

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 HJ¹, Mitchell C², Balch WM², University of Massachusetts Amherst¹, Bigelow Laboratory for Ocean Sciences²

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

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

Genco GW¹, Koeksoy E², Emerson D²

¹Colby College, ²Bigelow 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 PS¹, Jones RM² and Orcutt BN²

Universidad de Puerto Rico¹, Bigelow Laboratory for Ocean Sciences²

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

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

Dawydiak W¹, Lubelczyk L², Archer SD², Posman K², Poulton NJ²

University of Pennsylvania¹, Bigelow Laboratory for Ocean Sciences²

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

Seasonal Variation in dsDNA Eukaryotic Viruses in Antarctic Ace Lake

Hagggett, E. ^{1,2}, Cretoiu, S.², Bhattacharjee, A.², Martínez Martínez, J.²

Southern Maine Community College¹, Bigelow Laboratory²

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

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

Arndt HT¹, Lomas MW², Front Range Community College¹, Bigelow Laboratory for Ocean Sciences²

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

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

DMSP metabolism and Antarctic microbial community structure

Greenlee SM¹, Countway PD²

Colby College¹, Bigelow Laboratory for Ocean Sciences²

11:00 Josh Brycki - Juniata College, Huntingdon PA

Haloperoxidase-mediated gas exchange in two diatom species

Brycki JD^{1,2}, Posman KM¹, Archer SD¹

Bigelow Laboratory for Ocean Sciences¹, Juniata College²

11:15 Adelaida Arjona - Harvard University, Cambridge MA

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

Arjona AV¹, Mayot N², Matrai P², Harvard University¹, Bigelow Laboratory for Ocean Sciences²

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

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

McDermith EJ¹, Whitney LP¹, Lomas MW¹

Bigelow Laboratory for Ocean Sciences¹, University of Rhode Island²

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^{1,2}, Aeppli, C²

¹Chemistry Department, Hamilton College, NY ²Bigelow 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 TR¹, Aeppli C², Bowdoin College¹, Bigelow Laboratory for Ocean Sciences²

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^{1,2}, Archer SD², Burnell C², Tupper B², Record NR² Southern Maine Community College¹, Bigelow Laboratory for Ocean Sciences²

Southern Maine Community College¹, Bigelow Laboratory for Ocean Sciences²

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^{1,2}, Neimisto M^{1,3} Wahle R³, Fields DM¹

Bigelow Laboratory for Ocean Sciences, East Boothbay, ME¹, Palomar Community College² U-Maine³

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^{1,2}, , Suskiewicz TS², Rasher DB²

Stony Brook University School of Marine and Atmospheric Sciences (SoMAS)¹, Bigelow Laboratory for Ocean Sciences²

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^{1,2}, Honisch B¹, Price NN¹

Bigelow Laboratory for Ocean Sciences¹, Mount Holyoke College²

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^{1,2}, Rauschenberg S¹, Twining B¹

Bigelow Laboratory for Ocean Sciences¹, University of Idaho²

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^{1,2}, Neal BP¹, Honisch BL¹, Price NN¹

Bigelow Laboratory for Ocean Sciences¹, Northeastern University²

Abstracts and Posters

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

Steinmetz HJ¹, Mitchell C², Balch WM², University of Massachusetts Amherst¹, Bigelow Laboratory for Ocean Sciences²

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.

Using Hyperspectral and Multispectral Radiometry to Predict Forel-Ule Ocean Color

Halley Steinmetz^{1,2}, Catherine Mitchell², William M. Balch²

UMassAmherst School of Natural Sciences, University of Massachusetts Amherst, Amherst MA¹, Bigelow Laboratory for Ocean Sciences, Boothbay ME²

Introduction:

Ocean color provides scientists with information about optically-active constituents suspended near the sea surface, such as phytoplankton chlorophyll and colored dissolved organic matter. Two methods to measure ocean color are:

- Colorimetry: assigning a numeric value to the color of a sample using color comparator scales (Mobley, 1994), like the Forel-Ule scale, for which there are historic datasets dating back to the 1800s.
- Radiometry: measuring electromagnetic energy (Mobley, C. D., 1994) using multispectral and hyperspectral radiometric sensors, the former of which have been in use since the 1970s.

This project examines the relationship between colorimetry and radiometry, specifically with regard to providing continuity between interpreting historical Forel-Ule measurements and more recent radiometric measurements.

Methods: Data Processing

R software was used to perform data manipulation and analysis. The hyperspectral remote sensing reflectance (R_{rs}) data and derived multispectral remote sensing reflectance data were converted to C.I.E. color coordinates (Mobley, 1994) using the equations:

$$X = K_m \int_{\lambda} F(\lambda) S(\lambda) d\lambda$$

$$Y = K_m \int_{\lambda} F(\lambda) Y(\lambda) d\lambda$$

$$Z = K_m \int_{\lambda} F(\lambda) Z(\lambda) d\lambda$$

Where: $K_m = 618 \text{ (m}^2 \text{ W}^{-1})$ the maximum luminous efficacy, λ , Y , and Z are tristimulus functions standard (Mobley, 1994), F is the hyperspectral radiometry data and X , Y , and Z represents the red, green, and blue (respectively) components of F .

