest ground oyster shell fraction size will impact pH and carbonate chemistry more than larger fraction sizes



- Project Methods**
1. Field Monitoring
 - Deployed sensors inside and outside of Mook Sea Farm
 - SAMI (O₂, pH, T, S); SeapHox (pCO₂, T, S)
 2. Laboratory Experiments
 - Ground shell size fractions (250µm, 500µm, >1000µm) were subjected to six pCO₂ treatments
 - 250 (pre-industrial levels)
 - 500, 1000, 1500 (present day levels)
 - 2000, 4500 (extreme levels)
 - Mesocosm chambers were filled with filtered (0.2µm) seawater
 - Carbonate chemistry parameters: calculated using CO₂sys
 - Dissolution rate curves were fitted using R-studio

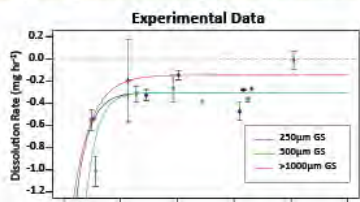


Figure 2. Dissolution rate as a function of aragonite saturation state (Ω_{ar}) for 250µm ground shell (blue), 500µm ground shell (green), and >1000µm ground shell (red). Horizontal line represents the precipitation-dissolution divide. 250µm GS: y₁ = 0.3183x - 2.5462; r² = 0.7131; R²_{adj} = 0.6549; 500µm GS: y₁ = -0.3152x + 9.9378; r² = 0.9491; R²_{adj} = 0.8937; >1000µm GS: y₁ = -0.1443x + 1.9052; r² = 0.4397; R²_{adj} = 0.03609.

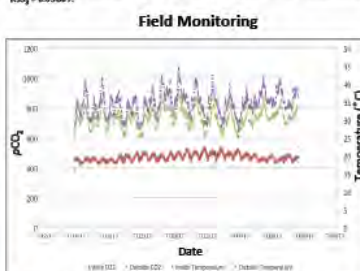


Figure 3. pCO₂ levels inside (green) and outside (purple) as well as temperature inside (blue) and outside (red) of Mook Sea Farm in the Damariscotta River between July 13-July 27, 2017.

Repurposing Shell Waste Problems

*Current regulations prevent commercial ground shell remediation as a strategy to bioengineer coastal waters

Maine Legislation: Title 12: Conservation, Part 9: Marine Resources; §652L. Dumping of dead marine animals or scaled finfish

- Not permitted to deposit or discard any dead marine animal or its parts in intertidal zones, harbors, or rivers

Clean Water Act of 1972: CWA § 502

- Pollutant is biological materials discharged into water
- Pollution is the man-made or man-induced alteration of the integrity of water

- Conclusions**
- A longer time series needs to be recorded to determine the conditions inside of an oyster farm
 - Lab conditions where pCO₂ is the main driver of changes in Ω differs from estuary conditions. Additional experiments testing effects of other drivers of acidification are warranted
 - Generally smaller size fractions (250µm, 500µm) had greater dissolution than larger size fractions (>1000µm)
 - Future work should investigate the effect of ground shells on oyster growth—important for investment from industry

Size Fractionation and GS Pricing

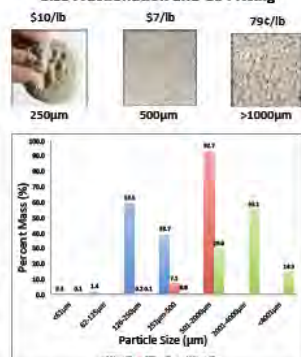


Figure 2. Ground shells were sieved for 20 minutes through a sieve stack (4000-61µm) to calculate a percent mass of particle sizes for each size fraction.

