

# 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)**

**WE ADVANCE BOLD SCIENCE  
FOR OUR BLUE PLANET.**



## 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<sup>1,2</sup>, William Balch<sup>1</sup>, Sunny Pinkham<sup>1</sup>

<sup>1</sup>Bigelow Laboratory for Ocean Sciences, <sup>2</sup>Colby College

**9:15 SOLUTION TO DILUTION: NOVEL SATURATION METHOD FOR THE ESTIMATION OF PHYTOPLANKTON GROWTH AND GRAZING RATES**

Michael Staiger<sup>1,2</sup>, Nicole J. Poulton<sup>2</sup>, Laura Lubelczyk<sup>2</sup>, Steve Archer<sup>2</sup>

Colby College<sup>1</sup>, Waterville, Maine, Bigelow Laboratory for Ocean Sciences<sup>2</sup>, East Boothbay, Maine

**9:30 ASSESSING CELLULAR POLYPHOSPHATE OF PHYTOPLANKTON GROWN ON PHOSPHONATES**

Briana Mays<sup>1</sup>, LeAnn Whitney<sup>1,2</sup>, Debra A. Lomas<sup>2</sup>, Michael W. Lomas<sup>2</sup>

<sup>1</sup>Maine Maritime Academy. <sup>2</sup>Bigelow Laboratory for Ocean Sciences

**9:45 Can microplastic be filtered out of wastewater using biofilm forming algae?**

Andre Martin<sup>1</sup>, Dr. Mike Lomas<sup>2</sup>, Robert Schmedike<sup>2</sup>

<sup>1</sup>Haverford College, <sup>2</sup>Bigelow Laboratory for Ocean Sciences

**10:00 IMPLEMENTING AND OPTIMIZING AN APPROVED METHOD FOR MONITORING DIARRHETIC SHELLFISH POISONING (DSP)**

Finn Dworkin<sup>1</sup>, Carmen Cartisano<sup>2</sup>, Craig Burnell<sup>2</sup>, Stephen Archer<sup>2</sup>

Grinnell College<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>

**10:15 Discovering the Presence of Ethylene within *Cyanophora paradoxa* Algae and its Pathway**

Matthew Yost<sup>1,2</sup>, Baptiste Genot<sup>2</sup>, John Burns<sup>2</sup>

<sup>1</sup>University of Maine, Orono, School of Marine Sciences, <sup>2</sup>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<sup>1,2</sup>, Brian DiMento<sup>1</sup>, Christoph Aepli<sup>1</sup>, David Fields<sup>1</sup>

<sup>1</sup>Bigelow Laboratory for Ocean Sciences, <sup>2</sup>University of Maryland, College Park

**10:45 – 11:00 Break**

## **Bigelow's REU Program 2021**

### **11:00 Feeding Cows Seaweed to Reduce Methane Emissions: Iodine in Seaweed and Milk**

Owen T. Keleher<sup>2</sup>, & Benjamin S. Twining, PhD.<sup>2</sup>

<sup>1</sup> Bates College, <sup>2</sup> 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<sup>1,2</sup>, Cath Mitchell<sup>2</sup>, Nichole Price<sup>2</sup>, Brittney Honisch<sup>2</sup>, Jessie Muhlin<sup>3</sup>, Hannah Webber<sup>4</sup>, Peter Nelson<sup>4</sup>, Stefan Claesson<sup>5</sup>

University of South Carolina-Columbia<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>, Maine Maritime Academy<sup>3</sup>, Schoodic Institute<sup>4</sup>, Nearview LLC<sup>5</sup>

### **11:30 BIODEGRADATION OF PHOTO-OXIDIZED OIL PRODUCTS: IMPLICATIONS FOR THE FATE OF MARINE WEATHERED OIL SPILLS**

Hannah Sterling<sup>1</sup>, Christoph Aeppli<sup>2</sup>

<sup>1</sup>Roger Williams University, <sup>2</sup>Bigelow Laboratory for Ocean Sciences

### **11:45 COPEPOD INGESTION RATE AT SUBLETHAL OIL CONCENTRATIONS**

Sam McNeely<sup>1,2</sup>, Maura Niemisto<sup>2</sup>, Christoph Aeppli, PhD.<sup>2</sup>, & David Fields, PhD.<sup>2</sup>

<sup>1</sup> University of North Carolina Wilmington, <sup>2</sup> 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<sup>1,2</sup>, Maura Niemisto<sup>2</sup>, Christoph Aeppli<sup>2</sup>, David Fields<sup>2</sup>

The College of Wooster<sup>1</sup>, Bigelow Laboratory of Ocean Sciences<sup>2</sup>

### **12:15 A Survey of Bivalve Transmissible Neoplasia in *Mya arenaria* Along the Casco Bay ME (USA)**

Satyatejas G. Reddy<sup>1,2</sup>, Dr. Michael J. Metzger<sup>3</sup>, Rachael M. Giersch<sup>3</sup>, Dr. José A. Fernández Robledo<sup>1</sup>, Dr. Peter D. Countway<sup>1</sup>

<sup>1</sup>Bigelow Laboratory for Ocean Sciences; <sup>2</sup>Odum School of Ecology, University of Georgia;

<sup>3</sup>Pacific Northwest Research Institute

**12:30-1:00 Lunch**

## **Bigelow's REU Program 2021**

### **1:00 IS ATP A SUFFICIENT PROXY FOR BIOMASS FOR USE IN DEEP SEA MINING REGULATION?**

Asher Platts<sup>1</sup>, Dr. Beth Orcutt, PhD<sup>2</sup>

Southern Maine Community College<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>

### **1:15 TRACE METAL COMPOSITION OF DEEP-SEA SEDIMENTS: AN ANALYSIS OF RARE EARTH ELEMENTS IN THE N. PACIFIC**

Christiana Okafor<sup>1</sup>, Jim McManus<sup>2</sup>

Bowdoin College<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>

### **1:30 VIRUSES DRIVE BACTERIAL EVOLUTION IN THE LOST CITY HYDROTHERMAL FIELD SUBSURFACE MORE THAN IN THE OCEAN**

Dani Buchheister<sup>1,2</sup>, Julia McGonigle<sup>1</sup>, Beth Orcutt<sup>1</sup>

Bigelow Laboratory for Ocean Sciences<sup>1</sup>, University of Colorado Boulder<sup>2</sup>

### **1:45 INVESTIGATING PILI STRUCTURES IN THE MARINE SUBSURFACE**

Autumn Pope<sup>1</sup>, Stephanie Carr<sup>1</sup>, Beth Orcutt<sup>2</sup>, Michael Rappé<sup>3</sup>, Olivia Nigro<sup>4</sup>

<sup>1</sup>Hartwick College, <sup>2</sup>Bigelow Laboratory for Ocean Sciences, <sup>3</sup>Hawaii Institute of Marine Biology, University of Hawaii at Manoa, <sup>4</sup>Hawaii Pacific University

### **2:00 ASSESSING STAGE-SPECIFIC THERMAL TOLERANCE OF *HOMARUS AMERICANUS* LARVAE**

Alexis Mullen<sup>1,2</sup>, Doug Rasher<sup>2</sup>, Eric Annis<sup>3</sup>, Aubrey Jane<sup>2,4</sup>

Bowdoin College<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>, Hood College<sup>3</sup>, University of New England<sup>4</sup>

### **2:15 DIVERSITY OF SULFATE REDUCING MICROBES IN SUBSURFACE FLUIDS OF THE LOST CITY HYDROTHERMAL VENT FIELD**

Grace Beery<sup>1</sup>, Julia McGonigle<sup>2</sup>, Beth Orcutt<sup>2</sup>

Boston University<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>

**2:30-2:45 Break**

## **Bigelow's REU Program 2021**

### **2:45 A SINGLE CELL GENOMIC APPROACH TO UNDERSTANDING VIRAL IMPACT ON PHENOTYPIC TRAITS IN MARINE MICROORGANISMS.**

Paxton Tomko<sup>1,2</sup>, Jacob Munson-McGee<sup>1</sup>, Julia Brown<sup>1</sup>, Nicole Poulton<sup>1</sup>, Ramunas Stepanauskas<sup>1</sup>

Bigelow Laboratory for Ocean Sciences<sup>1</sup>, Purdue University<sup>2</sup>

### **3:00 Automating A Visual Screening Task Using Deep Learning**

Julia Brown<sup>1</sup> Ben Tupper<sup>1</sup> Nick Record<sup>1</sup> Jace Innis<sup>1,2</sup> Jonathan Evanilla<sup>1</sup>

<sup>2</sup> California State University, Monterey Bay

<sup>1</sup>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<sup>1,2</sup>, José A. Fernández Robledo<sup>1</sup>

<sup>1</sup>Bigelow Laboratory for Ocean Sciences, 60 Bigelow Dr., East Boothbay ME. <sup>2</sup>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<sup>1,2</sup>, Doug Rasher<sup>2</sup>, Eric Annis<sup>3</sup>, Aubrey Jane<sup>2,4</sup>

Vassar College<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>, Hood College<sup>3</sup>, University of New England<sup>4</sup>

### **3:45 INGESTION RATE OF EARLY-STAGE LARVAL LOBSTER, *HOMARUS AMERICANUS*, ON THE DOMINANT COPEPOD GENUS, *ACARTIA*, IN THE GULF OF MAINE**

Molly Spencer<sup>1</sup>, Rachel Lasley Rasher<sup>1</sup>, Evie Layland<sup>2</sup>, Maura Niemisto<sup>3</sup>, David Fields<sup>3</sup>

University of Southern Maine, Portland, ME<sup>1</sup>, University of Maine, Orono<sup>2</sup>, Bigelow Laboratories for Ocean Sciences<sup>3</sup>

### **4:00 INGESTION RATES OF MARINE CLADOCERANS**

Allegra Rocha<sup>1</sup>, Maura Niemisto<sup>2</sup>, David Fields, Ph.D.<sup>2</sup>

<sup>1</sup>University of the Pacific, Stockton, CA, <sup>2</sup>Bigelow Laboratory for Ocean Sciences, East Boothbay, ME

**4:15 END**

# **Bigelow's REU Program 2021**


## **Abstracts and Posters**

# Bigelow's REU Program 2021

## DIATOM NUTRIENT LIMITATION IN A SOUTHERN OCEAN EDDY

Benjamin Gustafson<sup>1,2</sup>, William Balch<sup>1</sup>, Sunny Pinkham<sup>1</sup>  
<sup>1</sup>Bigelow Laboratory for Ocean Sciences, <sup>2</sup>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 μm and 100 μm) 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 Laboratory**  
NSF

## Diatom nutrient limitation in a Southern Ocean Eddy

Benjamin Gustafson<sup>1,2</sup>, William Balch<sup>1</sup>, Sunny Pinkham<sup>1</sup>

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



**Colby College**

### Abstract

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 μm and 100 μm) 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.

### Intrinsic Growth Rates

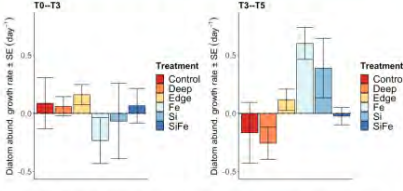


Fig. 5. Diatom intrinsic growth rates (day<sup>-1</sup>) from days 0-3 and days 3-5. Error bars are standard errors about the mean.

### Methods

- Collect eddy water

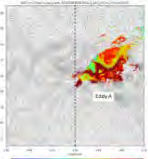


Fig. 1. Absolute dynamic topography (ADT) contours and log chlorophyll of a Southern Ocean eddy, the site of sample collection; 54°S x 142°W. (Figure courtesy of D. McGillicuddy, Woods Hole Oceanographic Institution, Woods Hole, MA).

- Incubate samples




Fig. 2. Surface samples were incubated for 5 days and sampled every 48 hours. Eddy center water was used, with 6 treatments: control, +Fe, +silicate, +Fe+silicate, plus two natural water additions from the eddy edge and deep water (311m).

- FlowCam sample analysis


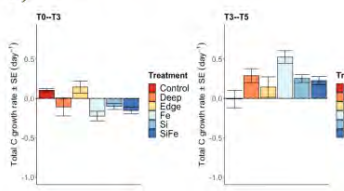


Fig. 3. All samples were run through a FlowCam, an imaging cytometer, for particle identification and sizing<sup>2</sup>. FlowCam files were sorted by phytoplankton group and size (0-12μm, >12μm). Analysis then provides sample cell abundance, C biomass using the Menden-Deuer & Lessard method<sup>3</sup>, and particle size distribution. Image: FlowCam Blog<sup>4</sup>

### C Growth Rates

a)



b)

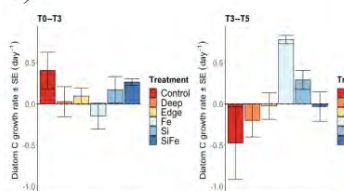


Fig. 4. C growth rates (day<sup>-1</sup>) for all treatments from days 0-3 and days 3-5 for a) all phytoplankton between 5μm and 100μm diameter and b) diatoms. Error bars are standard errors about the mean.

### Particle Size Distribution

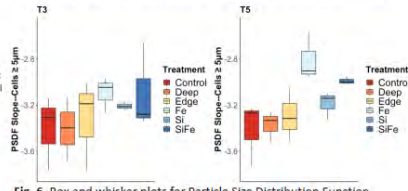


Fig. 6. Box and whisker plots for Particle Size Distribution Function (PSDF) slopes at T3 and T5 for all treatments. PSDF measures the abundance of cells as a function of size—a steep negative slope means relatively fewer large cells, and relatively more small cells, while a less steep slope (closer to 0) means the opposite.

### Key findings

- Fe promotes growth of total biomass and diatoms in a Southern Ocean eddy.
- Fe promotes relatively faster growth of larger cells and slower growth of small cells.
- Assemblages showed a 3-day lag time while cells adjusted to new conditions.
- Potential interaction effect in the +Fe+silicate treatment

### Citations

1. Sarinam, J. L., Gruber, N., Bratton, M. A., & Dumas, J. P. (2004). High-latitude controls of thermocline extinction and low latitude biological productivity. *Letters in Nature*, 427(6969), 56-60. <https://doi.org/10.1038/nature02127>

2. Pinkham, N. J., and J. L. Marra (2010). Imaging flow cytometry for quantitative phytoplankton analysis - FlowCAM: Microscopic and molecular methods for quantitative phytoplankton analysis. B. G. Balch, C. Coull, and E. Struelens, Paris, France. International Oceanographic Commission of UNESCO 35: 49-54.

3. Menden-Deuer, S., & Lessard, E. J. (2000). Carbon to volume relationships for diatoms, green, diatoms, and other marine plankton. *Limnology and Oceanography*, 45(2), 169-179. <https://doi.org/10.1002/llo.1110>

4. Devos, M. (n.d.). *FlowCam: Imaging Technology with Count Studies for Cells in Flow*. Mainz.

### Acknowledgements

Thanks to the Balch Lab Group for their help and guidance. Thank you to the officers, crew and science party of R/V Roger Revelle RR2004 for their hard work for sample collection. 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 NSF Grant OCE 1735664, as well as funding from the Linda Packman Biosciences Lab.


# Bigelow's REU Program 2021

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

Michael Staiger<sup>1,2</sup>, Nicole J. Poulton<sup>2</sup>, Laura Lubelczyk<sup>2</sup>, Steve Archer<sup>2</sup>


Colby College<sup>1</sup>, Waterville, Maine, Bigelow Laboratory for Ocean Sciences<sup>2</sup>, 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.




