

# Bigelow | Laboratory for Ocean Sciences

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

**REU Symposium Program & Abstracts  
Thursday, August 2, 2018**



# Oral Program

8:50 Opening Comments

9:00 Halley Steinmetz - University of Massachusetts Amherst, Amherst MA

**Using multispectral versus hyperspectral radiometry to predict Forel-Ule ocean color:  
Converting radiometry to colorimetry and implications for ocean color analysis**

Steinmetz HJ<sup>1</sup>, Mitchell C<sup>2</sup>, Balch WM<sup>2</sup>, University of Massachusetts Amherst<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>

9:15 Garret Genco - Colby College, Waterville, ME

**Geomicrobiology of Iron: Composition of Biogenic Iron (III) Mineral Stalks**

Genco GW<sup>1</sup>, Koeksoy E<sup>2</sup>, Emerson D<sup>2</sup>

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

9:30 Patricia Montalvo - Universidad de Puerto Rico, Mayagüez, PR

**Microbes eating rocks at the bottom of the ocean: Determining chemical preferences of basalt-attached microbial communities.**

Montalvo-Rodríguez PS<sup>1</sup>, Jones RM<sup>2</sup> and Orcutt BN<sup>2</sup>

Universidad de Puerto Rico<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>

9:45 Walter Dawydiak - University of Pennsylvania, Philadelphia, PA

**A Prey Saturation Approach as an Improved Method for Estimating Microzooplankton Grazing Rates**

Dawydiak W<sup>1</sup>, Lubelczyk L<sup>2</sup>, Archer SD<sup>2</sup>, Posman K<sup>2</sup>, Poulton NJ<sup>2</sup>

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

10:00 Emily Haggett - Southern Maine Community College South Portland, ME

**Seasonal Variation in dsDNA Eukaryotic Viruses in Antarctic Ace Lake**

Haggett, E. <sup>1,2</sup>, Cretoiu, S. <sup>2</sup>, Bhattacharjee, A. <sup>2</sup>, Martínez Martínez, J. <sup>2</sup>

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

10:15 Henry Arndt - Front Range Community College, Fort Collins CO

**Enhancing the Understanding of Picoplankton's Role in the Biogeochemical Carbon Pump**

Arndt HT<sup>1</sup>, Lomas MW<sup>2</sup>, Front Range Community College<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>

**10:30-10:45 Break (15 minutes) \*\*\*\*\***

10:45 Sydney Greenlee - Colby College, Waterville, ME

**DMSP metabolism and Antarctic microbial community structure**

Greenlee SM<sup>1</sup>, Countway PD<sup>2</sup>

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

11:00 Josh Brycki - Juniata College, Huntingdon PA

**Haloperoxidase-mediated gas exchange in two diatom species**

Brycki JD<sup>1,2</sup>, Posman KM<sup>1</sup>, Archer SD<sup>1</sup>

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

11:15 Adelaida Arjona - Harvard University, Cambridge MA

**The Role of Sea Ice Extent on Chlorophyll Patterns in the Greenland Sea**

Arjona AV<sup>1</sup>, Mayot N <sup>2</sup>, Matrai P <sup>2</sup>, Harvard University <sup>1</sup>, Bigelow Laboratory for Ocean Sciences <sup>2</sup>

11:30 Emily McDermith - University of Rhode Island, Kingston, Rhode Island

**Unsuspecting players in the dissolved organic phosphorus pool: phosphonates and eukaryotic phytoplankton**

McDermith EJ<sup>1</sup>, Whitney LP<sup>1</sup>, Lomas MW<sup>1</sup>

Bigelow Laboratory for Ocean Sciences<sup>1</sup>, University of Rhode Island<sup>2</sup>

11:45 Phoebe Keyes - Hamilton College, Clinton, New York

**An initial exploration into the biodegradation of photo-oxidized oil in the marine environment**

Keyes, PK<sup>1,2</sup>, Aeppli, C<sup>2</sup>

<sup>1</sup>Chemistry Department, Hamilton College, NY <sup>2</sup>Bigelow Laboratory for Ocean Sciences, ME

12:00 Tom Regan - Bowdoin College, Brunswick, ME

**How does oil photo-oxidation influence the toxicity of oil? Predicting the toxicity of oil photo-products**

Regan TR<sup>1</sup>, Aeppli C<sup>2</sup>, Bowdoin College<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>

**12:15 Break (1:00 hr) \*\*\*\*\***

1:15 Isabella Grasso - Southern Maine Community College, South Portland, ME

**Shellfish Toxicity Forecast in the Gulf of Maine using Neural Networks**

Grasso IF<sup>1,2</sup>, Archer SD<sup>2</sup>, Burnell C<sup>2</sup>, Tupper B<sup>2</sup>, Record NR<sup>2</sup> Southern Maine Community College<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>

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

1:30 Adrian Contreras - Palomar Community College, San Marcos, CA

**Effects of Ocean Acidification and High Temperatures on Metabolic Rates of Lobster Larvae**

Contreras AJ<sup>1,2</sup>, Neimisto M<sup>1,3</sup>, Wahle R<sup>3</sup>, Fields DM<sup>1</sup>

Bigelow Laboratory for Ocean Sciences, East Boothbay, ME<sup>1</sup>, Palomar Community College<sup>2</sup> U-Maine<sup>3</sup>

1:45 Courtney Stuart - Stony Brook University, Stony Brook, NY

**Kelp—A Comeback Story: A geospatial analysis of Maine's kelp forests over the past quarter century**

Stuart CE<sup>1,2</sup>, , Suskiewicz TS<sup>2</sup>, Rasher DB<sup>2</sup>

Stony Brook University School of Marine and Atmospheric Sciences (SoMAS)<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>

2:00 Sabrina Groves - Mount Holyoke College, South Hadley MA

**Physiological Impacts of Cultivating Mussels on a Kelp Farm A Solution to Ocean Acidification?**

Groves SL<sup>1,2</sup>, Honisch B<sup>1</sup>, Price NN<sup>1</sup>

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

2:15 Erika Alvarado - University of Idaho, Moscow ID

**Total and Inorganic Arsenic in Kelp Marketed for Human Consumption Using HPLC-ICP-MS Techniques**

Alvarado EA<sup>1,2</sup>, Rauschenberg S<sup>1</sup>, Twining B<sup>1</sup>

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

2:30 Sahana Simonetti - Northeastern University, Boston, MA.

**Complex interactions in changing seas: an emerging relationship between a host and a marine fungal pathogen**

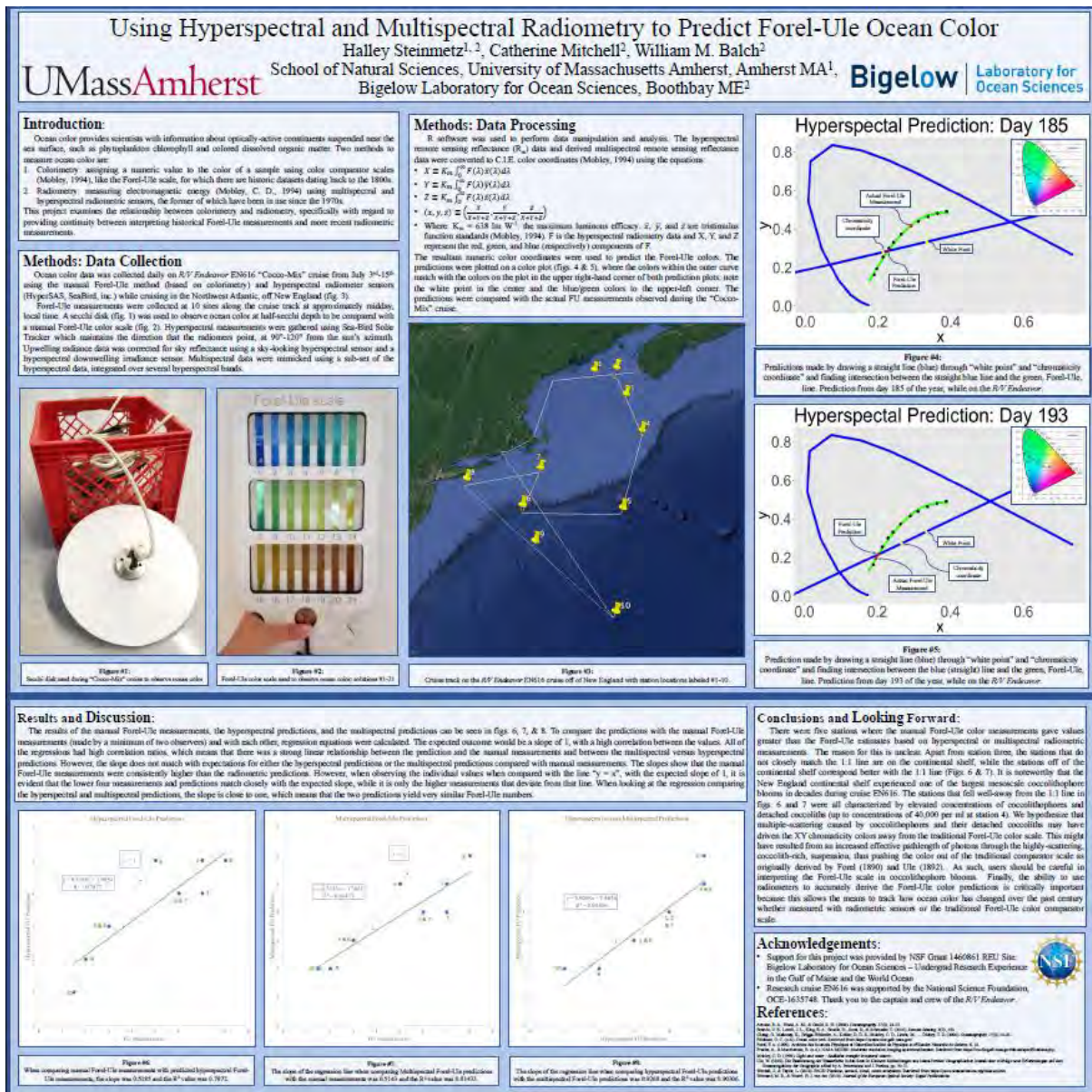
Simonetti SM<sup>1,2</sup>, Neal BP<sup>1</sup>, Honisch BL<sup>1</sup>, Price NN<sup>1</sup>

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

## **Abstracts and Posters**



Ocean color provides information about optically-active constituents suspended near the sea surface. Two methods of measuring ocean color are colorimetry (the science of assigning a numeric value to the color of a sample using color comparator scales, like the Forel-Ule (FU) scale) and radiometry (the science of measuring electromagnetic energy using hyperspectral and multispectral radiometric sensors). Colorimetry has been used since the late 1800s but radiometry has been more common since the 1970s. Here we examine the relationship between colorimetry and radiometry to provide continuity between interpreting historical FU measurements and radiometric measurements. Colorimetric and radiometric measurements were made during a 12-day research cruise aboard R/V Endeavor (EN616) in the Northwest Atlantic (July 2018) at 10 stations. The cruise coincided with one of the largest mesoscale coccolithophore blooms seen in the region in decades. We saw strong correspondence between multispectral and hyperspectral radiometric measurements. However, when comparing manual FU measurements with FU predictions based on radiometric data, five stations showed a 1:1 correspondence, while five stations within the bloom showed that manual FU measurements gave greater values than predictions based on radiometry. We hypothesize that multiple-scattering caused by coccolithophores and their detached coccoliths may have driven the XY chromaticity colors away from the traditional FU color scale as originally derived by Forel (1890) and Ule (1892). The ability to use radiometers to accurately derive the FU color predictions is critically important because this allows the means to track how ocean color has changed over the past century, whether measured with radiometric sensors or the traditional FU color comparator scale.