The resultant numeric color coordinates were used to predict the Forel-Ule colors. The predictions were plotted on a color plot (figs. 4 & 5), where the colors within the outer curve match with the colors on the plot in the upper right-hand corner of both prediction plots, near the white point in the center and the blue/green colors to the upper-left corner. The predictions were compared with the actual FU measurements observed during the "Coccolith-Mix" cruise.

Hyperspectral Prediction: Day 185

Figure 4: Predictions made by drawing a straight line (blue) through "white point" and "chromaticity coordinates" and finding intersection between the straight blue line and the green, Forel-Ule, line. Prediction from day 185 of the year, while on the R/V Endeavor.

Methods: Data Collection

Ocean color data was collected daily on R/V Endeavor EN616 "Coccolith-Mix" cruise from July 3rd-15th using the manual Forel-Ule method (based on colorimetry) and hyperspectral radiometer sensors (HyperAS, SeaWiFS, Inc.) while cruising in the Northwest Atlantic, off New England (fig. 3).

Forel-Ule measurements were collected at 10 sites along the cruise track at approximately midday, local time. A secchi disk (fig. 1) was used to observe ocean color at half-secchi depth to be compared with a manual Forel-Ule color scale (fig. 2). Hyperspectral measurements were gathered using Sea-Viewing Wide-Of-Area-of-Observe Satellite (SeaWiFS) which maintains the direction that the radiometer points, at 90°-120° from the sun's azimuth. Upwelling radiance data was converted for sky reflectance using a sky-viewing hyperspectral sensor and a hyperspectral downwelling irradiance sensor. Multispectral data were mimicked using a sub-set of the hyperspectral data, integrated over several hyperspectral bands.

Hyperspectral Prediction: Day 193

Figure 5: Prediction made by drawing a straight line (blue) through "white point" and "chromaticity coordinates" and finding intersection between the blue (straight) line and the green, Forel-Ule, line. Prediction from day 193 of the year, while on the R/V Endeavor.

Figure 1: Secchi disk used during "Coccolith-Mix" cruise to observe ocean color.

Figure 2: Forel-Ule color scale used to observe ocean color at station #1-10.

Figure 3: Cruise track on the R/V Endeavor EN616 cruise of New England with station locations labeled #1-10.

Results and Discussion:

The results of the manual Forel-Ule measurements, the hyperspectral predictions, and the multispectral predictions can be seen in figs. 6, 7, & 8. To compare: the predictions with the manual Forel-Ule measurements (made by a minimum of two observers) and with each other, regression equations were calculated. The expected outcome would be a slope of 1, with a high correlation between the values. All of the regressions had high correlation ratios, which means that there was a strong linear relationship between the prediction and the manual measurements; but the multispectral versus hyperspectral predictions. However, the slope does not match with expectations for either the hyperspectral predictions or the multispectral predictions compared with manual measurements. The slopes show that the manual Forel-Ule measurements were consistently higher than the radiometric predictions. However, when observing the individual values when compared with the line "y = x", it is evident that the lower data measurements and predictions match closely with the expected slope, while it is only the higher measurements that deviate from that line. When looking at the regression comparing the hyperspectral and multispectral predictions, the slope is close to one, which means that the two predictions yield very similar Forel-Ule numbers.

Figure 6: When comparing manual Forel-Ule measurements with predicted hyperspectral Forel-Ule measurements, the slope was 0.5201 and the R² value was 0.9972.

Figure 7: The slope of the regression that relates comparing Multispectral Forel-Ule predictions with the manual measurements was 0.5144 and the R² value was 0.9933.

Figure 8: The slope of the regression that relates comparing hyperspectral Forel-Ule predictions with the multispectral Forel-Ule predictions was 0.9848 and the R² value was 0.9936.

Conclusions and Looking Forward:

There were five stations where the manual Forel-Ule color measurements gave values greater than the Forel-Ule estimates based on hyperspectral or multispectral radiometric measurements. The reason for this is unclear. Apart from station three, the stations that do not closely match the 1:1 line are on the continental shelf, while the stations off of the continental shelf correspond better with the 1:1 line (Figs. 6 & 7). It is noteworthy that the New England continental shelf experienced one of the largest mesoscale coccolithophore blooms in decades during cruise EN616. The stations that fell away from the 1:1 line in Figs. 6 and 7 were all characterized by elevated concentrations of coccolithophores and detached coccoliths (up to concentrations of 40,000 per ml at station 4). We hypothesize that multiple-scattering caused by coccolithophores and their detached coccoliths may have driven the XY chromaticity colors away from the traditional Forel-Ule color scale. This might have resulted from an increased effective pathlength of photons through the highly-scattering, coccolith-rich, suspensions, thus pushing the color out of the traditional colorimetry scale as originally derived by Forel (1890) and Ule (1892). As such, users should be careful in interpreting the Forel-Ule scale in coccolithophore blooms. Finally, the ability to use radiometry to accurately derive the Forel-Ule 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 Forel-Ule color comparator scale.