### Solution to Dilution: A novel method for the estimation of planktonic growth & grazing

Mike Staiger<sup>1</sup> Nicole Poulton<sup>2</sup> Laura Lubelczyk<sup>2</sup> Steve Archer<sup>2</sup>  
 1. Colby College, Waterville Maine, 2. Bigelow Laboratories of Ocean Science, East Boothbay Maine.







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#### Introduction

- Oceanic phytoplankton account for 1/2 of carbon fixation on earth. (Figure 1)
- Grazing on phytoplankton – mainly by microzooplankton – accounts for ~12% of oceanic carbon cycling annually (Falkowski 1994, Calbet 2003).
- The dilution method (Landry and Hassett 1982) has been the standard for estimating grazing rates.
- But has issues with implementation, with failure rates as high as 60% (Dolan & Mckee 2005).
- A proposed saturation method may offer a more reliable alternative.
- The saturation method operates on the same principle, of reducing encounter rate, as the dilution method. In the saturation method grazers are inundated with surrogate prey, reducing their ability to graze on natural phytoplankton.

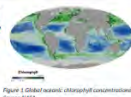


Figure 1. Global oceanic chlorophyll concentrations. Source: NASA

#### Dilution Method vs Saturation Method

Experimental Setup

Decreasing % Unfiltered Seawater

100% 75% 50% 25%

Increasing bead concentration/ml

0 x 10<sup>6</sup> 5 x 10<sup>6</sup> 5 x 10<sup>6</sup> 5 x 10<sup>6</sup>

Increasing Growth

● = Phytoplankton  
○ = Grazer  
○ = Surrogate Prey

Increasing Growth

Decreasing Predation

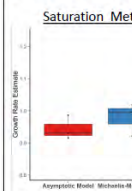
Decreasing Predation

Figure 1. Visual comparison of the dilution and saturation method for the estimation of phytoplankton growth and planktonic grazing rates. Phytoplankton and grazer population are not to scale.

#### Comparison of Results

**The Saturation approach may offer a more consistent estimate for both grazing and growth**

Saturation Method



Dilution Method

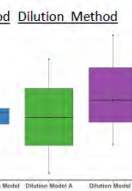


Figure 3. Boxplots comparing the spread of grazing and growth rate estimates between saturation and dilution methods. Points represent experimental estimates and boxes include middle 50% of data spread. Notice that the two models for the saturation method have much tighter distributions than the dilution methods. Dilution method A contains data from all dilution levels, dilution method B omits the potentially problematic 10% dilution level.

#### Methods

- Parallel dilution and saturation experiments:
- Experimental set up shown in Figure 1
- 2 μm yellow-green fluorescently labeled polystyrene beads used as surrogate prey
- Samples taken at initial and final time points
- 24-hour incubation period
- Population determined via flow cytometry
- Primary analysis of *Micromonas* population




Image 1. Incubation tank for parallel experiments. Bottles were sealed and covered by a shade cloth that reduces incoming solar radiation by 40%. Bottles were continuously mixed by inflow tube that kept the tank at ambient ocean temperature.




Image 2. Image of the gradient of bead saturated incubations. Moving from left to right the bottles increase in bead concentration from 0 beads/ml to 10<sup>7</sup> beads/ml.

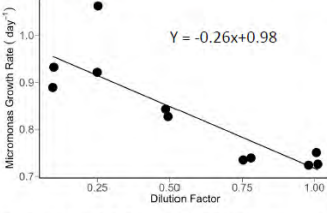


Figure 3. Linear regression for the dilution method. The x-axis from left to right is increasing percent of unfiltered seawater. The y-intercept is the growth rate of *Micromonas* without grazing pressure and the slope of the line is the grazing rate.

$Y = -0.26x + 0.98$

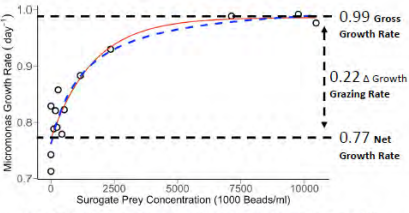


Figure 4. Comparing fits of Michaelis-Menton (blue dashed) and asymptotic regression (red) models for saturation grazing results. The point on the x-axis where both models flatten out is the growth rate of *Micromonas* without grazing pressure. The difference between this and the net growth rate is the grazing rate.

0.99 Gross Growth Rate  
0.22 Δ Growth = Grazing Rate  
0.77 Net Growth Rate

#### Take-Aways

**Potential Pros of the Saturation Method**

- More consistent results
- Potential for a two point method may reduce experimental set-up time
- Only provides population-specific grazing – not 'community' rates
- May allow for the chemical analysis of the effects of grazing

**Potential Cons of The Saturation Method**

- Surrogate prey bead waste
- Negative effect of beads on growth
- Achieving definitive saturation – i.e. zero grazing

**Acknowledgements:** We thank the Linda Packman Lab for Biosciences Innovation for sponsoring this REU summer internship. We also thank the National Science Foundation's support for this project under NSF project no. OCE 1760461 to Steve Archer and Nicole Poulton. I would like to especially thank Steve Archer and the lab for their assistance in conducting experiments and Steve Archer for his assistance in data analysis. Finally, to thank Nicole Poulton for her continued support and guidance throughout this project.

**References:**  
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 Falkowski, P. (1994) The oceanic carbon cycle. *Journal of Geophysical Research* 99: 15,331-15,340.  
 Landry, M.R. & Hassett, R.P. (1982) Estimating grazing rates of microzooplankton: a comparison of dilution and direct counts. *Journal of Plankton Research* 4: 11-20.




# Bigelow's REU Program 2021

## ASSESSING CELLULAR POLYPHOSPHATE OF PHYTOPLANKTON GROWN ON PHOSPHONATES

Briana Mays<sup>1</sup>, LeAnn Whitney<sup>1,2</sup>, Debra A. Lomas<sup>2</sup>, Michael W. Lomas<sup>2</sup>

<sup>1</sup>Maine Maritime Academy. <sup>2</sup>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, *Emiliana 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  $P_i$  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



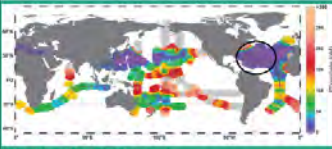
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### ASSESSING CELLULAR POLYPHOSPHATE OF PHYTOPLANKTON GROWN ON PHOSPHONATES

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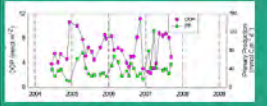



#### Phosphorus in the ocean



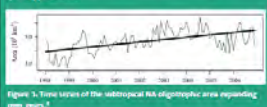
- Phosphorus (P) is an essential element for cellular function and activity.
- P is found in different forms, phosphate ( $P_i$ ) being the preferred form for phytoplankton.
- In the oligotrophic subtropical North Atlantic (NA), surface  $P_i$  is low (Figure 1).

Figure 1. Satellite P<sub>i</sub> surface concentrations.<sup>1</sup>



- Dissolved organic phosphorus (DOP) links primary production in oligotrophic regions (Figure 2) such as the subtropical NA.


Figure 2. Time series plot of integrated DOP concentrations and primary production.<sup>2</sup>



- The subtropical NA is expanding (Figure 3), suggesting the role of DOP in supporting primary production may increase.

Figure 3. Time series of the subtropical NA oligotrophic area expanding over years.<sup>3</sup>

#### Emiliana huxleyi grown on methylphosphonate



- Cells given MPN reached cell concentrations nearly two-fold greater with lower cellular P when compared to -P.
- P concentrations were similar between MPN and -P treatments.


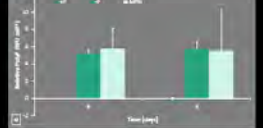


Figure 4. Growth curves for *E. huxleyi* grown under 0, -P, MPN conditions. [1] Growth curves, [2] Dissolved P concentrations, and [3] relative P for *E. huxleyi* [4] Polyp relative to 0P at day 11.



- Polyp levels increased in the MPN and -P conditions relative to +P.
- Relative polyp was similar between MPN and -P.

#### Phosphonate utilization in eukaryotic organisms

- The DOP pool is comprised of P esters (80%) and phosphonates (10%).<sup>4</sup>
- It was previously thought only prokaryotes can grow with phosphonates; we have shown widespread but not universal utilization by eukaryotic phytoplankton (Figure 4).

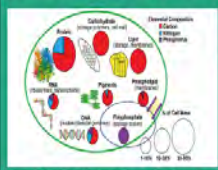
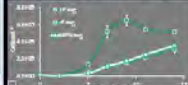


Figure 5. Assimilation of carbon, nitrogen, phosphorus by microorganisms. Pie chart size relates to total cell mass.<sup>5</sup>



- 12% increase in cell concentration in MPN treatments compared to -P.
- Growth on MPN results in reduced cellular P.




Figure 6. Growth curves for *I. galbana* grown under 0, -P, MPN conditions. [1] Growth curves, [2] Dissolved P concentrations, and [3] relative P for *I. galbana* [4] Polyp relative to 0P at day 11.

- Similar to *E. huxleyi*, *I. galbana* increases polyp in -P and MPN relative to +P.

#### Conclusions and implications

- MPN supports growth of *E. huxleyi* and *I. galbana*; resulting in reduced cellular P when compared to -P.
- Polyp increases in -P and MPN relative to +P.
- Taken together, this suggest polyp is not a source of P for growth; and that growth in MPN is due to phosphonate utilization.
- As oceans warm and oligotrophic regions expand the role of phosphonates may increase; utilization by eukaryotic phytoplankton may influence P and carbon cycling in future oceans.

How is cellular polyp changing in cells when given a phosphonate as the sole source of P?

#### Acknowledgements and Citations

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 [4] Lomas et al., 2018, *PLoS One* 13(10): e0201111  
 [5] Lomas et al., 2018, *PLoS One* 13(10): e0201111  
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# Bigelow's REU Program 2021

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

Andre Martin<sup>1</sup>, Dr. Mike Lomas<sup>2</sup>, Robert Schmedike<sup>2</sup>

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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 chrysoplata*, 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.

### Background

Authors: Andre Martin<sup>1</sup>, Dr. Mike Lomas<sup>2</sup>, Robert Schmedike<sup>2</sup>

- A lot of microplastic pollution goes through our waste water system
- Even the best water treatment plants miss 10% of microplastics
- The 10% need a solution that can be added to existing plants
- Algae that produce EPS (exopolysaccharide) can adhere to plastics
- Utilizing this adhesion could help stem the flow of microplastics into the environment by grabbing them out of waste water

### Methods

- To test if the EPS can remove plastics a filtration raceway was built
- The raceway is a 2" wide canal where strips of 300 um mesh seeded with algae could be placed and tested
- 1 liter of microplastic rich solution (about 6200000 MPs) was run over the algae, followed by a 1 liter salt water elution
- Removal was tracked by analysing the number of particles in the stock/ saltwater per ml before and after running over the raceway
- Algae seeded mesh was tested after 2, 4, & 6 days of growth

### Results

At 2 days of growth, microplastic retention was minimal, but at the 4 and 6 day mark the retention was much higher. This indicates that once the algae has time to grow on mesh it can be an effective substrate for microplastic removal. This is an encouraging proof of concept, but much more work is necessary to quantify the role of EPS in this process.

## Sticky algae can be used to capture microplastics in water

### Next Steps

- Develop a method to quantify the exact amount biomass and EPS in a given sample
- Preform spectroscopy on bound plastics
- Test if different elutions can unbind microplastics
- Study scalable ways to cultivate seeded mesh

#### Sent vs Retained by Growth Day

Growth Day	Sent (MPs)	Retained (MPs)	Retention %
2 Days	1280	895	12%
4 Days	22005	21912	72%
6 Days	4091	3121	62%

#### Retained vs Eluted by Growth Day

Growth Day	Retained (MPs)	Eluted (MPs)	Elution %
2 Days	831	245	23%
4 Days	40183	621	1%
6 Days	2331	363	16%


Support for this project was provided by NSF Grant 1850443 (REU Site: Bigelow Laboratory for Ocean Sciences – Undergrad Research Experience in the Gulf of Maine and the World Ocean)

# Bigelow's REU Program 2021

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

Finn Dworkin<sup>1</sup>, Carmen Cartisano<sup>2</sup>, Craig Burnell<sup>2</sup>, Stephen Archer<sup>2</sup>  
Grinnell College<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>

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 Laboratory for Ocean Sciences

### Implementing and Optimizing an Approved Method for Monitoring Diarrhetic Shellfish Poisoning (DSP)

Finn Dworkin<sup>1</sup>, Carmen Cartisano<sup>2</sup>, Craig Burnell<sup>2</sup>, Stephen Archer<sup>2</sup>

Grinnell College<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>



GRINNELL COLLEGE

#### Introduction

- The Maine shellfish industry is a vital sector of the Maine economy, but marine toxins jeopardize consumer safety and expanding industries.
- DSP causing marine toxins are the metabolite byproducts of microalgae, *Dinophysis* and *Prorocentrum*.
- Three monitored phycotoxins, Okadaic Acid (OA), Dinophysistoxin-1 (DTX-1), Dinophysistoxin-2 (DTX-2) cause DSP in the cilia lining of the large intestine.
- OA, DTX-1, and DTX-2 are lipophilic poly-ether acids that can accumulate at toxic levels in bivalve mollusks.


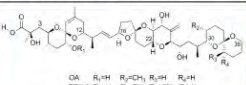



Figure 1: *D. acuta*      Figure 2: Chemical formulae for DSP toxins

OA:  $R_1=H, R_2=CH_2, R_3=H, R_4=H, R_5=H$   
DTX-1:  $R_1=H, R_2=CH_2, R_3=CH_2, R_4=H, R_5=H$   
DTX-2:  $R_1=H, R_2=H, R_3=H, R_4=H, R_5=CH_2$

LC-MS/MS: molecules of specific m/z are fragmented – the m/z of the parent and fragments consolidates identification.

#### Objectives

- Implement ISSC approved LC-MS/MS method
- Recognize issues in procedure and instrument settings to maximize sensitivity
- Apply adjustments to method and quantify improvements
- Use optimized method for DSP test kit comparison




Figure 3: Agilent LC-MS

#### Sampling and Extraction

**Sample Collection:**  
Blue Mussel, *Mytilus edulis* samples were collected by the Department of Marine Resources (DMR), spanning all of 2020. Each sample contained 12 mussels shucked and homogenized by DMR and stored in a -22°C freezer.