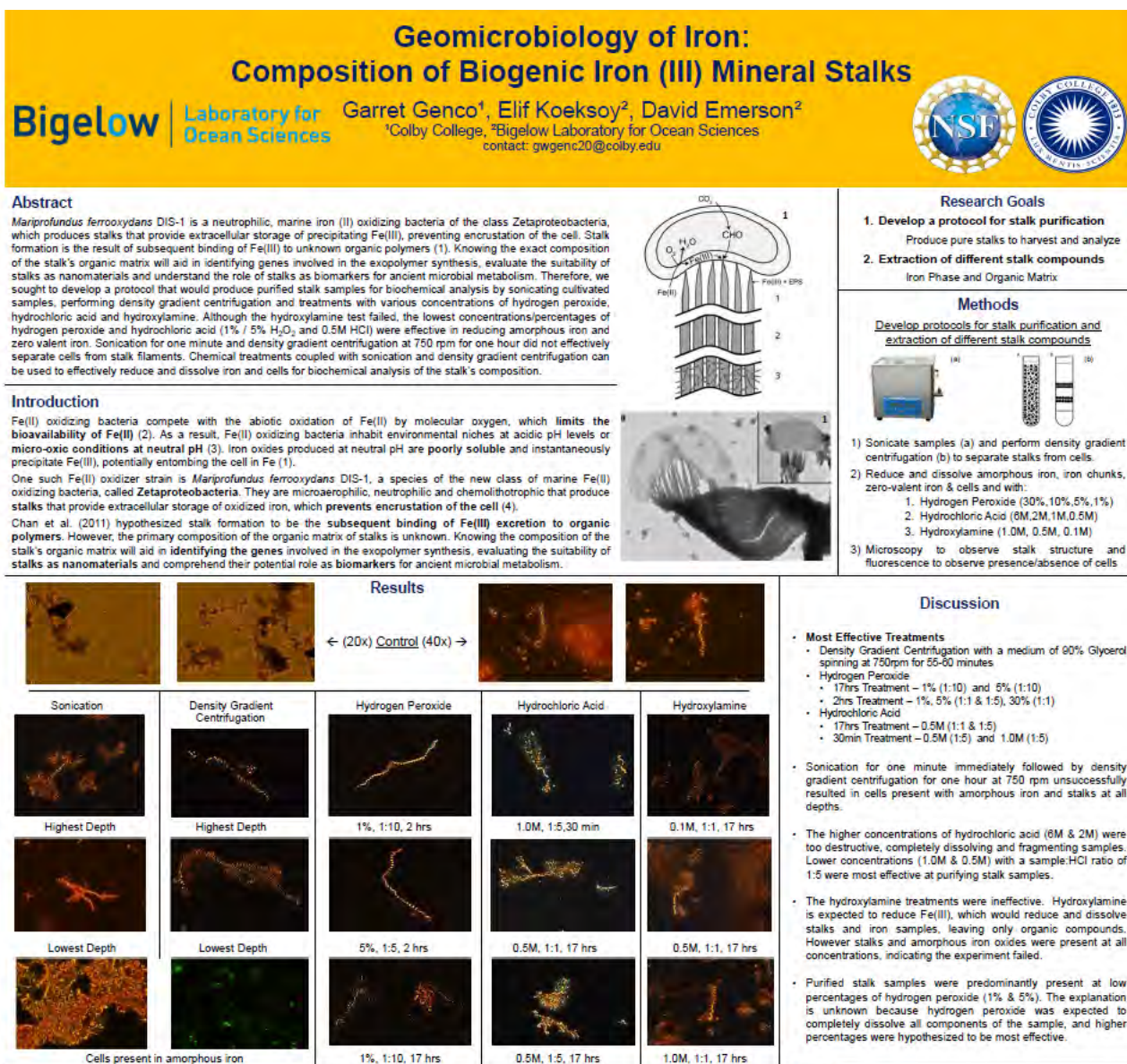


# Geomicrobiology of Iron: Composition of Biogenic Iron (III) Mineral Stalks

Genco GW<sup>1</sup>, Koeksoy E<sup>2</sup>, Emerson D<sup>2</sup>

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

*Mariprofundus ferrooxydans* is a neutrophilic, chemolithotrophic, marine iron (II) oxidizing bacteria of the class Zetaproteobacteria, which produces stalks that provide extracellular storage of instantaneously precipitating ferric iron, preventing encrustation of the cell. Stalk formation is the result of ferric iron excretion from discrete locations on the cell surface and subsequent binding of the ferric iron to organic polymers, however, the composition of the organic polymers is unknown. Knowing the exact composition of the stalk's organic matrix will aid in identifying genes involved in the exopolymer synthesis, to evaluate the suitability of stalks as nanomaterials and to understand the role of stalks as biomarkers for ancient microbial metabolism. Therefore, the experiment sought to develop a protocol that would produce purified stalk samples by treating cultivated samples through sonication, density gradient centrifugation and various concentrations of hydrogen peroxide, hydrochloric acid and hydroxylamine. Although the hydroxylamine test failed, the lowest concentrations/percentages of hydrogen peroxide and hydrochloric acid (1%/5% H<sub>2</sub>O<sub>2</sub> and 0.5M HCl) were effective in reducing amorphous iron and zero valent iron. Sonication for one minute and density gradient centrifugation at 750 rpm for one hour were did not effectively separate cells from stalk filaments. Chemical treatments coupled with sonication and density gradient centrifugation can be used to dissolve and reduce the amorphous iron and cells for biochemical analysis of the stalk's composition.



### Acknowledgements

Support for this project was provided by NSF Grant 1450881 REU Site: Bigelow Laboratory for Ocean Sciences – Undergrad Research Experience in the Gulf of Maine and the World Ocean. Special thanks to Dr. David Emerson's lab for permitting me access to conduct my research and for advising me throughout my research process. Also a special thanks to all REU interns, Bigelow staff and Colby College.

### Literature Cited

(1) Chan CS, Fakra SC, Emerson D, Fleming EJ, Edwards KJ (2011) Lithophilic iron-oxidizing bacteria produce organic stalks to control mineral growth: implications for biomineralization. *ISME J* 5:717-727. (2) Melton ED, Swanner ED, Beverss S, Schmidt C, Kasper A (2014) The interplay of microbially mediated and abiotic reactions in the biogeochemical Fe cycle. *Nat Rev Microbiol* 12:797-808. (3) Kasper A, Emerson D, Granick JA, Roden EE, Muehe EM (2016) Chapter 17 Geomicrobiology of Iron 343-399. (4) Chan CS, Fakra SC, Edwards DC, Emerson D, Benfield JF (2009) Iron polyhydroxide mineralization on microbial extracellular polysaccharides. *Geochim Cosmochim Acta* 73:3807-3819.



## Microbes eating rocks at the bottom of the ocean: Determining chemical preferences of basalt-attached microbial communities.

Montalvo-Rodríguez PS<sup>1</sup>, Jones RM<sup>2</sup> and Orcutt BN<sup>2</sup>

Universidad de Puerto Rico<sup>1</sup>, Bigelow Laboratory for Ocean Sciences<sup>2</sup>

Most of the oceanic seafloor biosphere remains a mystery to science. Microbial life is a key component of biogeochemical cycles at the bottom of the ocean. Understanding more about the microbes that make up subsea layers and their uptake of energy can serve as a baseline to study geochemical processes and environment stability at extreme depths. Microbes are able to use rocks and minerals as substrates that they can convert to energy sources through redox reactions. Although we have an idea of how microbes are able to obtain energy sources, their preferences and the effect of different compounds on these microbes is yet to be described. In order to learn more about metabolic preferences of microbes in the seafloor, we added rocks incubated in seafloor crustal fluid to different microcosms that contained excess of an organic or inorganic substrate. In comparison to a no-substrate control, cell counts taken from the microcosm fluid revealed the efficiency of organic substrates, like glucose and pyruvate, in facilitating microbial growth, whereas iron did not generate microbial growth. Oxygen and ferrozine levels measured in the beginning and end of the experiment indicated that non-valent iron underwent a rapid change into abiotic iron which reduced the amount of respiration. This was mediated by subjecting iron to microaerophilic conditions, which resulted in a slower consumption of abiotic iron. DNA extraction revealed the presence of cell growth in rocks and fluid, which factored in the cells that weren't accounted for in the cell counts. Overall biomass change indicated that there was a preference toward organic substrates and hydrogen gas, rather than inorganic substrates.

Bigelow Laboratory for Ocean Sciences

## Microbes eating rocks at the bottom of the ocean: Determining chemical preferences of basalt-attached microbial communities

Patricia Montalvo-Rodríguez<sup>1</sup>, Rose Jones<sup>2</sup> and Beth Orcutt<sup>2</sup>

Universidad de Puerto Rico, Mayagüez, PR<sup>1</sup>; Bigelow Laboratory for Ocean Sciences, East Boothbay, ME<sup>2</sup>



### Abstract

Most of the oceanic seafloor biosphere remains a mystery to science. Microbial life is a key component of biogeochemical cycles at the bottom of the ocean. Understanding more about the microbes that make up subsea layers and their uptake of energy can serve as a baseline to study geochemical processes and environment stability at extreme depths. Microbes are able to use rocks and minerals as substrates that they can convert to energy sources through redox reactions. Although we have an idea of how microbes are able to obtain energy sources, their preferences and the effect of different compounds on these microbes is yet to be described. In order to learn more about metabolic preferences of microbes in the seafloor, we added rocks incubated in seafloor crustal fluid to different microcosms that contained excess of an organic or inorganic substrate. In comparison to a no-substrate control, cell counts taken from the microcosm fluid revealed the efficiency of organic substrates, like glucose and pyruvate, in facilitating microbial growth, whereas iron did not generate microbial growth. Oxygen levels measured in the beginning and end of the experiment indicated that non-valent iron underwent a rapid change into abiotic iron which possibly reduced the amount of respiration. This was mediated by subjecting iron to microaerophilic conditions, which resulted in a slower consumption of abiotic iron. DNA extraction revealed the presence of cell growth in rocks and fluid, which factored in the cells that weren't accounted for in the cell counts. Overall biomass change indicated that there was a preference toward organic substrates and hydrogen gas, rather than other inorganic substrates.

### Sampling Site

North Pond is a young sediment pond found in the western flank of the Mid-Atlantic Ridge. With its highly oxygenated water and low temperatures, North Pond serves as a designated drilling site and a perfect substrate for the placement of CORKS.

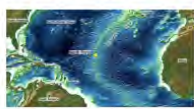


Figure 1: Location of North Pond at the Mid-Atlantic Ridge. Credit: IODP

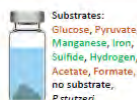
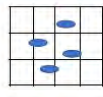


Figure 2: CORK at the seafloor. Credit: Modified from K. Edwards et al. (2012) Proc. IODP

### Methods

Microcosms were set up in 10 mL vials. The vials were inoculated with North Pond rock crush. North pond sterile water and an organic or inorganic substrate were also added. Microcosms were incubated at 48°C. A no substrate control and a positive control with *P. stutzeri* were used.

Cell counts were taken from the fluid of every vial to determine cell growth.



DNA was extracted from rocks in each vial which complements cell count data taken from the fluid.

Oxygen levels were measured to determine respiration levels. Ferrozine levels were measured in order to determine uptake of iron.

**Objective:** To explore different inorganic and organic substrates in the presence of oxygen and their individual effect on deep subsea microbial life based on changes in biomass, oxygen, pH, DNA and ferrozine levels.

- Microbes depend on oxygenated, cold crustal fluids that move around the rocks at the basalt crust.
- Microbes use available compounds as electron donors that can be turned into useful energy sources.

### Results



Figure 3: Changes in cell density over 24 days for various treatment types.

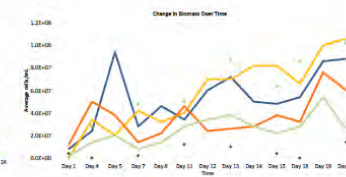


Figure 4: Changes in cell density over 20 days for various treatment types.

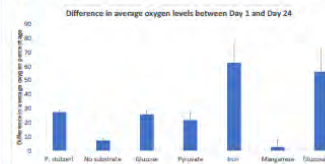


Figure 5: Changes in oxygen levels per treatment between Day 1 and Day 24

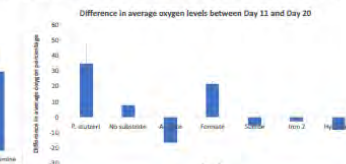


Figure 5: Changes in oxygen levels per treatment between Day 11 and Day 20

Samples	DNA in Fluid (ng/mL)	DNA in Rocks (ng/cm <sup>2</sup> )
<i>P. stutzeri</i>	38.67	51
No substrate	0.028	39.8
Glucose	Too low	39.6
Pyruvate	Too low	179
Glucosamine	0.295	45.4
Manganese	0.567	115
Iron	0.18	204
Iron (Microaerophilic)	0.142	25.8
Iron	Too low	54.8
Iron	Too low	123
Iron	Too low	129

Table 1: DNA concentrations from rocks and fluid in microcosms with different substrates

Samples	DNA in Fluid (ng/mL)	DNA in Rocks (ng/cm <sup>2</sup> )
<i>P. stutzeri</i>	TBD	785
No substrate	TBD	135
Acetate	TBD	189
Formate	TBD	132
Sulfide	TBD	136
Hydrogen	TBD	152
Iron (Microaerophilic)	TBD	174
Iron	TBD	149
Iron	TBD	184
Iron	TBD	147
Iron	TBD	146
Iron	TBD	92
Iron	TBD	89.2

Table 2: DNA concentrations from rocks and fluid in microcosms with different substrates

### Conclusions

- In concert with our hypothesis, the most complex organic compounds stimulated higher microbial growth in the microcosm fluid. (Figure 3)
- Hydrogen was the inorganic substrate that supported most continuous microbial growth. (Figure 4)
- DNA data indicates the presence of cell growth in rocks. (Table 1, Table 2)
- Changes in oxygen were most significant in samples with iron or glucosamine substrates. This could represent high respiration rates for glucosamine and drastic abiotic changes in iron. (Figure 5)
- Future directions include exploring the energetics of these reactions and performing DNA fingerprinting on the samples.

### Acknowledgements

Support for this project was provided by NSF grants #0939564 Center for Dark Energy Biosphere Investigations (C-DEBI), #1460861 REU Site Bigelow Laboratory of Ocean Sciences - Undergraduate Research Experience in the Gulf of Maine and the World Ocean, and #1536539 Collaborative Research: Completing North Pond Borehole Experiments to Elucidate the Hydrology of Young, Slow-Spread Crust. A special thank you to the members of Orcutt lab for support and guidance throughout this project.