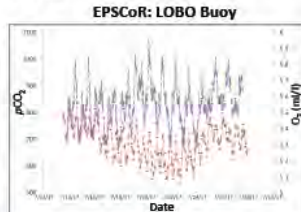


Figure 4. pCO₂ data (purple) and oxygen (red) recorded by the EPSCoR 1,000 buoy in the upper Damariscotta River from July 13-July 27 (lat: 43.59, 97.72, long: 69.2, 47.00).

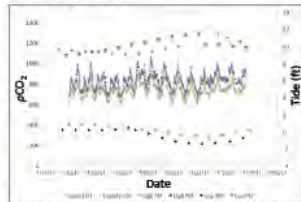


Figure 5. Low and high tide (H) published by the US Harbor tide charts from July 13-July 27 in Boothbay, Maine. pCO₂ measurements displayed to track water flow through the oyster farm and sensors region.

Acknowledgements

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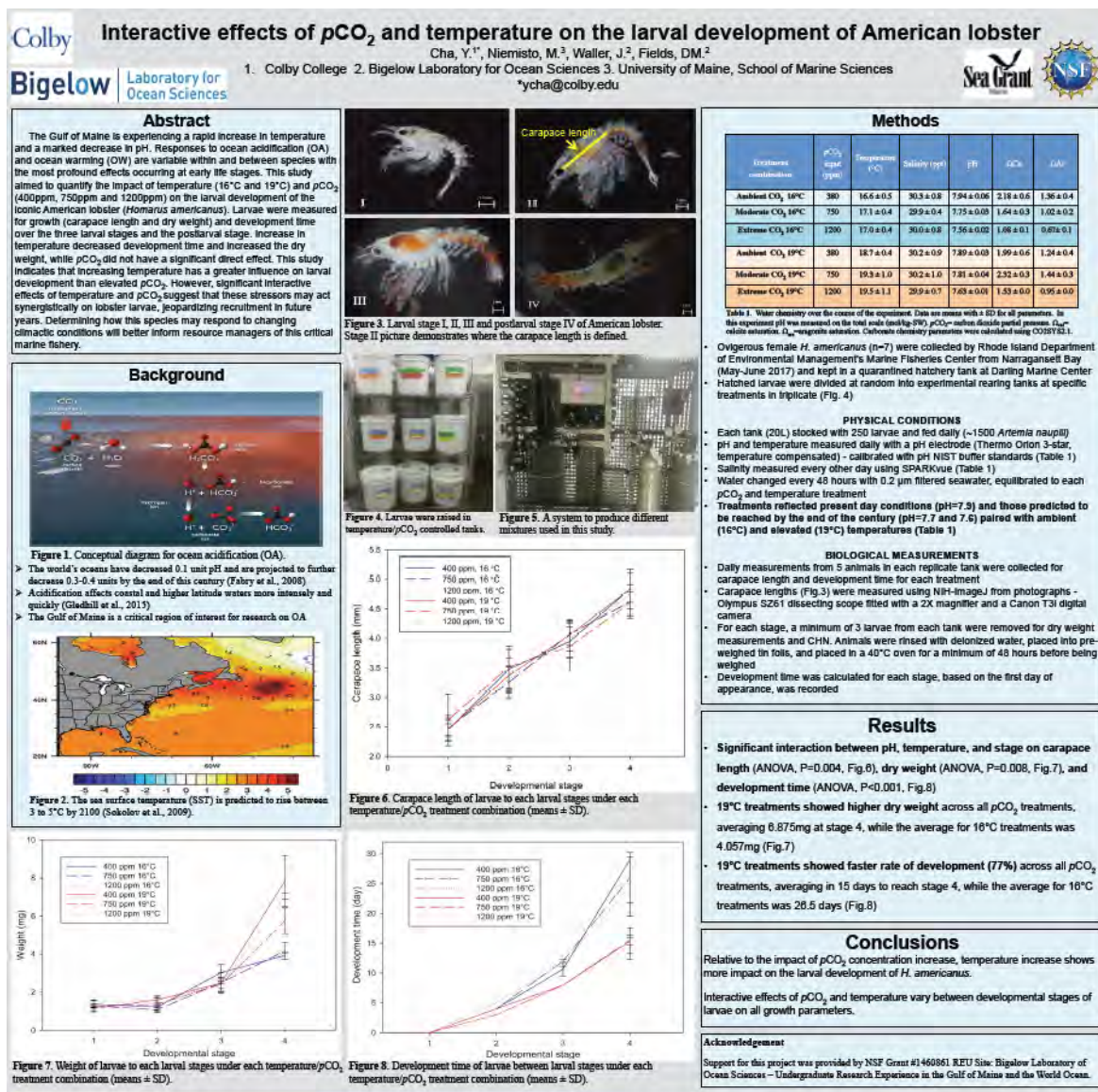
Funding: Bigelow Center for the Venture Research for Seafloor Solutions and NSF Grant #1460861 REU site: Bigelow Laboratory of Ocean Sciences

