Bigelow | Laboratory for Ocean Sciences

Research Experience for Undergraduates
The Gulf of Maine and the World Ocean
2021

REU Symposium Program & Abstracts
Wednesday (Posters) - Thursday, (Talks)

Bigelow's REU Program 2021

8:45 Opening Comments

9:00 Benjamin Gustafson – Colby College, ME
DIATOM NUTRIENT LIMITATION IN A SOUTHERN OCEAN EDDY
Benjamin Gustafson[1,2], William Balch[1], Sunny Pinkham[1]
[1]Bigelow Laboratory for Ocean Sciences, [2]Colby College

9:15 SOLUTION TO DILUTION: NOVEL SATURATION METHOD FOR THE ESTIMATION OF PHYTOPLANKTON GROWTH AND GRAZING RATES
Michael Staiger[1,2], Nicole J. Poulton[2], Laura Lubelczyk[2], Steve Archer[2]
Colby College[1], Waterville, Maine, Bigelow Laboratory for Ocean Sciences[2], East Boothbay, Maine

9:30 ASSESSING CELLULAR POLYPHOSPHATE OF PHYTOPLANKTON GROWN ON PHOSPHONATES
Briana Mays[1], LeAnn Whitney[1,2], Debra A. Lomas[2], Michael W. Lomas[2]
[1]Maine Maritime Academy. [2]Bigelow Laboratory for Ocean Sciences

9:45 Can microplastic be filtered out of wastewater using biofilm forming algae?
Andre Martin[1], Dr. Mike Lomas[2], Robert Schmedike[2]
[1]Haverford College, [2]Bigelow Laboratory for Ocean Sciences

10:00 IMPLEMENTING AND OPTIMIZING AN APPROVED METHOD FOR MONITORING DIARRHETIC SHELLFISH POISONING (DSP)
Finn Dworkin[1], Carmen Cartisano[2], Craig Burnell[2], Stephen Archer[2]
Grinnell College[1], Bigelow Laboratory for Ocean Sciences[2]

10:15 Discovering the Presence of Ethylene within *Cyanophora paradoxa* Algae and its Pathway
Matthew Yost[1,2], Baptiste Genot[2], John Burns[2]
[1]University of Maine, Orono, School of Marine Sciences, [2]Bigelow Laboratory for Ocean Sciences

10:30 Investigating the Effects of Dissolved Organic Matter on the Bioaccumulation of Short Chained Chlorinated Paraffins in the Copepod Species *Calanus finmarchicus*
Elizabeth Westbrook[1,2], Brian DiMento[1], Christoph Aeppli[1], David Fields[1]
[1]Bigelow Laboratory for Ocean Sciences, [2]University of Maryland, College Park

10:45 – 11:00 Break

11:00 Feeding Cows Seaweed to Reduce Methane Emissions: Iodine in Seaweed and Milk
Owen T. Keleher[2],& Benjamin S. Twining , PhD.[2]
1 Bates College , 2 Bigelow Laboratory for Ocean Sciences

11:15 ESTIMATION OF BIOMASS AND DESICCATION OF INTERTIDAL SEAWEEDS USING REFLECTANCE FOR FUTURE UNMANNED AERIAL SYSTEM SURVEY APPLICATIONS
Lydia Duncan[1,2] , Cath Mitchell[2], Nichole Price[2], Brittney Honisch[2], Jessie Muhlin[3], Hannah Webber[4], Peter Nelson[4], Stefan Claesson[5]
University of South Carolina-Columbia[1] , Bigelow Laboratory for Ocean Sciences[2] , Maine Maritime Academy[3] , Schoodic Institute[4] , Nearview LLC[5]

11:30 BIODEGRADATION OF PHOTO-OXIDIZED OIL PRODUCTS: IMPLICATIONS FOR THE FATE OF MARINE WEATHERED OIL SPILLS
Hannah Sterling[1], Christoph Aeppli[2]
[1]Roger Williams University, [2]Bigelow Laboratory for Ocean Sciences

11:45 COPEPOD INGESTION RATE AT SUBLETHAL OIL CONCENTRATIONS
Sam McNeely[1,2], Maura Niemisto[2], Christoph Aeppli, PhD.[2], & David Fields, PhD.[2]
1 University of North Carolina Wilmington, 2 Bigelow Laboratory for Ocean Sciences

12:00 Microplastics as carriers of PAHs released from oil spills: Measuring ingestion rates and bioaccumulation of PAHs in copepods
Manasi Desai[1,2], Maura Niemisto[2], Christoph Aeppli[2], David Fields[2]
The College of Wooster[1], Bigelow Laboratory of Ocean Sciences[2]

12:15 A Survey of Bivalve Transmissible Neoplasia in *Mya arenaria* Along the Casco Bay ME (USA)
Satyatejas G. Reddy[1,2], Dr. Michael J. Metzger[3], Rachael M. Giersch[3], Dr. José A. Fernández Robledo[1], Dr. Peter D. Countway[1]
[1]Bigelow Laboratory for Ocean Sciences; [2]Odum School of Ecology, University of Georgia; [3]Pacific Northwest Research Institute

12:30-1:00 Lunch

1:00 IS ATP A SUFFICIENT PROXY FOR BIOMASS FOR USE IN DEEP SEA MINING REGULATION?
Asher Platts[1], Dr. Beth Orcutt, PhD[2]
Southern Maine Community College[1], Bigelow Laboratory for Ocean Sciences[2]

1:15 TRACE METAL COMPOSITION OF DEEP-SEA SEDIMENTS: AN ANALYSIS OF RARE EARTH ELEMENTS IN THE N. PACIFIC
Christiana Okafor[1], Jim McManus[2]
Bowdoin College[1], Bigelow Laboratory for Ocean Sciences[2]

1:30 VIRUSES DRIVE BACTERIAL EVOLUTION IN THE LOST CITY HYDROTHERMAL FIELD SUBSURFACE MORE THAN IN THE OCEAN
Dani Buchheister[1,2], Julia McGonigle[1], Beth Orcutt[1]
Bigelow Laboratory for Ocean Sciences[1], University of Colorado Boulder[2]

1:45 INVESTIGATING PILI STRUCTURES IN THE MARINE SUBSURFACE
Autumn Pope[1], Stephanie Carr[1], Beth Orcutt[2], Michael Rappé[3], Olivia Nigro[4]
[1]Hartwick College, [2]Bigelow Laboratory for Ocean Sciences, [3] Hawaii Institute of Marine Biology, University of Hawaii at Manoa, [4]Hawaii Pacific University

2:00 ASSESSING STAGE-SPECIFIC THERMAL TOLERANCE OF *HOMARUS AMERICANUS* LARVAE
Alexis Mullen[1,2], Doug Rasher[2], Eric Annis[3], Aubrey Jane[2,4]
Bowdoin College[1], Bigelow Laboratory for Ocean Sciences[2], Hood College[3], University of New England[4]

2:15 DIVERSITY OF SULFATE REDUCING MICROBES IN SUBSURFACE FLUIDS OF THE LOST CITY HYDROTHERMAL VENT FIELD
Grace Beery[1], Julia McGonigle[2], Beth Ocrutt[2]
Boston University[1], Bigelow Laboratory for Ocean Sciences[2]

2:30-2:45 Break

2:45 A SINGLE CELL GENOMIC APPROACH TO UNDERSTANDING VIRAL IMPACT ON PHENOTYPIC TRAITS IN MARINE MICROORGANISMS.
Paxton Tomko[1,2], Jacob Munson-McGee[1], Julia Brown[1], Nicole Poulton[1], Ramunas Stepanauskas[1]
Bigelow Laboratory for Ocean Sciences[1], Purdue University[2]

3:00 Automating A Visual Screening Task Using Deep Learning
Julia Brown[1] Ben Tupper[1] Nick Record[1] Jace Innis[1,2] Jonathan Evanilla[1]
[2] California State University, Monterey Bay
[1]Bigelow Laboratory for Ocean Sciences, East Boothbay, ME

3:15 Expression of SARS-CoV-2 RBD gene in the marine protozoan *Perkinsus marinus*
Orellana María José Orellana Rosales[1, 2], José A. Fernández Robledo[1]
[1]Bigelow Laboratory for Ocean Sciences, 60 Bigelow Dr., East Boothbay ME. [2]Southern Maine Community College, 2 Fort Rd., South Portland ME.

3:30 EFFECT OF LARVAL TEMPERATURE ACCLIMATION ON THE THERMAL TOLERANCE OF AMERICAN LOBSTER (*HOMARUS AMERICANUS*) LARVAE
Hannah O'Loughlin[1,2], Doug Rasher[2], Eric Annis[3], Aubrey Jane[2,4]
Vassar College[1], Bigelow Laboratory for Ocean Sciences[2], Hood College[3], University of New England[4]

3;45 INGESTION RATE OF EARLY-STAGE LARVAL LOBSTER, *HOMARUS AMERICANUS*, ON THE DOMINANT COPEPOD GENUS, *ACARTIA*, IN THE GULF OF MAINE
Molly Spencer[1] , Rachel Lasley Rasher[1], Evie Layland[2], Maura Niemisto[3], David Fields[3]
University of Southern Maine, Portland, ME[1], University of Maine, Orono[2], Bigelow Laboratories for Ocean Sciences[3]

4:00 INGESTION RATES OF MARINE CLADOCERANS
Allegra Rocha[1], Maura Niemisto[2], David Fields, Ph.D.[2]
[1]University of the Pacific, Stockton, CA, [2]Bigelow Laboratory for Ocean Sciences, East Boothbay, ME

4:15 END

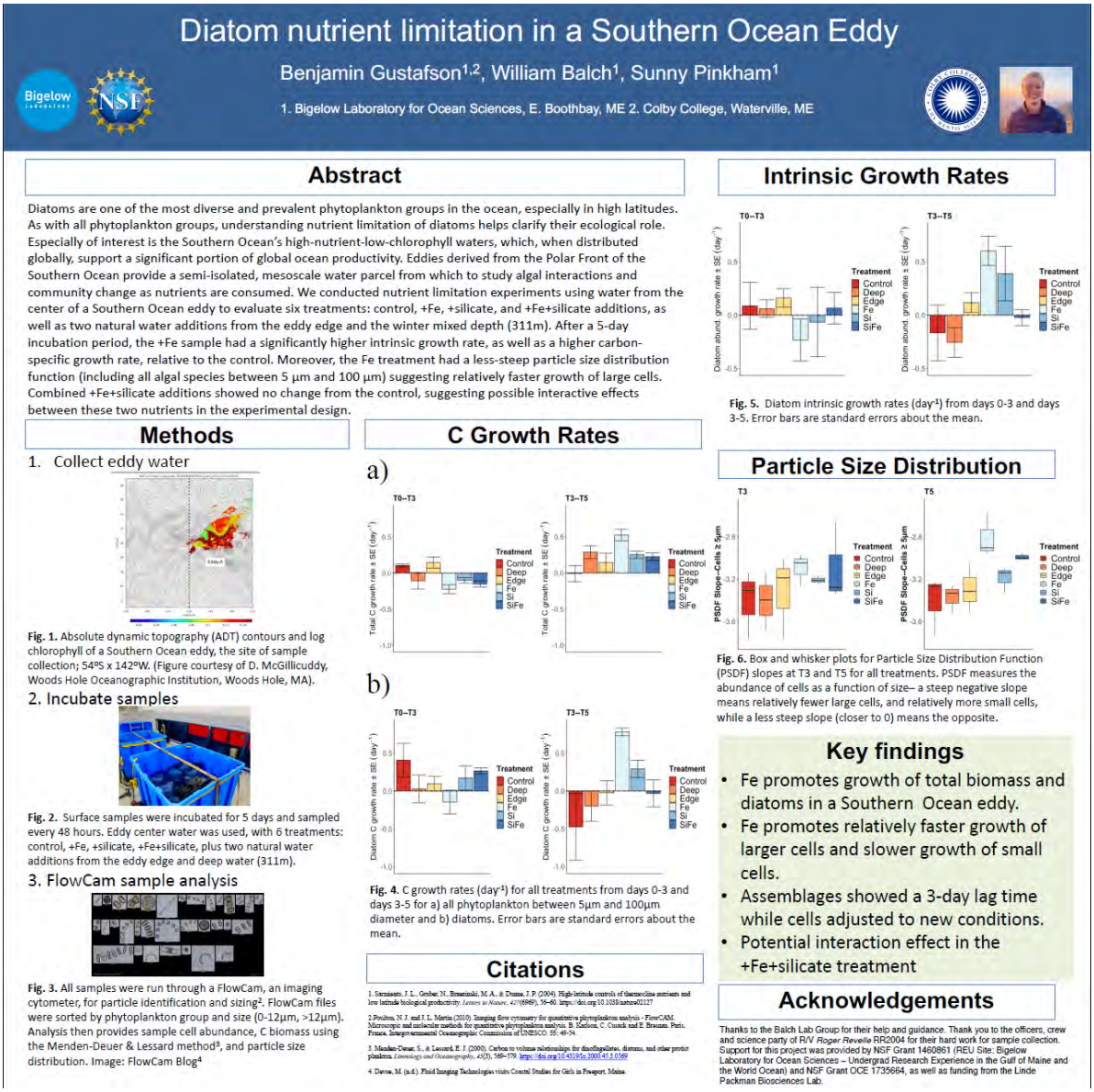
Abstracts and Posters

Bigelow's REU Program 2021

DIATOM NUTRIENT LIMITATION IN A SOUTHERN OCEAN EDDY

Benjamin Gustafson[1,2], William Balch[1], Sunny Pinkham[1]
[1]Bigelow Laboratory for Ocean Sciences, [2]Colby College

Diatoms are one of the most diverse and prevalent phytoplankton groups in the ocean, especially in high latitudes. As with all phytoplankton groups, understanding nutrient limitation of diatoms helps clarify their ecological role. Especially of interest is the Southern Ocean's high-nutrient-low-chlorophyll waters, which, when distributed globally, support a significant portion of global ocean productivity. Eddies derived from the Polar Front of the Southern Ocean provide a semi-isolated, mesoscale water parcel from which to study algal interactions and community change as nutrients are consumed. We conducted nutrient limitation experiments using water from the center of a Southern Ocean eddy to evaluate six treatments: control, +Fe, +silicate, and +Fe+silicate additions, as well as two natural water additions from the eddy edge and the winter mixed depth (311m). After a 5-day incubation period, the +Fe sample had a significantly higher intrinsic growth rate, as well as a higher carbon-specific growth rate, relative to the control. Moreover, the Fe treatment had a less-steep particle size distribution function (including all algal species between 5 um and 100 um) suggesting relatively faster growth of large cells. Combined +Fe+silicate additions showed no change from the control, suggesting possible interactive effects between these two nutrients in the experimental design.

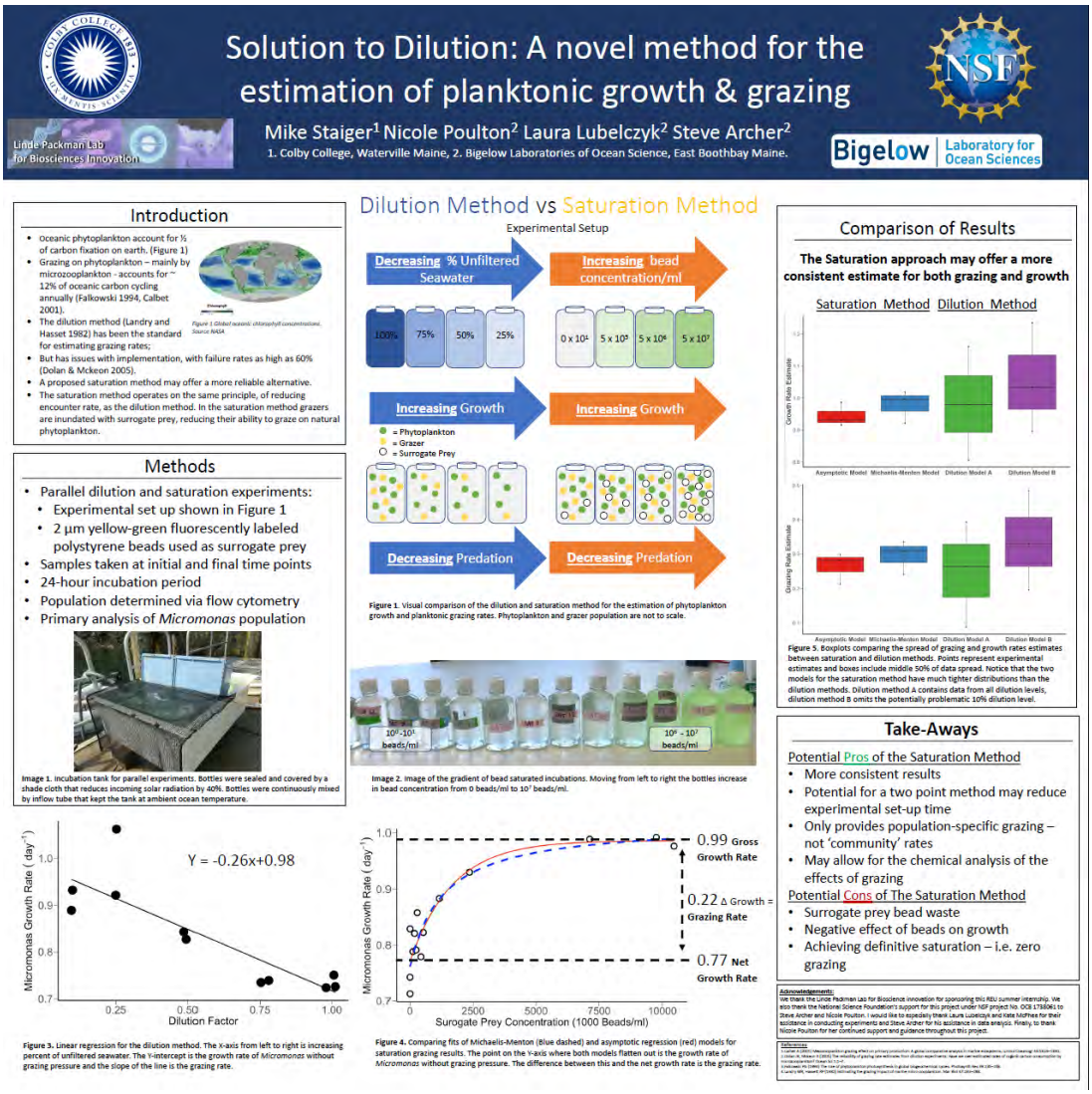


Bigelow's REU Program 2021

SOLUTION TO DILUTION: NOVEL SATURATION METHOD FOR THE ESTIMATION OF PHYTOPLANKTON GROWTH AND GRAZING RATES

Michael Staiger[1,2], Nicole J. Poulton[2], Laura Lubelczyk[2], Steve Archer[2]
Colby College[1], Waterville, Maine, Bigelow Laboratory for Ocean Sciences[2], East Boothbay, Maine