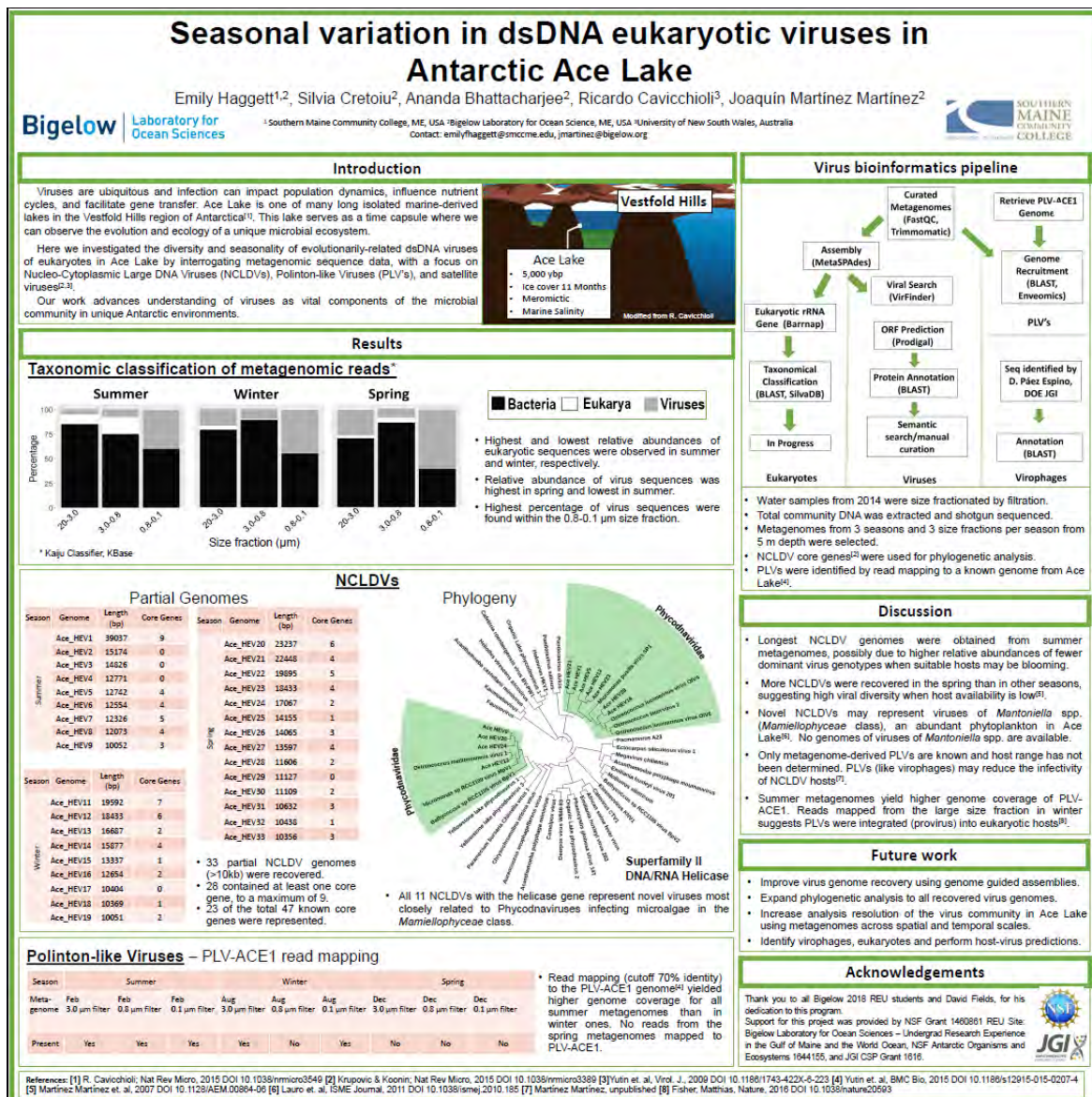






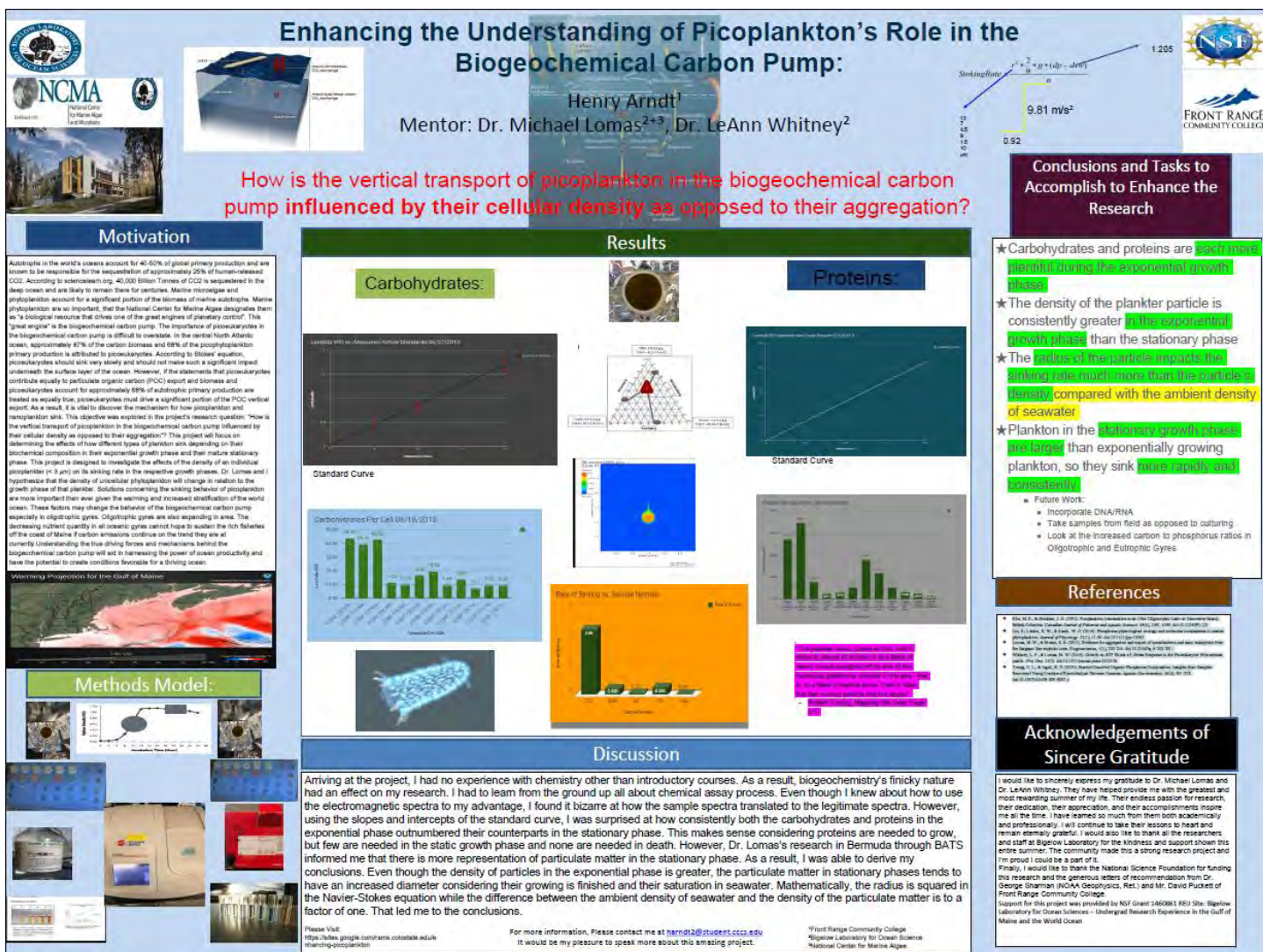


Viruses are ubiquitous, and infection can impact population dynamics, influence nutrient cycles, and facilitate gene transfer. Ace Lake is one of many long isolated marine-derived lakes in the Vestfold Hills region of Antarctica that serves as a time capsule for investigating the evolution and ecology of a unique microbial ecosystem. Here we studied the diversity and seasonality of evolutionarily-related dsDNA viruses of eukaryotes in Ace Lake by interrogating metagenomic sequence data from size fractionated water samples. We focused on Nucleo-Cytoplasmic Large DNA Viruses (NCLDV), Polinton-like viruses (PLVs), and virophages. The data revealed seasonal variation in the virus community, suggesting a pattern of high diversity and low abundance when eukaryotes are in lower abundance, and lower diversity but higher abundances of specific viruses when hosts are present in higher numbers. We recovered 33 partial NCLDV genomes. Phylogenetic analysis of the 11 NCLDVs containing the Superfamily II DNA/RNA Helicase core gene revealed novel viruses closely related to Phycodnaviruses of phytoplankton in the *Mamiellophyceae* class. The *Mantoniella* genus in this class is abundant in Ace Lake and is likely a host to those NCLDVs. Mapping to the reference PLV-ACE1 genome showed diverse PLVs with higher genome coverage for summer metagenomes. In winter metagenomes the higher mapping from the large size fraction reads suggests PLVs might be found as proviruses in eukaryotes during low host abundance periods. Only metagenome-derived PLVs are known and host range has not been determined. PLVs similarities to virophages make it possible to suggest PLVs may also depend on co-infection with NCLDVs for replication in eukaryotic hosts. This study lays the foundation for further investigation on the diversity and impacts of viruses on microbial ecology and evolution across temporal and spatial scales within Ace Lake as well as across other marine derived Antarctic Lakes.





Autotrophs in the world's oceans account for 40-50% of global primary production and are known to be responsible for the sequestration of approximately 25% of human-released CO<sub>2</sub>. Approximately 40,000 Billion Tonnes of CO<sub>2</sub> is sequestered in the deep ocean and are likely to remain there for centuries. Marine microalgae and phytoplankton account for a significant portion of the biomass of marine autotrophs. The National Center for Marine Algae designates them as "a biological resource that drives one of the great engines of planetary control". This "great engine" is the biogeochemical carbon pump. The importance of picoeukaryotes in the biogeochemical carbon pump is difficult to overstate. In the central North Atlantic ocean, approximately 87% of the carbon biomass and 68% of the primary production is attributed to picoeukaryotes. According to Stokes' equation, picoeukaryotes should not make such a significant impact underneath the surface layer of the ocean. However, evidence shows that picoeukaryotes must drive a significant portion of the POC vertical export. This project will focus on determining the effects of how different types of plankton sink depending on their biochemical composition in their exponential growth phase and their mature stationary phase. This project is designed to investigate the effects of the density of an individual picoplankter (< 3 μm) on its sinking rate and carbon sequestration potential in the respective growth phases. The warming and increased stratification of the world ocean may change the behavior of the biogeochemical carbon pump especially within oligotrophic gyres. The decreasing nutrient quantity in all oceanic gyres cannot sustain the valuable marine ecosystems that economies depend on for fisheries and tourism. Understanding the true driving mechanisms behind the biogeochemical carbon pump will aid in harnessing the power of ocean productivity and have the potential to create conditions favorable for a thriving ocean.





Seasonal algal blooms in the Southern Ocean surrounding Antarctica are dominated by dimethylsulfoniopropionate (DMSP)-producing algae. Some bacteria consume DMSP and catabolize it into dimethyl sulfide via DMSP lyases. Since little is known about how the presence of DMSP affects the abundance and diversity of DMSP consumers, this project aims to determine whether DMSP alters the structure of Antarctic microbial communities and the abundance of DMSP degradation genes. We optimized quantitative PCR (qPCR) reaction conditions for the *dddL F* and *dddL R* primer set to quantify the *dddL* gene in unknown samples. After an initial increase in *dddL* abundance, qPCR results revealed a decrease in *dddL* gene copies per milliliter over the course of the incubation experiment. Additionally, we have identified a novel Antarctic clade of the *dddL* gene which is most similar to a *dddL* clade identified from the Arctic. We investigated the identities of bacteria living in association with the DMSP-producing alga *Phaeocystis antarctica*. Bacterial sequences from this algal culture matched sequences from *Glaciecola spp.* While *Glaciecola spp.* are not DMSP consumers, they are known to be DMS consumers. This suggests the presence of an active sulfur-cycling community in co-culture. We investigated the trends in the relative abundance of a number of the most abundant bacterial OTUs from DMSP incubation experiments, noting the presence of types that participate in DMSP cycling. Our study suggests that DMS/P can influence that the relative proportions of some of the most abundant bacterial genera in Antarctic microbial communities as well as the genes that participate in DMS/P metabolism.

## DMSP metabolism and Antarctic microbial community structure

Sydney Greenlee<sup>1,2</sup>, Peter Countway<sup>2</sup>

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

## Abstract

Seasonal algal blooms at the Southern Ocean surrounding Antarctica are dominated by diethylsulphoniopropionate (DSP)-producing algae. Some bacteria consume DSP and catabolize it into dimethyl sulphide via the DMSP pathway. Since little is known about how the bacterial diversity of DSP-producing algae and the DMSP pathway varies in the Southern Ocean, we determined whether DSP alters the structure of Antarctic microbial communities and the abundance of DMSP degradation genes. We optimized quantitative PCR (qPCR) reaction conditions for the detection of the *dsrA* gene, which encodes the first step in the DMSP pathway, and used this to determine whether *dsrA* abundance varied in Antarctic microbial communities. Samples, after an initial increase in odd-chain, *n*-alkane:qPCR results revealed a decrease in odd-chain gene copies per milliliter over the course of the incubation experiment. Additionally, we have identified a novel Antarctic isolate of the *dsrA* gene which is most similar to the *dsrA* gene identified in the *Halorubrum* species. We also determined whether there was an association with the DMSP-producing alga *Phaeocystis antarctica*. Bacterial sequences from the algal culture matched sequences from *Glioclella* spp. While *Glioclella* spp. are not DMSP-producing, they are known to be important in the DMSP pathway. We also determined whether sulfury-sulfuric community in co-culture. We investigated the trends in the relative abundance of a number of the most abundant bacterial OTUs from DMSP incubation experiments, noting the presence of types that participate in DMSP cycling. Our study suggests that DMSP can alter the structure of Antarctic microbial communities as well as the genes that participate in DMSP metabolism.

## Background

- Seasonal algal blooms in the Southern Ocean surrounding Antarctica are dominated by DMSP-producing microalgae<sup>4</sup>(Fig. 1)
- There is a possible coupling of DMSP-producing phytoplankton and DMSP-degrading bacteria
- DMSP lyase pathways result in the production of the potentially climate-regulating gas dimethyl sulfide (DMS)<sup>2</sup>

## Methods

- Environmental samples were collected from the Western Antarctic Peninsula and incubated in an Ecostat experimental chamber with varying DMSP concentrations
- *dddL* endpoint PCR: first step to develop qPCR standards
- Primer set: *dddL\_F*: CTGGGAATACGGCTACGAGA  
*dddL\_R*: GTTCAACAGTACAGATCGCG (234 bp)<sup>9</sup>
- Clones were sequenced to ensure product was on target
- A qPCR standard curve for *dddL* was developed using a dilution series of cloned *dddL* to quantify gene copy number in environmental samples (Fig. 2)

## References

## Results

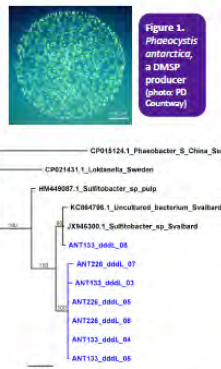


Figure 3. Phylogenetic tree of *dddL* clones and organisms known to possess *dddL* genes.