Interactive effects of $p\text{CO}_2$ and temperature on the larval development of American lobster

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The Gulf of Maine is experiencing a rapid increase in temperature and a marked decrease in pH. Responses to ocean acidification (OA) and ocean warming (OW) are variable within and between species with the most profound effects occurring at early life stages. This study aimed to quantify the impact of temperature (16°C and 19°C) and $p\text{CO}_2$ (400ppm, 750ppm and 1200ppm) on the larval development of the iconic American lobster (*Homarus americanus*). Larvae were measured for growth (carapace length and dry weight) and development time over the three larval stages and the postlarval stage. Increase in temperature decreased development time and increased the dry weight, while $p\text{CO}_2$ did not have a significant direct effect. This study indicates that increasing temperature has a greater influence on larval development than elevated $p\text{CO}_2$. However, significant interactive effects of temperature and $p\text{CO}_2$ suggest that these stressors may act synergistically on lobster larvae, jeopardizing recruitment in future years. Determining how this species may respond to changing climatic conditions will better inform resource managers of this critical marine fishery.

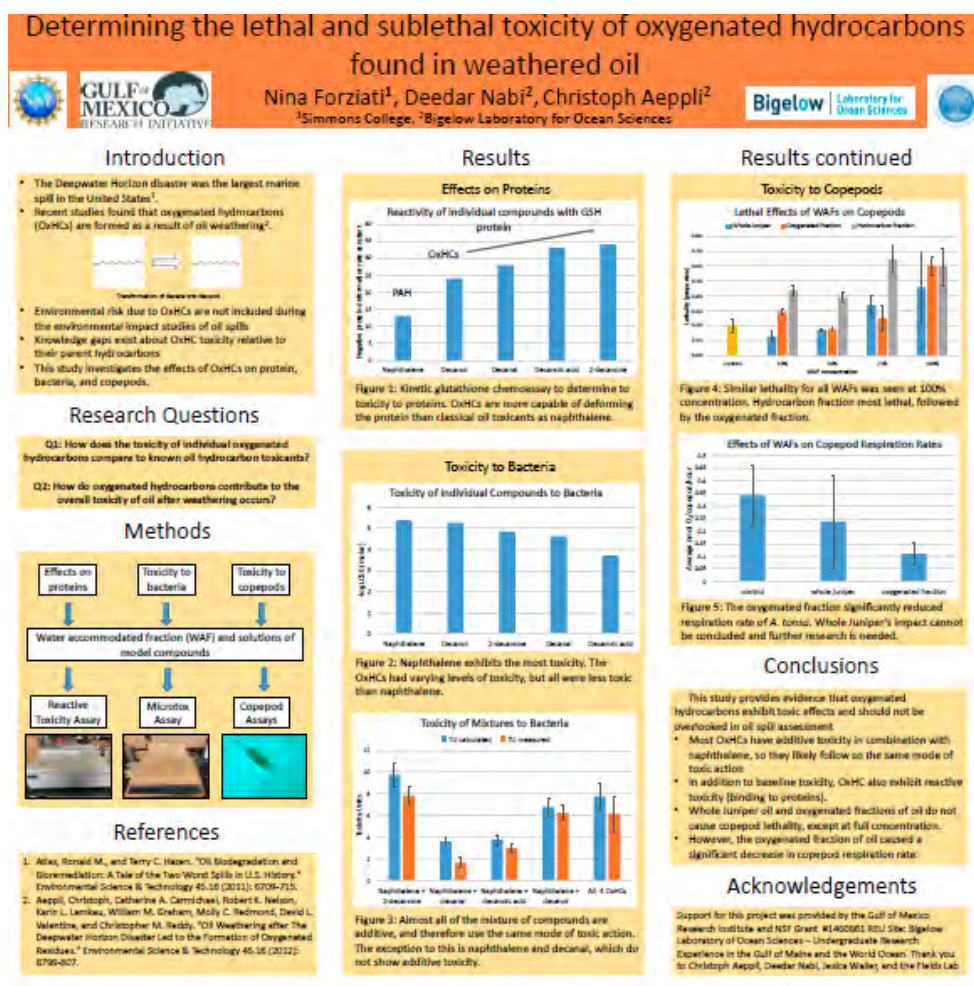


Determining the lethal and sublethal toxicity of oxygenated hydrocarbons found in weathered oil