Oceanic phytoplankton account for nearly half of the global carbon fixation in the ocean. Grazing on phytoplankton accounts for 12% of oceanic carbon cycling annually. A standard method for estimating community growth and grazing rates, was first described by Landry and Hassett in 1982, and has become known as the 'dilution method'. While commonly used, the dilution method is known for its issues with reproducibility and has potential failure rates as high as 60%. We propose an alternative approach, whereby reducing grazing pressure experimentally through prey saturation. In this new method, grazing pressure is reduced by saturating grazers with a surrogate prey limiting their ability to prey on the natural phytoplankton community. Fluoresbrite yellow-green 2 µm polystyrene beads were used as surrogate prey. A picoeukaryote population (predominately *Micromonas spp.)* was analyzed via flow cytometry as the primary phytoplankton subpopulation for determining growth and grazing rates. Parallel dilution and saturation experiments were conducted to compare growth and grazing rate estimates of the picoeukaryote population. Our results indicate that the saturation method may be less variable at estimating phytoplankton growth rates and planktonic grazing rates. This novel method allows for both a faster set up and implementation and for chemical analyses of the impacts of grazing, as it does not require water filtration or alteration to water chemistry. The saturation method may be an important alternative method for the determination of planktonic growth and grazing rates.



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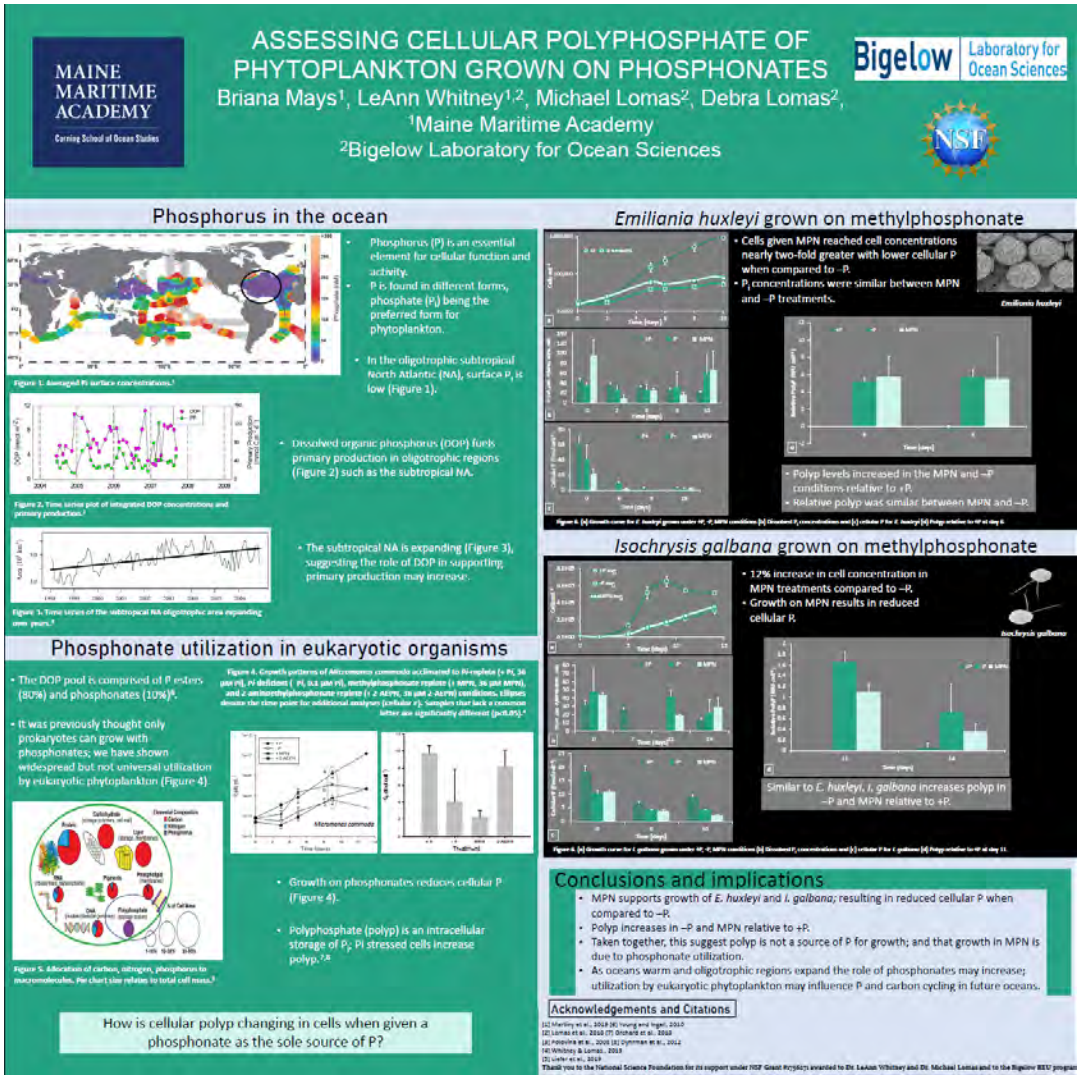
ASSESSING CELLULAR POLYPHOSPHATE OF PHYTOPLANKTON GROWN ON PHOSPHONATES

Briana Mays[1], LeAnn Whitney[1,2], Debra A. Lomas[2], Michael W. Lomas[2]
[1]Maine Maritime Academy. [2]Bigelow Laboratory for Ocean Sciences

Phosphate (P_i) is the form of phosphorus (P) most readily assimilated by phytoplankton. In large oceanic regions, such as the subtropical North Atlantic, surface P_i concentrations are sufficiently low as to limit rates of growth and production. Phytoplankton found in these oligotrophic regions depend on the dissolved organic phosphorus (DOP) pool to meet their P needs. Phosphonates, which are 10% of the DOP pool, were formerly thought to be utilized for growth by prokaryotes only. We have previously demonstrated that the eukaryotic phytoplankton, *Emiliania huxleyi* and *Isochrysis galbana,* when given phosphonates as the sole source of P, can achieve higher cell concentrations than cells grown under P deplete conditions. Both species were found to reduce their cellular P content when grown on methylphosphonate (MPN), when compared to P deplete cells. This suggests cells may be modulating their P storage in response to P source and/or availability. Polyphosphate is an intracellular storage pool of Pi that can account for up to 10% of total cell mass. In this study, we investigated changes in cellular polyphosphate in *E. huxleyi* and *I. galbana* when grown under various phosphorus conditions. We found that in all treatments, polyphosphates decreased over time. When compared to P deplete cells, cells grown on MPN as the sole source of P experienced a greater reduction in cellular polyphosphate. This suggests cells modulate their polyphosphate differently depending on the amount and type of P available. Oligotrophic oceans are expanding and are predicted to become more stratified, which could increase the importance of DOP, including phosphonates, as a nutrient source. Understanding DOP utilization could improve our understanding of future oceans and phytoplankton community composition

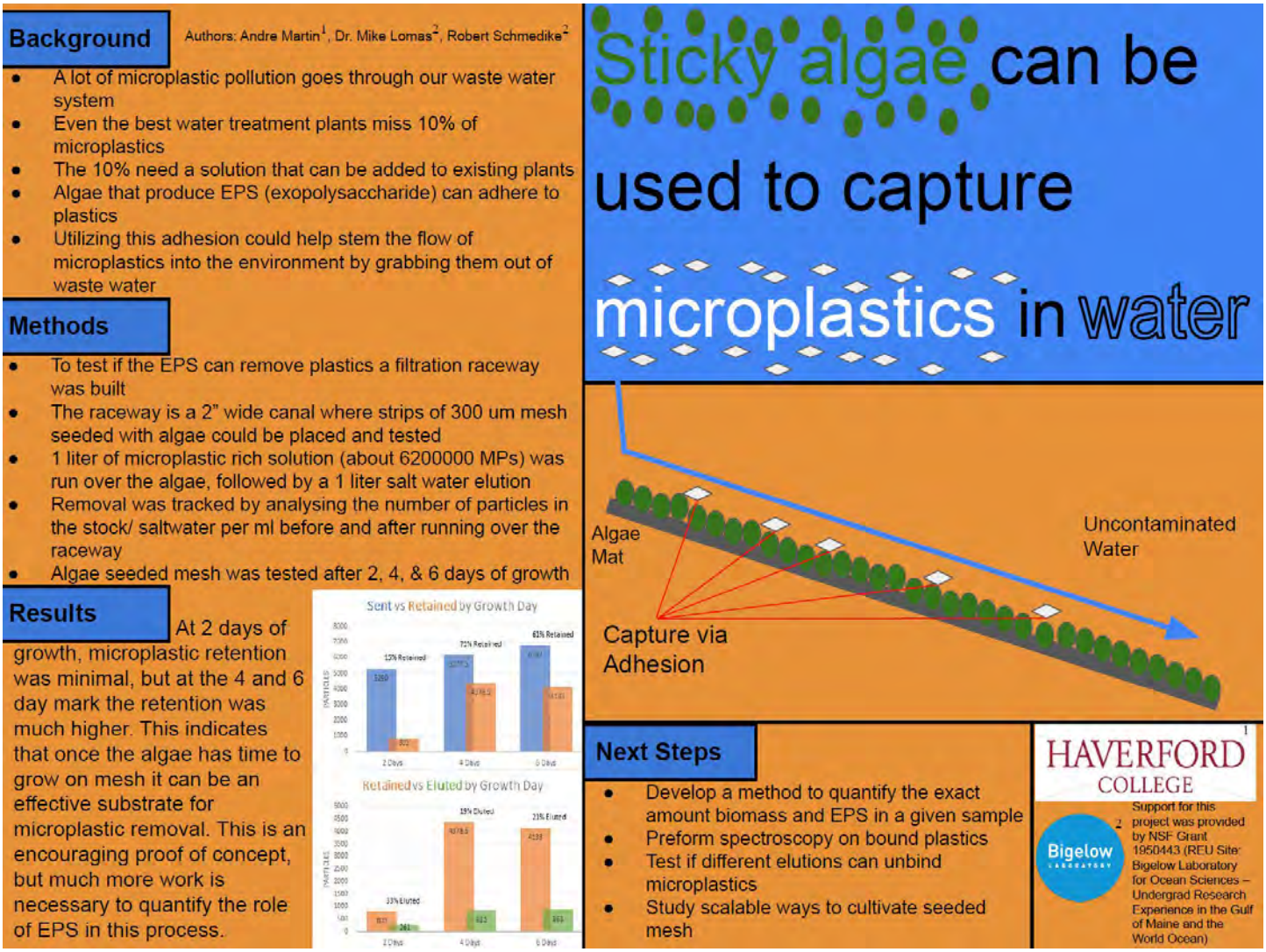


Bigelow's REU Program 2021

Can microplastic be filtered out of wastewater using biofilm forming algae?

Andre Martin[1], Dr. Mike Lomas[2], Robert Schmedike[2]
[1]Haverford College, [2]Bigelow Laboratory for Ocean Sciences

Current water filtration and treatment methods are not sufficient to capture all microplastics in our wastewater, allowing untold amounts of microplastics to escape into the environment. This research project sought to test a novel method for capturing these microplastics in the form of exposure to mats of *Glossomastix chrysoplasta,* an algae with the ability to adhere to plastic in marine environments by using a secreted external polysaccharide (EPS). We built a proof of concept raceway to run microplastic rich solutions over a film of *Glossomastix* at a fixed rate and count the number of particles in the solution before and after runs in order to measure retention. We then used the raceway to test strips of mesh seeded with *Glossomastix* that had been growing for a variable amount of time (2, 4, and 6 days) in the raceway. We discovered that older mats with presumably more EPS & biomass were significantly more efficient at retaining microplastics. While this proof of concept is a promising first step, to be certain about the mechanisms responsible for the increase in retention the next step is to couple raceway testing data with measurements of other metrics like biomass and EPS levels. The level of microplastic retention in algal mats is significant and indicates that this novel methodology merits more study as a potential tool in the effort to reduce microplastic pollution.



Bigelow's REU Program 2021

IMPLEMENTING AND OPTIMIZING AN APPROVED METHOD FOR MONITORING DIARRHETIC SHELLFISH POISONING (DSP)

Finn Dworkin[1], Carmen Cartisano[2], Craig Burnell[2], Stephen Archer[2]
Grinnell College[1], Bigelow Laboratory for Ocean Sciences[2]

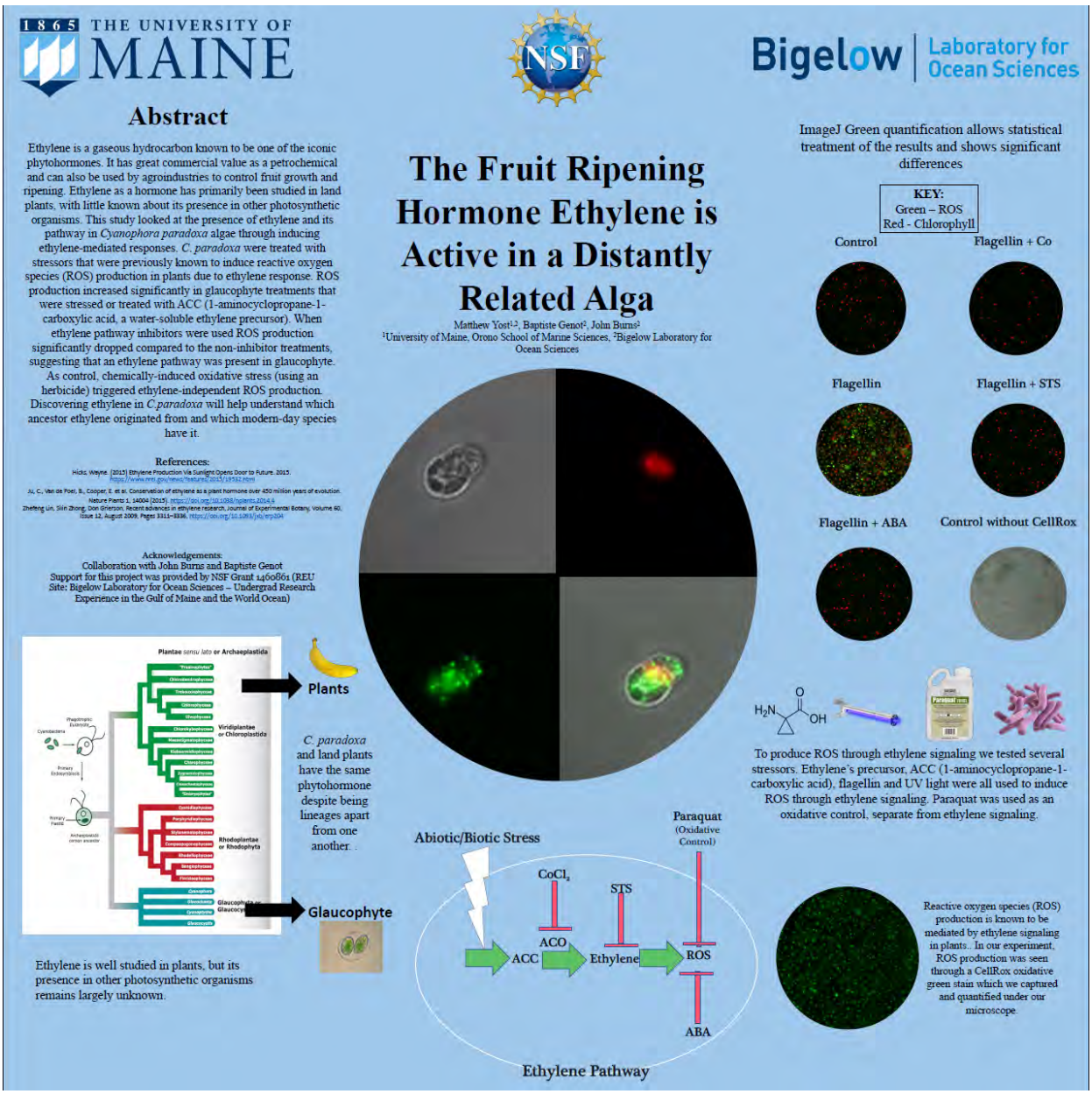
Marine toxins threaten consumer safety, and the Maine shellfish industry's longevity. Bivalve shellfish filter feed, imbibing plankton species in the water. Planktonic microalgal species, *Dinophysis* and *Prorocentrum*, produce poly-ether acid metabolites that can accumulate in the tissue of shellfish. Consuming shellfish transfers the toxins to a human, causing diarrhetic shellfish poisoning (DSP). DSP is an acute illness with low morbidity symptoms: diarrhea, nausea, and stomach pain. Currently, the Food and Drug Administration requires an LC-MS/MS method approved by the Interstate Shellfish Sanitation Conference, for regulatory testing of shellfish DSP. The approved LC-MS/MS method is costly and demands technical knowledge for operation. Our study aims to, first, optimize the current approved LC-MS/MS method and second, compare the approved method with cheaper, more accessible commercial test kits. The results show that addition of a matrix blank conditioning step and removal of a hexane wash step improves sensitivity and reproducibility and makes the extraction method faster, cheaper, and greener for future comparative research.