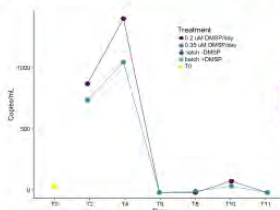


Figure 5. Copies of *dddL* per milliliter of seawater for unknown samples over the course of the incubation. Values determined using *dddL* qPCR standard curve.

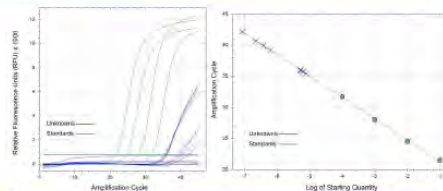


Figure 2. Amplification of *dddL* standards and unknowns (right) and *dddL* standard curve with plotted unknowns (left) to estimate gene copy numbers in seawater.

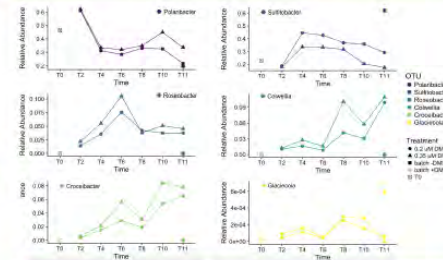


Figure 4. Mean relative abundance of the five most abundant operational taxonomic units (OTUs) and *Glaciecola* over the course of the incubation as determined by next-gen sequencing.

## Conclusions

- Novel Antarctic *dddL* clade found (Fig. 3)
- DMSP-degrading bacteria increase in relative abundance with the continuous addition of DMSP (Fig. 4)
- Optimization of Raina et al. *dddL* primer set was successful for Antarctic microbes
- For all treatments, the number of copies of *dddL* decreased over the experiment (Fig. 5)
- Cloned 16S PCR products from *Phaeocystis antarctica* matched with the DMS consumer<sup>4</sup> *Glaciecola* spp. in GenBank

**Contact:** Sydney Greenlee (Sydney.Greenlee@colby.edu)

## Acknowledgements

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REU Site: Bigelow Laboratory for Ocean Sciences – Undergraduate Research Experience in the Gulf of Maine and the World Ocean

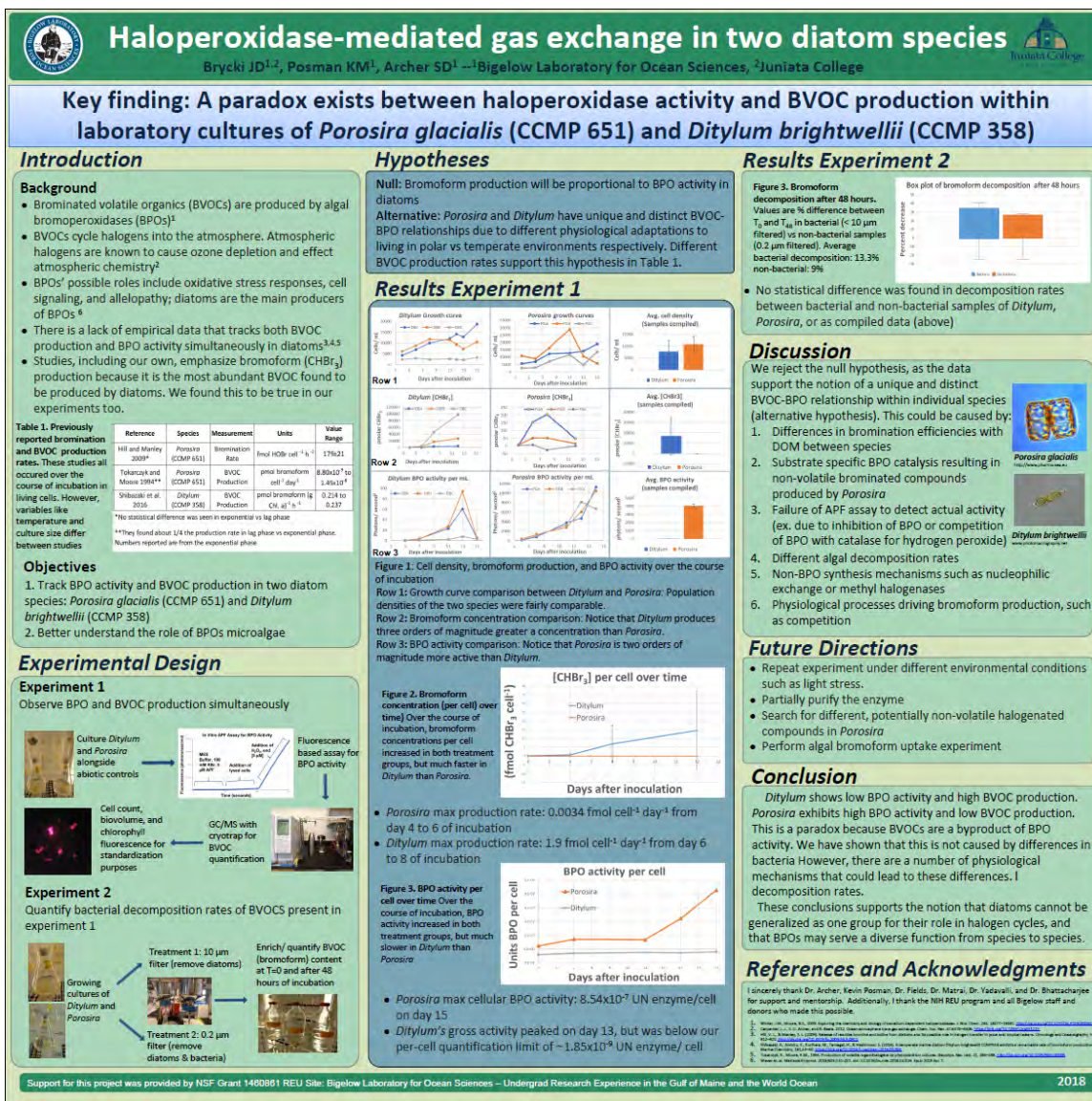


# Haloperoxidase-mediated gas exchange in two diatom species

Brycki JD<sup>1,2</sup>, Posman KM<sup>1</sup>, Archer SD<sup>1</sup>

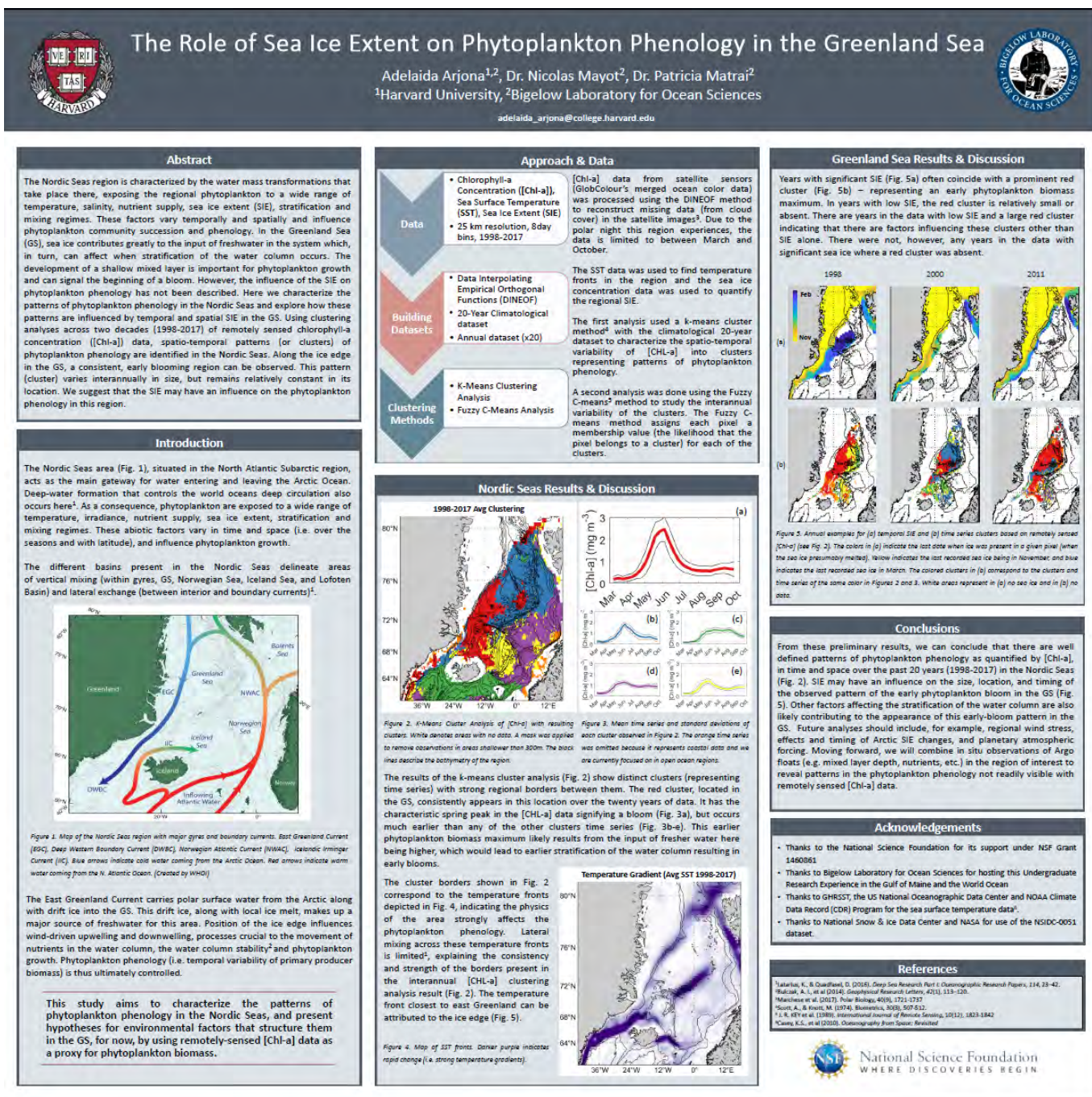
Bigelow Laboratory for Ocean Sciences<sup>1</sup>, Juniata College<sup>2</sup>

Brominated volatile organic compounds (BVOCs) are produced abundantly by marine algal enzymes called bromoperoxidases (BPOs).<sup>1</sup> Importantly, BVOCs and BPOs influence Earth's biogeochemical cycles as major sources of atmospheric halogens, which affect atmospheric chemistry including ozone depletion.<sup>2</sup> BPOs physiological role within microalgae, primarily diatoms, is thought to involve oxidative stress response, cell signaling, and allelopathy.<sup>1</sup> In diatoms, previous studies have observed BPO activity and BVOC production, but there is a lack of empirical data that tracks them simultaneously.<sup>3,4,5</sup> The objective of this experiment is to display BPO activity in relation to BVOC production in two diatom species: *Porosira glacialis* (CCMP 651) and *Ditylum brightwellii* (CCMP 358). In non-axenic cultures, BVOC production was tracked using GC/MS, and BPO activity was quantified using a novel, highly sensitive assay developed by Archer et al. (unpublished). Our results display a paradox between BPO activity and BVOC production rates. *Ditylum* shows unquantifiably low BPO activity, but abundant BVOC production ( $1.9 \text{ fmol cell}^{-1} \text{ day}^{-1}$ ). On the other hand, *Porosira* exhibits high bromoperoxidase activity ( $8.54 \times 10^{-7} \text{ UN enzyme/cell}$ , Sigma-Aldrich B2170-10UN standard) and an order of magnitude less BVOC production than *Porosira* (maximum  $0.0034 \text{ fmol cell}^{-1} \text{ day}^{-1}$ ). To understand this relationship, we enriched  $10 \mu\text{m}$  filtered samples (diatom-free) with the primary BVOC found, bromoform, to see if bacterial consumption was causing the paradox. We found no significant difference. These results indicate that BPO-BVOC relationships differ in various species of diatoms, likely a result of species physiology. This research has built on our understanding of the functional role of BPOs in microalgae. Additionally, this study demonstrates that diatoms cannot be generalized as one group for their role in halogen cycles.