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. Research cruise EN616 was supported by the National Science Foundation, OCE-1633748. Thank you to the captain and crew of the R/V Endeavor.

References:

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

Genco GW¹, Koeksoy E², Emerson D²

Colby College¹, Bigelow Laboratory for Ocean Sciences²

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% H₂O₂ 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.

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

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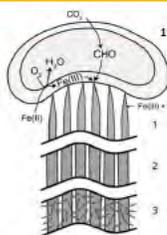
Garret Genco¹, Elif Koeksoy², David Emerson²

¹Colby College, ²Bigelow Laboratory for Ocean Sciences

contact: gwgenco20@colby.edu

Abstract

Mariprofundus ferrooxydans DIS-1 is a neutrophilic, marine iron (II) oxidizing bacteria of the class Zetaproteobacteria, which produces stalks that provide extracellular storage of precipitating Fe(III), preventing encrustation of the cell. Stalk formation is the result of subsequent binding of Fe(III) to unknown organic polymers (1). Knowing the exact composition of the stalk's organic matrix will aid in identifying genes involved in the exopolymer synthesis, evaluate the suitability of stalks as nanomaterials and understand the role of stalks as biomarkers for ancient microbial metabolism. Therefore, we sought to develop a protocol that would produce purified stalk samples for biochemical analysis by sonicating cultivated samples, performing density gradient centrifugation and treatments with 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% H₂O₂ 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 did not effectively separate cells from stalk filaments. Chemical treatments coupled with sonication and density gradient centrifugation can be used to effectively reduce and dissolve iron and cells for biochemical analysis of the stalk's composition.



Research Goals

1. Develop a protocol for stalk purification
Produce pure stalks to harvest and analyze
2. Extraction of different stalk compounds
Iron Phase and Organic Matrix

Methods

Develop protocols for stalk purification and extraction of different stalk compounds



- 1) Sonicate samples (a) and perform density gradient centrifugation (b) to separate stalks from cells.
- 2) Reduce and dissolve amorphous iron, iron chunks, zero-valent iron & cells and with:
 1. Hydrogen Peroxide (30%, 10%, 5%, 1%)
 2. Hydrochloric Acid (6M, 2M, 1M, 0.5M)
 3. Hydroxylamine (1.0M, 0.5M, 0.1M)
- 3) Microscopy to observe stalk structure and fluorescence to observe presence/absence of cells

Introduction

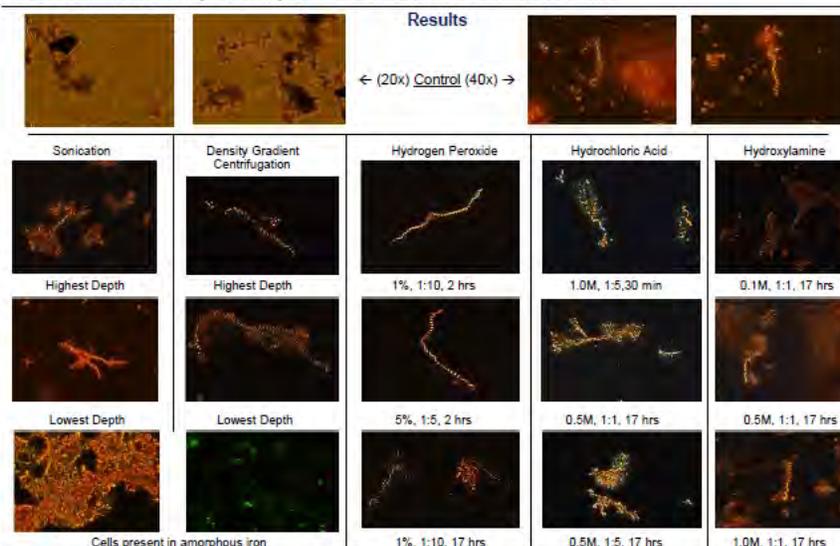
Fe(II) oxidizing bacteria compete with the abiotic oxidation of Fe(II) by molecular oxygen, which limits the bioavailability of Fe(II) (2). As a result, Fe(II) oxidizing bacteria inhabit environmental niches at acidic pH levels or micro-oxic conditions at neutral pH (3). Iron oxides produced at neutral pH are poorly soluble and instantaneously precipitate Fe(III), potentially entombing the cell in Fe (1).