Bigelow's REU Program 2021

Discovering the Presence of Ethylene within *Cyanophora paradoxa* Algae and its Pathway

Matthew Yost[1,2], Baptiste Genot[2], John Burns[2]
[1]University of Maine, Orono, School of Marine Sciences, [2]Bigelow Laboratory for Ocean Sciences

Ethylene is a gaseous hydrocarbon known to be one of the iconic phytohormones. It has great commercial value as a petrochemical and can also be used by agroindustries to control fruit growth and ripening. Ethylene as a hormone has primarily been studied in land plants, with little known about its presence in other photosynthetic organisms. This study looked at the presence of ethylene and its pathway in *Cyanophora paradoxa* algae through inducing ethylene-mediated responses. *C. paradoxa* were treated with stressors that were previously known to induce reactive oxygen species (ROS) production in plants due to ethylene response. ROS production increased significantly in glaucophyte treatments that were stressed or treated with ACC (1-aminocyclopropane-1-carboxylic acid, a water soluble ethylene precursor). When ethylene pathway inhibitors were used ROS production significantly dropped compared to the non-inhibitor treatments, suggesting that an ethylene pathway was present in glaucophyte. As control, chemically-induced oxidative stress (using an herbicide) triggered ethylene-independent ROS production. Discovering ethylene in *C.paradoxa* will help understand which ancestor ethylene originated from and which modern-day species have it.

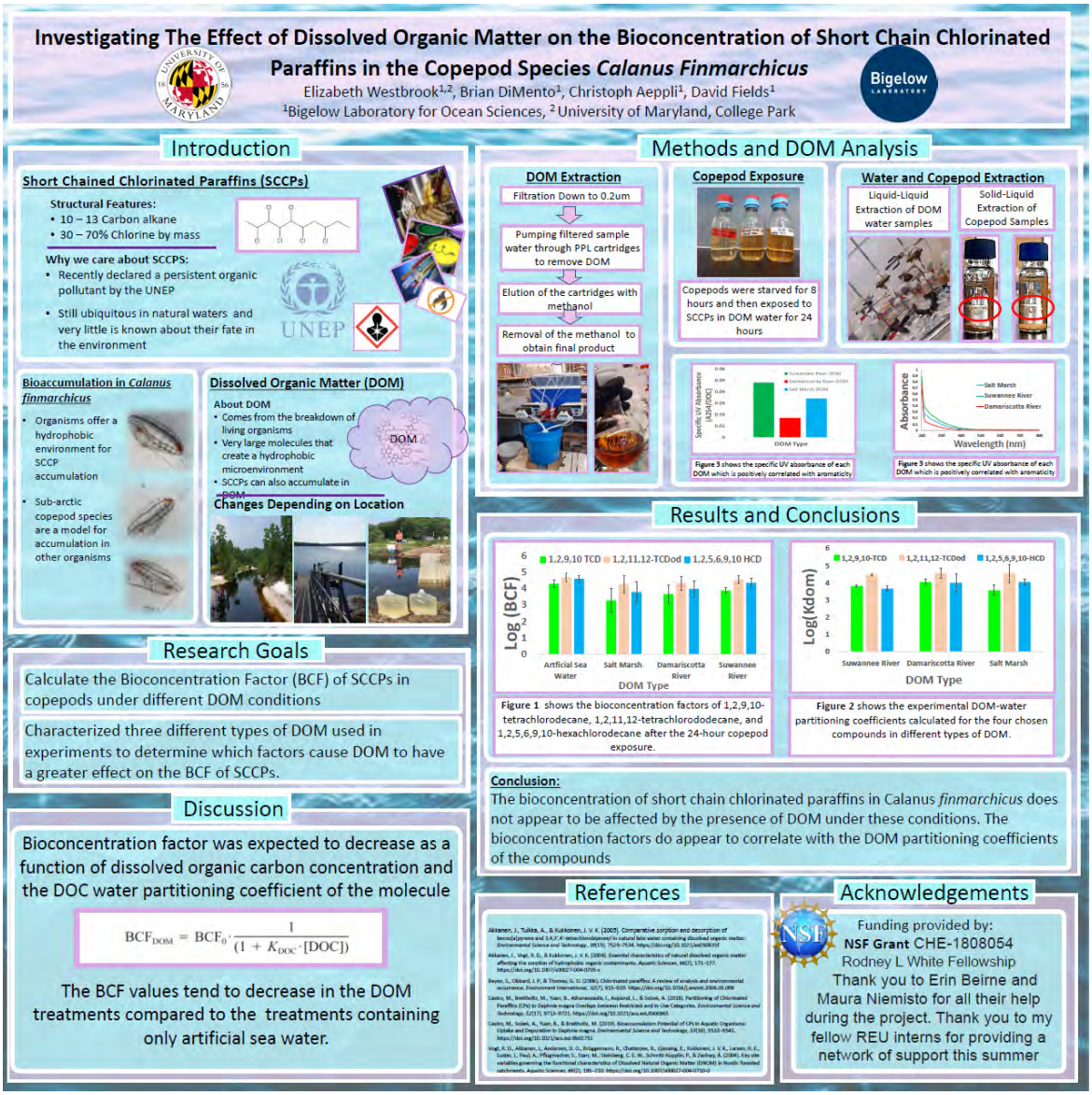


Bigelow's REU Program 2021

Investigating the Effects of Dissolved Organic Matter on the Bioaccumulation of Short Chained Chlorinated Paraffins in the Copepod Species *Calanus finmarchicus*

Elizabeth Westbrook[1,2], Brian DiMento[1], Christoph Aeppli[1], David Fields[1]
[1]Bigelow Laboratory for Ocean Sciences, [2]University of Maryland, College Park

Short chain chlorinated paraffins (SCCPs) are a class of polychlorinated alkanes that have been used in industrial processes since around the 1930s, particularly as metal working lubricants and plasticizers. In 2017, SCCPs were declared a persistent organic pollutant by the United Nations Environment Programme due to their toxicity, their potential for bioaccumulation, and their potential to be a carcinogen. Unfortunately, SCCPs remain ubiquitous in natural waters. To better understand their fate in the environment, we investigated how the partitioning of SCCPs in to three different types of dissolved organic matter (DOM) affected their bioaccumulation in a model organism, which was the subarctic copepod species *Calanus finmarchicus*. DOM creates a hydrophobic microenvironment wherein smaller hydrophobic molecules like SCCPs can sorb, potentially influencing their ability to accumulate in biota. The bioconcentration factor (BCF), a measure of the amount of SCCPs cumulated from the water by organisms, was expected to decrease as a function of the DOM concentration and the DOM water partitioning coefficient of the SCCPs. Results showed that the BCF values tend to decrease in the presence of DOM. High levels of DOM therefore appear to have an impact on the accumulation of SCCPs in aquatic organisms.

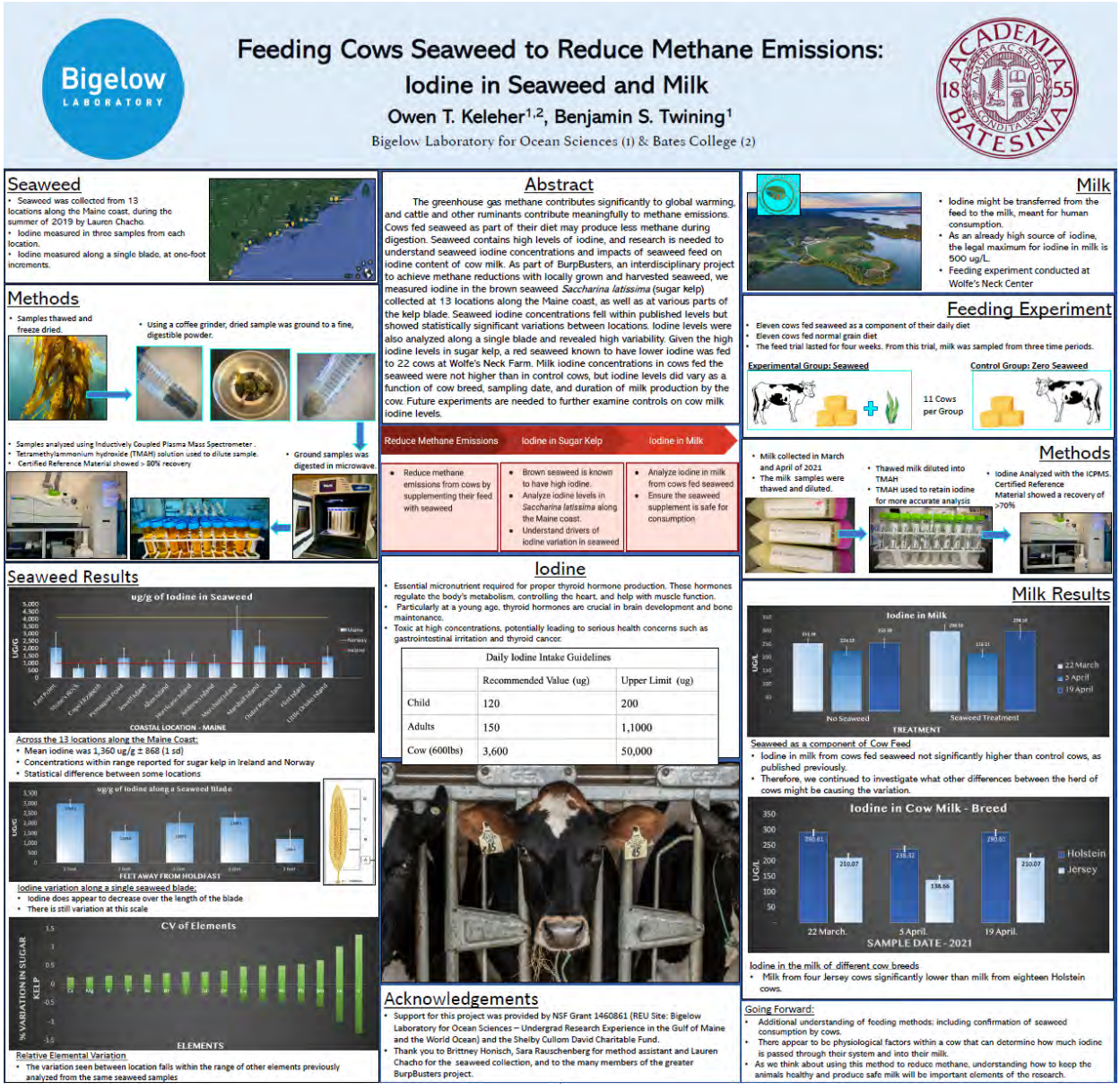


Bigelow's REU Program 2021

Feeding Cows Seaweed to Reduce Methane Emissions: Iodine in Seaweed and Milk

Owen T. Keleher[2],& Benjamin S. Twining , PhD.[2]
1 Bates College , 2 Bigelow Laboratory for Ocean Sciences

The greenhouse gas methane contributes significantly to global warming, and cattle and other ruminants contribute meaningfully to methane emissions. Cows fed seaweed as part of their diet may produce less methane during digestion. Seaweed contains high levels of iodine, and research is needed to understand seaweed iodine concentrations and impacts of seaweed feed on iodine content of cow milk. As part of BurpBusters, an interdisciplinary project to achieve methane reductions with locally grown and harvested seaweed, we measured iodine in the brown seaweed *Saccharina latissima* (sugar kelp) collected at 13 locations along the Maine coast, as well as at various parts of the kelp blade. Seaweed iodine concentrations fell within published levels but showed statistically significant variations between locations. Iodine levels were also analyzed along a single blade and revealed high variability. Given the high iodine levels in sugar kelp, a red seaweed known to have lower iodine was fed to 22 cows at Wolfe's Neck Farm. Milk iodine concentrations in cows fed the seaweed were not higher than in control cows, but iodine levels did vary as a function of cow breed, sampling date, and duration of milk production by the cow. Future experiments are needed to further examine controls on cow milk iodine levels.



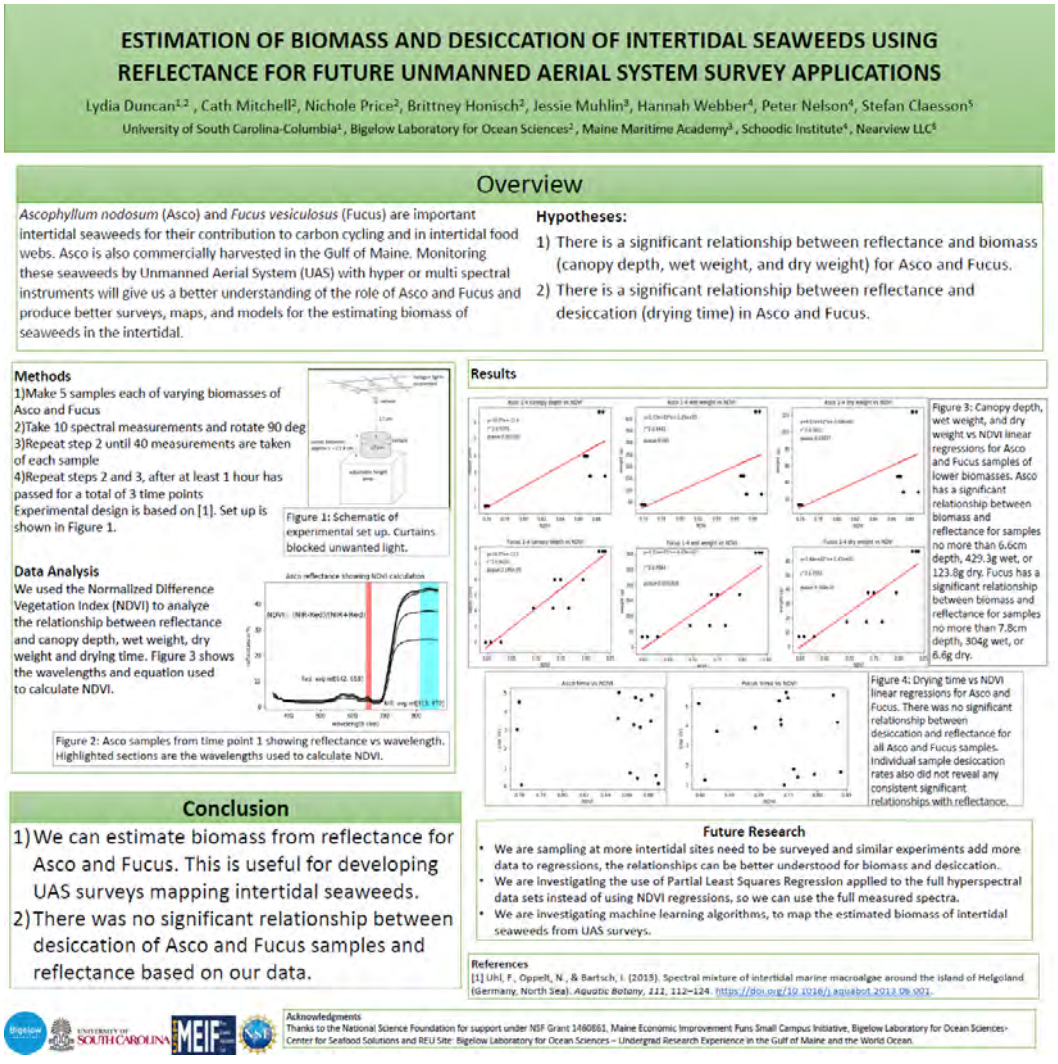
Support for this project was provided by NSF Grant 1460861 (REU Site: Bigelow Laboratory for Ocean Sciences – Undergrad Research Experience in the Gulf of Maine and the World Ocean) and the Shelby Cullom David Charitable Fund.
Thank you to Brittney Honisch, Sara Rauschenberg for method assistant and Lauren Chacho for the seaweed collection, and to the many members of the greater BurpBusters project.