**Extraction Method:**




Figure 5: Schematic of extraction method

**Homogenize** → **Methanol Extraction** → **Hydrolysis** → **Filtration** → **Hexane Wash** → **LC-MS-MS**

Figure 4: *M. edulis*

#### LC-MS/MS Approach

**Reverse Phase Liquid Chromatography:**  
Mobile Phase A: 2mM  $NH_4HCO_3$ , 50 mM  $CH_3OH$ , 100% Water  
Mobile Phase B: 2mM  $NH_4HCO_3$ , 50 mM  $CH_3OH$ , 95% Acetonitrile  
40°C

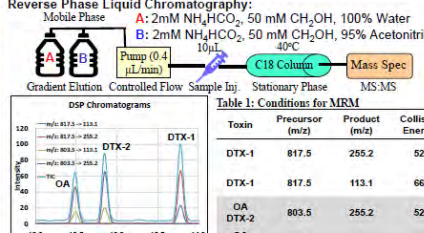


Figure 6: Chromatogram for DSP toxins

**Table 1: Conditions for MRM**

Toxin	Precursor (m/z)	Product (m/z)	Collision Energy
DTX-1	817.5	255.2	52
DTX-1	817.5	113.1	66
OA	803.5	255.2	52
DTX-2	803.5	113.1	66

Figure 6: Chromatogram for DSP toxins

**Tandem Mass Spectrometry:**

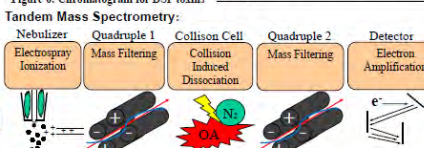


Figure 7: Tandem mass spectrometry diagram

**Table 2: Conditions for DSP FDA Method for LCMS-MS**

Gas Temp.	Gas Flow	Sheath Gas Temp.	Sheath Gas Flow	Collision Cell Volt.	Capillary Volt.	Nozzle Volt.	Delta EMV	Polarity	Dwell
300°C	3.0 L/min	125°C	3.9 L/min	4200 V	500 V	500 V	Negative	60	

#### Results

**Initial Issues**

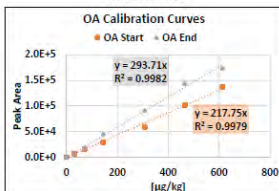


Figure 7: Initial calibration curves for OA

**Table 3: Comparison of calibration curve slopes of initial runs**

Toxin	Curve	Slope	% Diff
OA	Start	217.8	29.7
	End	293.7	
DTX-2	Start	215.6	48.4
	End	324.6	
DTX-1	Start	155.2	12.0
	End	175.1	

**Experiments**

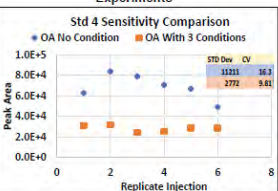


Figure 8: Multiple runs of STD 4 with and without conditioning (Three Matrix Blank Injections)

**Table 4: Comparison of spiked sample extract 0003-F with and without hexane step (P.A. = Peak Area)**

Toxin	Treatment	Average (P.A.)	SD (P.A.)	CV (P.A.)	% Diff
OA	Hexane	12445	573	5	3.74
	No Hexane	12919	705	5	
DTX-2	Hexane	12864	364	3	4.04
	No Hexane	13395	590	5	
DTX-1	Hexane	5381	165	3	8.85
	No Hexane	4925	147	3	

**Resolution**

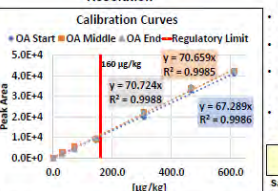


Figure 9: Final calibration curves with conditioning

**Table 5: Comparison of calibration curve slopes of final runs after conditioning**

Toxin	Curve	Slope	% Diff
OA	Start	67.3	4.93
	End	70.7	
DTX-2	Start	64.0	3.01
	End	62.1	
DTX-1	Start	61.9	7.61
	End	66.8	

#### Conclusions

- Introducing three matrix blanks as a condition each queue prevents initial elution interference in the instrument.
- The conditioning reduced intensity variance for start and final calibration curves, improving confidence in samples and curves.
- Removing the hexane wash reduces extraction time, saves resources, and prevents hazardous waste without sensitivity loss.
- Procedural optimization determined the Limit of Detection (LOD) (1.6, 1.4, 5.6 OA Eq. ug/kg) and Limit of Quantification (LOQ) (4.8, 4.1, 15.2 OA Eq. ug/kg) for future naturally incurred testing.
- Further study will utilize the modified extraction method and LC-MS/MS running procedure to compare with low-cost commercial test kits options for monitoring DSP.

#### Acknowledgements

**Sampling and Data:** All samples were provided by the Maine Department of Marine Resources (DMR). The Maine DMR provided data on Dinophysistoxin cell count, and mussel sampling stations.

**Funding:** NSF Grant 1460861 and NOAA Grant NA17NO04780179

**Site:** Bigelow Laboratory for Ocean Sciences, 80 Bigelow Dr. East Boothbay, Maine – Undergrad Research Experience in the Gulf of Maine and the World Ocean.

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- Sourced Photos: Agilent (Figure 3)

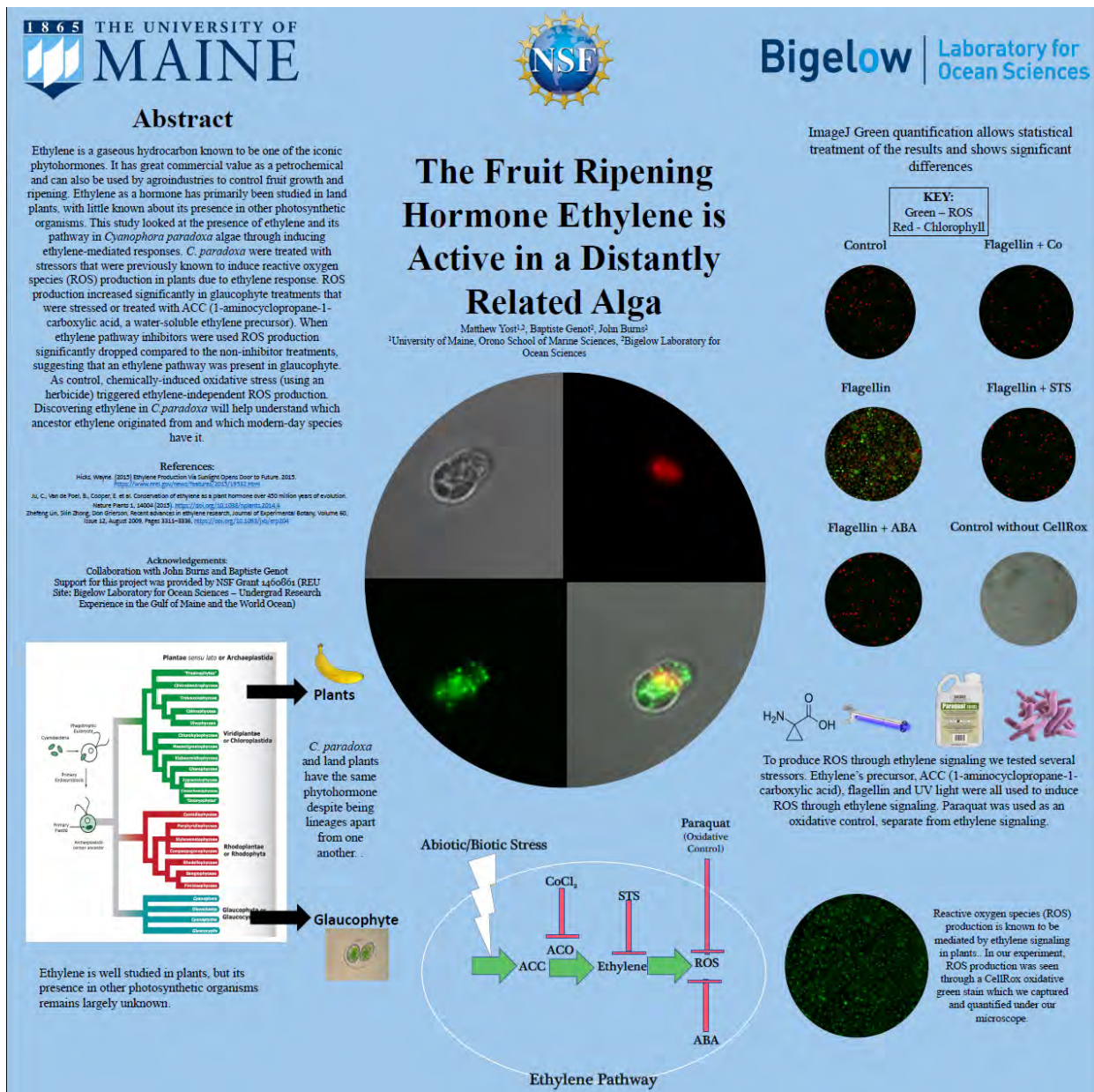
# Bigelow's REU Program 2021

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

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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<sup>1,2</sup>, Brian DiMento<sup>1</sup>, Christoph Aeppli<sup>1</sup>, David Fields<sup>1</sup>

<sup>1</sup>Bigelow Laboratory for Ocean Sciences, <sup>2</sup>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.

**Investigating The Effect of Dissolved Organic Matter on the Bioconcentration of Short Chain Chlorinated Paraffins in the Copepod Species *Calanus Finmarchicus***  
 Elizabeth Westbrook<sup>1,2</sup>, Brian DiMento<sup>1</sup>, Christoph Aeppli<sup>1</sup>, David Fields<sup>1</sup>  
<sup>1</sup>Bigelow Laboratory for Ocean Sciences, <sup>2</sup>University of Maryland, College Park

**Introduction**

**Short Chained Chlorinated Paraffins (SCCPs)**

**Structural Features:**

- 10 – 13 Carbon alkane
- 30 – 70% Chlorine by mass

**Why we care about SCCPs:**

- Recently declared a persistent organic pollutant by the UNEP
- Still ubiquitous in natural waters and very little is known about their fate in the environment

**Bioaccumulation in *Calanus finmarchicus***

- Organisms offer a hydrophobic environment for SCCP accumulation
- Sub-arctic copepod species are a model for accumulation in other organisms

**Dissolved Organic Matter (DOM)**

**About DOM**

- Comes from the breakdown of living organisms
- Very large molecules that create a hydrophobic microenvironment
- SCCPs can also accumulate in DOM

**DOM Changes Depending on Location**

**Methods and DOM Analysis**

**DOM Extraction**

Filtration Down to 0.2µm

Pumping filtered sample water through PPL cartridges to remove DOM

Elution of the cartridges with methanol

Removal of the methanol to obtain final product

**Copepod Exposure**

Copepods were starved for 8 hours and then exposed to SCCPs in DOM water for 24 hours

**Water and Copepod Extraction**

Liquid-Liquid Extraction of DOM water samples

Solid-Liquid Extraction of Copepod Samples

Figure 3 shows the specific UV absorbance of each DOM which is positively correlated with aromaticity.

Figure 3 shows the specific UV absorbance of each DOM which is positively correlated with aromaticity.

**Results and Conclusions**

Figure 1 shows the bioconcentration factors of 1,2,9,10-tetrachlorodecane, 1,2,11,12-tetrachlorododecane, and 1,2,5,6,9,10-hexachlorodecane after the 24-hour copepod exposure.

Figure 2 shows the experimental DOM-water partitioning coefficients calculated for the four chosen compounds in different types of DOM.

**Conclusion:**

The bioconcentration of short chain chlorinated paraffins in *Calanus finmarchicus* does not appear to be affected by the presence of DOM under these conditions. The bioconcentration factors do appear to correlate with the DOM partitioning coefficients of the compounds

**Research Goals**

Calculate the Bioconcentration Factor (BCF) of SCCPs in copepods under different DOM conditions

Characterized three different types of DOM used in experiments to determine which factors cause DOM to have a greater effect on the BCF of SCCPs.

**Discussion**

Bioconcentration factor was expected to decrease as a function of dissolved organic carbon concentration and the DOC water partitioning coefficient of the molecule

$$BCF_{DOM} = BCF_0 \cdot \frac{1}{(1 + K_{DOC} \cdot [DOC])}$$

The BCF values tend to decrease in the DOM treatments compared to the treatments containing only artificial sea water.

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# Bigelow's REU Program 2021

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

Owen T. Keleher<sup>2</sup>, & Benjamin S. Twining, PhD.<sup>2</sup>

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.




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

Owen T. Keleher<sup>1,2</sup>, Benjamin S. Twining<sup>1</sup>

Bigelow Laboratory for Ocean Sciences (1) & Bates College (2)




ACADEMIA  
BATESINA

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#### Seaweed

- Seaweed was collected from 13 locations along the Maine coast, during the summer of 2019 by Lauren Chacho.
- Iodine measured in three samples from each location.
- Iodine measured along a single blade, at one-foot increments.



#### Methods

- Samples thawed and freeze dried.
- Using a coffee grinder, dried sample was ground to a fine, digestible powder.
- Samples analyzed using Inductively Coupled Plasma Mass Spectrometer.
- Tetramethylammonium hydroxide (TMAH) solution used to dilute sample.
- Certified Reference Material showed ~90% recovery.
- Ground samples were digested in microwave.



#### Abstract

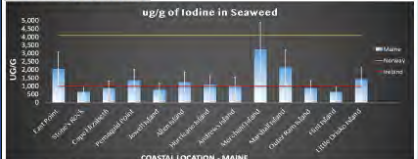
The greenhouse gas methane contributes significantly to global warming, and cattle and other ruminants contribute meaningfully to methane emissions. Cows fed seaweed as part of their diet may produce less methane during digestion. Seaweed contains high levels of iodine, and research is needed to understand 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.

#### Milk

- Iodine might be transferred from the feed to the milk, meant for human consumption.
- As an already high source of iodine, the legal maximum for iodine in milk is 500 ug/L.
- Feeding experiment conducted at Wolfe's Neck Center.


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#### Seaweed Results




Across the 13 locations along the Maine Coast:

- Mean iodine was 1,350 ug/g ± 568 (1 sd)
- Concentrations within range reported for sugar kelp in Ireland and Norway
- Statistical difference between some locations



Iodine variation along a single seaweed blade:

- Iodine does appear to decrease over the length of the blade
- There is still variation at this scale




Relative Elemental Variation

- The variation seen between location falls within the range of other elements previously analyzed from the same seaweed samples


#### Feeding Experiment

- Eleven cows fed seaweed as a component of their daily diet
- Eleven cows fed normal grain diet
- The feed trial lasted for four weeks. From this trial, milk was sampled from three time periods.

Experimental Group: Seaweed




Control Group: Zero Seaweed



11 Cows per Group

#### Methods

- Milk collected in March and April of 2021
- The milk samples were TMAH
- TMAH used to retain iodine for more accurate analysis
- Iodine analyzed with the ICPMS. Certified Reference Material showed a recovery of ~90%



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#### Reduce Methane Emissions

- Reduce methane emissions from cows by supplementing their feed with seaweed

#### Iodine in Sugar Kelp

- Brown seaweed is known to have high iodine.
- Analyze iodine levels in *Saccharina latissima* along the Maine coast.
- Understand drivers of iodine variation in seaweed

#### Iodine in Milk

- Analyze iodine in milk from cows fed seaweed
- Ensure the seaweed supplement is safe for consumption

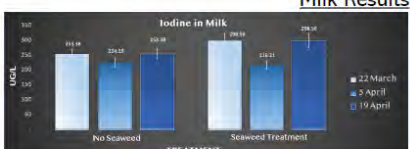
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#### Iodine

- Essential micronutrient required for proper thyroid hormone production. These hormones regulate the body's metabolism, controlling the heart, and help with muscle function.
- Particularly at a young age, thyroid hormones are crucial in brain development and bone maintenance.
- Toxic at high concentrations, potentially leading to serious health concerns such as gastrointestinal irritation and thyroid cancer.


	Daily Iodine Intake Guidelines	
	Recommended Value (ug)	Upper Limit (ug)
Child	120	200
Adults	150	1,100
Cow (600lbs)	3,600	50,000

#### Milk Results



Seaweed as a component of Cow Feed

- Iodine in milk from cows fed seaweed not significantly higher than control cows, as published previously.
- Therefore, we continued to investigate what other differences between the herd of cows might be causing the variation.



Iodine in the milk of different cow breeds

- Milk from four Jersey cows significantly lower than milk from eighteen Holstein cows.

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#### Acknowledgements

- 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 Davis Charitable Fund.
- Thank you to Brittney Honich, Sara Rauschenberg for method assistant and Lauren Chacho for the seaweed collection, and to the many members of the greater BurpBusters project.