One such Fe(II) oxidizer strain is *Mariprofundus ferrooxydans* DIS-1, a species of the new class of marine Fe(II) oxidizing bacteria, called Zetaproteobacteria. They are microaerophilic, neutrophilic and chemolithotrophic that produce stalks that provide extracellular storage of oxidized iron, which prevents encrustation of the cell (4).

Chan et al. (2011) hypothesized stalk formation to be the subsequent binding of Fe(III) excretion to organic polymers. However, the primary composition of the organic matrix of stalks is unknown. Knowing the composition of the stalk's organic matrix will aid in identifying the genes involved in the exopolymer synthesis, evaluating the suitability of stalks as nanomaterials and comprehend their potential role as biomarkers for ancient microbial metabolism.



Results



Discussion

- Most Effective Treatments
 - Density Gradient Centrifugation with a medium of 90% Glycerol spinning at 750rpm for 50-80 minutes
 - Hydrogen Peroxide
 - 17hrs Treatment – 1% (1:10) and 5% (1:10)
 - 2hrs Treatment – 1%, 5% (1:1 & 1:5), 30% (1:1)
 - Hydrochloric Acid
 - 17hrs Treatment – 0.5M (1:1 & 1:5)
 - 30min Treatment – 0.5M (1:5) and 1.0M (1:5)
- Sonication for one minute immediately followed by density gradient centrifugation for one hour at 750 rpm unsuccessfully resulted in cells present with amorphous iron and stalks at all depths.
- The higher concentrations of hydrochloric acid (6M & 2M) were too destructive, completely dissolving and fragmenting samples. Lower concentrations (1.0M & 0.5M) with a sample:HCl ratio of 1:5 were most effective at purifying stalk samples.
- The hydroxylamine treatments were ineffective. Hydroxylamine is expected to reduce Fe(III), which would reduce and dissolve stalks and iron samples, leaving only organic compounds. However stalks and amorphous iron oxides were present at all concentrations, indicating the experiment failed.
- Purified stalk samples were predominantly present at low percentages of hydrogen peroxide (1% & 5%). The explanation is unknown because hydrogen peroxide was expected to completely dissolve all components of the sample, and higher percentages were hypothesized to be most effective.

Acknowledgements

Support for this project was provided by NSF Grant 1450861 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 BJ, Edwards KJ (2011) Lithotrophic iron-oxidizing bacteria produce organic stalks to control mineral growth: implications for biogeochemistry. *ISME J* 5:717-727. (2) Nelson ED, Swanner ED, Brenners S, Schmidt C, Kasper A (2014) The intensity of microbially mediated and abiotic reactions in the biogeochemical Fe cycle. *Nat Rev Microbiol* 12:797-808. (3) Kasper A, Emerson D, Girnick JA, Roden EE, Muehe BM (2016) Chapter 17 Geomicrobiology of Iron 343-399. (4) Chan CS, Fakra SC, Emerson D, Edwards DC, Emerson D, Barfield JF (2009) Iron oxyhydroxide 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 PS¹, Jones RM² and Orcutt BN²

Universidad de Puerto Rico¹, Bigelow Laboratory for Ocean Sciences²

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.

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

Patricia Montalvo-Rodríguez¹, Rose Jones² and Beth Orcutt²

Universidad de Puerto Rico, Mayagüez, PR¹; Bigelow Laboratory for Ocean Sciences, East Boothbay, ME²



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 4°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.

Substrates:
Glucose, Pyruvate, Manganese, Iron, Sulfide, Hydrogen, Acetate, Formate, P. stutzeri.

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

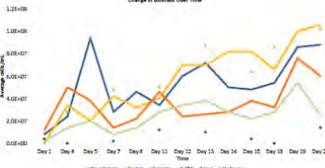



Figure 3: Changes in cell density over 24 days for various treatment types. Figure 4: Changes in cell density over 20 days for various treatment types.

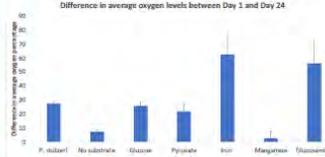
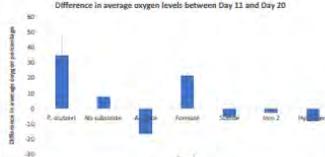



Figure 5: Changes in oxygen levels per treatment between Day 1 and Day 24. Figure 5: Changes in oxygen levels per treatment between Day 11 and Day 20.