Bigelow's REU Program 2021

ESTIMATION OF BIOMASS AND DESICCATION OF INTERTIDAL SEAWEEDS USING REFLECTANCE FOR FUTURE UNMANNED AERIAL SYSTEM SURVEY APPLICATIONS

Lydia Duncan[1,2] , Cath Mitchell[2], Nichole Price[2], Brittney Honisch[2], Jessie Muhlin[3], Hannah Webber[4], Peter Nelson[4], Stefan Claesson[5]
University of South Carolina-Columbia[1] , Bigelow Laboratory for Ocean Sciences[2] , Maine Maritime Academy[3] , Schoodic Institute[4] , Nearview LLC[5]

Intertidal seaweeds, *Ascophyllum nodosum (A. nodosum)* and *Fucus vesiculosus (F. vesiculosus)*, are vital to carbon cycling and intertidal food webs in the Gulf of Maine. *A. nodosum* is commercially harvested as a Maine fishery with economic value. To monitor the sustainability of harvest limits, a project began to develop methods to estimate biomass of seaweeds from Unmanned Aerial System surveys. This study focuses on understanding the relationship between reflectance and biomass as given by canopy depth or wet and dry weight. We also investigated the relationship between reflectance and desiccation between tidal cycles. For both *A. nodosum* and *F. vesiculosus,* 5 samples of increasing levels of biomass were prepared and the reflectance was measured over 3 time points to replicate hours left exposed during low tide. Each sample's reflectance was represented by the calculated Normalized Difference Vegetation Index (NDVI). *A. nodosum* samples were found to have a significant relationship between NDVI and biomass below canopy depth of 6.6cm, wet weight of 429.3g, and dry weight of 123.8g (p values: 0.0032, 0.045, and 0.039). *F. vesiculosus* samples were found to have a significant relationship between NDVI and biomass below canopy depth of 304cm, wet weight of 66.6g, and dry weight of 7.8g (p values: 2.1e-6, 1.8e-4, and 9.8e-5). There was no overall significant relationship between NDVI and desiccation for each seaweed, and no consistent relationship among individual samples. These results show potential in estimating biomass of intertidal seaweeds with future UAS survey methods.

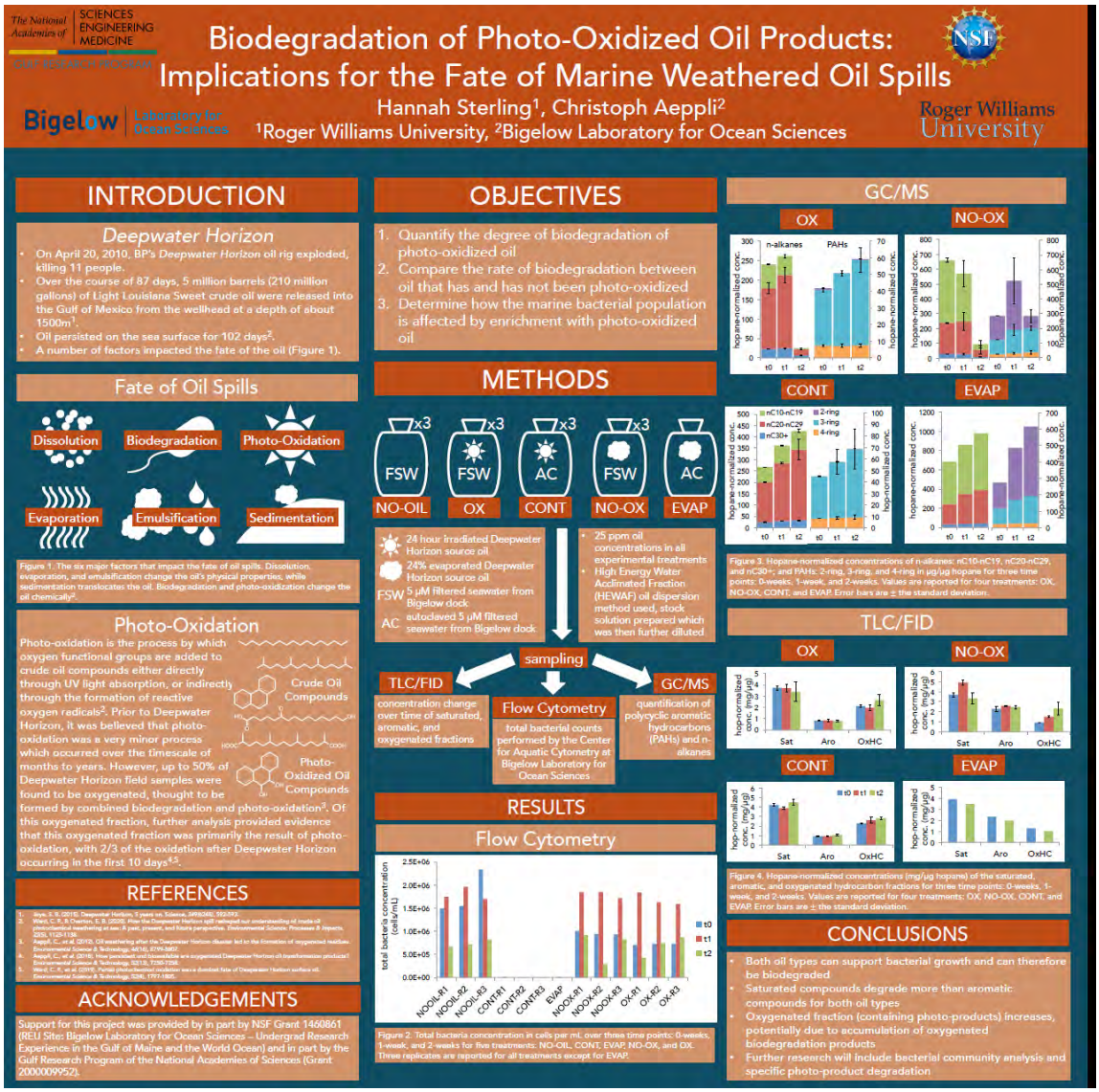


Bigelow's REU Program 2021

BIODEGRADATION OF PHOTO-OXIDIZED OIL PRODUCTS: IMPLICATIONS FOR THE FATE OF MARINE WEATHERED OIL SPILLS

Hannah Sterling[1], Christoph Aeppli[2]
[1]Roger Williams University, [2]Bigelow Laboratory for Ocean Sciences

Photo-oxidation is a process that weathers oil in the marine environment, where light either directly or indirectly oxidizes crude oil compounds. It was believed that photo-oxidation had only a minimal effect on the fate of oil, however new research in the aftermath of Deepwater Horizon, the largest accidental marine oil spill, has shown oxidized photo-products form a much larger proportion of the total oil mass than previously understood. Still, the fate of these photoproducts, particularly how they are biodegraded, is poorly understood. A series of experimental treatments were prepared to compare the rates of biodegradation in the marine environment between oil that has and has not been photo-oxidized in order to further understand how oil spills can be best mitigated by natural microbial communities. Photo-oxidized and evaporated treatments were prepared, including sterile controls and no oil controls. Over the course of two weeks, samples collected for GC/MS, TLC/FID, and flow cytometry analysis provided evidence that both types of oil can support bacterial growth and can therefore be biodegraded. Saturated compounds degraded more readily than aromatic compounds in both types of oil, but oxygenated products were formed, potentially indicating the presence of biodegradation products. Overall, photo-oxidation does not inhibit biodegradation of saturated hydrocarbons. In contrast, the observed increase in oxygenated compounds indicates that these products may not be as readily degraded.

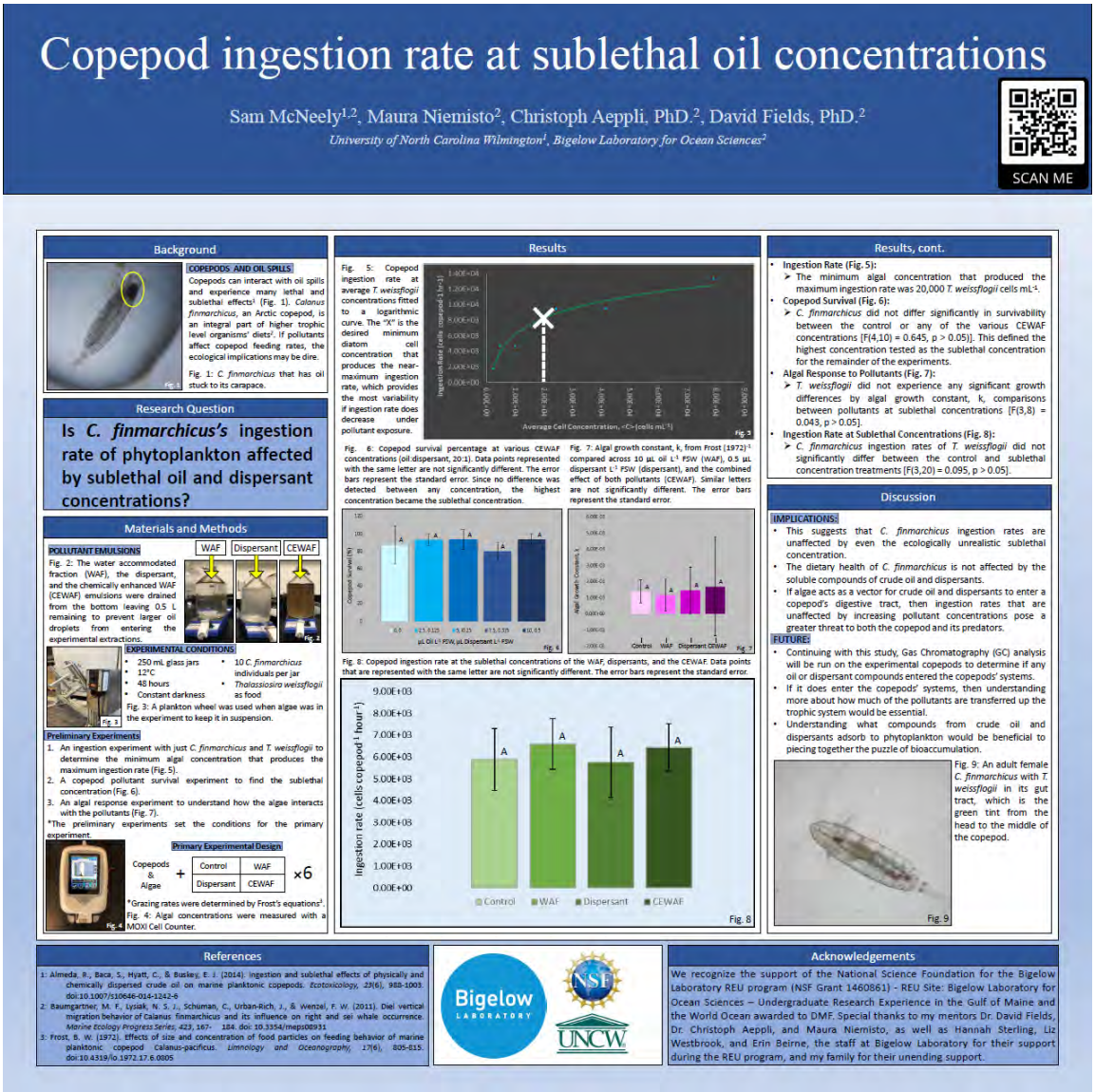


Bigelow's REU Program 2021

COPEPOD INGESTION RATE AT SUBLETHAL OIL CONCENTRATIONS

Sam McNeely[1,2], Maura Niemisto[2], Christoph Aeppli, PhD.[2], & David Fields, PhD.[2]
1 University of North Carolina Wilmington, 2 Bigelow Laboratory for Ocean Sciences

Receding sea ice in the Arctic opens the gate for exploitation of Arctic oil reserves as well as transportation, thereby increasing the likelihood for an oceanic oil spill to occur. When exposed to sublethal crude oil and dispersant concentrations, copepods experience decreases in offspring production and fecal pellet production rates, suggesting a decrease in ingestion rates. This study examined the effects that sublethal crude oil and dispersant concentrations have on ingestion rates in a common North Atlantic copepod, *Calanus finmarchicus*, a valuable component in the Arctic food web. The survival rate of adult *C. finmarchicus* was examined on a concentration gradient for a water accommodated fraction (WAF), dispersants, and a chemically enhanced water accommodated fraction (CEWAF). The highest sublethal concentration became the concentration at which the copepods were exposed to when examining their ingestion rates of a centric diatom, *Thalassiosira weissflogii*. Even at a high WAF concentration of 10 µL oil L^{-1} water and a dispersant concentration of 0.5 µL dispersant L^{-1} water, *C. finmarchicus* did not show a significant difference in survivability. *C. finmarchicus* ingestion rates at the sublethal WAF, dispersant, and CEWAF concentrations did not differ significantly. This suggests that the dietary health of *C. finmarchicus* would not be affected by the soluble components of crude oil and dispersants. However, *T. weissflogii* may act as a vector for the pollutants to enter the digestive tract of *C. finmarchicus*.

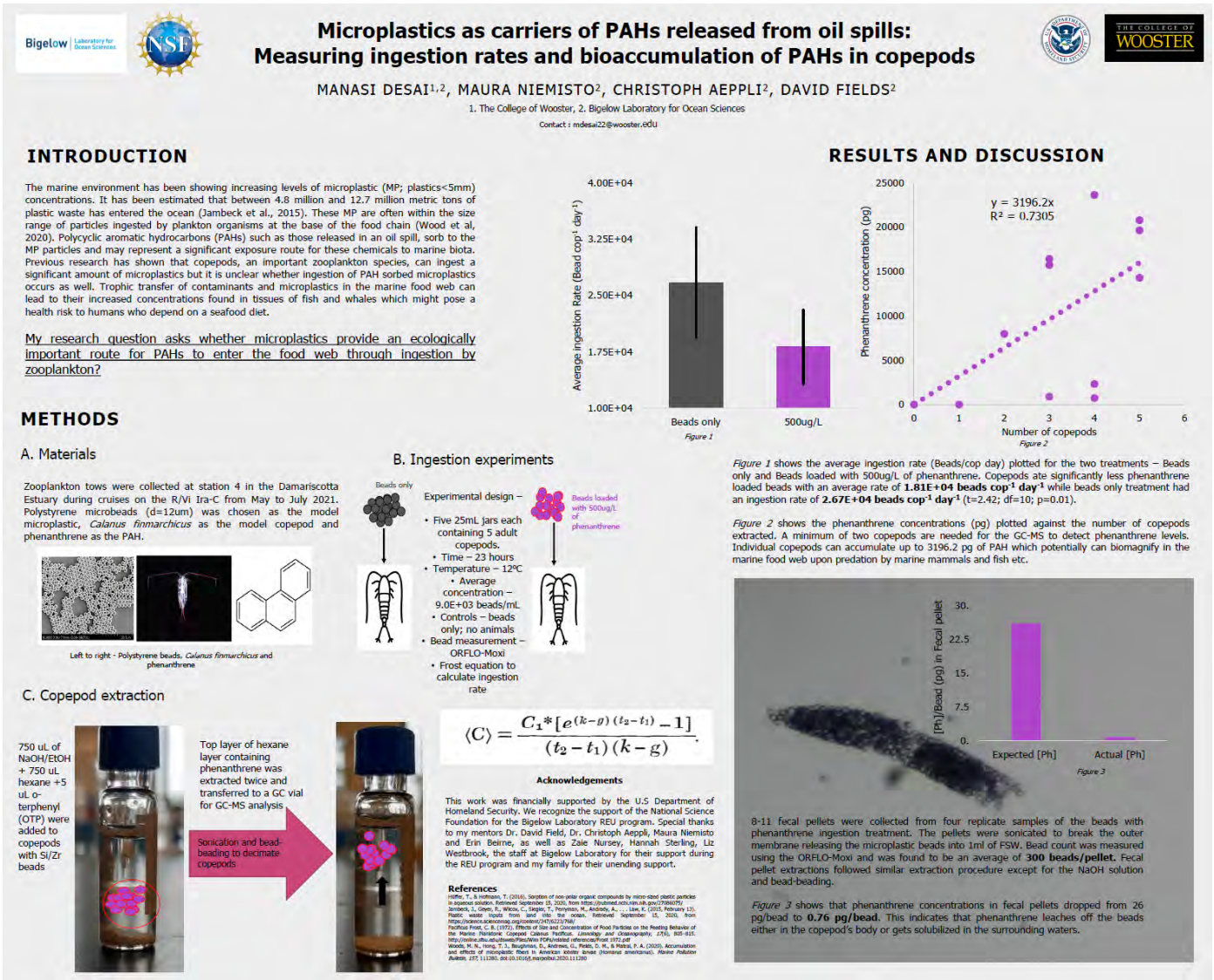


Bigelow's REU Program 2021

Microplastics as carriers of PAHs released from oil spills: Measuring ingestion rates and bioaccumulation of PAHs in copepods

Manasi Desai[1,2], Maura Niemisto[2], Christoph Aeppli[2], David Fields[2]
The College of Wooster[1], Bigelow Laboratory of Ocean Sciences[2]

Microplastics (MP; plastics < 5mm) are a growing problem in the marine environment due to a large influx of plastics entering the marine environment through rivers, wastewater and litter from land. (Ziccardi et al., 2016). MP are known to adsorb nonpolar compounds in the water including polycyclic aromatic hydrocarbons (PAHs) released from oil spills. In this study, we quantified the ingestion rates of a marine copepod (*Calanus finmarchicus)* on clean polystyrene MP beads (12 um) and PAH loaded MP beads (26 pg PAH pellet $^{-1}$) to measure the role of MP as a vector for PAHs into marine food webs. Copepods ate significantly lower PAH loaded MP (1.81e+04 beads cop^{-1} day^{-1}) than microplastics only (2.67E+04 beads cop^{-1} day^{-1}) suggesting that copepods can reject particles based on chemical content. Accumulation of phenanthrene within the copepods (GC-MS analysis) showed individual copepods can accumulate up to 3196 pg of PAH which potentially can biomagnify in the marine food web upon predation. Fecal pellet analysis showed that PAH concentrations within the beads decreased from 26 pg/bead to 0.76 pg/bead which suggests the PAH desorb off and are retained in either the copepod's body or solubilizes in the surrounding waters.