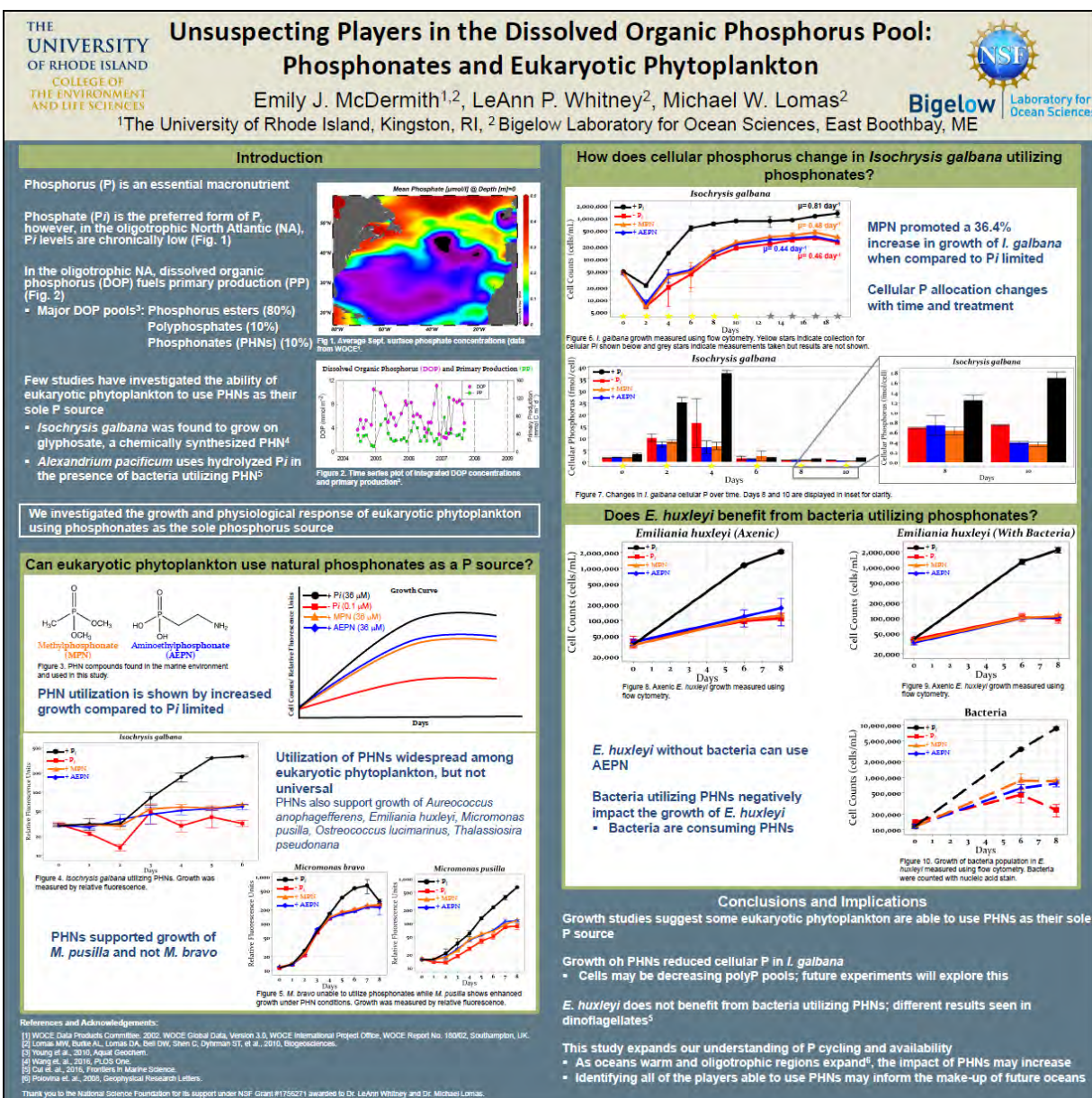


The Nordic Seas region is characterized by the water mass transformations that take place there, exposing the regional phytoplankton to a wide range of temperature, salinity, nutrient supply, sea ice extent (SIE), stratification and mixing regimes. These factors vary temporally and spatially and influence phytoplankton community succession and phenology. In the Greenland Sea (GS), sea ice contributes greatly to the input of freshwater in the system which, in turn, can affect when stratification of the water column occurs. The development of a shallow mixed layer is important for phytoplankton growth and can signal the beginning of a bloom. However, the influence of the SIE on phytoplankton phenology has not been described. Here we characterize the patterns of phytoplankton phenology in the Nordic Seas and explore how these patterns are influenced by temporal and spatial SIE in the GS. Using clustering analyses across two decades (1998-2017) of remotely sensed chlorophyll-a concentration ([Chl-a]) data, spatio-temporal patterns (or clusters) of phytoplankton phenology are identified in the Nordic Seas. Along the ice edge in the GS, a consistent, early blooming region can be observed. This pattern (cluster) varies interannually in size, but remains relatively constant in its location. We suggest that the SIE may have an influence on the phytoplankton phenology in this region.





Phosphorus (P) is an essential macronutrient for life as it is found in genetic material, cell walls and ATP. In the marine environment, phosphate (Pi) is the preferred form of P as it can be directly assimilated. In oligotrophic oceans such as the North Atlantic (NA), Pi concentrations are chronically low contributing to low primary production. Phytoplankton that reside in the oligotrophic NA utilize alternative P forms for growth, such as dissolved organic P (DOP). P esters, making up the majority of the DOP pool, are used by both prokaryotic and eukaryotic organisms. Phosphonates (PHNs) making up 10% of the DOP pool are thought to solely be utilized by prokaryotic organisms. However, this tenet is being challenged as several species of eukaryotic phytoplankton have been shown to use a chemically synthesized PHN to support growth. In this study, the growth response of eight species of eukaryotic phytoplankton supplied with two forms of PHN naturally found in the marine environment as the sole source of P was investigated. Five species were found to grow on at least one form of PHN as determined by an increase in cell abundance when compared to cells grown under Pi-limiting conditions. During exponential growth utilization of PHNs resulted in a reduction in cellular P when compared to cells grown under Pi-replete and Pi-limiting conditions. This study demonstrates that some eukaryotic phytoplankton can use PHNs to support growth, however this ability is not universal. This work will enhance our understanding of P availability and P cycling in the oceans. Furthermore, as oceans warm and oligotrophic regions expand, the importance of the DOP pool, including PHNs may be enhanced. Identifying all of the players that are able to utilize PHNs may help inform which organisms will make up our future oceans.



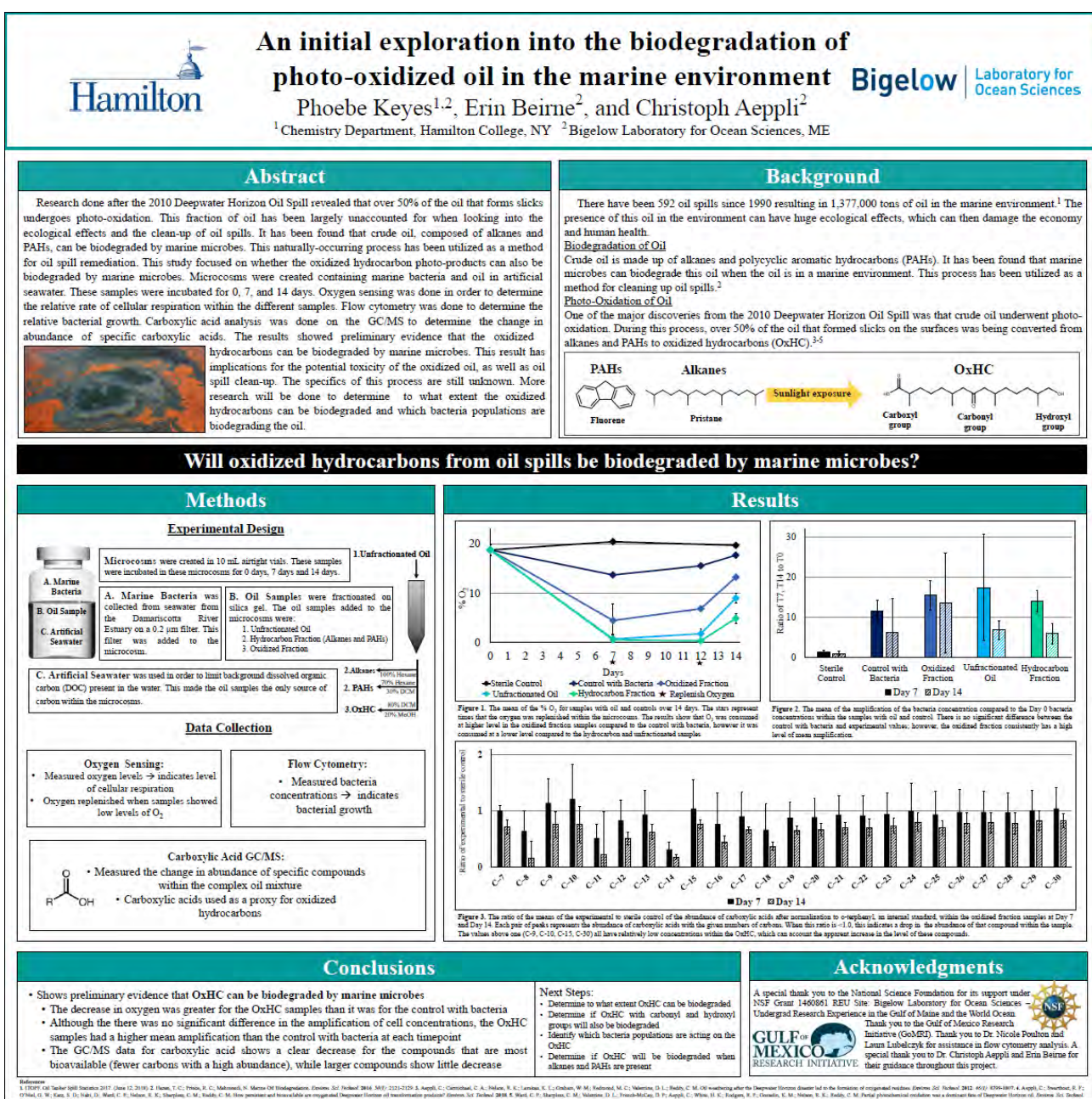


# An initial exploration into the biodegradation of photo-oxidized oil in the marine environment

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It has been found that crude oil, made up of alkanes and PAHs, can be biodegraded by marine microbes. This naturally occurring process has been utilized as a method for oil spill remediation. Research done after the 2010 Deepwater Horizon Oil Spill revealed that over 50% of the oil that forms slicks undergoes photo-oxidation. This fraction of oil has been largely unaccounted for when looking into the ecological effects and the clean-up of oil spills. This study focused on whether the oxidized hydrocarbon photo-products can also be biodegraded by marine microbes. Weather oil can be fractionated on silica gel into hydrocarbon and oxidized hydrocarbon fractions. Oil samples were taken from both the unfractionated weather oil and the fractions of this oil. Microcosms were created containing marine bacteria and an oil sample in artificial seawater. These samples were incubated for 0, 7, and 14 days. Oxygen sensing was done to determine the relative rate of cellular respiration within the different samples. Flow cytometry was done to determine the relative bacterial growth. Carboxylic acid analysis was done on the GC/MS to determine the change in abundance of specific carboxylic acids within the samples. The results showed preliminary evidence that the oxidized hydrocarbons can be biodegraded by marine microbes. This result has implications for the potential toxicity of the oxidized oil, as well as oil spill clean-up. The specifics of this process are still unknown. More research should be done into to what extent the oxidized hydrocarbons can be biodegraded and what bacteria populations are biodegrading the oil.





Eight years after the Deepwater Horizon Oil Spill of 2010, oil remains across the U.S. Gulf Coast. Because of the high prevalence and scope of oil spills, one must analyze the environmental fate of oil in coastal areas even years after the initial spill. When exposed to sunlight on the shore or ocean surface, oil oxides to form oxygenated photo-products. While the structures of these products are widely unknown, *ab initio* software (COSMOtherm and COSMOmic) and analytical methods (Microtox bioassays and solid phase microextraction (SPME)) can be used to predict the baseline toxicity (EC50) of possible photo-products in marine environments. Computational chemical calculations by COSMOmic and COSMOtherm for aliphatic oxygenated compounds and n-alkanes (up to carbon number 30), showed that there is a logarithmic increase in toxicity and logarithmic decrease in solubility with increasing carbon number. Also, COSMOmic calculations showed that oxygenated compounds with adjacent functional groups (e.g. 2-hydroxy carboxylic acids) are more toxic than compounds with functional groups bonded to opposite ends of the carbon chain (e.g. omega hydroxy acids). Computational data additionally showed that alkanes (found in unweathered oil) were more toxic yet less soluble than all of the studied aliphatic oxygenated compounds. Microtox measurements showed higher toxicity levels in all analytic compounds as compared to the toxicity levels calculated by COSMOmic (200 mM body burden for EC50 calculation). This disparity may be caused by some offset or toxic effects apart from bioaccumulation. Though, toxicity levels of analytic compounds from Microtox and COSMOmic calculations do show a similar trend. Additionally, initial SPME results suggest that toxicity levels of analytic compounds from SPME and COSMOmic calculations show a similar trend.

# How does oil photo-oxidation influence the toxicity of oil?

## Predicting the toxicity of oil photo-products

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

### Background

U.S. Coastal Waters Affected by the Gulf Oil Spill

-Oil persists in oil spill regions years after incidents e.g. Deepwater Horizon Spill of 2010 (Figure 1)

-Oil oxidizes when exposed to sunlight to create oxygenated photo-products (Appell et al., 2012)

-Oxidation occurs quickly and prevalently, with one study determined 2/3 of oil oxygenates within ten days of exposure (Ward et al., 2018)

-The environmental fate, toxicity, and structure of these oil photo-products is widely unknown

**Possible Solution and Goal:**

- Analyze possible photo-products by computational methods and compare to empirically measured baseline toxicity values (EC50)

**Measureable Toxicity Properties:**

- Water solubility
- Log<sub>10</sub> 1-octanol-water partition coefficient (K<sub>OW</sub>)
- K<sub>OW</sub> and K<sub>OW</sub> - membrane/liposome water partition coefficient (K<sub>ML</sub>)

### Results

#### QUESTION #1

COSMOtherm solubility for various aliphatic organic molecules in seawater

COSMOmic EC50 values for various aliphatic organic molecules

Figure 5 & 6 – As the size of these aliphatic compounds increases, their toxicity logarithmically increases and their solubility logarithmically decreases. Alkanes show the highest toxicity, yet their solubility is about two log units less than the least soluble oxygenated compounds.

### Research Questions

**QUESTION #1:** How does the toxicity of oil photo-products compare to that of unweathered oil?

**QUESTION #2:** How do COSMOmic calculations of toxicity compare to Microtox bioassay results of toxicity?

**QUESTION #3:** How do COSMOmic calculations of toxicity compare to solid phase microextraction results of toxicity?

### Methods

#### Ab Initio Computational Methods -

Computational methods based on quantum theory can be used to understand the fate of these photo-products

**TURBOMOLE** - optimizes the geometry of molecules (model creation), SMILES code of each molecule is converted to SDF files using Open Babel

**COSMOmic** - predicts membrane/liposome-water partitioning (K<sub>ML</sub>-w), accounts for potential aliphatic bilayer

**COSMOtherm** - predicts 1-octanol-water partitioning (K<sub>OW</sub>), water solubility

#### COSMOmic EC50 values of Alcohols, Aldehydes, Ketones, Carboxylic acids

Figure 7 - From COSMOmic calculations of the aliphatic compounds analyzed with a single oxygenated functional group, aldehydes are most toxic. Following aldehydes in descending toxicity are alcohols, ketones, and carboxylic acids. C-8 molecules are more than ten times toxic than C-10 molecules in the same organic class.

#### COSMOmic EC50 values of Oxo-acid compounds

Figure 8 – Oxo-acid derivatives are greater in toxicity when a hydroxy or oxo group are in the adjacent 2 position as compared to that in the 2 position. 1,2 diols also show greater toxicity than 1, 3 diols.