Samples	DNA in Fluid (ng/mL)	DNA in Rocks (ng/cm ²)	Samples	DNA in Fluid	DNA in Rocks (ng/cm ²)
<i>P. stutzeri</i>	38.67	51	<i>P. stutzeri</i>	TBD	785
No substrate	0.028	39.8	No substrate	TBD	135
Glucose	---	39.6	Glucose	TBD	189
Pyruvate	Too low	179	Pyruvate	TBD	132
Glucosamine	0.295	115	Formate	TBD	136
Manganese	0.567	204	Sulfide	TBD	174
Iron	0.18	25.8	Sulfide	TBD	149
	0.142	54.8	Hydrogen	TBD	184
	Too low	123	Hydrogen	TBD	147
	Too low	129	Iron (Microaerophilic)	TBD	146
			Iron (Microaerophilic)	TBD	92
					89.2

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

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

Dawydiak W¹, Lubelczyk L², Archer SD², Posman K², Poulton NJ²
 University of Pennsylvania¹, Bigelow Laboratory for Ocean Sciences²

Microzooplankton play a significant role in nutrient recycling within the marine microbial loop. In order to better characterize and quantify grazing and nutrient turnover, it's important to understand the dynamics between the microzooplankton and their prey. The most widely accepted technique currently used to measure microzooplankton grazing, the dilution method, is difficult and laborious to execute and can provide questionable results. This is because this method significantly alters the water chemistry due to the filtration of seawater and relies on cell concentrations from very dilute samples. This project proposes an innovative approach to determine grazing rates that attempts to improve upon the drawbacks of the dilution method. This new technique, the saturation method, uses increasing concentrations of a surrogate prey to minimize grazing stress on the target prey within a natural plankton community. The saturation method was assessed using model laboratory communities using specific predators (*Ochromonas danica* or *Oxyrrhis marina*) and prey cultures (*Micromonas pusilla*). Known concentrations of surrogate prey, such as latex fluorescent beads or GFP-labeled bacteria, were added at several concentrations across replicate samples. The surrogate prey served as substitute prey for the microzooplankton. The grazing rate was estimated by comparing the apparent growth of the prey in the presence and absence of surrogate prey. Experiments were incubated for 24 hours and concentrations of target and surrogate prey were determined at multiple timepoints using flow cytometry. In addition to laboratory cultures, the saturation method was applied to field samples at six stations during a research cruise aboard the RV Endeavor in July 2018. Both laboratory and field results indicated the potential for this new method to determine grazing rates by microzooplankton, but further experimentation is necessary to refine the protocol and compare the results to other methods.

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

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Abstract

Microzooplankton play a significant role in nutrient recycling within the marine microbial loop. In order to better characterize and quantify grazing and nutrient turnover, it's important to understand the dynamics between the microzooplankton and their prey. The most widely accepted technique currently used to measure microzooplankton grazing, the dilution method, is difficult and laborious to execute and can provide questionable results. This is because this method significantly alters the water chemistry due to the filtration of seawater and relies on cell concentrations from very dilute samples. This project proposes an innovative approach to determine grazing rates that attempts to improve upon the drawbacks of the dilution method. This new technique, the saturation method, uses increasing concentrations of a surrogate prey to minimize grazing stress on the target prey within a natural plankton community. The saturation method was assessed using model laboratory communities using specific predators (*Ochromonas danica* or *Oxyrrhis marina*) and prey cultures (*Micromonas pusilla*). Known concentrations of surrogate prey, such as latex fluorescent beads or GFP-labeled bacteria, were added at several concentrations across replicate samples. The surrogate prey served as substitute prey for the microzooplankton. The grazing rate was estimated by comparing the apparent growth of the prey in the presence and absence of surrogate prey. Experiments were incubated for 24 hours and concentrations of target and surrogate prey were determined at multiple timepoints using flow cytometry. In addition to laboratory cultures, the saturation method was applied to field samples at six stations during a research cruise aboard the RV Endeavor in July 2018. Both laboratory and field results indicated the potential for this new method to determine grazing rates by microzooplankton, but further experimentation is necessary to refine the protocol and compare the results to other methods.

Project Goals

- 1) Develop a method to estimate microzooplankton grazing rates by applying a prey saturation approach to model communities created in the laboratory
- 2) Apply this new saturation method to diverse communities from a variety of field locations

Saturation Approach

$$\text{Apparent Growth} = \ln(\text{final prey concentration} / \text{initial prey concentration}) / (T_f - T_i)$$

Gross growth: growth rate without grazing stress (containers with highest surrogate prey concentration)
Net growth: growth rate with grazing (containers without surrogate prey)
Grazing: Difference between gross and net

Introduction

- Large amount of nutrient recycling in microbial loop which has broad implications for global marine processes, such as primary production
- Microzooplankton mediate the amount of nutrients recycled within the microbial loop as opposed to being passed on to higher trophic levels
- To better characterize the loop, we must understand ecological interactions between major players (including microzooplankton and their prey)
- Current method to quantify microzooplankton grazing, the dilution method¹, is unreliable and difficult to execute