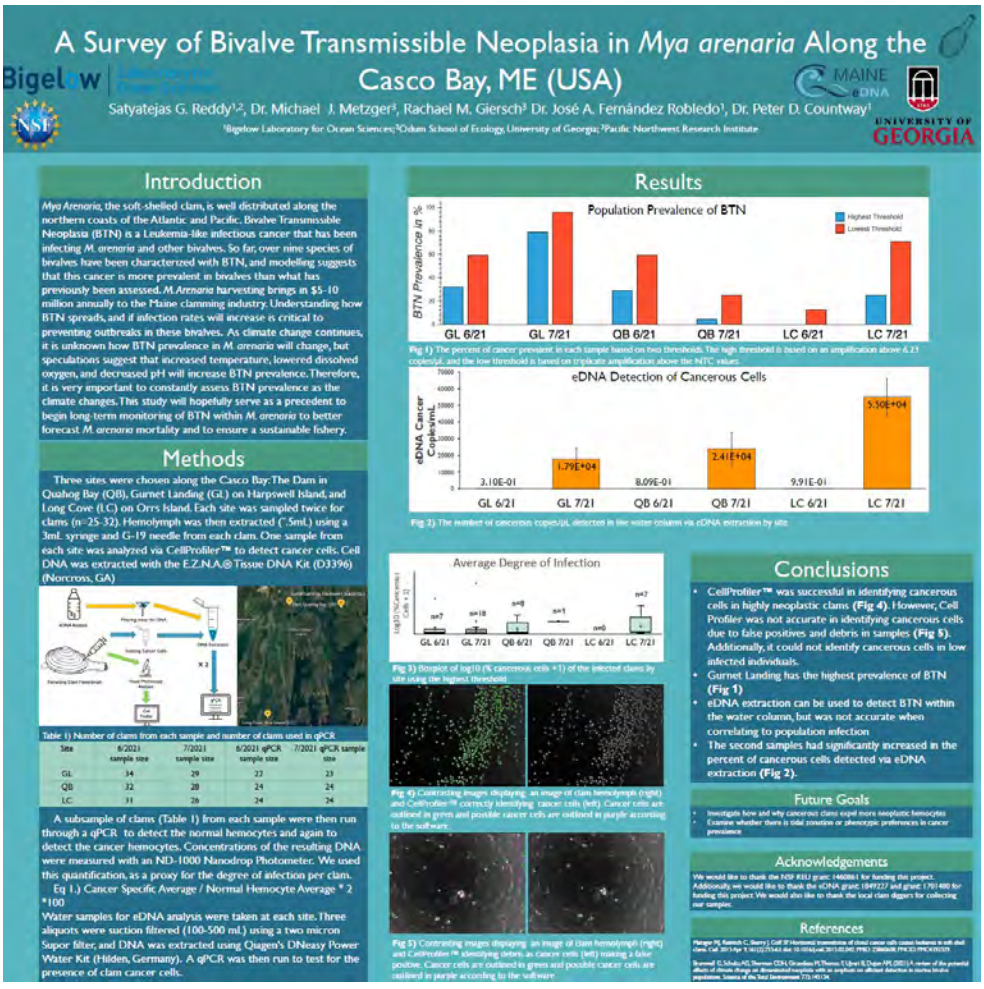
Bigelow's REU Program 2021

A Survey of Bivalve Transmissible Neoplasia in *Mya arenaria* Along the Casco Bay ME (USA)

Satyatejas G. Reddy[1,2], Dr. Michael J. Metzger[3], Rachael M. Giersch[3], Dr. José A. Fernández Robledo[1], Dr. Peter D. Countway[1]

[1]Bigelow Laboratory for Ocean Sciences; [2]Odum School of Ecology, University of Georgia; [3]Pacific Northwest Research Institute

Bivalve Transmissible Neoplasia (BTN) is a Leukemia-like infectious cancer described in *Mya arenaria* and other marine bivalves. *M. arenaria* harvesting brings $5-10 million annually to the Maine clamming industry and serves as a vital nutrient cycler in estuarine systems. It is unknown how climate change will affect BTN prevalence and intensity. Still, speculations suggest that increased temperature, lowered dissolved oxygen, and decreased pH will increase BTN prevalence and severity. This study conducted a BTN survey in three sites in Maine: The Dam in Quahog Bay, Gurnet Landing on Harpswell Island, and Long Cove on Orrs Island. Each site was sampled twice over two months in the summer of 2021 using three methods to detect the cancer cells. We used the software, CellProfiler, to numerically identify cancer cells from microscope images of clam hemolymph, ran two qPCRs to quantify the cancer cells and normal hemocytes within each clam, and ran qPCRs on eDNA from the water surrounding the clams to see if the BTN cell prevalence in the environment would correlate to the prevalence in the clam population. We found that BTN was most prevalent in Gurnet Landing sample two (79-96% prevalence) and least present in Long Cove sample one (0-12.5% prevalence). CellProfiler was able to identify cancerous cells; however, it did not perform well in low-infected individuals and hemolymph with large amounts of cellular debris. For the first time, eDNA was used to detect BTN cells within the water column. Although cancer cell prevalence in the water did not align with the BTN prevalence in the clams, all detections of BTN in July (18-55 copies/µL) amplified at a significantly higher copy number than that of June (0.0003-0.001 copies/µL). Altogether, our results indicate that BTN is still present, and eDNA can be used to monitor the presence of neoplastic cells.

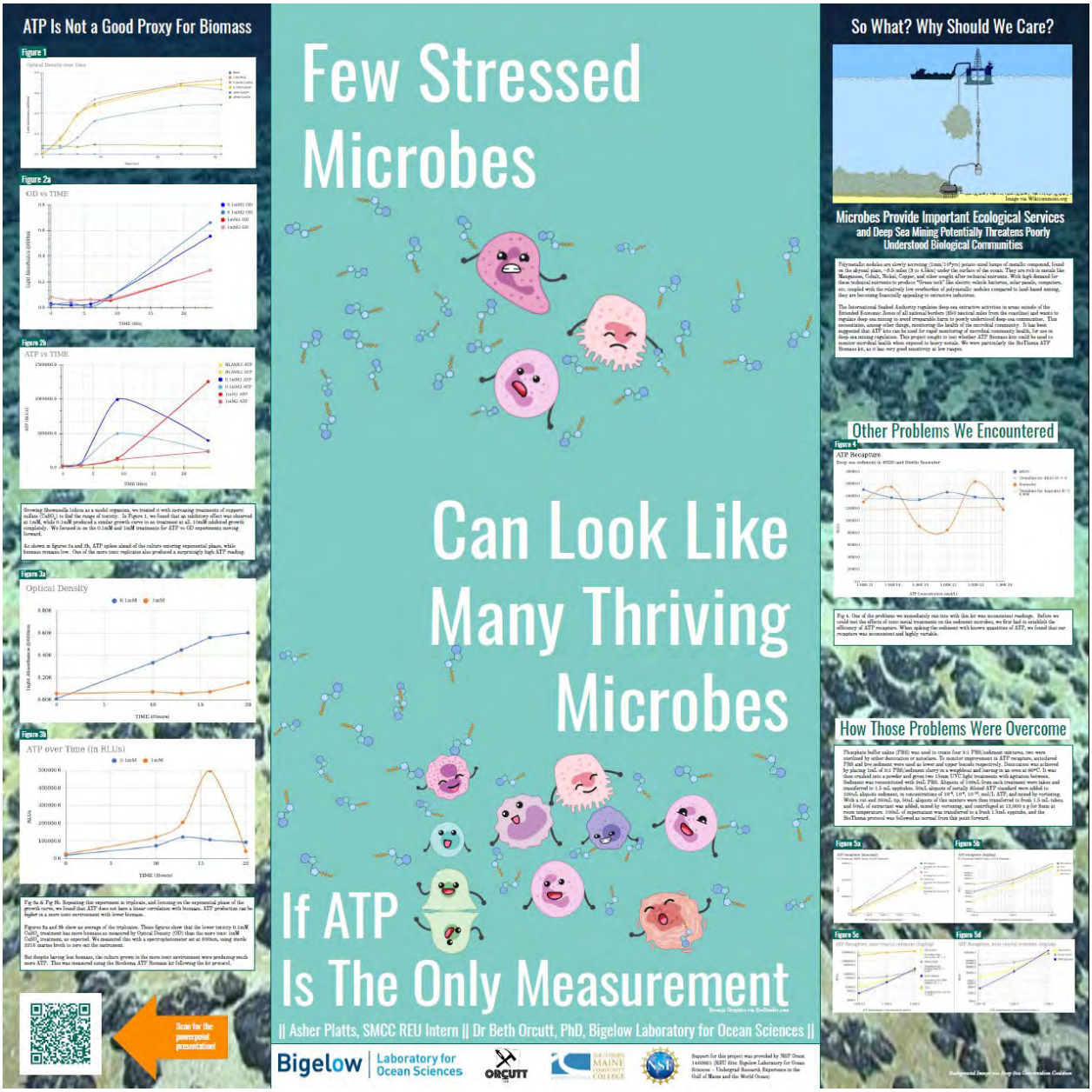


Bigelow's REU Program 2021

IS ATP A SUFFICIENT PROXY FOR BIOMASS FOR USE IN DEEP SEA MINING REGULATION?

Asher Platts[1], Dr. Beth Orcutt, PhD[2]
Southern Maine Community College[1], Bigelow Laboratory for Ocean Sciences[2]

Deep Sea Mining poses a potential threat to abyssal plain microbial communities, and the ecological services they provide, both of which are poorly understood. This research project sought to understand the effects of mining-related heavy metal toxicity on these microbial communities, and if those effects could be rapidly detected, using an ATP assessment approach recommended by the agency that regulates deep-sea mining. We discovered that ATP assessment of sediment microbial communities required method improvement to overcome ATP binding to sediment. The issue of mining-related heavy metal toxicity was not resolved, but using a model organism in culture, we found that ATP does not have a linear correlation to biomass, and low populations of bacteria in toxic conditions can look similar to high populations of bacteria in less toxic conditions. Therefore, ATP is not a good proxy for biomass for rapid diagnostics of deep-sea mining impacts to sediment.

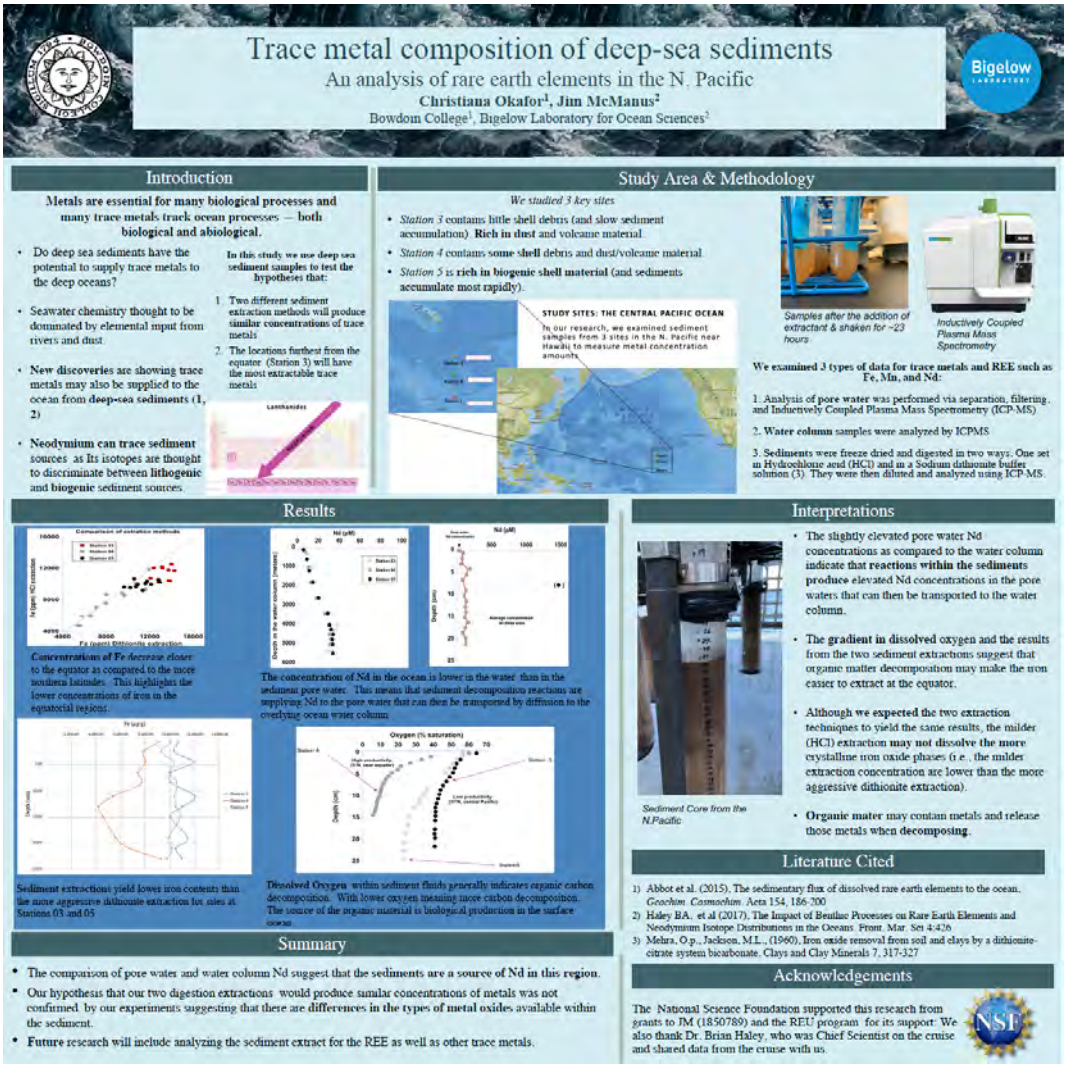


Bigelow's REU Program 2021

TRACE METAL COMPOSITION OF DEEP-SEA SEDIMENTS: AN ANALYSIS OF RARE EARTH ELEMENTS IN THE N. PACIFIC

Christiana Okafor[1], Jim McManus[2]
Bowdoin College[1], Bigelow Laboratory for Ocean Sciences[2]

Many trace metals in the ocean are important for biological processes and tracing the physical, biological, and chemical characteristics of the ocean. These elements are thought to be predominately input to the ocean from rivers or atmospheric deposition. However, new research points to the idea that some trace metals are supplied to the ocean from deep-sea sediments. This notion suggests that solid phase sediment particles undergo dissolution within the seafloor sediment package and that these solids could be derived from atmospheric inputs. One trace metal that we are studying, the rare earth element (REE) neodymium, serves as an ocean tracer that can decipher between lithogenic and biogenic sources of the dissolved concentration of the element. In our research, we examined sediment samples from three sites in the central north Pacific between Hawaii and the equator. These sites vary in sediment composition from rich biological deposits to sediment that is heavily influenced by dust deposition. Our research is informed done using three types of data: water column, pore water, and sediment data. Water column, or ocean water data, show that with increasing depth in the ocean, trace metal concentrations like neodymium, increase. In pore fluids, which are the fluids from within the sediment below the sea floor, oxygen concentrations show decreases with increasing depth, which indicates that organic carbon is undergoing decomposition. In these sediments, iron concentrations are lower near the equator and highest at our two more northern sites. In upcoming research, we will continue to analyze trace metal concentrations in our samples to further determine whether deep sea sediments are truly a source of trace metals.