#### COSMOmic EC50 values of PAHs and αPAHs

Figure 9 - The PAHs fluorene, phenanthrene, anthracene show greater toxicity than their oxygenated derivatives. Naphthalene is more toxic than 1,4-naphthoquinone and 2-naphthol yet is less toxic than 2,3-dihydroxy-naphthalene.

#### EC50 values (baseline toxicity) are calculated from COSMOmic K<sub>OW</sub> values, with an assumed bubble burden of 200 mmol/L bioaccumulation

EC50 = (C<sub>bubble burden</sub>) / (K<sub>OW</sub>)

Figure 2 – Visual representation of dodecanoic acid partitioning when dissolved in the phospholipid bilayer DMPC and water

### QUESTION #2

#### Comparison of COSMOmic and Microtox EC50 values

Figure 10 – The toxicity, as calculated by Microtox, for each listed compound is greater than that calculated by COSMOmic, excluding error. COSMOmic and Microtox EC50 values show a similar trend.

### QUESTION #3

#### Comparison of COSMOmic and SPME Toxicities

Figure 11 – Preliminary solid phase microextraction data suggest that SPME toxicity measurements and COSMOmic toxicity calculations show a similar trend. Five SPME measurements were made: 7-octanoic acid, 1,2-octanediol, 1,8-octanediol, 2-naphthol, and 2-octanone (left to right). GC-FID response was assumed to be proportional to toxicity.

### Microtox Bioassays

-Microtox measures the light output of the exposed bacteria at different concentrations of analytes and calculates the EC50 as a percent of the known analyte concentration

-Analyte mixes with *Vibrio fischeri*, a species of bioluminescent bacteria.

-81.9% screening tests were performed (nine dilutions of the analyte in total)

-DMSO was used as a cosolvent

### Solid Phase Microextraction (SPME)

-A polydimethylsiloxane (PDMS) fiber is submerged in an analyte solution (Figure 4)

-PDMS is similar to the phospholipid bilayer DMPC used for COSMOmic calculations

-Gas chromatography (GC) paired with mass spectrometry (MS) or flame ionization detection (FID) measures the concentration of analyte present in PDMS

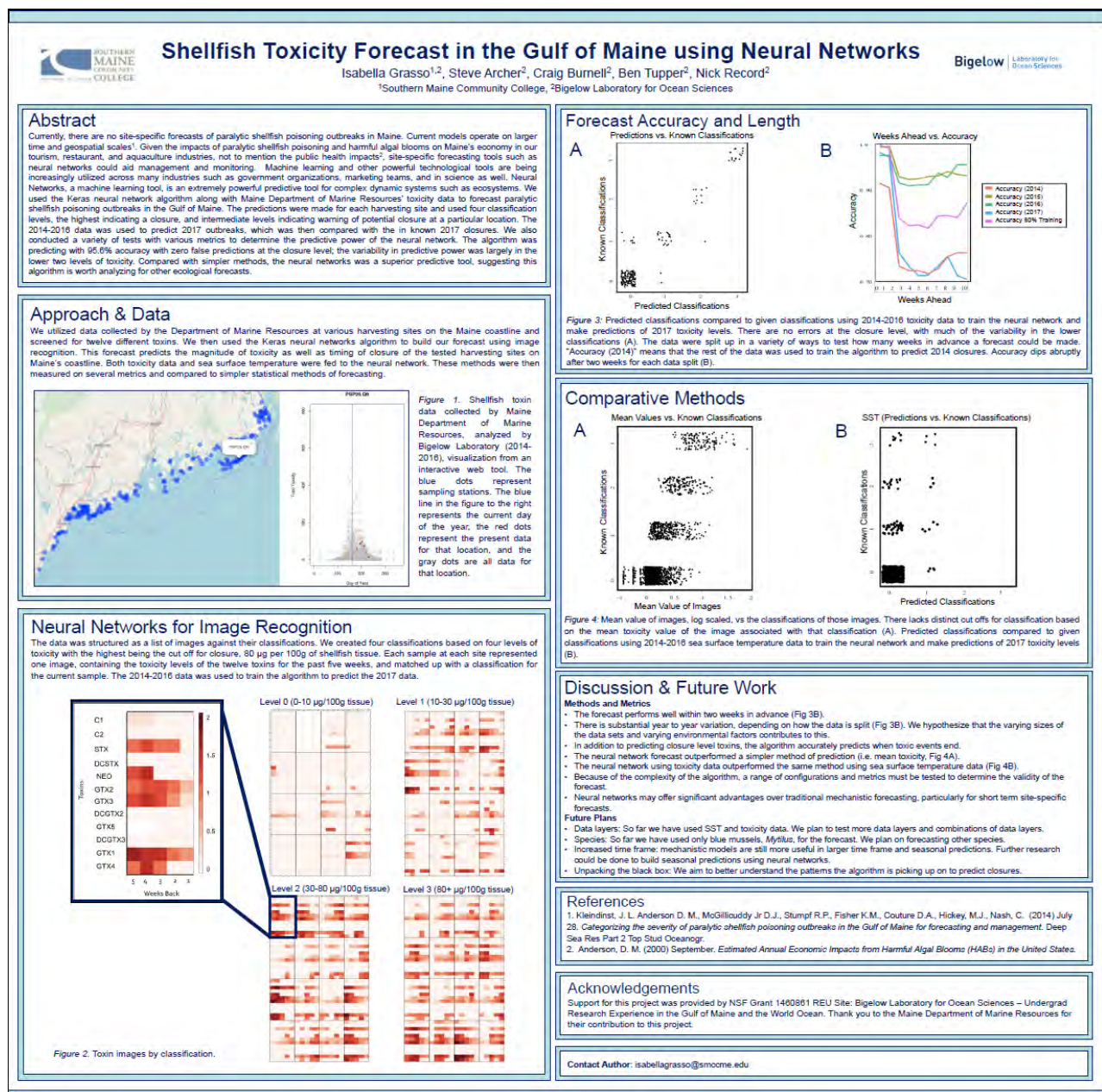
-DMSO was used as a cosolvent

Figure 4 – Target Compounds for Analysis Toxicity Analysis by Microtox and SPME (in synthetic seawater solution)

Index	Compound Name	Class	Concentration (μg/L)
0001	1-octanol	Alcohol	1000
0002	2-octanol	Alcohol	1000
0003	3-octanol	Alcohol	1000
0004	4-octanol	Alcohol	1000
0005	5-octanol	Alcohol	1000
0006	6-octanol	Alcohol	1000
0007	7-octanol	Alcohol	1000
0008	8-octanol	Alcohol	1000
0009	9-octanol	Alcohol	1000
0010	10-octanol	Alcohol	1000
0011	11-octanol	Alcohol	1000
0012	12-octanol	Alcohol	1000
0013	13-octanol	Alcohol	1000
0014	14-octanol	Alcohol	1000
0015	15-octanol	Alcohol	1000
0016	16-octanol	Alcohol	1000
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0022	22-octanol	Alcohol	1000
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0025	25-octanol	Alcohol	1000
0026	26-octanol	Alcohol	1000
0027	27-octanol	Alcohol	1000
0028	28-octanol	Alcohol	1000
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0031	31-octanol	Alcohol	1000
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0033	33-octanol	Alcohol	1000
0034	34-octanol	Alcohol	1000
0035	35-octanol	Alcohol	1000
0036	36-octanol	Alcohol	1000
0037	37-octanol	Alcohol	1000
0038	38-octanol	Alcohol	1000
0039	39-octanol	Alcohol	1000
0040	40-octanol	Alcohol	1000
0041	41-octanol	Alcohol	1000
0042	42-octanol	Alcohol	1000
0043	43-octanol	Alcohol	1000
0044	44-octanol	Alcohol	1000
0045	45-octanol	Alcohol	1000
0046	46-octanol	Alcohol	1000
00			

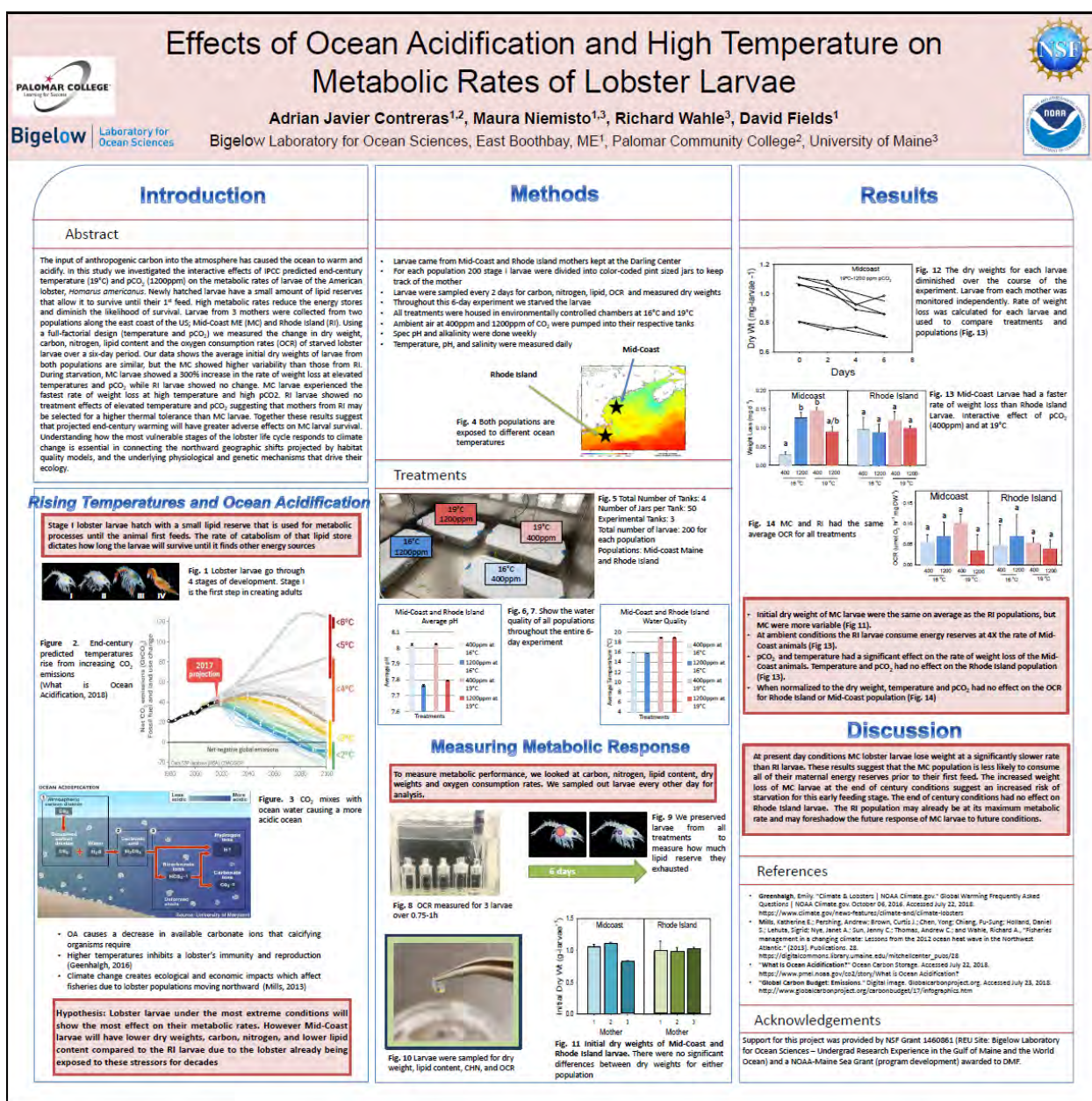


Currently, there are no site-specific forecasts of paralytic shellfish poisoning outbreaks in Maine. Recent mechanistic models operate on larger time and geospatial scales<sup>1</sup>. Given the impacts of paralytic shellfish poisoning and harmful algal blooms on Maine's economy in management and monitoring, losses to commercial fisheries, tourism and recreation and public health costs<sup>2</sup>, site-specific forecasting tools such as neural networks could aid both harvesters and regulatory agencies. Machine learning is being increasingly utilized across many industries such as government organizations, marketing teams, and in science as well. Neural networks is an extremely powerful predictive machine learning tool for complex dynamic systems such as ecosystems. We used the Keras neural network algorithm along with Maine Department of Marine Resources toxicity data to forecast paralytic shellfish poisoning outbreaks in coastal Maine. The predictions were made for each harvesting site and used four classification levels, the highest indicating a closure. The 2014-2016 data was used to predict 2017 outbreaks, which was then compared with the in known 2017 closures. We also conducted a variety of tests with various metrics and time frames to determine the predictive power of the neural network. The algorithm was predicting with 95.6% accuracy with zero false predictions at the closure level; the variability in predictive power was largely in the lower two levels of toxicity. The neural network outperformed simpler statistical methods, suggesting this algorithm is worth analyzing for other ecological forecasts.