#### Going Forward:

- Additional understanding of feeding methods, including confirmation of seaweed consumption by cows.
- There appear to be physiological factors within a cow that can determine how much iodine is passed through their system and into their milk.
- As we think about using this method to reduce methane, understanding how to keep the animals healthy and produce safe milk will be important elements of the research.

# Bigelow's REU Program 2021

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

Lydia Duncan<sup>1,2</sup>, Cath Mitchell<sup>2</sup>, Nichole Price<sup>2</sup>, Brittney Honisch<sup>2</sup>, Jessie Muhlin<sup>3</sup>, Hannah Webber<sup>4</sup>, Peter Nelson<sup>4</sup>, Stefan Claesson<sup>5</sup>

University of South Carolina-Columbia<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>, Maine Maritime Academy<sup>3</sup>, Schoodic Institute<sup>4</sup>, Nearview LLC<sup>5</sup>

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.

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

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University of South Carolina-Columbia<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>, Maine Maritime Academy<sup>3</sup>, Schoodic Institute<sup>4</sup>, Nearview LLC<sup>5</sup>

#### Overview

*Ascophyllum nodosum* (Asco) and *Fucus vesiculosus* (Fucus) are important intertidal seaweeds for their contribution to carbon cycling and in intertidal food webs. Asco is also commercially harvested in the Gulf of Maine. Monitoring these seaweeds by Unmanned Aerial System (UAS) with hyper or multi spectral instruments will give us a better understanding of the role of Asco and Fucus and produce better surveys, maps, and models for the estimating biomass of seaweeds in the intertidal.

#### Hypotheses:

- 1) There is a significant relationship between reflectance and biomass (canopy depth, wet weight, and dry weight) for Asco and Fucus.
- 2) There is a significant relationship between reflectance and desiccation (drying time) in Asco and Fucus.

#### Methods

- 1) Make 5 samples each of varying biomasses of Asco and Fucus
  - 2) Take 10 spectral measurements and rotate 90 deg
  - 3) Repeat step 2 until 40 measurements are taken of each sample
  - 4) Repeat steps 2 and 3, after at least 1 hour has passed for a total of 3 time points
- Experimental design is based on [1]. Set up is shown in Figure 1.

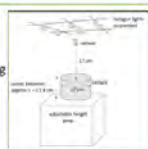


Figure 1: Schematic of experimental set up. Curtains blocked unwanted light.

#### Data Analysis

We used the Normalized Difference Vegetation Index (NDVI) to analyze the relationship between reflectance and canopy depth, wet weight, dry weight and drying time. Figure 3 shows the wavelengths and equation used to calculate NDVI.

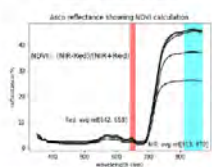


Figure 2: Asco samples from time point 1 showing reflectance vs wavelength. Highlighted sections are the wavelengths used to calculate NDVI.

#### Results

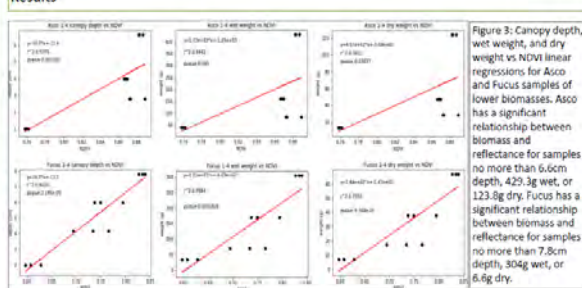


Figure 3: Canopy depth, wet weight, and dry weight vs NDVI linear regressions for Asco and Fucus samples of lower biomasses. Asco has a significant relationship between biomass and reflectance for samples no more than 6.6cm depth, 429.3g wet, or 123.8g dry. Fucus has a significant relationship between biomass and reflectance for samples no more than 7.8cm depth, 304g wet, or 6.6g dry.

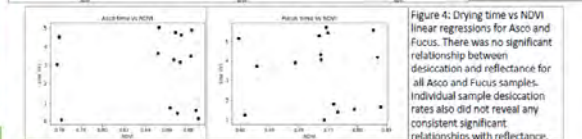


Figure 4: Drying time vs NDVI linear regressions for Asco and Fucus. There was no significant relationship between desiccation and reflectance for all Asco and Fucus samples. Individual sample desiccation rates also did not reveal any consistent significant relationships with reflectance.

#### Conclusion

- 1) We can estimate biomass from reflectance for Asco and Fucus. This is useful for developing UAS surveys mapping intertidal seaweeds.
- 2) There was no significant relationship between desiccation of Asco and Fucus samples and reflectance based on our data.

#### Future Research

- We are sampling at more intertidal sites need to be surveyed and similar experiments add more data to regressions, the relationships can be better understood for biomass and desiccation.
- We are investigating the use of Partial Least Squares Regression applied to the full hyperspectral data sets instead of using NDVI regressions, so we can use the full measured spectra.
- We are investigating machine learning algorithms, to map the estimated biomass of intertidal seaweeds from UAS surveys.

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# Bigelow's REU Program 2021

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

Hannah Sterling<sup>1</sup>, Christoph Aeppli<sup>2</sup>

<sup>1</sup>Roger Williams University, <sup>2</sup>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.

The National Academies of  
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MEDICINE

Bigelow Laboratory for  
Ocean Sciences

### Biodegradation of Photo-Oxidized Oil Products: Implications for the Fate of Marine Weathered Oil Spills

Hannah Sterling<sup>1</sup>, Christoph Aeppli<sup>2</sup>

<sup>1</sup>Roger Williams University, <sup>2</sup>Bigelow Laboratory for Ocean Sciences

Roger Williams  
University

#### INTRODUCTION

**Deepwater Horizon**

- On April 20, 2010, BP's Deepwater Horizon oil rig exploded, killing 11 people.
- Over the course of 87 days, 5 million barrels (210 million gallons) of Light Louisiana Sweet crude oil were released into the Gulf of Mexico from the wellhead at a depth of about 1500m<sup>1</sup>.
- Oil persisted on the sea surface for 102 days<sup>2</sup>.
- A number of factors impacted the fate of the oil (Figure 1).

Fate of Oil Spills

Dissolution

Evaporation

Biodegradation

Emulsification

Photo-Oxidation

Sedimentation

Figure 1. The six major factors that impact the fate of oil spills. Dissolution, evaporation, and emulsification change the oil's physical properties, while sedimentation traps the oil. Biodegradation and photo-oxidation change the oil's chemistry<sup>3</sup>.

**Photo-Oxidation**

Photo-oxidation is the process by which oxygen functional groups are added to crude oil compounds either directly through UV light absorption, or indirectly through the formation of reactive oxygen radicals<sup>4</sup>. Prior to Deepwater Horizon, it was believed that photo-oxidation was a very minor process which occurred over the timescale of months to years. However, up to 50% of Deepwater Horizon field samples were found to be oxygenated, thought to be formed by combined biodegradation and photo-oxidation<sup>5</sup>. Of this oxygenated fraction, further analysis provided evidence that this oxygenated fraction was primarily the result of photo-oxidation, with 2/3 of the oxidation after Deepwater Horizon occurring in the first 10 days<sup>4,5</sup>.

#### OBJECTIVES

- Quantify the degree of biodegradation of photo-oxidized oil
- Compare the rate of biodegradation between oil that has and has not been photo-oxidized
- Determine how the marine bacterial population is affected by enrichment with photo-oxidized oil

#### METHODS

NO-OIL

OX

CONT

NO-OX

EVAP

24 hour irradiated Deepwater Horizon source oil  
 24% evaporated Deepwater Horizon source oil  
 FSW 5 µM filtered seawater from Bigelow dock  
 AC autoclaved 5 µM filtered seawater from Bigelow dock

25 ppm oil concentrations in all experimental treatments  
 High Energy Water Accumulated Fraction (H2WAF) oil dispersion method used, stock solution prepared which was then further diluted

sampling

TLC/FID

concentration change over time of saturated, aromatic, and oxygenated fractions

Flow Cytometry

total bacterial counts performed by the Center for Aquatic Cytometry at Bigelow Laboratory for Ocean Sciences

GC/MS

quantification of polycyclic aromatic hydrocarbons (PAHs) and n-alkanes

#### GC/MS

OX

NO-OX

Hopane-normalized concentrations of n-alkanes: nC10-nC19, nC20-nC29, and PAHs: 2-ring, 3-ring, and 4-ring in µg/µg hopane for three time points: 0-week, 1-week, and 2-weeks. Values are reported for four treatments: OX, NO-OX, CONT, and EVAP. Error bars are ± the standard deviation.

CONT

EVAP

Hopane-normalized concentrations of n-alkanes: nC10-nC19, nC20-nC29, and PAHs: 2-ring, 3-ring, and 4-ring in µg/µg hopane for three time points: 0-week, 1-week, and 2-weeks. Values are reported for four treatments: OX, NO-OX, CONT, and EVAP. Error bars are ± the standard deviation.

#### TLC/FID

OX

NO-OX

Hopane-normalized concentrations (mg/µg hopane) of the saturated, aromatic, and oxygenated hydrocarbon fractions for three time points: 0-week, 1-week, and 2-weeks. Values are reported for four treatments: OX, NO-OX, CONT, and EVAP. Error bars are ± the standard deviation.

CONT

EVAP

Hopane-normalized concentrations (mg/µg hopane) of the saturated, aromatic, and oxygenated hydrocarbon fractions for three time points: 0-week, 1-week, and 2-weeks. Values are reported for four treatments: OX, NO-OX, CONT, and EVAP. Error bars are ± the standard deviation.

#### RESULTS

##### Flow Cytometry

total bacteria concentration in cells per mL over three time points: 0-week, 1-week, and 2-weeks for five treatments: NO-OIL, CONT, EVAP, NO-OX, and OX. Three replicates are reported for all treatments except for EVAP.

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#### CONCLUSIONS

- Both oil types can support bacterial growth and can therefore be biodegraded
- Saturated compounds degrade more than aromatic compounds for both oil types
- Oxygenated fraction (containing photo-products) increases, potentially due to accumulation of oxygenated biodegradation products
- Further research will include bacterial community analysis and specific photo-product degradation



## COPEPOD INGESTION RATE AT SUBLETHAL OIL CONCENTRATIONS

Sam McNeely<sup>1,2</sup>, Maura Niemisto<sup>2</sup>, Christoph Aeppli, PhD.<sup>2</sup>, & David Fields, PhD.<sup>2</sup>

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  $\mu\text{L oil L}^{-1}$  water and a dispersant concentration of 0.5  $\mu\text{L dispersant L}^{-1}$  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*.

## Copepod ingestion rate at sublethal oil concentrations

Sam McNeely<sup>1,2</sup>, Maura Niemisto<sup>2</sup>, Christoph Aeppli, PhD.<sup>2</sup>, David Fields, PhD.<sup>2</sup>

University of North Carolina Wilmington<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>



SCAN ME

### Background

**COPEPODS AND OIL SPILLS**  
Copepods can interact with oil spills and experience many lethal and sublethal effects (Fig. 1). *Calanus finmarchicus*, an Arctic copepod, is an integral part of higher trophic level organisms' diets. If pollutants affect copepod feeding rates, the ecological implications may be dire.

Fig. 1: *C. finmarchicus* that has oil stuck to its carapace.

### Research Question

**Is *C. finmarchicus*'s ingestion rate of phytoplankton affected by sublethal oil and dispersant concentrations?**

### Materials and Methods

**POLLUTANT EMULSIONS**  
Fig. 2: The water accommodated fraction (WAF), the dispersant, and the chemically enhanced WAF (CEWAF) emulsions were drained from the bottom leaving 0.5 L remaining to prevent larger oil droplets from entering the experimental extractions.

**EXPERIMENTAL CONDITIONS**

- 250 mL glass jars
- 10 *C. finmarchicus* individuals per jar
- 12°C
- 48 hours
- Constant darkness
- 10  $\mu\text{L oil L}^{-1}$  PSW
- 10  $\mu\text{L dispersant L}^{-1}$  PSW
- 10  $\mu\text{L CEWAF L}^{-1}$  PSW
- Thalassiosira weissflogii* as food

Fig. 3: A plankton wheel was used when algae was in the experiment to keep it in suspension.

### Preliminary Experiments

- An ingestion experiment with just *C. finmarchicus* and *T. weissflogii* to determine the minimum algal concentration that produces the maximum ingestion rate (Fig. 5).
- A copepod pollutant survival experiment to find the sublethal concentration (Fig. 6).
- An algal response experiment to understand how the algae interacts with the pollutants (Fig. 7).

\*The preliminary experiments set the conditions for the primary experiment.

**Primary Experimental Design**

Copepods & Algae	Control	WAF	Dispersant	CEWAF
------------------	---------	-----	------------	-------

x6

\*Grazing rates were determined by Frost's equations.  
Fig. 4: Algal concentrations were measured with a MOXII Cell counter.

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### Results

Fig. 5: Copepod ingestion rate at average *T. weissflogii* concentrations fitted to a logarithmic curve. The "X" is the desired minimum diatom cell concentration that produces the near-maximum ingestion rate, which provides the most variability if ingestion rate does decrease under pollutant exposure.

Fig. 6: Copepod survival percentage at various CEWAF concentrations (oil dispersant, 20:1). Data points represented with the same letter are not significantly different. The error bars represent the standard error. Since no difference was detected between any concentration, the highest concentration became the sublethal concentration.

Fig. 7: Algal growth constant, k, from Frost (1972) compared across 10  $\mu\text{L L}^{-1}$  PSW (WAF), 0.5  $\mu\text{L dispersant L}^{-1}$  PSW (Dispersant), and the combined effect of both pollutants (CEWAF). Similar letters are not significantly different. The error bars represent the standard error.

Fig. 8: Copepod ingestion rate at the sublethal concentrations of the WAF, dispersants, and the CEWAF. Data points that are represented with the same letter are not significantly different. The error bars represent the standard error.

Fig. 9: An adult female *C. finmarchicus* with *T. weissflogii* in its gut tract, which is the green tint from the head to the middle of the copepod.

### Results, cont.

- Ingestion Rate (Fig. 5):
  - The minimum algal concentration that produced the maximum ingestion rate was 20,000 *T. weissflogii* cells  $\text{mL}^{-1}$ .
- Copepod Survival (Fig. 6):
  - C. finmarchicus* did not differ significantly in survivability between the control or any of the various CEWAF concentrations [F(4,10) = 0.645, p > 0.05]. This defined the highest concentration tested as the sublethal concentration for the remainder of the experiments.
- Algal Response to Pollutants (Fig. 7):
  - T. weissflogii* did not experience any significant differences by algal growth constant, k, comparisons between pollutants at sublethal concentrations [F(3,8) = 0.043, p > 0.05].
- Ingestion Rate at Sublethal Concentrations (Fig. 8):
  - C. finmarchicus* ingestion rates of *T. weissflogii* did not significantly differ between the control and sublethal concentration treatments [F(3,20) = 0.095, p > 0.05].

### Discussion

#### IMPLICATIONS

- This suggests that *C. finmarchicus* ingestion rates are unaffected by even the ecologically unrealistic sublethal concentration.
- The dietary health of *C. finmarchicus* is not affected by the soluble components of crude oil and dispersants.
- If algae acts as a vector for crude oil and dispersants to enter a copepod's digestive tract, then ingestion rates that are unaffected by increasing pollutant concentrations pose a greater threat to both the copepod and its predators.