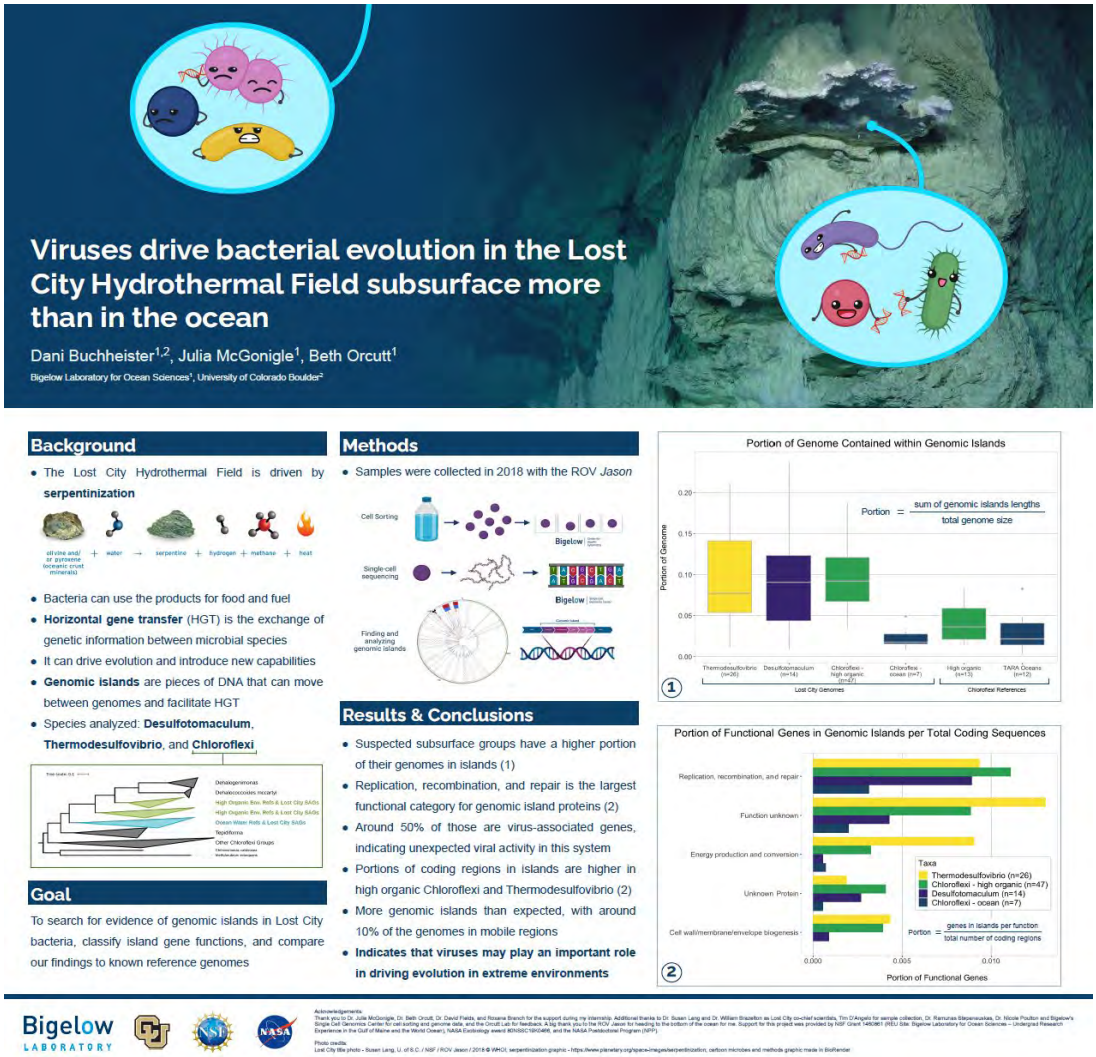


Bigelow's REU Program 2021

VIRUSES DRIVE BACTERIAL EVOLUTION IN THE LOST CITY HYDROTHERMAL FIELD SUBSURFACE MORE THAN IN THE OCEAN

Dani Buchheister[1,2], Julia McGonigle[1], Beth Orcutt[1]
Bigelow Laboratory for Ocean Sciences[1], University of Colorado Boulder[2]

The Lost City Hydrothermal Field (LCHF) is a hydrothermal system driven by interactions between seawater and crust minerals. This chemical reaction, called serpentinization, produces hot, alkaline fluids with high concentrations of hydrogen and methane. This system is astrobiologically interesting due to the ability of these environments to abiotically provide food and fuel for potential life on other planets. The LCHF has been studied for over 20 years, but little is known about the site's subsurface microbial life. This knowledge gap includes information about horizontal gene transfer, the exchange of genetic information between microbial species. It can allow microbes to gain new capabilities, useful in the harsh Lost City environment. Gene transfer often occurs via special regions of DNA known as genomic islands. To explore this topic, genomic islands were identified in both single-cell genomes sequenced from LCHF hydrothermal fluids collected in 2018 and reference genomes. Gene functions were classified and compared between groups. Our results indicate that genomes of Lost City's subsurface bacteria contain higher portions of genomic islands when compared to bacteria from more traditional marine environments. Genes associated with viral-mediated transfer indicate viral activity in the community. These results show stronger genetic movement signals than expected and provide a starting point for understanding how viruses introduce adaptations and aid in bacterial survival in extreme environments.

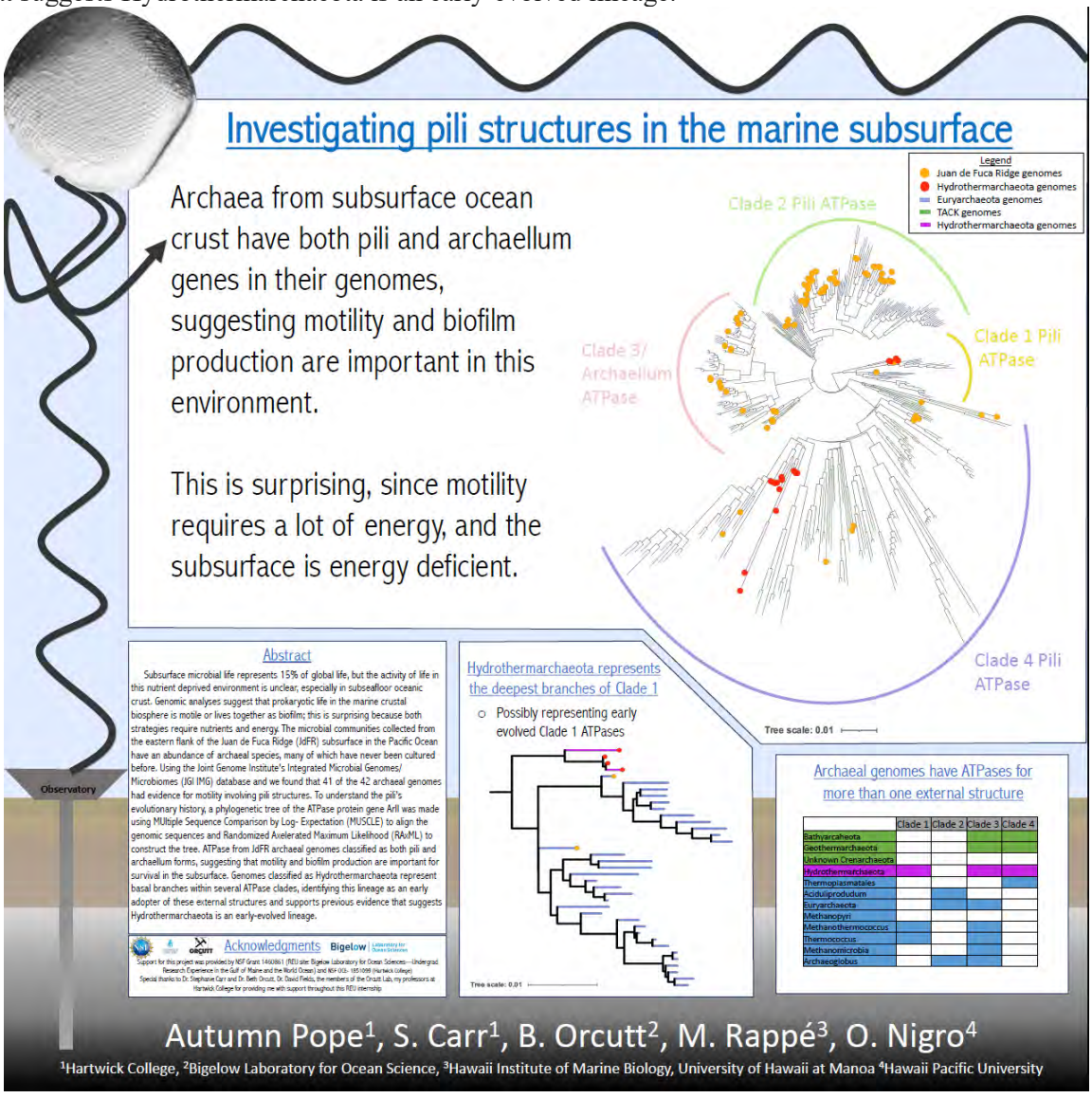


Bigelow's REU Program 2021

INVESTIGATING PILI STRUCTURES IN THE MARINE SUBSURFACE

Autumn Pope[1], Stephanie Carr[1], Beth Orcutt[2], Michael Rappé[3], Olivia Nigro[4]
[1]Hartwick College, [2]Bigelow Laboratory for Ocean Sciences, [3] Hawaii Institute of Marine Biology, University of Hawaii at Manoa, [4]Hawaii Pacific University

Subsurface microbial life represents 15% of global life, but the activity of life in this nutrient deprived environment is unclear, especially in subseafloor oceanic crust. Genomic analyses suggest that prokaryotic life in the marine crustal biosphere is motile or lives together as biofilm; this is surprising because both strategies require nutrients and energy. The microbial communities collected from the eastern flank of the Juan de Fuca Ridge (JdFR) subsurface in the Pacific Ocean have an abundance of archaeal species, many of which have never been cultured before. Using the Joint Genome Institute's Integrated Microbial Genomes/Microbiomes (JGI IMG) database and we found that 41 of the 42 archaeal genomes had evidence for motility involving pili structures. To understand the pili's evolutionary history, a phylogenetic tree of the ATPase protein gene ArlI was made using MUltiple Sequence Comparison by Log- Expectation (MUSCLE) to align the genomic sequences and Randomized Axelerated Maximum Likelihood (RAxML) to construct the tree. ATPase from JdFR archaeal genomes classified as both pili and archaellum forms, suggesting that motility and biofilm production are important for survival in the subsurface. Genomes classified as Hydrothermarchaeota represent basal branches within several ATPase clades, identifying this lineage as an early adopter of these external structures and supports previous evidence that suggests Hydrothermarchaeota is an early-evolved lineage.

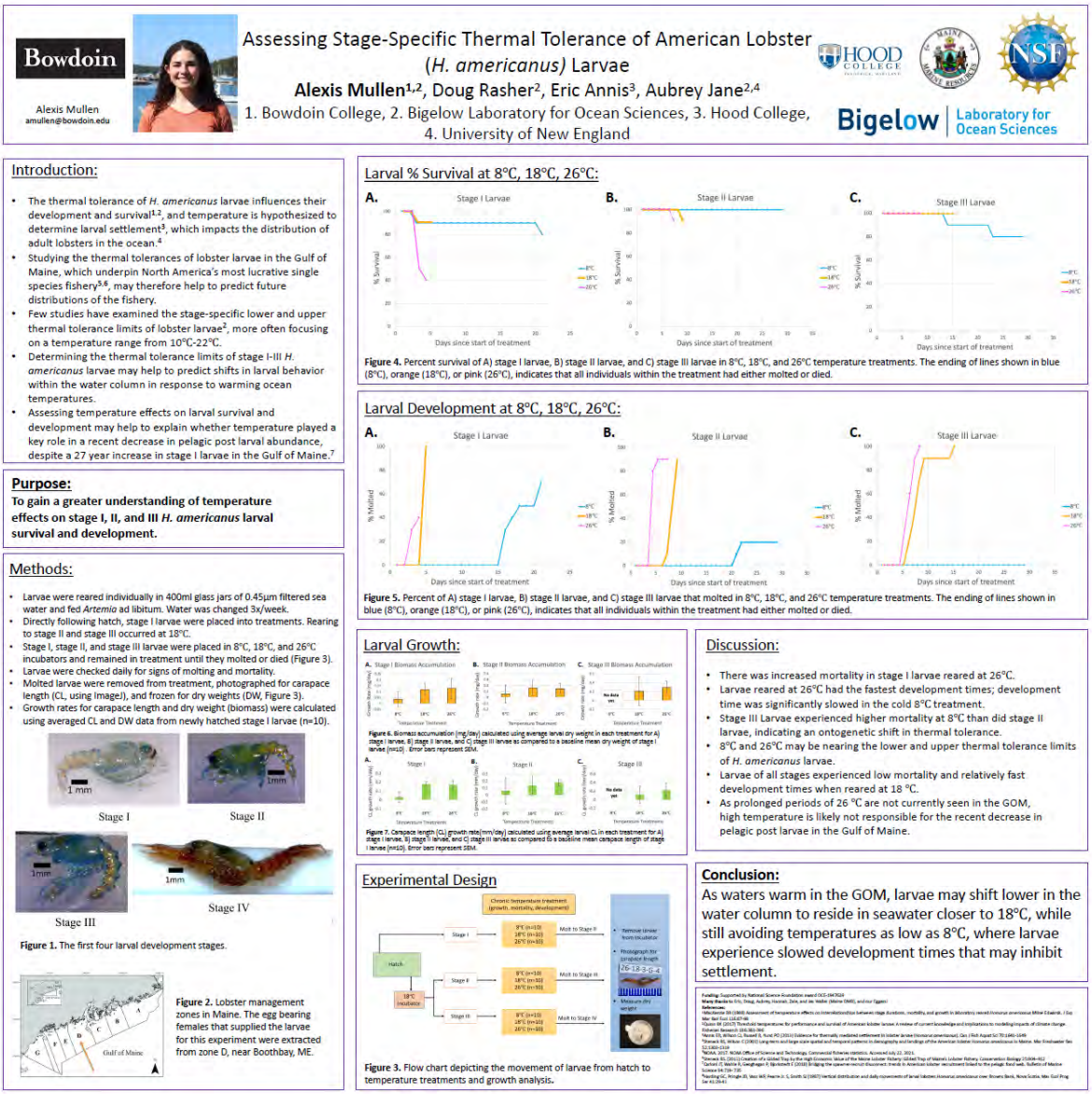


Bigelow's REU Program 2021

ASSESSING STAGE-SPECIFIC THERMAL TOLERANCE OF *HOMARUS AMERICANUS* LARVAE

Alexis Mullen[1,2], Doug Rasher[2], Eric Annis[3], Aubrey Jane[2,4]
Bowdoin College[1], Bigelow Laboratory for Ocean Sciences[2], Hood College[3], University of New England[4]

The thermal tolerance of lobster (*H. americanus)* larvae may influence their development and survival, therein impacting the distribution of juvenile and adult lobsters in the ocean. Studying the thermal tolerances of lobster larvae in the Gulf of Maine, which underpin North America's most lucrative single species fishery, may therefore help to predict future distributions of the fishery. Here, we quantified the growth, development, and mortality of *H. americanus* larvae reared under three seawater temperature treatments—8ºC, 18ºC, and 26ºC—to gauge stage-specific responses of larvae to potential lower and upper thermal threshold temperatures. Stage I larvae experienced high mortality in 26ºC, while stage I, stage II, and stage III larvae experienced slowed development times when reared at 8ºC, suggesting that these temperatures may be nearing the upper and lower thermal tolerance limits (respectively) of *H. americanus* larvae. Stage I, II, and III larvae experienced low mortality and relatively fast development time when reared at 18ºC. As waters warm in the GOM, larvae may shift lower in the water column to reside in seawater closer to 18°C, while still avoiding temperatures as low as 8°C, where larvae experience slowed development times that may inhibit settlement.