Figure 1: A simplified view of nutrient recycling in the microbial loop

Methods

Dilution Method

- Difficult to carry out: requires large amount of filtration which is time consuming
- Highly manipulative of seawater: water chemistry changes when cells are lysed during filtration
- Inherently questionable results: calculations rely on cell counts of most dilute samples where there may be very few cells, leading to high variation

Saturation Approach

- Less manipulative of seawater: water chemistry is less affected by input of relatively inert particles
- Much simpler protocol: only requires addition of surrogate prey
- Suitable for flow cytometry: since communities are never diluted out they are easy to count reliably using flow cytometers

Surrogate Prey

- Multiple surrogate prey were evaluated and used (fluorescent latex beads, GFP-labeled bacteria, PLGA beads)
- Necessary characteristics of surrogate prey:
 - Remain monodispersed over period of experiment
 - Reasonably similar to target prey
 - Easily isolated and counted using flow cytometry

Flow Cytometry

- Concentrations of predator, natural prey, and surrogate prey were determined at three or more timepoints using either a BD FACScan or BioRad ZES cell analyzer
- Collected light scatter and fluorescent properties for each cell

Figure 3: Lab setup with fluorescent beads

Theoretical Model Growth Curve

Example Field Experiment

Experimental Design

Figure 2: Experimental Design

Results

Figure 4: Results

Discussion and Conclusions

- The saturation method successfully decoupled grazing from natural prey growth in both laboratory and field experiments
- There was more variability in the field results due to adaptation to a different flow cytometer (carryover), and variable incubation conditions, especially water temperature and light intensity
- Further experimentation will include:
 - Comparing saturation approach results to the dilution method
 - Testing method with other types and sizes of surrogate prey
 - Replicate field and lab experiments with different predator and prey communities

Laboratory and Field Experiments

- Initial experiments were carried out in the lab using monocultures of both predator (*Oxyrrhis marina* or *Ochromonas danica*) and prey (*Micromonas pusilla*), and one of several surrogate prey
- The method was adapted to field samples collected from six stations aboard RV Endeavor research cruise EN-616 (July 3-15 2018)
- Latex fluorescent beads (1 or 2 µm) were added to 0.5 L samples of 200 µm pre-screened water which were then sampled and incubated for 24-hours
- Water samples were collected from a variety of ecosystems and water conditions (coastal, slope water, and Sargasso Sea)

Figure 4: Sample sites for field experiments

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Acknowledgements

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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 CO₂. Approximately 40,000 Billion Tonnes of CO₂ 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.

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

Henry Arndt¹
 Mentor: Dr. Michael Lomas^{2,3}, Dr. LeAnn Whitney²

How is the vertical transport of picoplankton in the biogeochemical carbon pump influenced by their cellular density as opposed to their aggregation?

Motivation

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 CO₂. According to scienteseearch.org, 40,000 Billion Tonnes of CO₂ 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. Marine phytoplankton are so important, that 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 sink very slowly and 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 is designed to investigate the effects of the density of an individual picoplankter (< 3 μm) on its sinking rate 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.

Results

Carbohydrates:

Proteins:

Standard Curve

Conclusions and Tasks to Accomplish to Enhance the Research

- ★ Carbohydrates and proteins are **each more dense during the exponential growth phase**
- ★ The density of the plankter particle is consistently greater in the **exponential growth phase** than the stationary phase
- ★ The **radius of the particle impacts the sinking rate** more than the **particle's density** compared with the ambient density of seawater
- ★ Plankton in the **stationary growth phase** are **larger** than exponentially growing plankton, so they sink **more rapidly and consistently**

Future Work:

- Incorporate DNA/RNA
- Take samples from field as opposed to culturing
- Look at the increased carbon to phosphorus ratios in Oligotrophic and Eutrophic Gyres

Methods Model:

Discussion

Arriving at the project, I had no experience with chemistry other than introductory courses. As a result, biogeochemistry's finicky nature had an effect on my research. I had to learn from the ground up all about chemical assay process. Even though I knew about how to use the electromagnetic spectra to my advantage, I found it bizarre at how the sample spectra translated to the legitimate spectra. However, using the slopes and intercepts of the standard curve, I was surprised at how consistently both the carbohydrates and proteins in the exponential phase outnumbered their counterparts in the stationary phase. This makes sense considering proteins are needed to grow, but few are needed in the static growth phase and none are needed in death. However, Dr. Lomas's research in Bermuda through BATS informed me that there is more representation of particulate matter in the stationary phase. As a result, I was able to derive my conclusions. Even though the density of particles in the exponential phase is greater, the particulate matter in stationary phases tends to have an increased diameter considering their growing is finished and their saturation in seawater. Mathematically, the radius is squared in the Navier-Stokes equation while the difference between the ambient density of seawater and the density of the particulate matter is to a factor of one. That led me to the conclusions.