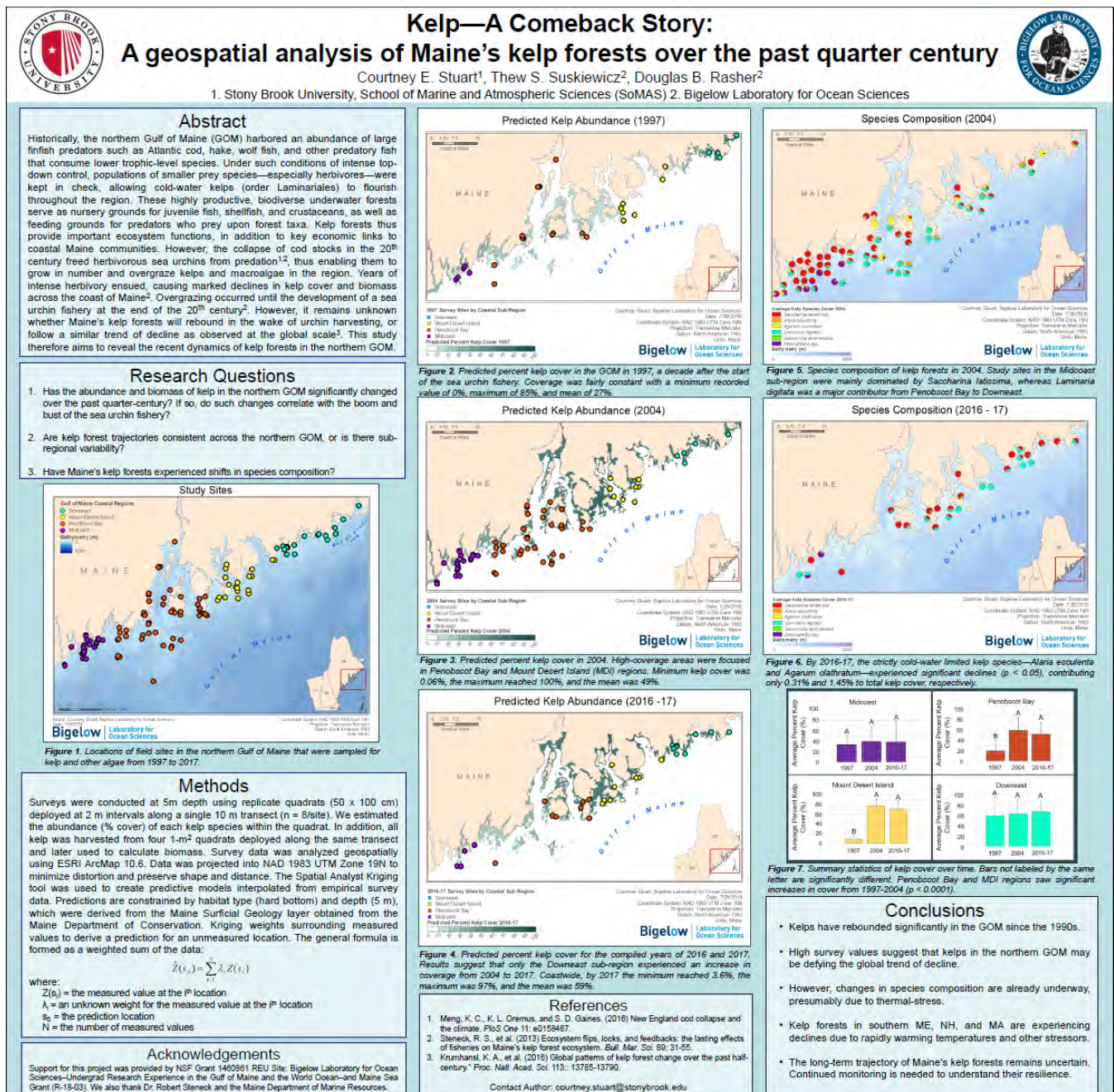


The input of anthropogenic carbon into the atmosphere has caused the ocean to warm and acidify. In this study we investigated the interactive effects of IPCC predicted end-century temperature (19°C) and pCO<sub>2</sub> (1200ppm) on the metabolic rates of larvae of the American lobster, *Homarus americanus*. Newly hatched larvae have a small amount of lipid reserves that allow it to survive until their 1<sup>st</sup> feed. High metabolic rates reduce the energy stores and diminish the likelihood of survival. Larvae from 3 mothers were collected from two populations along the east coast of the US; Mid-Coast ME (MC) and Rhode Island (RI). Using a full-factorial design (temperature and pCO<sub>2</sub>) we measured the change in dry weight, carbon, nitrogen, lipid content and the oxygen consumption rates (OCR) of starved lobster larvae over a six-day period. Our data shows the average initial dry weights of larvae from both populations are similar, but the MC showed higher variability than those from RI. During starvation, MC larvae showed a 300% increase in the rate of weight loss at elevated temperatures and pCO<sub>2</sub> while RI larvae showed no change. MC larvae experienced the fastest rate of weight loss at high temperature and high pCO<sub>2</sub>. RI larvae showed no treatment effects of elevated temperature and pCO<sub>2</sub> suggesting that mothers from RI may be selected for a higher thermal tolerance than MC larvae. Together these results suggest that projected end-century warming will have greater adverse effects on MC larval survival. Understanding how the most vulnerable stages of the lobster life cycle responds to climate change is essential in connecting the northward geographic shifts projected by habitat quality models, and the underlying physiological and genetic mechanisms that drive their ecology.





Historically, the northern Gulf of Maine (GOM) harbored an abundance of large finfish predators such as Atlantic cod, hake, wolf fish, and other predatory fish that consume lower trophic-level species. Under such conditions of intense top-down control, populations of smaller prey species—especially herbivores—were kept in check, allowing cold-water kelps (order Laminariales) to flourish throughout the region. These highly productive, biodiverse underwater forests serve as nursery grounds for juvenile fish, shellfish, and crustaceans, as well as feeding grounds for predators who prey upon forest taxa. Kelp forests thus provide important ecosystem functions, in addition to key economic links to coastal Maine communities. However, the collapse of cod stocks in the 20<sup>th</sup> century freed herbivorous sea urchins from predation<sup>1,2</sup>, thus enabling them to grow in number and overgraze kelps and macroalgae in the region. Years of intense herbivory ensued, causing marked declines in kelp cover and biomass across the coast of Maine<sup>2</sup>. Overgrazing occurred until the development of a sea urchin fishery at the end of the 20<sup>th</sup> century<sup>2</sup>. However, it remains unknown whether Maine's kelp forests will rebound in the wake of urchin harvesting, or follow a similar trend of decline as observed at the global scale<sup>3</sup>. This study therefore aims to reveal the recent dynamics of kelp forests in the northern GOM.



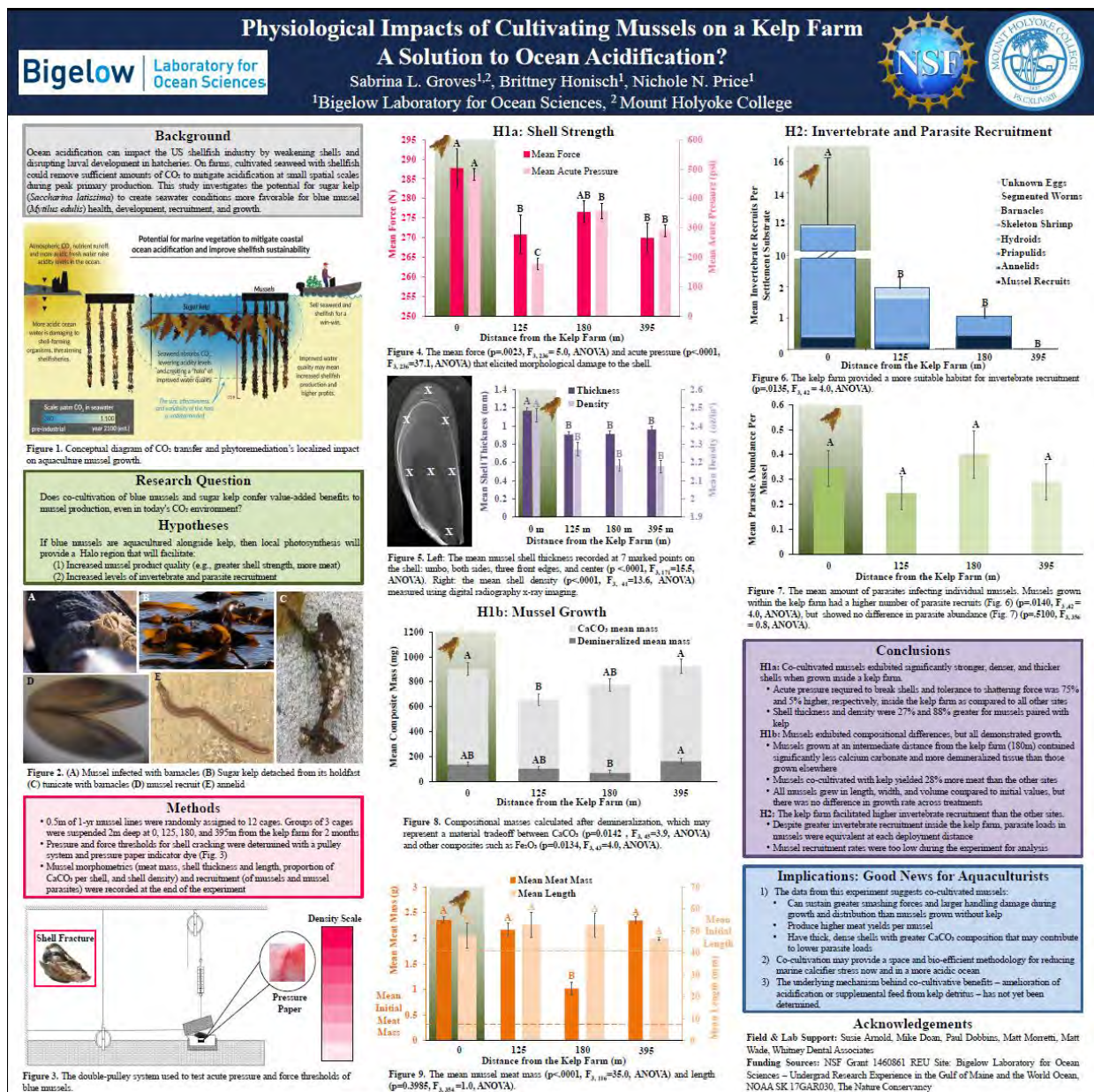


# Physiological Impacts of Cultivating Mussels on a Kelp Farm A Solution to Ocean Acidification?

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

Ocean acidification is thought to already be impacting productivity of the US shellfish industry via weakening of shells and disrupted larval development. Seaweed aquaculture could remove sufficient amounts of CO<sub>2</sub> to mitigate acidification at small spatial scales and over short periods of time. This study investigates the interactive potential for sugar kelp (*Saccharina latissima*) to create seawater conditions more favorable for blue mussel (*Mytilus edulis*) health, development, recruitment, and growth. We conducted a three-month manipulative field experiment where replicated year-old mussel lines were deployed in predator exclusion cages at increasing distances from the kelp farm (0, 125, 180, 395m), along with standardized recruitment substrata. Mussel shell metrics (length, width, mass, density, thickness, shell composition, and resistance to breakage), meat mass, total animal volume, recruitment, and parasite load were measured. Mussels cultivated on the kelp farm exhibited significantly greater meat mass (28%), shell thickness (27%), and acute pressure (75%) and force tolerances (5%). Despite higher rates of invertebrate recruitment inside the kelp farm, parasite loads in mussels were equivalent at each deployment distance, as were changes in mussel shell length and width. Mussel recruitment rates were too low during the experiment to determine impacts on this process. The exact underlying mechanism – amelioration of acidification or supplemental feed from kelp detritus – has not yet been determined. However, this study provides evidence to suggest that co-cultivative aquaculture practices provide a space and bio-efficient methodology for reducing marine calcifier stress, while simultaneously increasing mussel product quality (e.g., meat yields and resistance to breakage during shipping).