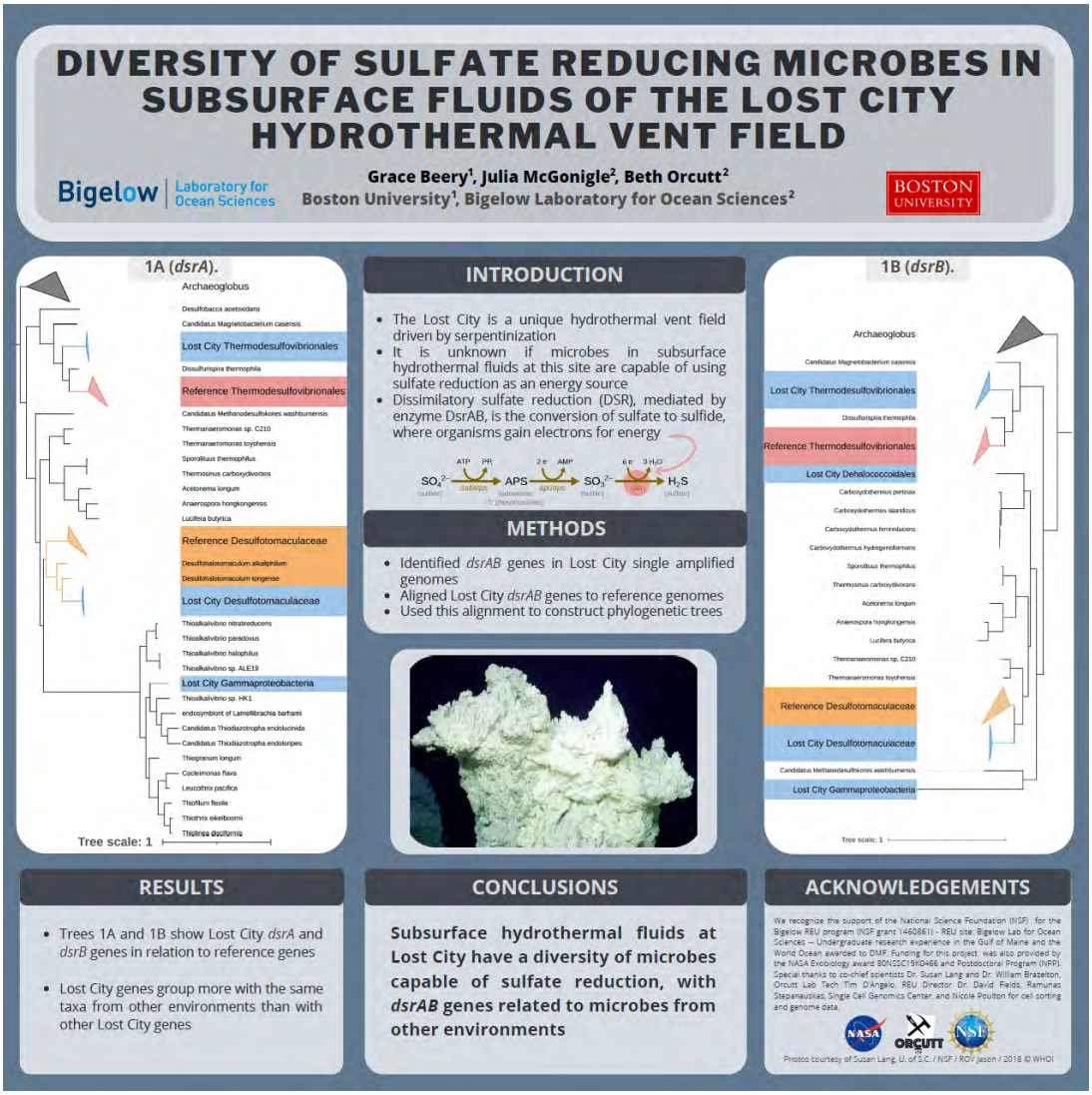


Bigelow's REU Program 2021

DIVERSITY OF SULFATE REDUCING MICROBES IN SUBSURFACE FLUIDS OF THE LOST CITY HYDROTHERMAL VENT FIELD

Grace Beery[1], Julia McGonigle[2], Beth Ocrutt[2]
Boston University[1], Bigelow Laboratory for Ocean Sciences[2]

The Lost City Hydrothermal Vent Field is a unique hydrothermal system driven by serpentinization, a rock-water reaction that produces fuel for microbial communities. Dissimilatory sulfate reduction (DSR) is the conversion of sulfate to sulfide, where organisms can gain electrons for energy. DSR is a major component of the global carbon cycle, helping drive the organic carbon to CO_2 flux in marine sediments. DSR was previously identified in Lost City chimney microbes, where hydrothermal fluids mix with seawater. It was unknown if subsurface microbes found in hydrothermal fluids could undergo sulfate reduction, and if so, what the diversity of genes in the DSR community is. This project leverages hydrothermal fluid samples collected in 2018 that were processed at Bigelow's Single Cell Genomics Center, giving us the opportunity to look at the Lost City community on a single-cell level. All single amplified genomes containing the *dsrAB* genes, a key protein involved in DSR, were identified and aligned to reference genes to construct phylogenetic trees. We found that Lost City hydrothermal fluids contain a diversity of sulfate reducing taxa, including Thermodesulfovibrionales, Desulfotomaculaceae, Gammaproteobacteria, and Dehalococcoidales. Their *dsrAB* genes are more closely related to the *dsrAB* genes found in these taxa in other environments than they are to other Lost City microbes. This suggests that sulfate reduction is a metabolic strategy in the Lost City subsurface, and that *dsrAB* genes evolved from multiple evolutionary events that reflect the history of DSR organisms.



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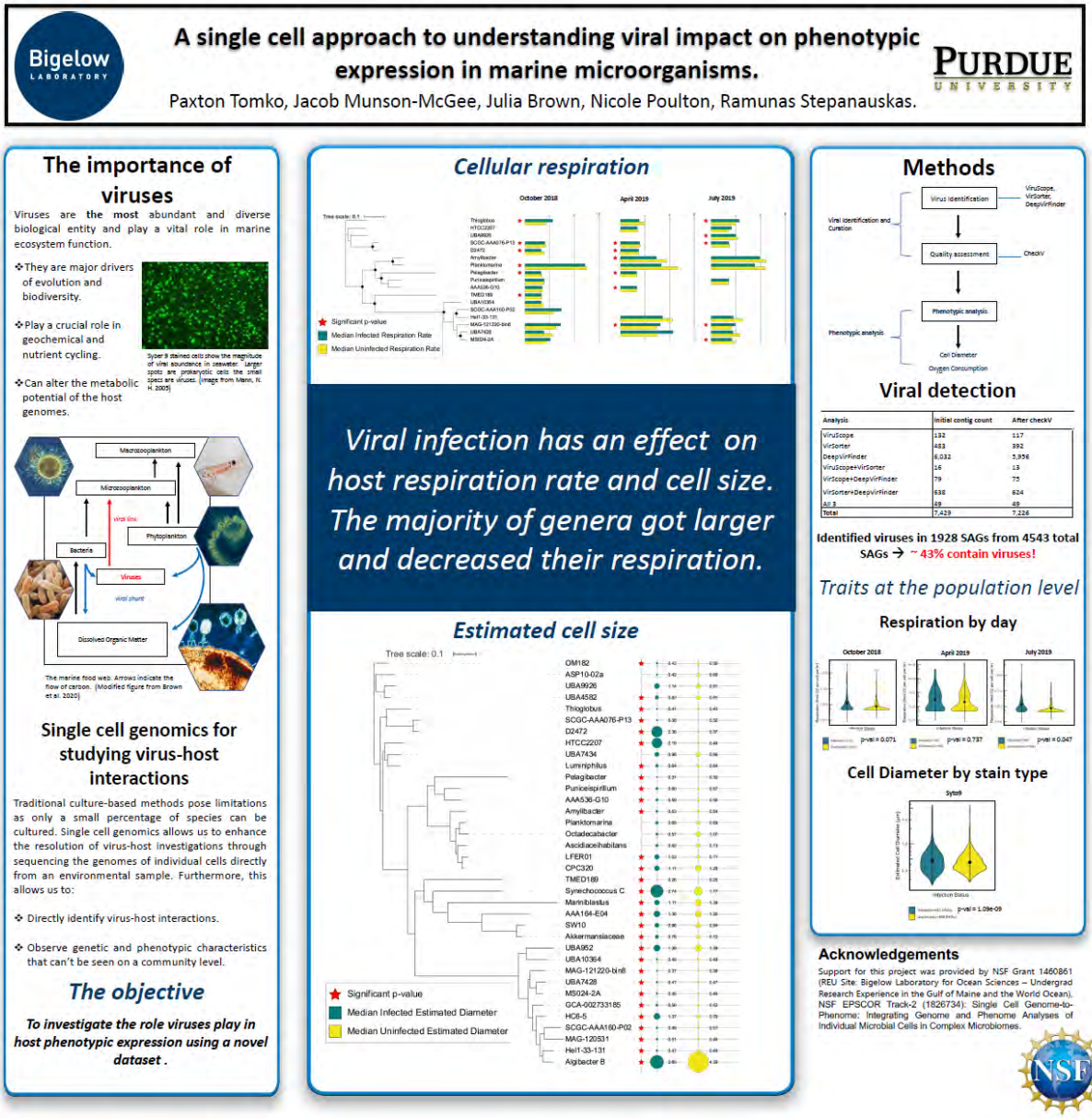
A SINGLE CELL GENOMIC APPROACH TO UNDERSTANDING VIRAL IMPACT ON PHENOTYPIC TRAITS IN MARINE MICROORGANISMS.

Paxton Tomko[1,2], Jacob Munson-McGee[1], Julia Brown[1], Nicole Poulton[1], Ramunas Stepanauskas[1]
Bigelow Laboratory for Ocean Sciences[1], Purdue University[2]

Viruses are the most abundant and diverse biological entities in marine ecosystems. They are major drivers of evolution and are key to ecosystem function through the preservation of marine biodiversity and their role in biochemical and nutrient cycling in these environments. Traditional methods of studying virus-host interactions (e.g., viral impact on host metabolic function) rely on studying them in aggregate at the community level or using culture-based approaches. This poses limitations as only a small percentage of species can be cultured; thus, these studies are not representative of natural environments. To increase the relevance of these studies, Single Cell Genomics (SCG) can be used to sequence the genome of individual cells hereby bypassing the need to culture host organisms. Furthermore, the use of SCG allows investigators to directly link the virus and host in the system and observe genetic and phenotypic characteristics of the pair that can't be seen on a community level. Here, we use a unique dataset of single cell genomes paired with phenotypic measurements of the individual cells to investigate the impact viruses have on the phenotypes of their hosts.



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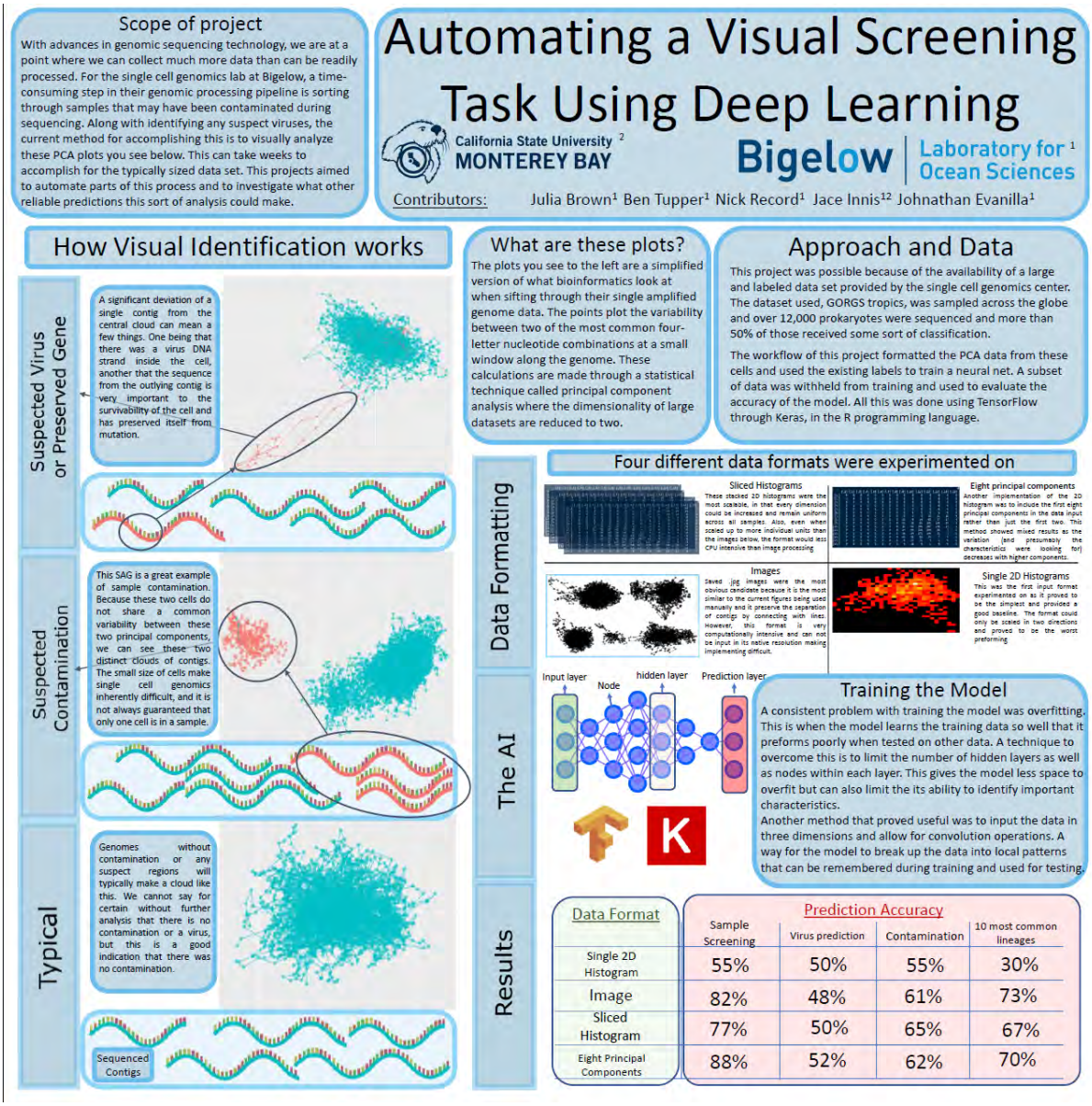
Automating A Visual Screening Task Using Deep Learning

Julia Brown[1] Ben Tupper[1] Nick Record[1] Jace Innis[12] Jonathan Evanilla[1]
[2] California State University, Monterey Bay
[1]Bigelow Laboratory for Ocean Sciences, East Boothbay, ME

Identifying characteristics of prokaryoplankton from single amplified genomes can be challenging due to the lack of reference data as well as the diversity within lineages and the possibility of contamination during the sequencing process. Alternative efforts have been made to make these identifications without directly comparing nucleotide sequences to a reference genome. One of these methods is a principal component analysis (PCA) of DNA tetramers (four letter nucleotide combinations). Specifically, a visual analysis of a plot that shows the variability between two components along the sample's genome. This method is effective at weeding out suspect genomes that could have been contaminated during processing as well as identifying outlying contigs in comparison to the rest of the genome. However, this process proves to be very labor intensive when working with large data sets and this study's aim is to automate parts of this using AI. Four different data input formats and labels from the GORGS tropics dataset were used to train the model on a number of different classification problems such as virus presence and lineage. Virus identification proved to be the most difficult with no model predicting better than chance while other classification achieved accuracies as high as 80%.



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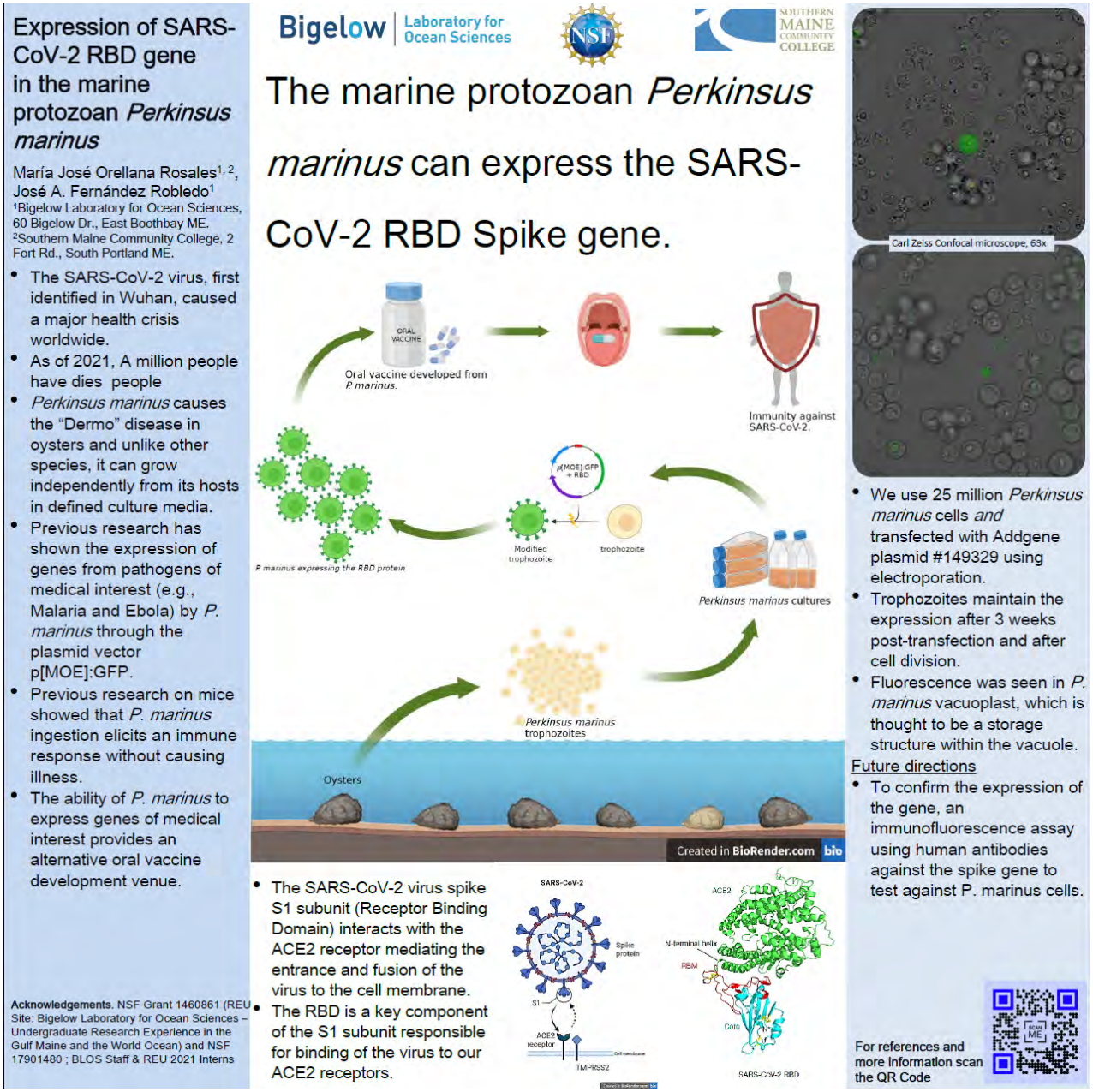
Expression of SARS-CoV-2 RBD gene in the marine protozoan *Perkinsus marinus*

Orellana María José Orellana Rosales[1, 2], José A. Fernández Robledo[1]
[1]Bigelow Laboratory for Ocean Sciences, 60 Bigelow Dr., East Boothbay ME. [2]Southern Maine Community College, 2 Fort Rd., South Portland ME.