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The Deepwater Horizon disaster was the largest marine oil spill in the United States. An unprecedented number of samples have been collected during and after the spill, which provides a unique opportunity to study the fate of oil in the environment. Recent studies found that persistent oxygenated hydrocarbons (OxHCs) form as a result of oil weathering. However, these compounds are generally not factored into risk assessment of oil spills, and little is known about how OxHCs are contributing to the toxicity of weathered oil. Therefore, we investigated toxicity of OxHC using three toxicity assays: Microtox toxicity assay, a reactive toxicity assay, and copepod toxicity experiments. Using the Microtox assay with pure OxHC model compounds, we found that OxHCs have significant baseline toxicity, and that their toxicity is additive with that of naphthalene, a representative polycyclic aromatic hydrocarbon (PAH). In addition to baseline toxicity, we found that OxHCs (and to a much lesser degree naphthalene) also exhibit reactive toxicity. Lastly, we found that OxHCs isolated from weathered oil lead to a reduction in respiration rate for *Acartia tonsa*. Our results suggest that Microtox, the standard method for determining baseline toxicity, does not capture all modes of toxic action of OxHCs such as the reactive toxicity. Furthermore, our results with water accommodated fraction (WAF) of the oxygenated fraction of weathered oil showed that the WAF caused respiration rate of *A. tonsa* to decline, suggesting that OxHCs may have a substantial effect on organisms that is currently not taken into consideration in oil spill damage and risk assessment.