#### FUTURE

- Continuing with this study, Gas Chromatography (GC) analysis will be run on the experimental copepods to determine if any oil or dispersant compounds entered the copepods' systems.
- If it does enter the copepods' systems, then understanding more about how much of the pollutants are transferred up the trophic system would be essential.
- Understanding what compounds from crude oil and dispersants adsorb to phytoplankton would be beneficial to piecing together the puzzle of bioaccumulation.



We recognize the support of the National Science Foundation for the Bigelow Laboratory REU program (NSF grant 1460861) - REU Site: Bigelow Laboratory for Ocean Sciences - Undergraduate Research Experience in the Gulf of Maine and the World Ocean awarded to DMF. Special thanks to my mentors Dr. David Fields, Dr. Christoph Aeppli, and Maura Niemisto, as well as Hannah Sterling, Liz Westbrook, and Erin Belme, the staff at Bigelow Laboratory for their support during the REU program, and my family for their unending support.

# Bigelow's REU Program 2021

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



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The College of Wooster<sup>1</sup>, Bigelow Laboratory of Ocean Sciences<sup>2</sup>

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<sup>-1</sup>) 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<sup>-1</sup> day<sup>-1</sup>) than microplastics only (2.67E+04 beads cop<sup>-1</sup> day<sup>-1</sup>) 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.

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

MANASI DESAI<sup>1,2</sup>, MAURA NIEMISTO<sup>2</sup>, CHRISTOPH AEPPLI<sup>2</sup>, DAVID FIELDS<sup>2</sup>  
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### INTRODUCTION

The marine environment has been showing increasing levels of microplastic (MP; plastics < 5mm) concentrations. It has been estimated that between 4.8 million and 12.7 million metric tons of plastic waste has entered the ocean (Jambeck et al., 2015). These MP are often within the size range of particles ingested by plankton organisms at the base of the food chain (Wood et al., 2020). Polycyclic aromatic hydrocarbons (PAHs) such as those released in an oil spill, sorb to the MP particles and may represent a significant exposure route for these chemicals to marine biota. Previous research has shown that copepods, an important zooplankton species, can ingest a significant amount of microplastics but it is unclear whether ingestion of PAH sorbed microplastics occurs as well. Trophic transfer of contaminants and microplastics in the marine food web can lead to their increased concentrations found in tissues of fish and whales which might pose a health risk to humans who depend on a seafood diet.

My research question asks whether microplastics provide an ecologically important route for PAHs to enter the food web through ingestion by zooplankton?

### RESULTS AND DISCUSSION

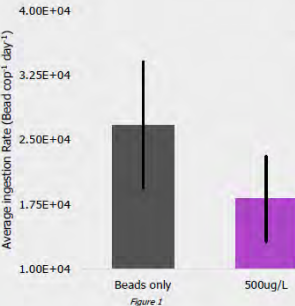
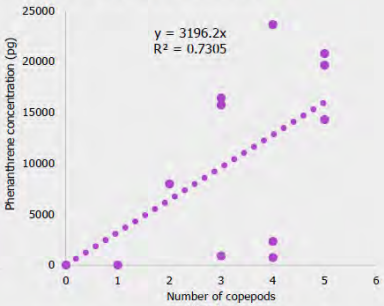



Figure 1 shows the average ingestion rate (Beads/cop day<sup>-1</sup>) plotted for the two treatments – Beads only and Beads loaded with 500ug/L of phenanthrene. Copepods ate significantly less phenanthrene loaded beads with an average rate of **1.81E+04 beads cop<sup>-1</sup> day<sup>-1</sup>** while beads only treatment had an ingestion rate of **2.67E+04 beads cop<sup>-1</sup> day<sup>-1</sup>** (t=2.42; df=10; p=0.01).


Figure 2 shows the phenanthrene concentrations (pg) plotted against the number of copepods extracted. A minimum of two copepods are needed for the GC-MS to detect phenanthrene levels. Individual copepods can accumulate up to 3196.2 pg of PAH which potentially can biomagnify in the marine food web upon predation by marine mammals and fish etc.

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### METHODS

#### A. Materials

Zooplankton tows were collected at station 4 in the Damariscotta Estuary during cruises on the R/V I Ra-C from May to July 2021. Polystyrene microbeads (d=12um) was chosen as the model microplastic, *Calanus finmarchicus* as the model copepod and phenanthrene as the PAH.

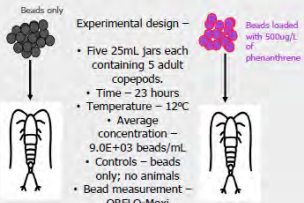


Left to right - Polystyrene beads, *Calanus finmarchicus* and phenanthrene

#### B. Ingestion experiments

**Experimental design –**


- Five 25mL jars each containing 5 adult copepods.
- Time – 23 hours
- Temperature – 12°C
- Average concentration – 9.0E+03 beads/mL
- Controls – beads only; no animals
- Bead measurement – ORFLO-Moxi
- Frost equation to calculate ingestion rate




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#### C. Copepod extraction

750 uL of NaOH/ETOH + 750 uL hexane + 5 uL o-terphenyl (OTP) were added to copepods with Si/Zr beads



Top layer of hexane layer containing phenanthrene was extracted twice and transferred to a GC vial for GC-MS analysis



Sonication and bead-beading to decimate copepods

$$\langle C \rangle = \frac{C_1 * [e^{(k-g)}(t_2 - t_1) - 1]}{(t_2 - t_1)(k - g)}$$

**Acknowledgements**

This work was financially supported by the U.S. Department of Homeland Security. We recognize the support of the National Science Foundation for the Bigelow Laboratory REU program. Special thanks to my mentors Dr. David Field, Dr. Christoph Aeppli, Maura Niemisto and Erin Beirne, as well as Zale Nursey, Hannah Sterling, Liz Westbrook, the staff at Bigelow Laboratory for their support during the REU program and my family for their unending support.

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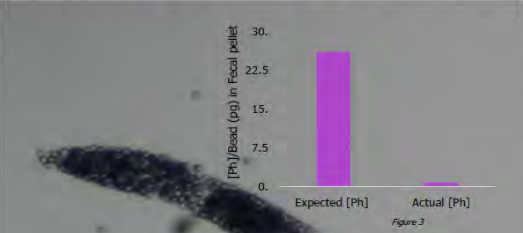


Figure 3 shows that phenanthrene concentrations in fecal pellets dropped from 26 pg/bead to **0.76 pg/bead**. This indicates that phenanthrene leaches off the beads either in the copepod's body or gets solubilized in the surrounding waters.

# Bigelow's REU Program 2021

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

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<sup>1</sup>Bigelow Laboratory for Ocean Sciences; <sup>2</sup>Odum School of Ecology, University of Georgia; <sup>3</sup>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 cyler 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/ $\mu\text{L}$ ) amplified at a significantly higher copy number than that of June (0.0003-0.001 copies/ $\mu\text{L}$ ). Altogether, our results indicate that BTN is still present, and eDNA can be used to monitor the presence of neoplastic cells.

**Introduction**

*Mya Arenaria*, the soft-shelled clam, is well distributed along the northern coasts of the Atlantic and Pacific. Bivalve Transmissible Neoplasia (BTN) is a Leukemia-like infectious cancer that has been infecting *M. arenaria* and other bivalves. So far over nine species of bivalves have been characterized with BTN, and modelling suggests that this cancer is more prevalent in bivalves than what has previously been assessed. *M. arenaria* harvesting brings in \$5-10 million annually to the Maine clamming industry. Understanding how BTN spreads, and if infection rates will increase is critical to preventing outbreaks in these bivalves. As climate change continues, it is unknown how BTN prevalence in *M. arenaria* will change, but speculations suggest that increased temperature, lowered dissolved oxygen, and decreased pH will increase BTN prevalence. Therefore, it is very important to constantly assess BTN prevalence as the climate changes. This study will hopefully serve as a precedent to begin long term monitoring of BTN within *M. arenaria* to better forecast *M. arenaria* mortality and to ensure a sustainable fishery.

**Methods**

Three sites were chosen along the Casco Bay: The Dam in Quahog Bay (QB), Gurnet Landing (GL) on Harpswell Island, and Long Cove (LC) on Orrs Island. Each site was sampled twice for clams (n=25-32). Hemolymph was then extracted (~5mL) using a 3mL syringe and G-19 needle from each clam. One sample from each site was analyzed via CellProfiler™ to detect cancer cells. Cell DNA was extracted with the EZ.N.A.® Tissue DNA Kit (D3396) (NORCROSS, GA)

**Results**

**Population Prevalence of BTN**

Site	Sample	Highest Threshold (%)	Lowest Threshold (%)
GL	6/21	~15	~10
	7/21	~75	~80
QB	6/21	~15	~10
	7/21	~15	~10
LC	6/21	~10	~10
	7/21	~15	~10

**eDNA Detection of Cancerous Cells**

Site	Sample	eDNA Cancer Copies/ $\mu\text{L}$
GL	6/21	3.10E-01
	7/21	1.76E+04
QB	6/21	8.09E-01
	7/21	2.41E+04
LC	6/21	9.91E-01
	7/21	5.30E+04

**Conclusions**

- CellProfiler™ was successful in identifying cancerous cells in highly neoplastic clams (Fig 4). However, Cell Profiler was not accurate in identifying cancerous cells due to false positives and debris in samples (Fig 1). Additionally, it could not identify cancerous cells in low infected individuals.
- Gurnet Landing has the highest prevalence of BTN (Fig 1)
- eDNA extraction can be used to detect BTN within the water column, but was not accurate when correlating to population infection
- The second samples had significantly increased in the percent of cancerous cells detected via eDNA extraction (Fig 2).

**Future Goals**

- Investigate how and why cancerous clams spread from neoplastic hemocytes.
- Explore whether there is a role for shell or siphon preferences in cancer prevalence.

**Acknowledgements**

We would like to thank the NOAA ES&D grant: 1408861 for funding this project. Additionally, we would like to thank the eDNA grant: 1849227 and grant: 1704893 for funding this project. We would also like to thank the local clam diggers for collecting our samples.

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# Bigelow's REU Program 2021

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

Asher Platts<sup>1</sup>, Dr. Beth Orcutt, PhD<sup>2</sup>

Southern Maine Community College<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>

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.

### ATP Is Not a Good Proxy For Biomass

**Figure 1**

**Figure 2a**

**Figure 2b**

**Figure 2c**

**Figure 3a**

**Figure 3b**

**Figure 3c**

# Few Stressed Microbes

# Can Look Like Many Thriving Microbes

# If ATP Is The Only Measurement

|| Asher Platts, SMCC REU Intern || Dr Beth Orcutt, PhD, Bigelow Laboratory for Ocean Sciences ||

### So What? Why Should We Care?

#### Microbes Provide Important Ecological Services and Deep Sea Mining Potentially Threatens Poorly Understood Biological Communities

**Other Problems We Encountered**

**How Those Problems Were Overcome**

20

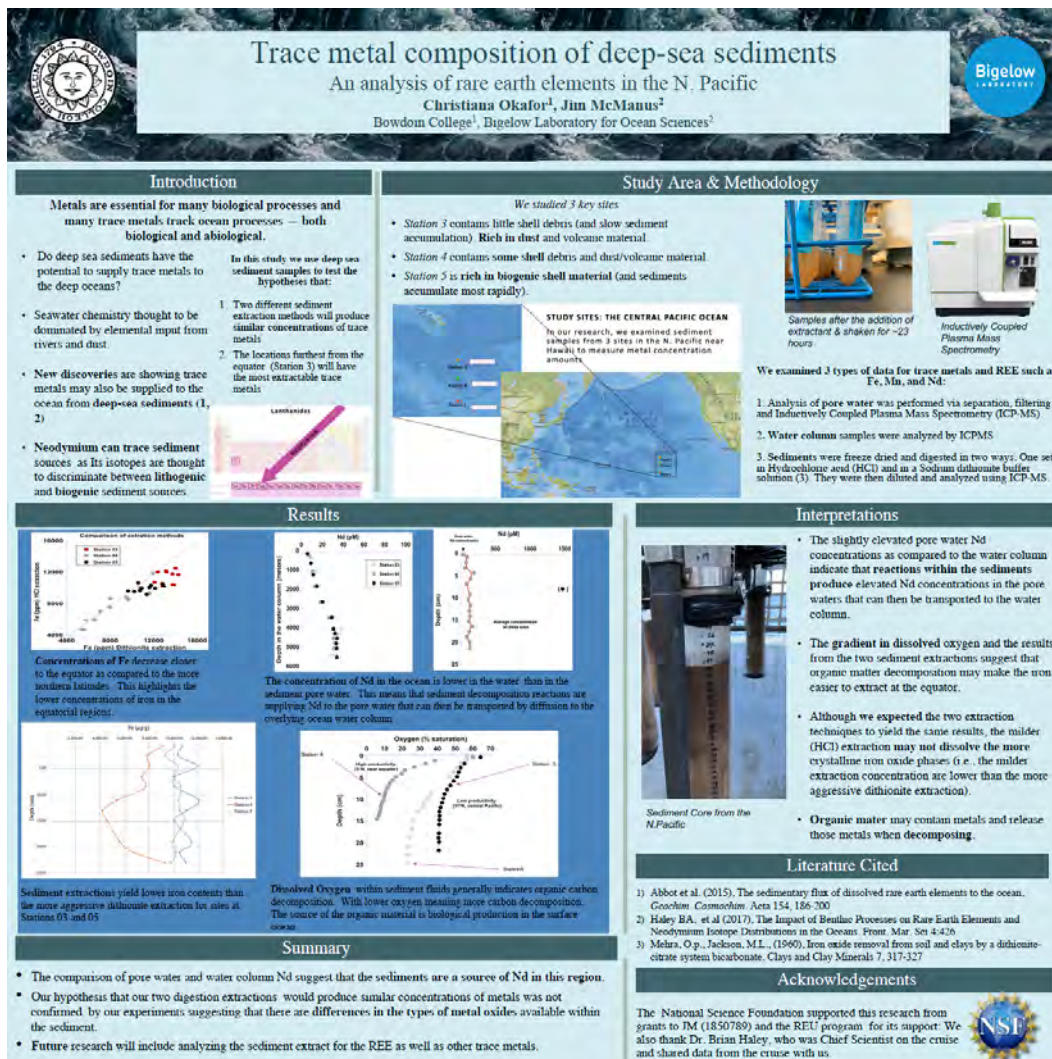
# Bigelow's REU Program 2021

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

Christiana Okafor<sup>1</sup>, Jim McManus<sup>2</sup>

Bowdoin College<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>

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.



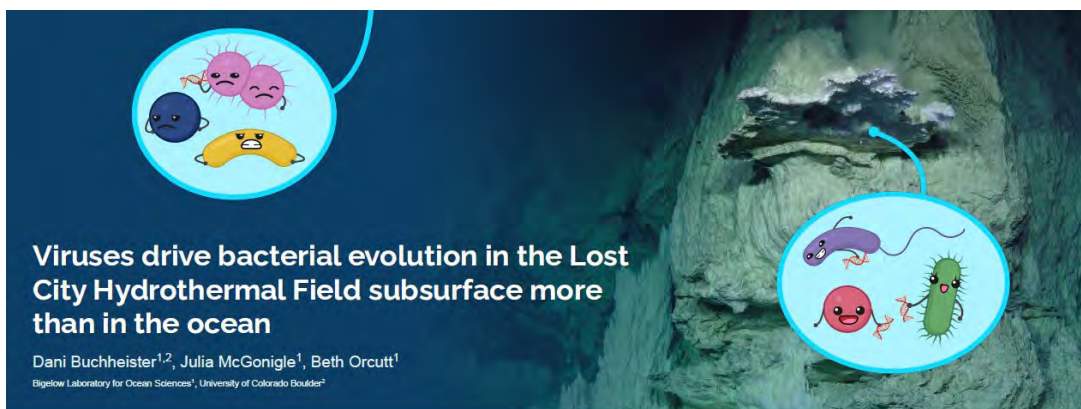
# Bigelow's REU Program 2021

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

Dani Buchheister<sup>1,2</sup>, Julia McGonigle<sup>1</sup>, Beth Orcutt<sup>1</sup>

Bigelow Laboratory for Ocean Sciences<sup>1</sup>, University of Colorado Boulder<sup>2</sup>

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.