Abstract. Perkinsus marinus is a marine protozoan parasite, which causes the "Dermo" disease in oysters. Unlike other parasites, Perkinsus marinus can grow independently from its hosts in defined culture media. The development of a transfection technique for P. marinus has made the protozoan a novel tool for genetic studies and protein expression. Previous research has shown the expression of genes from pathogens of medical interest (e.g., Malaria and Ebola) by P. marinus through the plasmid vector p[MOE]:GFP. The ability of P. marinus to express genes of medical interest provides an alternative oral vaccine development venue. We transfected *P. marinus* using the plasmid pDONR223 SARS-CoV-2 S (Addgene plasmid # 149329) with GFP tagged RBD Spike gene. Confocal microscopy was used to detect fluorescence even after 3 weeks of transfection.



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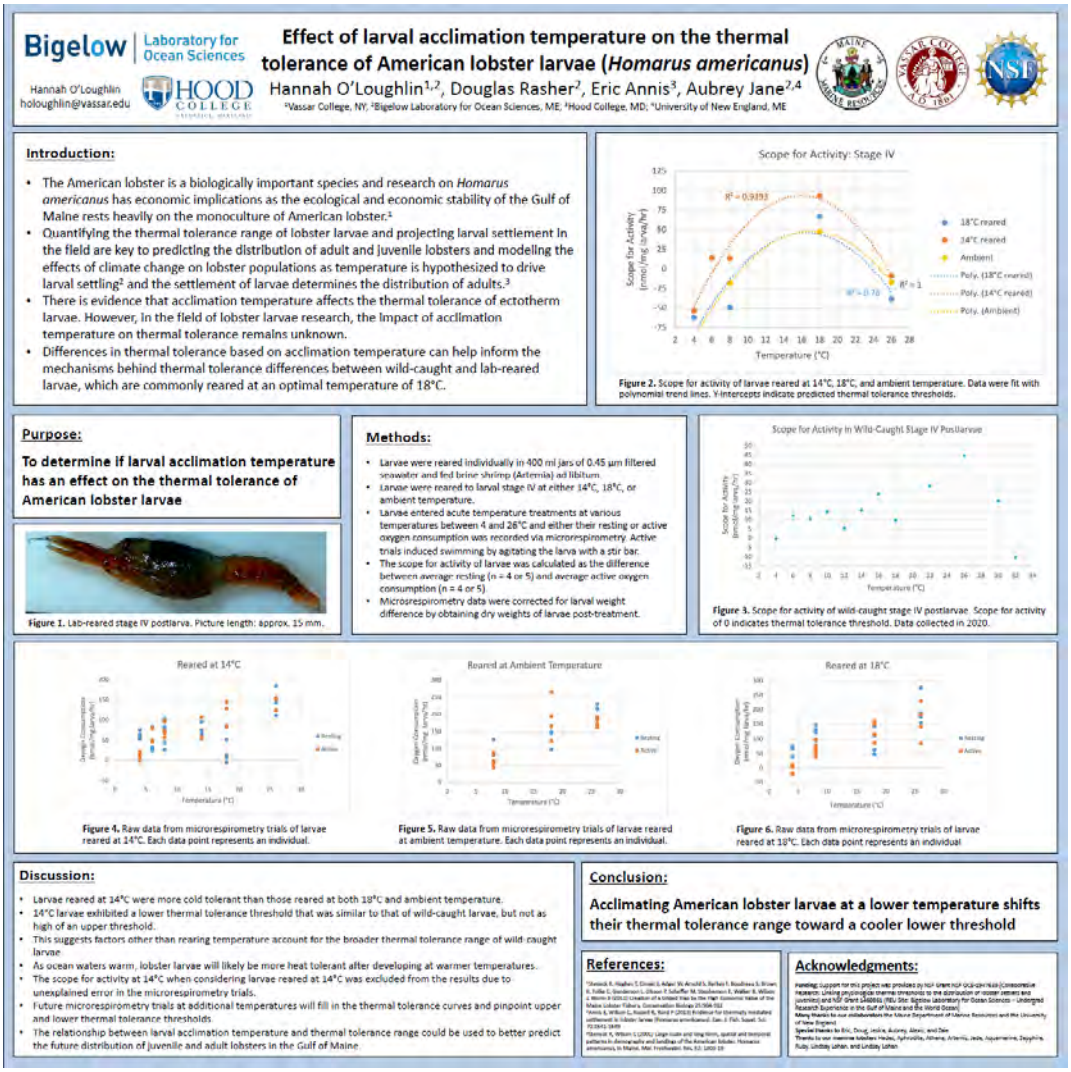
EFFECT OF LARVAL TEMPERATURE ACCLIMATION ON THE THERMAL TOLERANCE OF AMERICAN LOBSTER (*HOMARUS AMERICANUS*) LARVAE

Hannah O'Loughlin[1,2], Doug Rasher[2], Eric Annis[3], Aubrey Jane[2,4]
Vassar College[1], Bigelow Laboratory for Ocean Sciences[2], Hood College[3], University of New England[4]

The thermal tolerance of American lobster (*Homarus americanus*) larvae determines larval settlement in the field, which subsequently dictates the distribution of juvenile and adult lobsters. Defining the thermal tolerance of lobster larvae is important considering the Gulf of Maine is warming rapidly due to climate change and the distribution of lobster is shifting. There is evidence that acclimation temperature affects the thermal tolerance of ectotherm larvae. However, in the field of lobster larvae research, the impact of acclimation temperature on thermal tolerance remains unknown. To determine if larvae reared at different temperatures exhibit different thermal tolerance ranges, we reared larvae at 14°C, 18°C, and ambient temperature to larval stage IV and then measured scope for activity via respirometry during acute temperature treatments between 4°C and 26°C. We found that larvae reared at 14°C were more cold tolerant than those reared at both 18°C and ambient temperature. Larvae reared at 14°C exhibited a lower thermal tolerance threshold that was similar to that of wild-caught larvae, but not as high of an upper threshold. This suggests factors other than rearing temperature account for the broader thermal tolerance range of wild-caught larvae. Our findings indicate that as ocean waters warm, lobster larvae will likely be more heat tolerant after developing at warmer temperatures. This relationship between larval acclimation temperature and thermal tolerance range could be used to better predict the future distribution of juvenile and adult lobsters in the Gulf of Maine.



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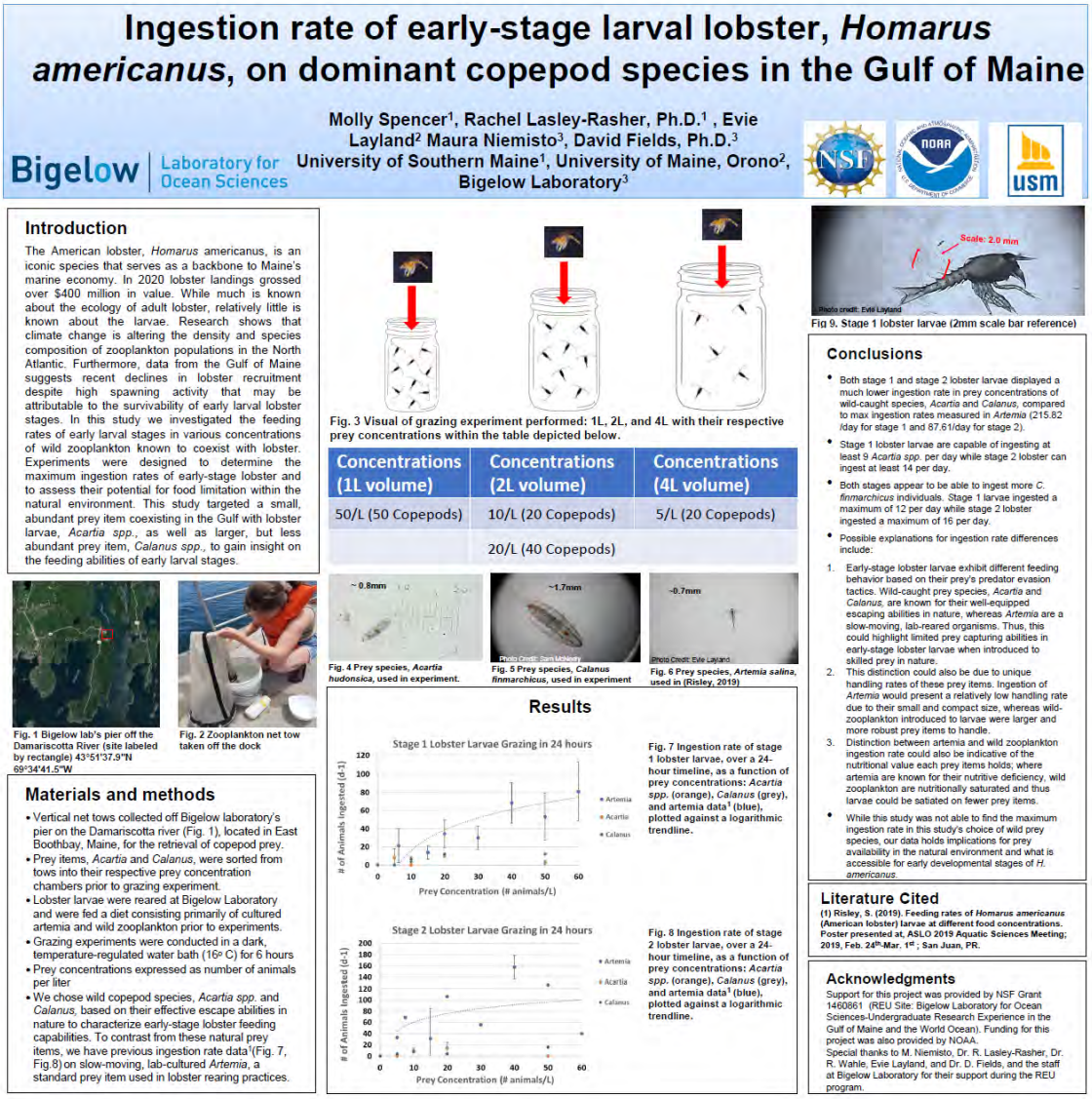
INGESTION RATE OF EARLY-STAGE LARVAL LOBSTER, *HOMARUS AMERICANUS*, ON DOMINANT COPEPOD SPECIES IN THE GULF OF MAINE

Molly Spencer[1] , Rachel Lasley Rasher[1], Evie Layland[2], Maura Niemisto[3], David Fields[3]
University of Southern Maine, Portland, ME[1], University of Maine, Orono[2], Bigelow Laboratories for Ocean Sciences[3]

The American lobster, *Homarus americanus*, is an iconic species that serves as a backbone to Maine's marine resource economy, with 2020 bringing in just over $400 million in landed value. but relatively little is known about their larval feeding ecology. Shifts in zooplankton density and species composition within the water column are known to negatively impact commercially important fish stocks, such as Atlantic herring, haddock, and cod, but studies concerning their impact on lobster populations have received far less attention. Recent data from the Gulf of Maine has revealed a decline in lobster recruitment despite high spawning activity, which is believed to be attributable to a decrease in larval survivorship. This study investigated the ingestion rate in early stages of the American lobster, *Homarus americanus*, in variable prey concentrations of copepod species to assess their potential for food limitation in their natural environment. Our study found that both stages I and II larvae were capable of feeding on prey concentrations of the dominant copepod, *Acartia spp.*, however, despite experiencing double the concentrations of prey found in nature, the larvae displayed lowered rates of ingestion. These results yield insight into the feeding ecology of larval lobster and highlight circumstances under which early lobster stages may become food-limited in nature.



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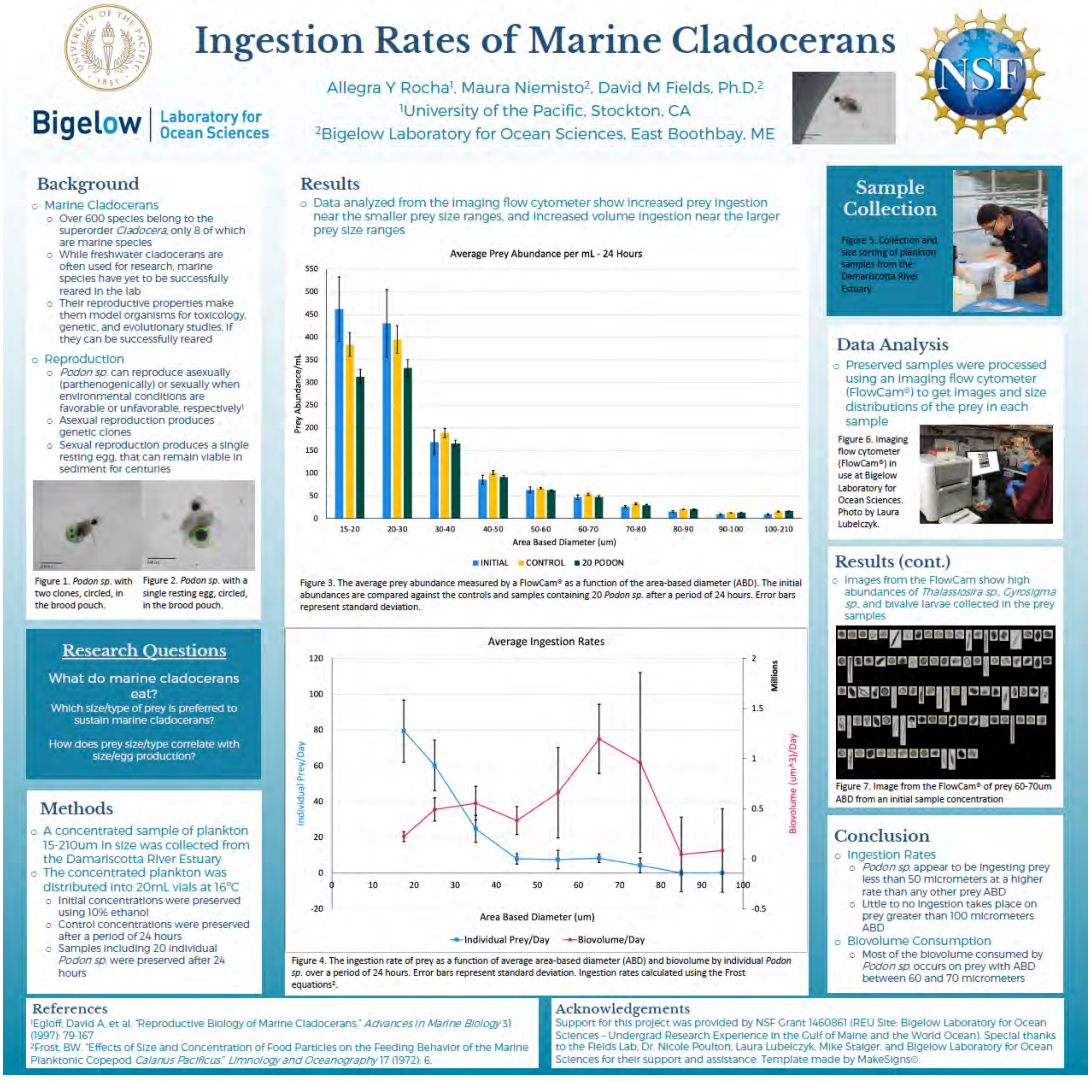
INGESTION RATES OF MARINE CLADOCERANS

Allegra Rocha[1], Maura Niemisto[2], David Fields, Ph.D.[2]
[1]University of the Pacific, Stockton, CA, [2]Bigelow Laboratory for Ocean Sciences, East Boothbay, ME

Cladocerans are small (mm) aquatic crustaceans that reproduce asexually (parthenogenically) producing populations of genetically identical individuals used in toxicology, genetic, and evolutionary studies. When facing suboptimal conditions, cladocerans produce males, that mate with females to create a resting egg that can remain viable for centuries in the sediment. Of the over 600 species of cladocerans, only 8 are marine. Freshwater species are often used in research, but marine species have yet to be successfully cultured, making them difficult to use in research. In this study we investigated the diets of a marine cladoceran, *Podon sp*., using imaging flow cytometry. Natural plankton 50-210 um were collected from the Damariscotta River Estuary and provided as prey for *Podon sp.* in a 16-degree Celsius water bath for 24 hours. Ingestion rates were calculated as a function of prey size and biovolume using Frost Equations (Frost 1972). The data suggest *Podon sp.* ingested the greatest number of prey in the 15-30 um range. Prey larger than 100 um were not consumed. When analyzed based on prey volume, the greatest ingestion occurred within the size range 60-70 um. Images collected from the feeding experiments suggest the dominant prey items include *Thalassiosira sp*., *Gyrosigma sp*., and bivalve larvae. These findings suggest there could be an individual or group of individuals within this size range that is a preferred prey item for *Podon sp*. in the Gulf of Maine. This data may be used to further future research in the effort to culture marine cladocerans in a controlled lab.



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Thanks to the National Science Foundation for its support under NSF Grant OCE 1950443 – REU Site: Bigelow Laboratory for Ocean Sciences – Undergraduate Research Experience in the Gulf of Maine and the World Ocean.