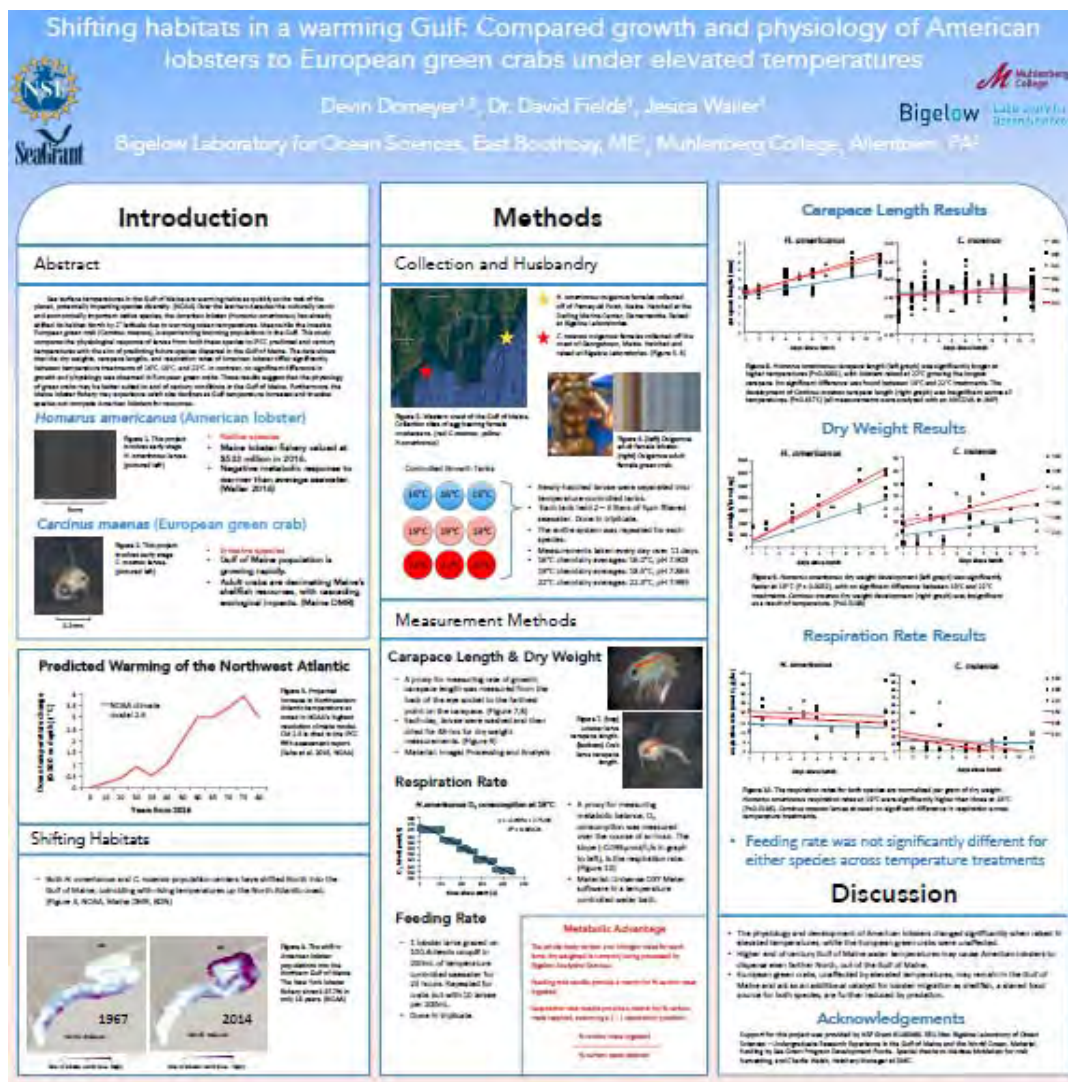


Shifting habitats in a warming Gulf: Compared growth and physiology of American lobsters to European green crabs under elevated temperatures.

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Sea surface temperatures in the Gulf of Maine are warming twice as quickly as the rest of the planet, potentially impacting species diversity. (NOAA) Over the last two decades the culturally iconic and economically important native species, the American lobster (*Homarus americanus*) has shifted its habitat range North by 2° latitude due to warming ocean temperatures. Meanwhile, the invasive European green crab (*Carcinus maenas*), is experiencing booming populations in the Gulf. This study compares the physiological response of larvae from both these species to IPCC predicted end-century temperatures with the aim of predicting future species dispersal in the Gulf of Maine. The data shows that the dry weights, carapace lengths, and respiration rates of American lobster differ significantly between temperature treatments of 16°C, 19°C, and 22°C. In contrast, no significant difference in the growth and physiology was observed in European green crabs. These results suggest that the physiology of green crabs may be better suited to end of century conditions in the Gulf of Maine. Furthermore, the Maine lobster fishery may experience catch size declines as Gulf temperature increases and invasive species out-compete American lobsters for resources.



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