## Viruses drive bacterial evolution in the Lost City Hydrothermal Field subsurface more than in the ocean

Dani Buchheister<sup>1,2</sup>, Julia McGonigle<sup>1</sup>, Beth Orcutt<sup>1</sup>

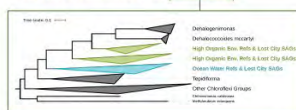
Bigelow Laboratory for Ocean Sciences<sup>1</sup>, University of Colorado Boulder<sup>2</sup>

### Background

- The Lost City Hydrothermal Field is driven by **serpentinization**



- Bacteria can use the products for food and fuel
- Horizontal gene transfer (HGT)** is the exchange of genetic information between microbial species
- It can drive evolution and introduce new capabilities
- Genomic islands** are pieces of DNA that can move between genomes and facilitate HGT
- Species analyzed: **Desulfotomaculum**, **Thermodesulfovibrio**, and **Chloroflexi**

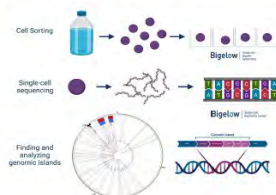


### Goal

To search for evidence of genomic islands in Lost City bacteria, classify island gene functions, and compare our findings to known reference genomes

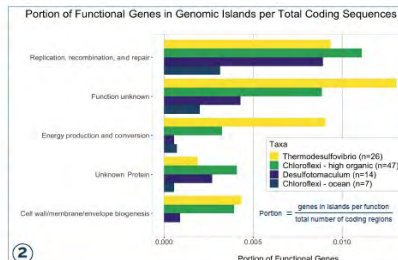
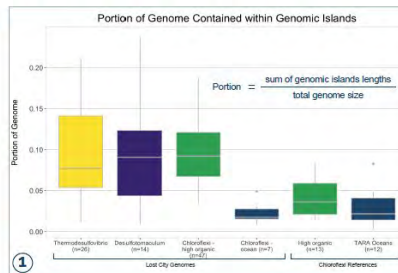
### Methods

- Samples were collected in 2018 with the ROV Jason



### Results & Conclusions

- Suspected subsurface groups have a higher portion of their genomes in islands (1)
- Replication, recombination, and repair is the largest functional category for genomic island proteins (2)
- Around 50% of those are virus-associated genes, indicating unexpected viral activity in this system
- Portions of coding regions in islands are higher in high organic Chloroflexi and Thermodesulfovibrio (2)
- More genomic islands than expected, with around 10% of the genomes in mobile regions
- Indicates that viruses may play an important role in driving evolution in extreme environments**



# Bigelow's REU Program 2021

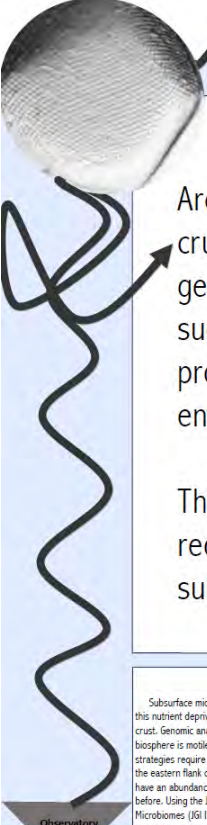
## INVESTIGATING PILI STRUCTURES IN THE MARINE SUBSURFACE

Autumn Pope<sup>1</sup>, Stephanie Carr<sup>1</sup>, Beth Orcutt<sup>2</sup>, Michael Rappé<sup>3</sup>, Olivia Nigro<sup>4</sup>

<sup>1</sup>Hartwick College, <sup>2</sup>Bigelow Laboratory for Ocean Sciences, <sup>3</sup>Hawaii Institute of Marine Biology, University of Hawaii at Manoa, <sup>4</sup>Hawaii Pacific University

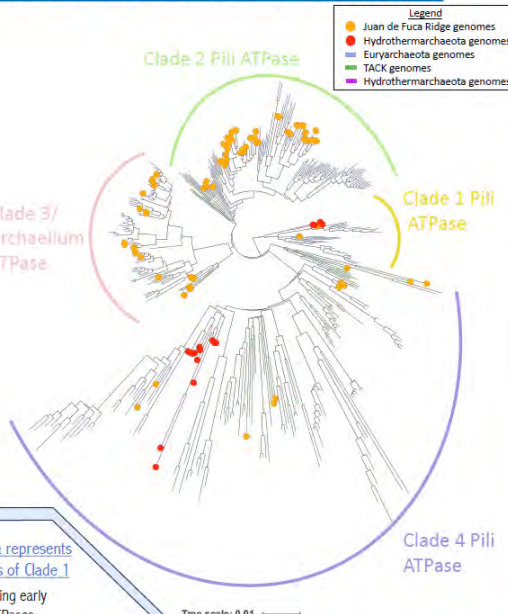
Subsurface microbial life represents 15% of global life, but the activity of life in this nutrient deprived environment is unclear, especially in subseafloor oceanic crust. Genomic analyses suggest that prokaryotic life in the marine crustal biosphere is motile or lives together as biofilm; this is surprising 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 ArII 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 archaeellum 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.

### Investigating pili structures in the marine subsurface



Archaea from subsurface ocean crust have both pili and archaeellum genes in their genomes, suggesting motility and biofilm production are important in this environment.

This is surprising, since motility requires a lot of energy, and the subsurface is energy deficient.




Tree scale: 0.01

**Abstract**

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 ArII 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 archaeellum 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.

**Hydrothermarchaeota represents the deepest branches of Clade 1**

○ Possibly representing early evolved Clade 1 ATPases



Tree scale: 0.01

**Archaeal genomes have ATPases for more than one external structure**

	Clade 1	Clade 2	Clade 3	Clade 4
Bathyarchaeota				
Electrothermarchaeota				
Unknown Crenarchaeota				
Hydrothermarchaeota				
Thermoplasmales				
Acidithiobaculum				
Euryarchaeota				
Methanopyrus				
Methanothermobacter				
Thermoplasma				
Methanocaldococcus				
Archaeoglobus				

**Acknowledgments** Bigelow | University of Hawaii

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Special thanks to Dr. Stephanie Carr and Dr. Beth Orcutt, Dr. David Parks, the members of the Orcutt Lab, my professors at Hartwick College for providing me with support throughout this REU internship.

Autumn Pope<sup>1</sup>, S. Carr<sup>1</sup>, B. Orcutt<sup>2</sup>, M. Rappé<sup>3</sup>, O. Nigro<sup>4</sup>

<sup>1</sup>Hartwick College, <sup>2</sup>Bigelow Laboratory for Ocean Science, <sup>3</sup>Hawaii Institute of Marine Biology, University of Hawaii at Manoa <sup>4</sup>Hawaii Pacific University


# Bigelow's REU Program 2021

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

Alexis Mullen<sup>1,2</sup>, Doug Rasher<sup>2</sup>, Eric Annis<sup>3</sup>, Aubrey Jane<sup>2,4</sup>

Bowdoin College<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>, Hood College<sup>3</sup>, University of New England<sup>4</sup>

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—8°C, 18°C, and 26°C—to gauge stage-specific responses of larvae to potential lower and upper thermal threshold temperatures. Stage I larvae experienced high mortality in 26°C, while stage I, stage II, and stage III larvae experienced slowed development times when reared at 8°C, 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 18°C. 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.






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### Assessing Stage-Specific Thermal Tolerance of American Lobster (*H. americanus*) Larvae

Alexis Mullen<sup>1,2</sup>, Doug Rasher<sup>2</sup>, Eric Annis<sup>3</sup>, Aubrey Jane<sup>2,4</sup>

1. Bowdoin College, 2. Bigelow Laboratory for Ocean Sciences, 3. Hood College, 4. University of New England

Bigelow Laboratory for Ocean Sciences

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**Introduction:**

- The thermal tolerance of *H. americanus* larvae influences their development and survival<sup>1,2</sup>, and temperature is hypothesized to determine larval settlement<sup>3,4</sup>, which impacts the distribution of adult lobsters in the ocean<sup>5</sup>.
- Studying the thermal tolerances of lobster larvae in the Gulf of Maine, which underpin North America's most lucrative single species fishery<sup>5,6</sup>, may therefore help to predict future distributions of the fishery.
- Few studies have examined the stage-specific lower and upper thermal tolerance limits of lobster larvae<sup>2</sup>, more often focusing on a temperature range from 10°C-22°C.
- Determining the thermal tolerance limits of stage I-III *H. americanus* larvae may help to predict shifts in larval behavior within the water column in response to warming ocean temperatures.
- Assessing temperature effects on larval survival and development may help to explain whether temperature played a key role in a recent decrease in pelagic post larval abundance, despite a 27 year increase in stage I larvae in the Gulf of Maine.<sup>7</sup>

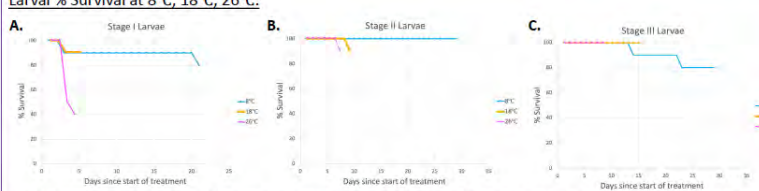
**Purpose:**

To gain a greater understanding of temperature effects on stage I, II, and III *H. americanus* larval survival and development.

**Methods:**

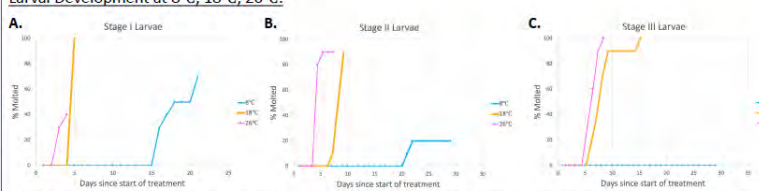
- Larvae were reared individually in 400ml glass jars of 0.45µm filtered sea water and fed *Artemia* ad libitum. Water was changed 3x/week.
- Directly following hatch, stage I larvae were placed into treatments. Rearing to stage II and stage III occurred at 18°C.
- Stage I, stage II, and stage III larvae were placed in 8°C, 18°C, and 26°C incubators and remained in treatment until they molted or died (Figure 3).
- Larvae were checked daily for signs of molting and mortality.
- Molted larvae were removed from treatment, photographed for carapace length (CL, using ImageJ), and frozen for dry weights (DW, Figure 3).
- Growth rates for carapace length and dry weight (biomass) were calculated using averaged CL and DW data from newly hatched stage I larvae (n=10).

**Larval % Survival at 8°C, 18°C, 26°C:**



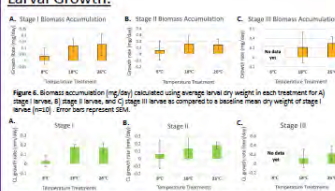
**Figure 4.** Percent survival of A) stage I larvae, B) stage II larvae, and C) stage III larvae in 8°C, 18°C, and 26°C temperature treatments. The ending of lines shown in blue (8°C), orange (18°C), or pink (26°C), indicates that all individuals within the treatment had either molted or died.

**Larval Development at 8°C, 18°C, 26°C:**

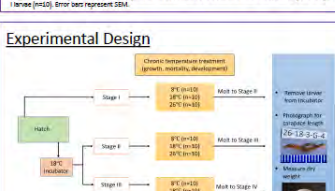


**Figure 5.** Percent of A) stage I larvae, B) stage II larvae, and C) stage III larvae that molted in 8°C, 18°C, and 26°C temperature treatments. The ending of lines shown in blue (8°C), orange (18°C), or pink (26°C), indicates that all individuals within the treatment had either molted or died.

**Larval Growth:**



**Figure 6.** Biomass accumulation (mg/dry wt) calculated using average larval dry weights in each treatment for A) stage I larvae, B) stage II larvae, and C) stage III larvae as compared to a baseline mean dry weight of stage I larvae (mg/dry wt). Error bars represent SEM.



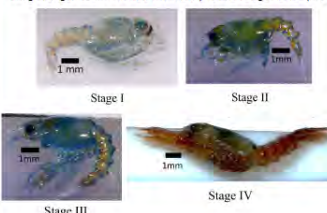
**Figure 7.** Carapace length (CL) growth rate (mm/day) calculated using average larval CL in each treatment for A) stage I larvae, B) stage II larvae, and C) stage III larvae as compared to a baseline mean carapace length of stage I larvae (mm/day). Error bars represent SEM.

**Discussion:**


- There was increased mortality in stage I larvae reared at 26°C.
- Larvae reared at 26°C had the fastest development times; development time was significantly slowed in the cold 8°C treatment.
- Stage III larvae experienced higher mortality at 8°C than did stage II larvae, indicating an ontogenetic shift in thermal tolerance.
- 8°C and 26°C may be nearing the lower and upper thermal tolerance limits of *H. americanus* larvae.
- Larvae of all stages experienced low mortality and relatively fast development times when reared at 18°C.
- As prolonged periods of 26°C are not currently seen in the GOM, high temperature is likely not responsible for the recent decrease in pelagic post larvae in the Gulf of Maine.

**Conclusion:**

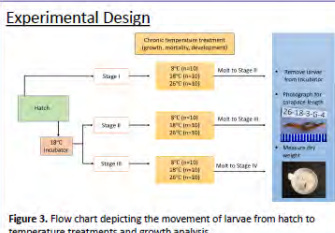
As waters warm in the GOM, larvae may shift lower in the water column to reside in seawater closer to 18°C, while still avoiding temperatures as low as 8°C, where larvae experience slowed development times that may inhibit settlement.



**Figure 1.** The first four larval development stages.



**Figure 2.** Lobster management zones in Maine. The egg bearing females that supplied the larvae for this experiment were extracted from zone D, near Boothbay, ME.