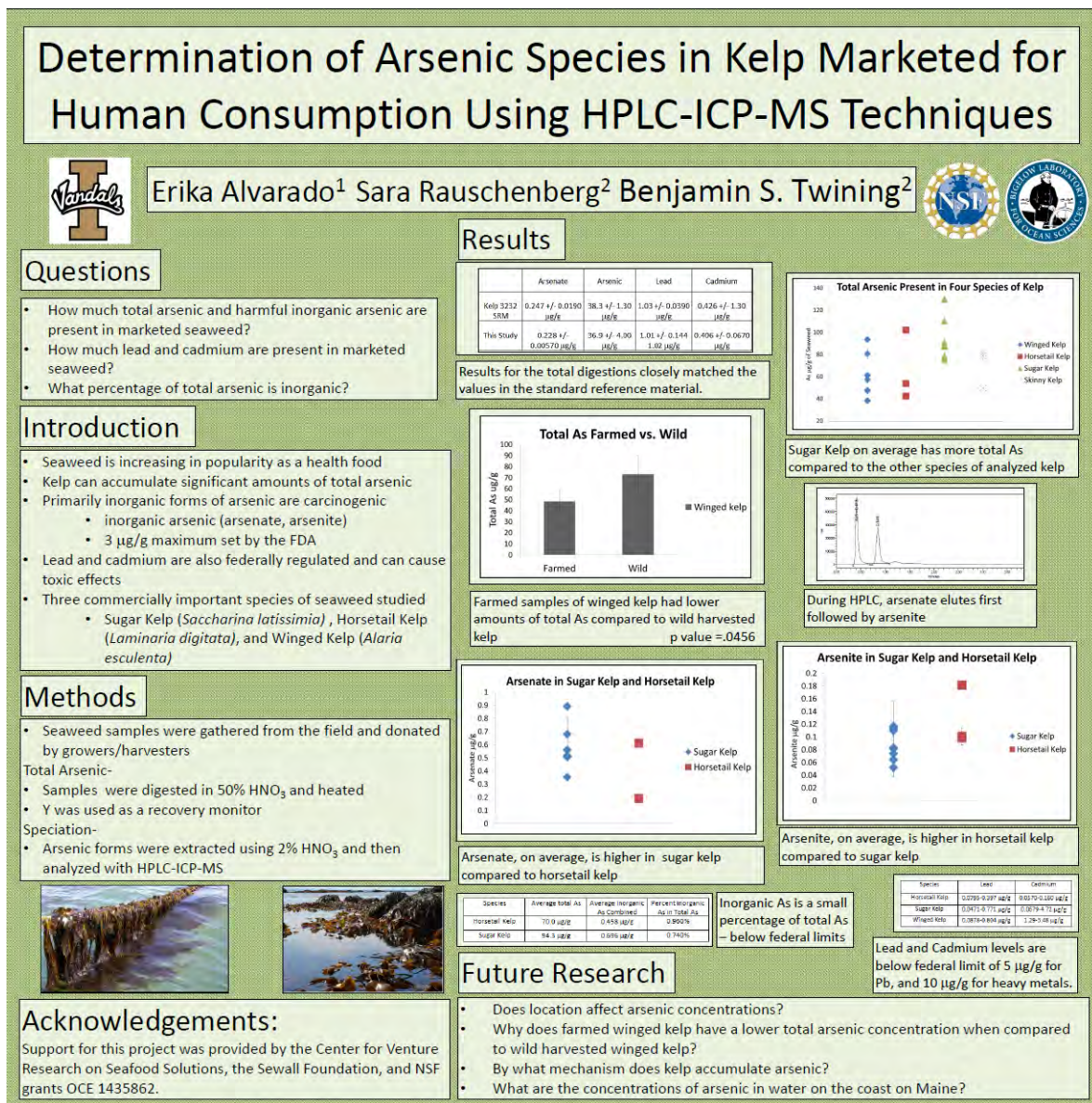


## Total and Inorganic Arsenic in Kelp Marketed for Human Consumption Using HPLC-ICP-MS Techniques

Alvarado EA<sup>1,2</sup>, Rauschenberg S<sup>1</sup>, Twining B<sup>1</sup>

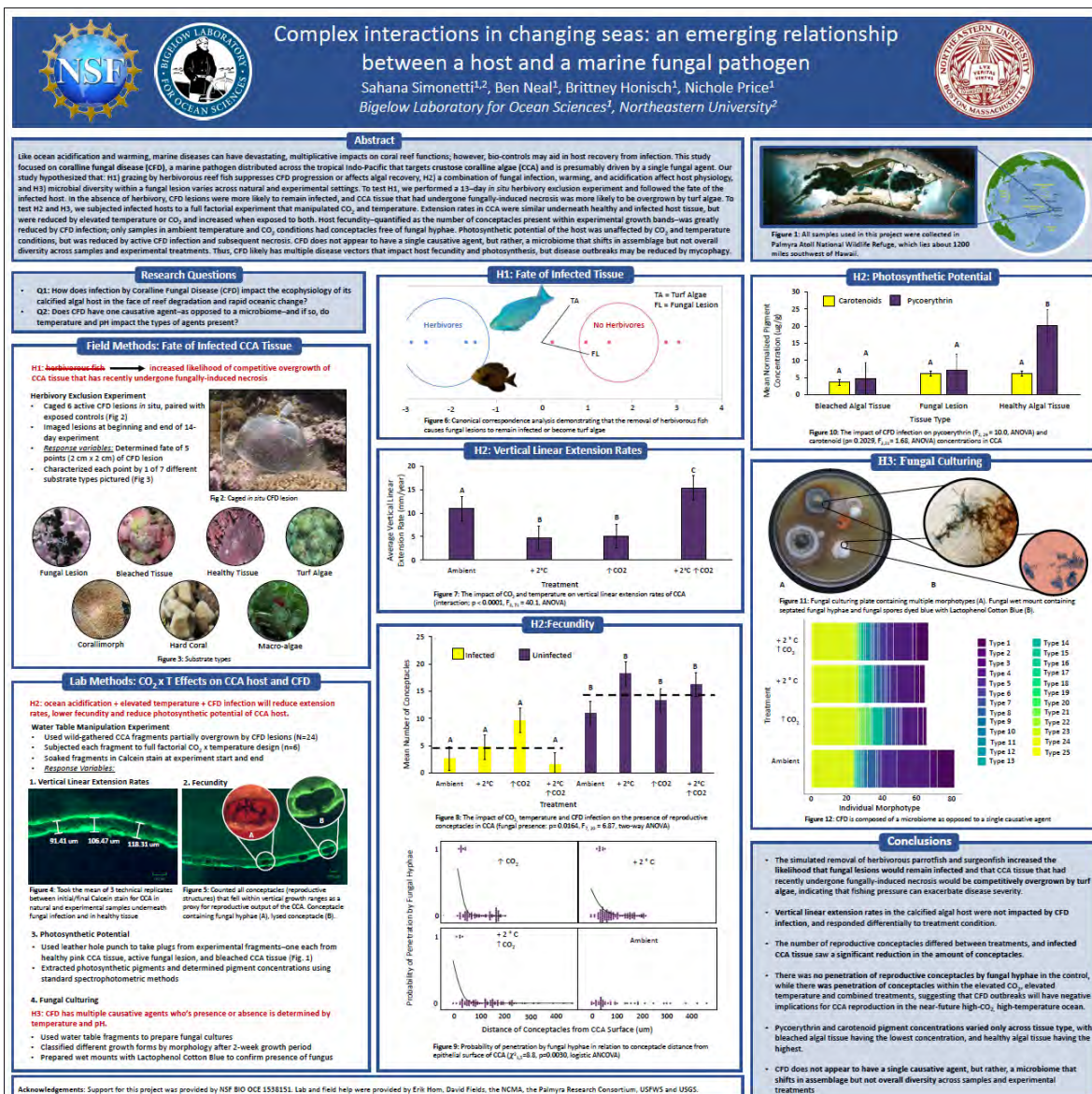
Bigelow Laboratory for Ocean Sciences<sup>1</sup>, University of Idaho<sup>2</sup>

Arsenic is present in the marine environment and can be accumulated by marine organisms, including macroalgae. Due to the health food status of seaweed and its increasing popularity, it is important to understand the concentrations of arsenic in its various forms in macroalgae. The inorganic forms of arsenic are the most toxic and are known carcinogens. Total and inorganic arsenic, as well as total lead and cadmium, concentrations were measured in three common commercial seaweed species from Maine: sugar kelp (*Saccharina lattissima*), winged kelp (*Alaria esculenta*), and horsetail kelp (*Laminaria digitata*). Dried commercial product was provided by growers. Contribution of arsenic by epiphytes and biofilms was analyzed using freshly-collected cleaned and uncleaned seaweed samples. Seaweed processing by drying outside at ambient temperature was compared against a freeze drying technique to understand how arsenic species change form with processing. Total arsenic concentrations in *Saccharina lattissima* varied from 75 to 123 mg/g (mean =  $87.6 \pm 19.3$  mg/g). *Alaria esculenta* and *Laminaria digitata* had total arsenic means of  $60.8 \pm 18.3$  and  $66.0 \pm 31.7$ , respectively. Lead content was highest in *Alaria esculenta* ( $0.398 \pm 0.281$  mg/g), and lowest in sugar kelp (with an average value of  $0.196 \pm 0.0100$  mg/g). Both *Saccharina lattissima* and *Alaria esculenta* had higher values of cadmium (mean =  $2.49 \pm 1.15$  mg/g and  $2.44 \pm 0.825$  mg/g, respectively) compared to *Laminaria digitata* (mean =  $0.117 \pm 0.0533$  mg/g). Data generated from this study was comparable to arsenate (within 7.69%), lead (within 1.94%), and cadmium (within 4.69%) levels reported for a kelp standard reference material (NIST Kelp 3232). All cadmium, lead, and inorganic arsenic concentrations present in the kelp were below the set federal limits.





Like ocean acidification and warming, marine diseases can have devastating, multiplicative impacts on coral reef functions; however, bio-controls may aid in host recovery from infection. This study focused on coralline fungal disease (CFD), a marine pathogen distributed across the tropical Indo-Pacific that targets crustose coralline algae (CCA) and is presumably driven by a single fungal agent. Our study hypothesized that: H1) grazing by herbivorous reef fish suppresses CFD progression or affects algal recovery, H2) a combination of fungal infection, warming, and acidification affect host physiology, and H3) microbial diversity within a fungal lesion varies across natural and experimental settings. To test H1, we performed a 13-day *in situ* herbivory exclusion experiment and followed the fate of the infected host. In the absence of herbivory, CFD lesions were more likely to remain infected, and CCA tissue that had undergone fungally-induced necrosis was more likely to be overgrown by turf algae. To test H2 and H3, we subjected infected hosts to a full factorial experiment that manipulated CO<sub>2</sub> and temperature. Extension rates in CCA were similar underneath healthy and infected host tissue, but were reduced by elevated temperature or CO<sub>2</sub> and increased when exposed to both. Host fecundity—quantified as the number of conceptacles present within experimental growth bands—was greatly reduced by CFD infection; only samples in ambient temperature and CO<sub>2</sub> conditions had conceptacles free of fungal hyphae. Photosynthetic potential of the host was unaffected by CO<sub>2</sub> and temperature conditions, but was reduced by active CFD infection and subsequent necrosis. CFD does not appear to have a single causative agent, but rather, a microbiome that shifts in assemblage but not overall diversity across samples and experimental treatments. Thus, CFD likely has multiple disease vectors that impact host fecundity and photosynthesis, but disease outbreaks may be reduced by mycophagy.



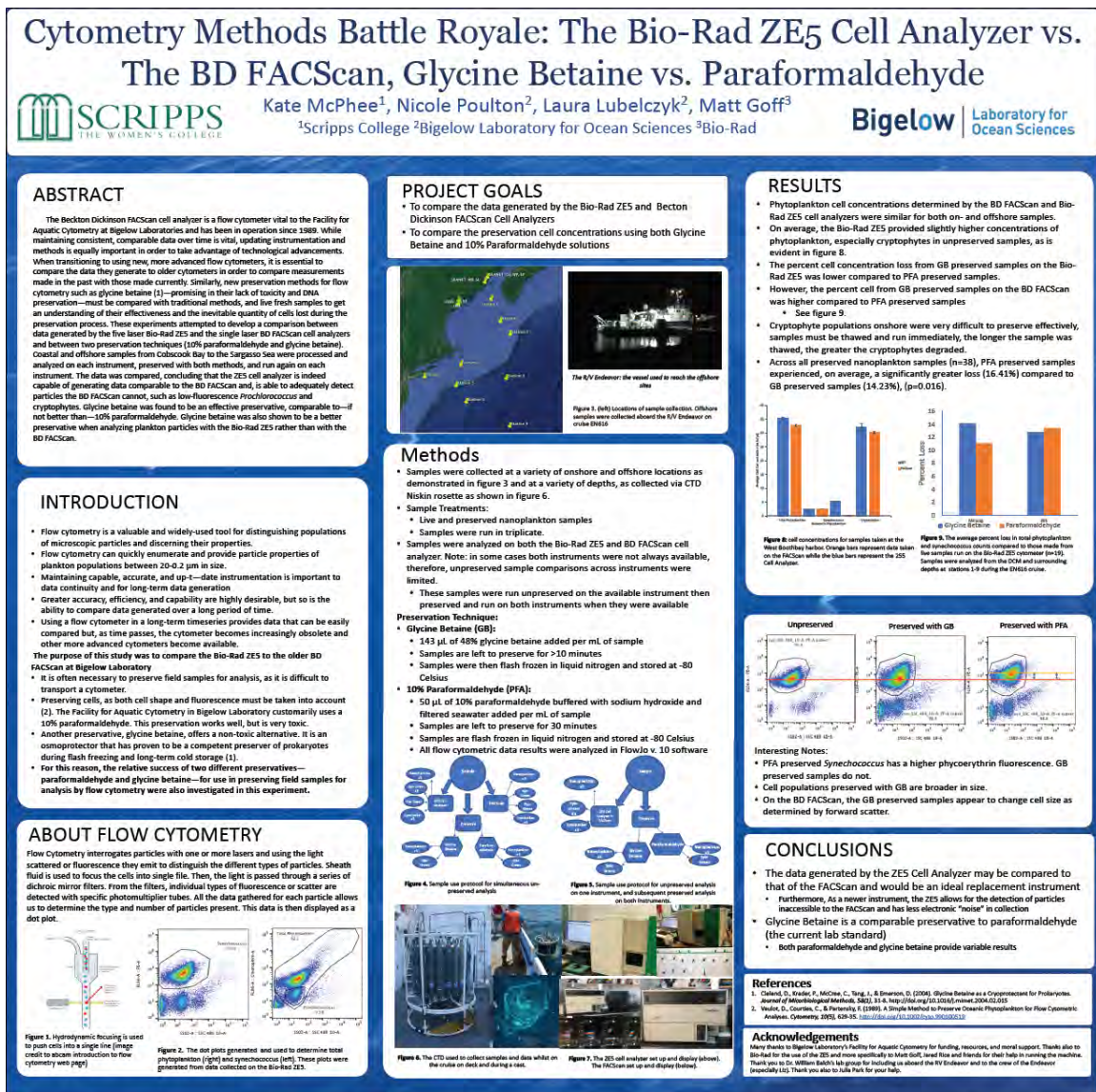


## Cytometry Methods Battle Royale: The Bio-Rad ZE5 Cell Analyzer vs. the BD FACScan, Glycine Betaine vs. Paraformaldehyde

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The Beckton Dickinson FACScan cell analyzer is a flow cytometer vital to the Facility for Aquatic Cytometry at Bigelow Laboratories and has been in operation since 1989. While maintaining consistent, comparable data over time is vital, updating instrumentation and methods is equally important in order to take advantage of technological advancements. When transitioning to using new, more advanced flow cytometers, it is essential to compare the data they generate to older cytometers in order to compare measurements made in the past with those made currently. Similarly, new preservation methods for flow cytometry such as glycine betaine (1)—promising in their lack of toxicity and DNA preservation—must be compared with traditional methods, and to live, fresh samples to get an understanding of their effectiveness and the inevitable quantity of cells lost during the preservation process. These experiments attempted to develop a comparison between data generated by the five laser Bio-Rad ZE5 and the single laser BD FACScan cell analyzers and between two preservation techniques (10% paraformaldehyde and glycine betaine). Coastal and offshore samples from Cobscook Bay to the Sargasso Sea were processed and analyzed on each instrument, preserved with both methods, and run again on each instrument. The data was compared, concluding that the ZE5 cell analyzer is indeed capable of generating data comparable to that of the BD FACScan and is able to adequately detect particles the BD FACScan cannot, such as low-fluorescence *Prochlorococcus* and cryptophytes. Glycine betaine was found to be an effective preservative, comparable to—if not better than—10% paraformaldehyde. Glycine betaine was also shown to be a better preservative when analyzing plankton particles with the Bio-Rad ZE5 rather than with the BD FACScan.



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