Please Visit:
<https://www.google.com/maps/@40.228444,-105.054444,15z>
<http://www.frontrangecc.edu>
<http://www.ncma.gov>

For more information, please contact me at arndt@student.frc.edu
 It would be my pleasure to speak more about this amazing project.

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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

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Abstract

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.

Background

- Seasonal algal blooms in the Southern Ocean surrounding Antarctica are dominated by DMSP-producing microalgae¹ (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)²

Methods

- Environmental samples were collected from the Western Antarctic Peninsula and incubated in an Ecostat experimental chamber with varying DMSP concentrations
- dddL* endpoint PCR products were cloned to develop qPCR standards
- Primer set: *dddL F*: CTGGGAATCGGCTACGAGA
dddL R: GTTCAAGATCAGCATCCGG (234 bp)³
- 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)

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Results

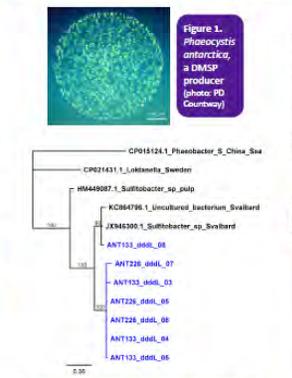


Figure 1. Phylogenetic tree of *dddL* clones and organisms known to possess *dddL* genes.

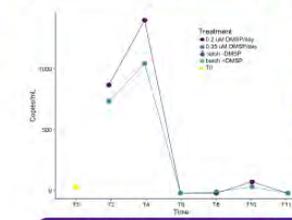


Figure 3. Copies of *dddL* per milliliter of seawater for unknown samples over the course of the incubation. Values determined using *dddL* qPCR standard curve.

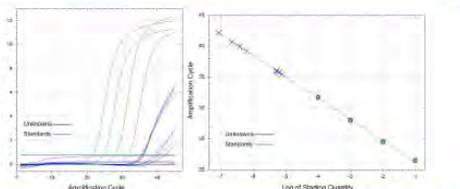


Figure 2. Amplification of *dddL* standards and unknowns (right) and *dddL* standard curve with plotted unknowns (left) to estimate gene copy numbers in seawater.

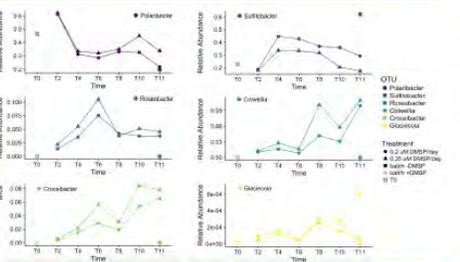


Figure 4. Mean relative abundance of the five most abundant operational taxonomic units (OTUs) and *Glaciecola* over the course of the incubation as determined by next-gen sequencing.

Acknowledgements

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

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 consumer⁴ *Glaciecola spp.* in GenBank

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Haloperoxidase-mediated gas exchange in two diatom species

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Brominated volatile organic compounds (BVOCs) are produced abundantly by marine algal enzymes called bromoperoxidases (BPOs).¹ Importantly, BVOCs and BPOs influence Earth's biogeochemical cycles as major sources of atmospheric halogens, which affect atmospheric chemistry including ozone depletion.² BPOs physiological role within microalgae, primarily diatoms, is thought to involve oxidative stress response, cell signaling, and allelopathy.¹ In diatoms, previous studies have observed BPO activity and BVOC production, but there is a lack of empirical data that tracks them simultaneously.^{3,4,5} 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 fmol cell⁻¹ day⁻¹). On the other hand, *Porosira* exhibits high bromoperoxidase activity (8.54x10⁻⁷ UN enzyme/cell, Sigma-Aldrich B2170-10UN standard) and an order of magnitude less BVOC production than *Porosira* (maximum 0.0034 fmol cell⁻¹ day⁻¹). To understand this relationship, we enriched 10 µm 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.

Haloperoxidase-mediated gas exchange in two diatom species

Brycki JD^{1,2}, Posman KM¹, Archer SD¹ ¹Bigelow Laboratory for Ocean Sciences, ²Juniata College

Key finding: A paradox exists between haloperoxidase activity and BVOC production within laboratory cultures of *Porosira glacialis* (CCMP 651) and *Ditylum brightwellii* (CCMP 358)

Introduction

Background

- Brominated volatile organics (BVOCs) are produced by algal bromoperoxidases (BPOs)¹
- BVOCs cycle halogens into the atmosphere. Atmospheric halogens are known to cause ozone depletion and effect atmospheric chemistry²
- BPOs' possible roles include oxidative stress responses, cell signaling, and allelopathy; diatoms are the main producers of BPOs⁶
- There is a lack of empirical data that tracks both BVOC production and BPO activity simultaneously in diatoms^{3,4,5}
- Studies, including our own, emphasize bromoform (CHBr₃) production because it is the most abundant BVOC found to be produced by diatoms. We found this to be true in our experiments too.