**Figure 3.** Flow chart depicting the movement of larvae from hatch to temperature treatments and growth analysis.



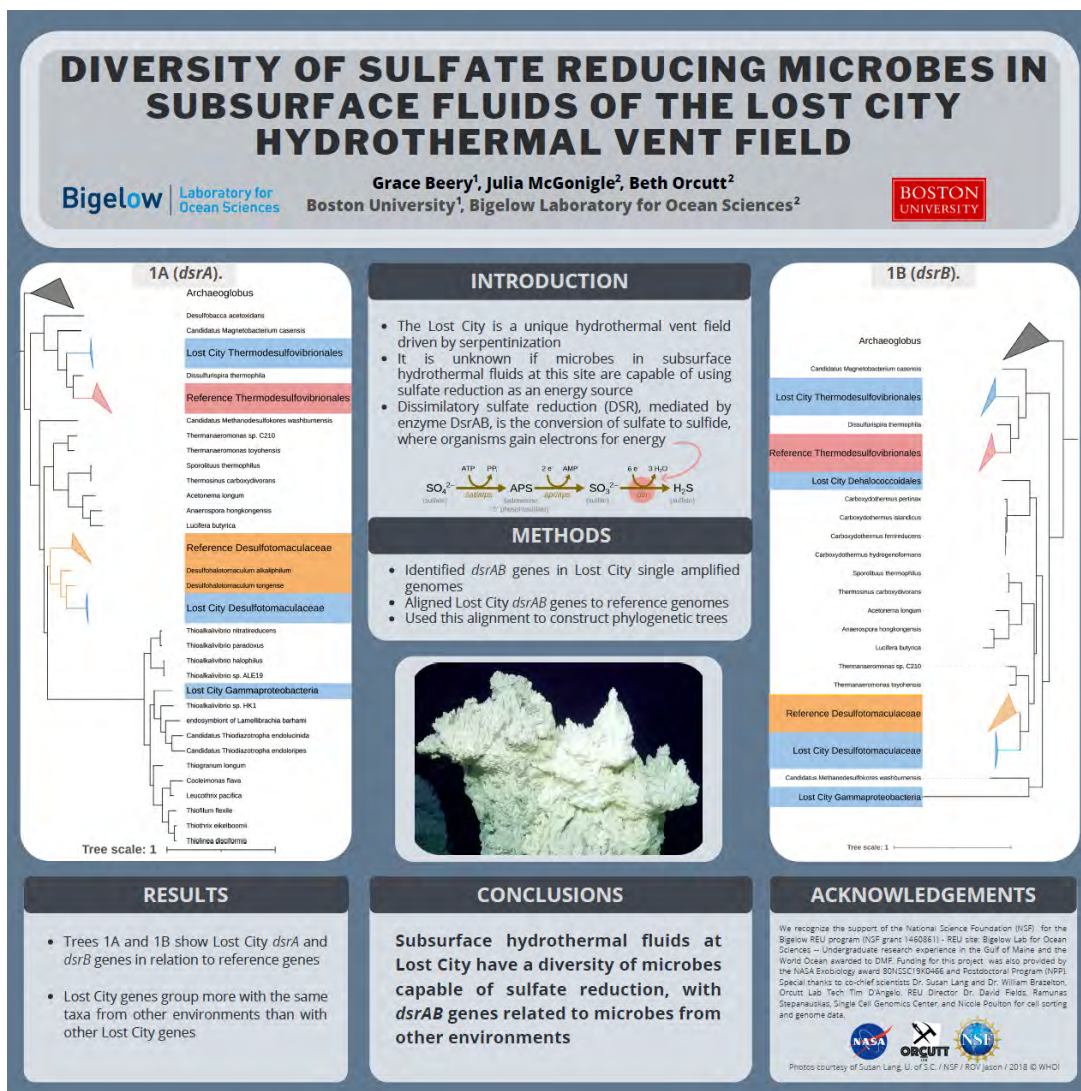
# Bigelow's REU Program 2021

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

Grace Beery<sup>1</sup>, Julia McGonigle<sup>2</sup>, Beth Orcutt<sup>2</sup>

Boston University<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>

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<sub>2</sub> 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 Thermodesulfobrivionales, 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.




# Bigelow's REU Program 2021

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


Paxton Tomko<sup>1,2</sup>, Jacob Munson-McGee<sup>1</sup>, Julia Brown<sup>1</sup>, Nicole Poulton<sup>1</sup>, Ramunas Stepanauskas<sup>1</sup>  
Bigelow Laboratory for Ocean Sciences<sup>1</sup>, Purdue University<sup>2</sup>

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.



### A single cell approach to understanding viral impact on phenotypic expression in marine microorganisms.

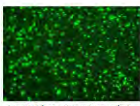
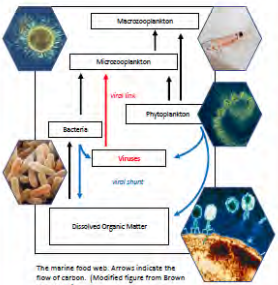
Paxton Tomko, Jacob Munson-McGee, Julia Brown, Nicole Poulton, Ramunas Stepanauskas.



#### The importance of viruses

Viruses are the most abundant and diverse biological entity and play a vital role in marine ecosystem function.

- They are major drivers of evolution and biodiversity.
- Play a crucial role in geochemical and nutrient cycling.
- Can alter the metabolic potential of the host genomes.

The marine food web. Arrows indicate the flow of carbon. (Modified figure from Brown et al. 2020)

#### Single cell genomics for studying virus-host interactions

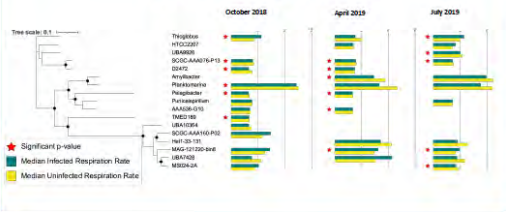
Traditional culture-based methods pose limitations as only a small percentage of species can be cultured. Single cell genomics allows us to enhance the resolution of virus-host investigations through sequencing the genomes of individual cells directly from an environmental sample. Furthermore, this allows us to:

- Directly identify virus-host interactions.
- Observe genetic and phenotypic characteristics that can't be seen on a community level.

#### The objective

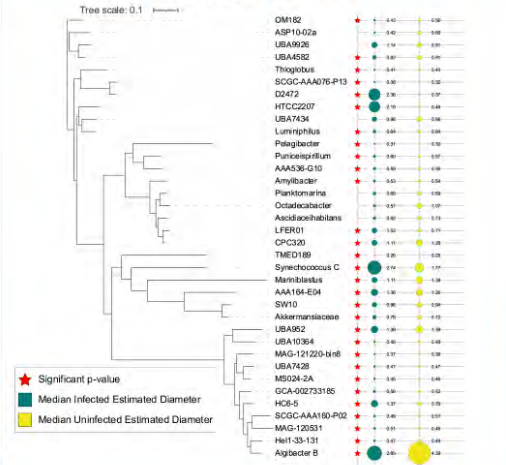
To investigate the role viruses play in host phenotypic expression using a novel dataset.

#### Cellular respiration

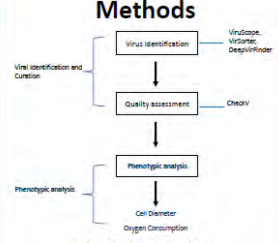


Viral infection has an effect on host respiration rate and cell size. The majority of genera got larger and decreased their respiration.

#### Estimated cell size



#### Methods



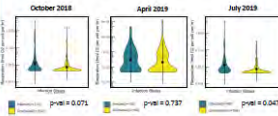
#### Viral detection

Analysis	Initial contig count	After checkV
VirusScope	132	117
VirSorter	463	392
DeepVirFinder	6232	5254
VirusScope+VirSorter	16	13
VirusScope+DeepVirFinder	79	73
VirSorter+DeepVirFinder	838	624
All 3	89	89
Total	7,429	7,228

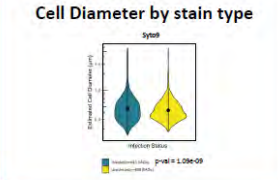
Identified viruses in 1928 SAGs from 4543 total SAGs → ~43% contain viruses!

#### Traits at the population level

##### Respiration by day




##### Cell Diameter by stain type



#### Acknowledgements

Support for this project was provided by NSF Grant 1440961 (REU Site: Bigelow Laboratory for Ocean Sciences – Undergrad Research Experience in the Gulf of Maine and the World Ocean), NSF EPSCOR Track-2 (1820734): Single Cell Genome-to-Phenome: Integrating Genome and Phenome Analyses of Individual Microbial Cells in Complex Microbiomes.



# Bigelow's REU Program 2021

## Automating A Visual Screening Task Using Deep Learning

Julia Brown<sup>1</sup> Ben Tupper<sup>1</sup> Nick Record<sup>1</sup> Jace Innis<sup>1,2</sup> Jonathan Evanilla<sup>1</sup>

<sup>2</sup> California State University, Monterey Bay

<sup>1</sup>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%.

### Scope of project

With advances in genomic sequencing technology, we are at a point where we can collect much more data than can be readily processed. For the single cell genomics lab at Bigelow, a time-consuming step in their genomic processing pipeline is sorting through samples that may have been contaminated during sequencing. Along with identifying any suspect viruses, the current method for accomplishing this is to visually analyze these PCA plots you see below. This can take weeks to accomplish for the typically sized data set. This projects aimed to automate parts of this process and to investigate what other reliable predictions this sort of analysis could make.

# Automating a Visual Screening Task Using Deep Learning

California State University <sup>2</sup>  
**MONTEREY BAY**

## Bigelow | Laboratory for <sup>1</sup> Ocean Sciences

Contributors: Julia Brown<sup>1</sup> Ben Tupper<sup>1</sup> Nick Record<sup>1</sup> Jace Innis<sup>1,2</sup> Johnathan Evanilla<sup>1</sup>

### How Visual Identification works

**Suspected Virus or Preserved Gene**

A significant deviation of a single contig from the central cloud can mean a few things. One being that there was a virus DNA strand inside the cell, another that the sequence from the outlying contig is very important to the survivability of the cell and has preserved itself from mutation.

**Suspected Contamination**

This SAG is a great example of sample contamination. Because these two cells do not share a common variability between these two principal components, we can see these two distinct 'clouds' of contigs. The small size of cells make single cell genomics inherently difficult, and it is not always guaranteed that only one cell is in a sample.

**Typical**

Genomes without contamination or any suspect regions will typically make a cloud like this. We cannot say for certain without further analysis that there is no contamination or a virus, but this is a good indication that there was no contamination.

### What are these plots?

The plots you see to the left are a simplified version of what bioinformatics look at when sifting through their single amplified genome data. The points plot the variability between two of the most common four-letter nucleotide combinations at a small window along the genome. These calculations are made through a statistical technique called principal component analysis where the dimensionality of large datasets are reduced to two.

### Approach and Data

This project was possible because of the availability of a large and labeled data set provided by the single cell genomics center. The dataset used, GORGS tropics, was sampled across the globe and over 12,000 prokaryotes were sequenced and more than 50% of those received some sort of classification.

The workflow of this project formatted the PCA data from these cells and used the existing labels to train a neural net. A subset of data was withheld from training and used to evaluate the accuracy of the model. All this was done using TensorFlow through Keras, in the R programming language.

### Four different data formats were experimented on

**Sliced Histograms**

These sliced 2D histograms were the most scalable, in that every dimension could be increased and remain uniform across all samples. Also, even when stacked up to more individual cells than the images show, the format would not be CPU intensive than image processing.

**Eight principal components**

Another implementation of the 2D histogram was to include the first eight principal components in the data input rather than just the first two. This method showed mixed results as the prediction (and presumably the characteristics were looking for) decreases with higher components.

**Images**

Saved .jpg images were the most obvious choices because it is the most similar to the current figure's data used manually and it preserve the separation of contigs by connecting with lines. However, this format is very computationally intensive and can not be input in its native resolution making implementing difficult.

**Single 2D Histograms**

This was the first input format experiment on as it proved to be the simplest and produced a good baseline. The format could only be looked in two directions and proved to be the worst performing.

### The AI

A consistent problem with training the model was overfitting. This is when the model learns the training data so well that it performs poorly when tested on other data. A technique to overcome this is to limit the number of hidden layers as well as nodes within each layer. This gives the model less space to overfit but can also limit its ability to identify important characteristics.

Another method that proved useful was to input the data in three dimensions and allow for convolution operations. A way for the model to break up the data into local patterns that can be remembered during training and used for testing

### Results

Data Format	Sample Screening	Prediction Accuracy		
		Virus prediction	Contamination	10 most common lineages
Single 2D Histogram	55%	50%	55%	30%
Image	82%	48%	61%	73%
Sliced Histogram	77%	50%	65%	67%
Eight Principal Components	88%	52%	62%	70%

# Bigelow's REU Program 2021

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

Orellana María José Orellana Rosales<sup>1,2</sup>, José A. Fernández Robledo<sup>1</sup>

<sup>1</sup>Bigelow Laboratory for Ocean Sciences, 60 Bigelow Dr., East Boothbay ME. <sup>2</sup>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.

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

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

- The SARS-CoV-2 virus, first identified in Wuhan, caused a major health crisis worldwide.
- As of 2021, A million people have died.
- *Perkinsus marinus* causes the “Dermo” disease in oysters and unlike other species, it can grow independently from its hosts in defined culture media.
- 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.
- Previous research on mice showed that *P. marinus* ingestion elicits an immune response without causing illness.
- The ability of *P. marinus* to express genes of medical interest provides an alternative oral vaccine development venue.

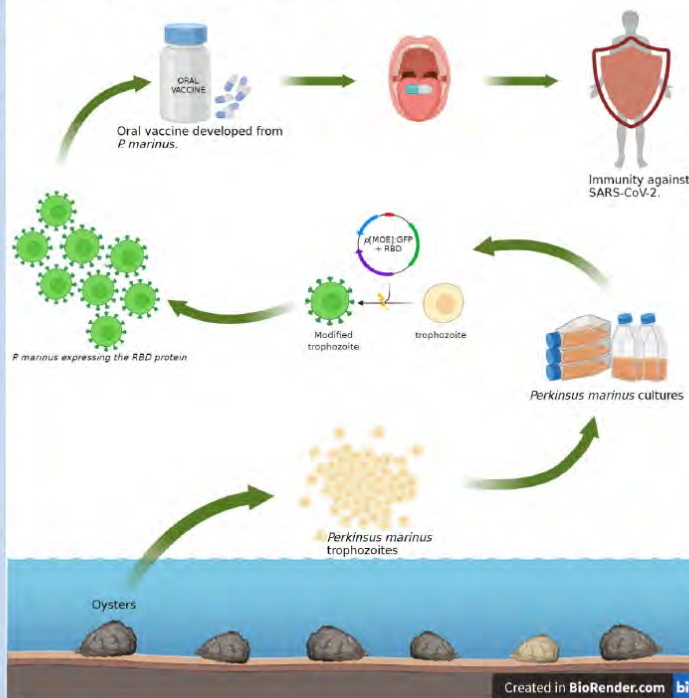
**Acknowledgements.** NSF Grant 1460861 (REU Site: Bigelow Laboratory for Ocean Sciences – Undergraduate Research Experience in the Gulf of Maine and the World Ocean) and NSF 17901480 ; BLOS Staff & REU 2021 Interns

Bigelow Laboratory for Ocean Sciences

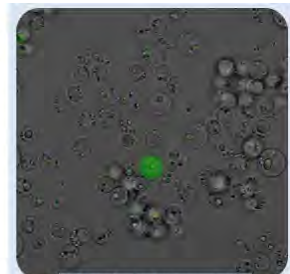
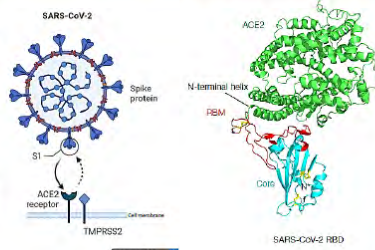


Southern Maine Community College

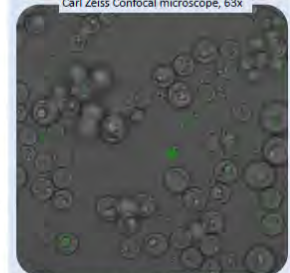
## The marine protozoan *Perkinsus marinus* can express the SARS-CoV-2 RBD Spike gene.



- The SARS-CoV-2 virus spike S1 subunit (Receptor Binding Domain) interacts with the ACE2 receptor mediating the entrance and fusion of the virus to the cell membrane.
- The RBD is a key component of the S1 subunit responsible for binding of the virus to our ACE2 receptors.



Carl Zeiss Confocal microscope, 63x



- We use 25 million *Perkinsus marinus* cells and transfected with Addgene plasmid #149329 using electroporation.
- Trophozoites maintain the expression after 3 weeks post-transfection and after cell division.
- Fluorescence was seen in *P. marinus* vacuoplast, which is thought to be a storage structure within the vacuole.

#### Future directions

- To confirm the expression of the gene, an immunofluorescence assay using human antibodies against the spike gene to test against *P. marinus* cells.



For references and more information scan the QR Code

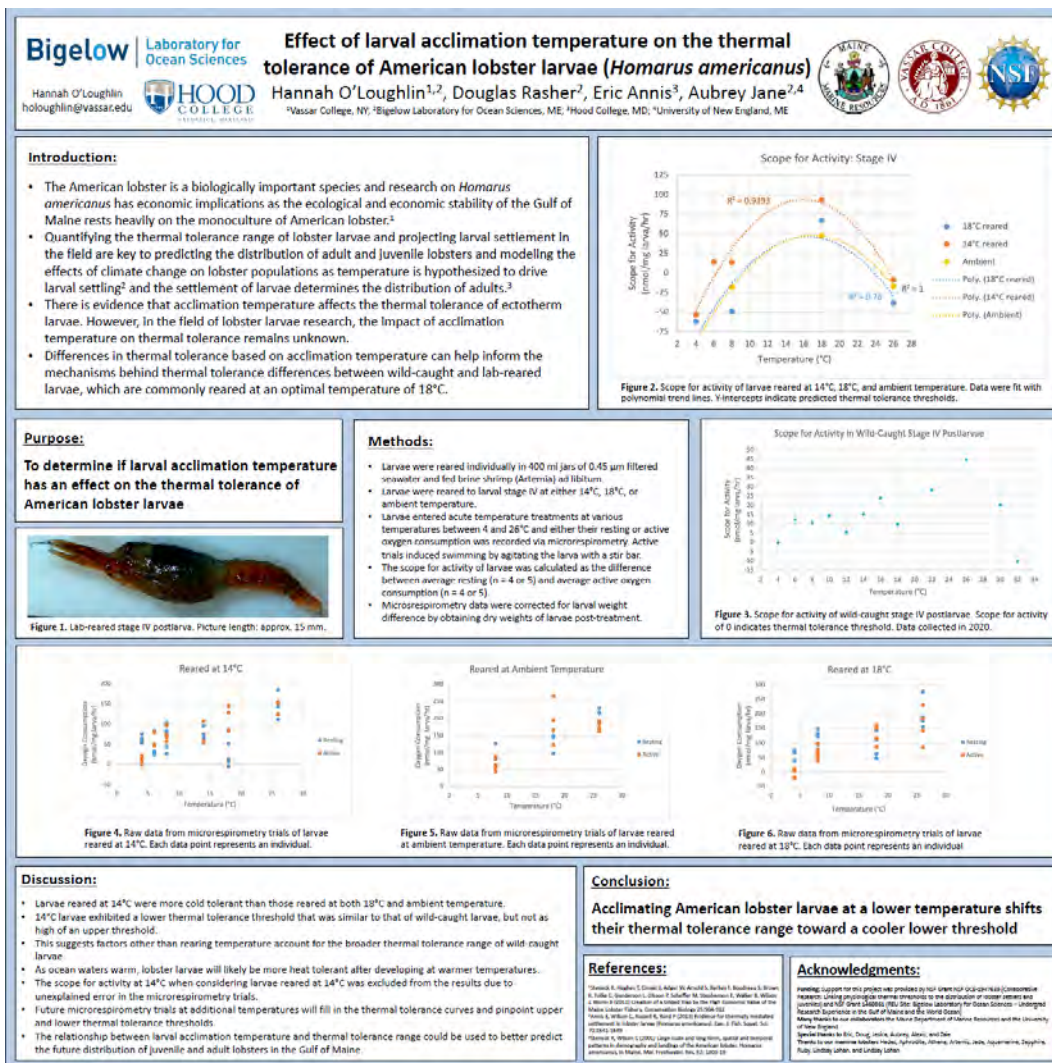
# Bigelow's REU Program 2021

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

Hannah O'Loughlin<sup>1,2</sup>, Doug Rasher<sup>2</sup>, Eric Annis<sup>3</sup>, Aubrey Jane<sup>2,4</sup>

Vassar College<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>, Hood College<sup>3</sup>, University of New England<sup>4</sup>

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.



# Bigelow's REU Program 2021

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

Molly Spencer<sup>1</sup>, Rachel Lasley Rasher<sup>1</sup>, Evie Layland<sup>2</sup>, Maura Niemisto<sup>3</sup>, David Fields<sup>3</sup>

University of Southern Maine, Portland, ME<sup>1</sup>, University of Maine, Orono<sup>2</sup>, Bigelow Laboratories for Ocean Sciences<sup>3</sup>

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.

### Ingestion rate of early-stage larval lobster, *Homarus americanus*, on dominant copepod species in the Gulf of Maine

Molly Spencer<sup>1</sup>, Rachel Lasley-Rasher, Ph.D.<sup>1</sup>, Evie Layland<sup>2</sup>, Maura Niemisto<sup>3</sup>, David Fields, Ph.D.<sup>3</sup>  
 University of Southern Maine<sup>1</sup>, University of Maine, Orono<sup>2</sup>, Bigelow Laboratory<sup>3</sup>



#### Introduction

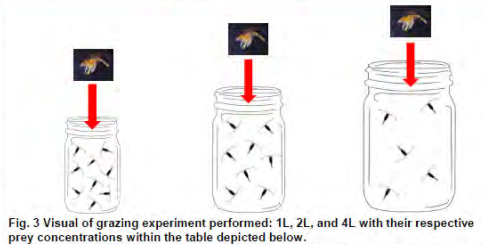
The American lobster, *Homarus americanus*, is an iconic species that serves as a backbone to Maine's marine economy. In 2020 lobster landings grossed over \$400 million in value. While much is known about the ecology of adult lobster, relatively little is known about the larvae. Research shows that climate change is altering the density and species composition of zooplankton populations in the North Atlantic. Furthermore, data from the Gulf of Maine suggests recent declines in lobster recruitment despite high spawning activity that may be attributable to the survivability of early larval lobster stages. In this study we investigated the feeding rates of early larval stages in various concentrations of wild zooplankton known to coexist with lobster. Experiments were designed to determine the maximum ingestion rates of early-stage lobster and to assess their potential for food limitation within the natural environment. This study targeted a small, abundant prey item coexisting in the Gulf with lobster larvae, *Acartia spp.*, as well as larger, but less abundant prey item, *Calanus spp.*, to gain insight on the feeding abilities of early larval stages.



Fig. 1 Bigelow lab's pier off the Damariscotta River (site labeled by rectangle) 43°51'37.9"N 69°34'41.5"W  
 Fig. 2 Zooplankton net tow taken off the dock

#### Materials and methods

- Vertical net tows collected off Bigelow laboratory's pier on the Damariscotta river (Fig. 1), located in East Boothbay, Maine, for the retrieval of copepod prey.
- Prey items, *Acartia* and *Calanus*, were sorted from tows into their respective prey concentration chambers prior to grazing experiment.
- Lobster larvae were reared at Bigelow Laboratory and were fed a diet consisting primarily of cultured *Artemia* and wild zooplankton prior to experiments.
- Grazing experiments were conducted in a dark, temperature-regulated water bath (16°C) for 6 hours
- Prey concentrations expressed as number of animals per liter
- We chose wild copepod species, *Acartia spp.* and *Calanus*, based on their effective escape abilities in nature to characterize early-stage lobster feeding capabilities. To contrast from these natural prey items, we have previous ingestion rate data (Fig. 7, Fig. 8) on slow-moving, lab-cultured *Artemia*, a standard prey item used in lobster rearing practices.



Concentrations (1L volume)	Concentrations (2L volume)	Concentrations (4L volume)
50/L (50 Copepods)	10/L (20 Copepods)	5/L (20 Copepods)
20/L (40 Copepods)		



Fig. 4 Prey species, *Acartia hudsonica*, used in experiment. Fig. 5 Prey species, *Calanus finmarchicus*, used in experiment. Fig. 6 Prey species, *Artemia salina*, used in (Risley, 2019)

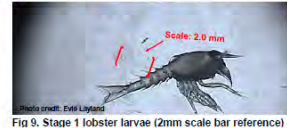
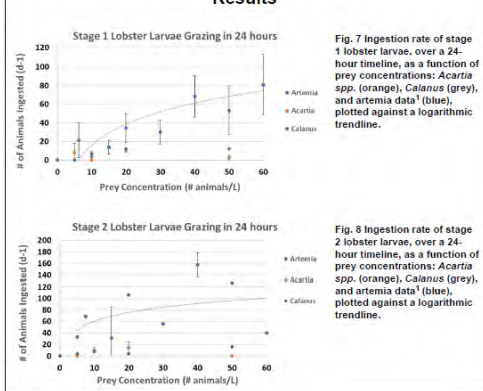


Fig. 9. Stage 1 lobster larvae (2mm scale bar reference)

#### Conclusions

- Both stage 1 and stage 2 lobster larvae displayed a much lower ingestion rate in prey concentrations of wild-caught species, *Acartia* and *Calanus*, compared to max ingestion rates measured in *Artemia* (215.82/day for stage 1 and 87.61/day for stage 2).
- Stage 1 lobster larvae are capable of ingesting at least 9 *Acartia spp.* per day while stage 2 lobster can ingest at least 14 per day.
- Both stages appear to be able to ingest more *C. finmarchicus* individuals. Stage 1 larvae ingested a maximum of 12 per day while stage 2 lobster ingested a maximum of 16 per day.
- Possible explanations for ingestion rate differences include:
  - Early-stage lobster larvae exhibit different feeding behavior based on their prey's predator evasion tactics. Wild-caught prey species, *Acartia* and *Calanus*, are known for their well-equipped escaping abilities in nature, whereas *Artemia* are a slow-moving, lab-reared organisms. Thus, this could highlight limited prey capturing abilities in early-stage lobster larvae when introduced to skilled prey in nature.
  - This distinction could also be due to unique handling rates of these prey items. Ingestion of *Artemia* would present a relatively low handling rate due to their small and compact size, whereas wild-zooplankton introduced to larvae were larger and more robust prey items to handle.
  - Distinction between *Artemia* and wild zooplankton ingestion rate could also be indicative of the nutritional value each prey items holds; where *Artemia* are known for their nutritive deficiency, wild zooplankton are nutritionally saturated and thus larvae could be satiated on fewer prey items.
- While this study was not able to find the maximum ingestion rate in this study's choice of wild prey species, our data holds implications for prey availability in the natural environment and what is accessible for early developmental stages of *H. americanus*.

#### Literature Cited

(1) Risley, S. (2019). Feeding rates of *Homarus americanus* (American lobster) larvae at different food concentrations. Poster presented at ASLO 2019 Aquatic Sciences Meeting, 2019, Feb. 24<sup>th</sup>-Mar. 1<sup>st</sup>; San Juan, PR.

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# Bigelow's REU Program 2021

## INGESTION RATES OF MARINE CLADOCERANS


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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.



## Ingestion Rates of Marine Cladocerans




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**Background**

- Marine Cladocerans
  - Over 600 species belong to the superorder Cladocera only 8 of which are marine species
  - While freshwater cladocerans are often used for research, marine species have yet to be successfully reared in the lab
  - Their reproductive properties make them model organisms for toxicology, genetic, and evolutionary studies, if they can be successfully reared
- Reproduction
  - Podon sp.* can reproduce asexually (parthenogenically) or sexually when environmental conditions are favorable or unfavorable, respectively
  - Asexual reproduction produces genetic clones
  - Sexual reproduction produces a single resting egg, that can remain viable in sediment for centuries



**Research Questions**

What do marine cladocerans eat?  
Which size/type of prey is preferred to sustain marine cladocerans?  
How does prey size/type correlate with size/egg production?

**Methods**

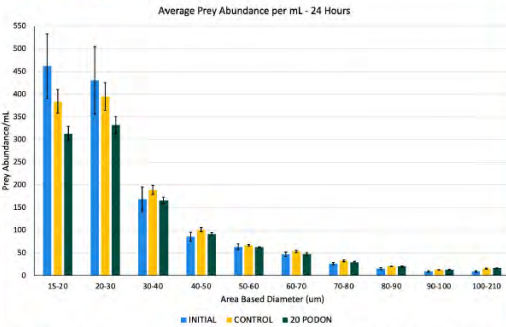
- A concentrated sample of plankton 15-210um in size was collected from the Damariscotta River Estuary
- The concentrated plankton was distributed into 20mL Vials at 16°C
  - Initial concentrations were preserved using 10% ethanol
  - Control concentrations were preserved after a period of 24 hours
  - Samples including 20 individual *Podon sp.* were preserved after 24 hours

**References**

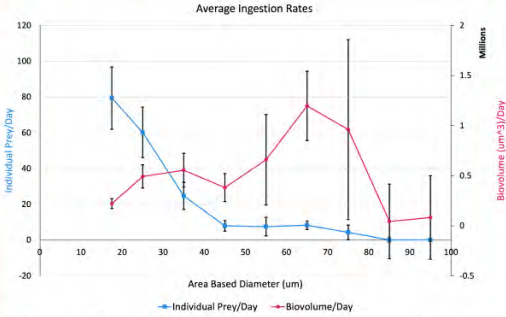
Egloff, David A. et al. "Reproductive Biology of Marine Cladocerans." *Advances in Marine Biology* 31 (1997): 79-167  
 Frost, BW. "Effects of Size and Concentration of Food Particles on the Feeding Behavior of the Marine Planktonic Copepod *Calanus Pacificus*." *Limnology and Oceanography* 17 (1972): 6.

**Results**

- Data analyzed from the imaging flow cytometer show increased prey ingestion near the smaller prey size ranges, and increased volume ingestion near the larger prey size ranges




**Figure 3.** The average prey abundance measured by a FlowCam<sup>®</sup> as a function of the area-based diameter (ABD). The initial abundances are compared against the controls and samples containing 20 *Podon sp.* after a period of 24 hours. Error bars represent standard deviation.




**Figure 4.** The ingestion rate of prey as a function of average area-based diameter (ABD) and biovolume by individual *Podon sp.* over a period of 24 hours. Error bars represent standard deviation. Ingestion rates calculated using the Frost equations<sup>2</sup>.

**Sample Collection**



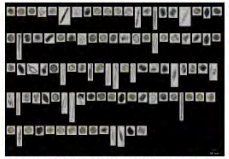
**Data Analysis**

- Preserved samples were processed using an imaging flow cytometer (FlowCam<sup>®</sup>) to get images and size distributions of the prey in each sample



**Results (cont.)**

- Images from the FlowCam show high abundances of *Thalassiosira sp.*, *Gyrosigma sp.*, and bivalve larvae collected in the prey samples



**Conclusion**

- Ingestion Rates**
  - Podon sp.* appear to be ingesting prey less than 50 micrometers at a higher rate than any other prey ABD
  - Little to no ingestion takes place on prey greater than 100 micrometers ABD
- Biovolume Consumption**
  - Most of the biovolume consumed by *Podon sp.* occurs on prey with ABD between 60 and 70 micrometers

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