Table 1. Previously reported bromination and BVOC production rates. These studies all occurred over the course of incubation in living cells. However, variables like temperature and culture size differ between studies

Reference	Species	Measurement	Units	Value Range
Hill and Whitney 2009 ³	<i>Porosira</i> (CCMP 651)	Bromination Rate	fmoles HCB cell ⁻¹ h ⁻¹	1.76x10 ⁻²
Tokarczyk and Moore 1994 ⁴	<i>Porosira</i> (CCMP 651)	BVOC Production	pico bromoform cell ⁻¹ day ⁻¹	8.89x10 ⁻² to 1.49x10 ⁻²
Shibasaki et al. 2016	<i>Ditylum</i> (CCMP 358)	BVOC Production	pico bromoform (µg cell ⁻¹ h ⁻¹)	0.234 to 0.237

*No statistical difference was seen in exponential vs lag phase. **They found about 1/4 the production rate in lag phase vs exponential phase. Numbers reported are from the exponential phase.

Objectives

1. Track BPO activity and BVOC production in two diatom species: *Porosira glacialis* (CCMP 651) and *Ditylum brightwellii* (CCMP 358)
2. Better understand the role of BPOs microalgae

Hypotheses

Null: Bromoform production will be proportional to BPO activity in diatoms

Alternative: *Porosira* and *Ditylum* have unique and distinct BVOC-BPO relationships due to different physiological adaptations to living in polar vs temperate environments respectively. Different BVOC production rates support this hypothesis in Table 1.

Results Experiment 1

Figure 1. Cell density, bromoform production, and BPO activity over the course of incubation

Row 1: Growth curve comparison between *Ditylum* and *Porosira*. Population densities of the two species were fairly comparable.

Row 2: Bromoform concentration comparison: Notice that *Ditylum* produces three orders of magnitude greater a concentration than *Porosira*.

Row 3: BPO activity comparison. Notice that *Porosira* is two orders of magnitude more active than *Ditylum*.

Results Experiment 2

Figure 3. Bromoform decomposition after 48 hours. Values are % difference between T₀ and T₄₈ in bacterial (< 10 µm filtered) vs non-bacterial samples (0.2 µm filtered). Average bacterial decomposition: 13.3% non-bacterial: 9%

• No statistical difference was found in decomposition rates between bacterial and non-bacterial samples of *Ditylum*, *Porosira*, or as compiled data (above)

Discussion

We reject the null hypothesis, as the data support the notion of a unique and distinct BVOC-BPO relationship within individual species (alternative hypothesis). This could be caused by:

1. Differences in bromination efficiencies with DOM between species
2. Substrate specific BPO catalysis resulting in non-volatile brominated compounds produced by *Porosira*
3. Failure of APF assay to detect actual activity (ex. due to inhibition of BPO or competition of BPO with catalase for hydrogen peroxide)
4. Different algal decomposition rates
5. Non-BPO synthesis mechanisms such as nucleophilic exchange or methyl halogenases
6. Physiological processes driving bromoform production, such as competition

Future Directions

- Repeat experiment under different environmental conditions such as light stress.
- Partially purify the enzyme
- Search for different, potentially non-volatile halogenated compounds in *Porosira*
- Perform algal bromoform uptake experiment

Conclusion

Ditylum shows low BPO activity and high BVOC production. *Porosira* exhibits high BPO activity and low BVOC production. This is a paradox because BVOCs are a byproduct of BPO activity. We have shown that this is not caused by differences in bacteria. However, there are a number of physiological mechanisms that could lead to these differences. I decomposition rates. These conclusions supports the notion that diatoms cannot be generalized as one group for their role in halogen cycles, and that BPOs may serve a diverse function from species to species.

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I sincerely thank Dr. Archer, Kevin Posman, Dr. Fields, Dr. Matrai, Dr. Yadavalli, and Dr. Bhattacharjee for support and mentorship. Additionally, I thank the NIH REU program and all Bigelow staff and donors who made this possible.

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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.

The Role of Sea Ice Extent on Phytoplankton Phenology in the Greenland Sea

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Abstract

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.

Introduction

The Nordic Seas area (Fig. 1), situated in the North Atlantic Subarctic region, acts as the main gateway for water entering and leaving the Arctic Ocean. Deep-water formation that controls the world oceans deep circulation also occurs here¹. As a consequence, phytoplankton are exposed to a wide range of temperature, irradiance, nutrient supply, sea ice extent, stratification and mixing regimes. These abiotic factors vary in time and space (i.e. over the seasons and with latitude), and influence phytoplankton growth.

The different basins present in the Nordic Seas delineate areas of vertical mixing (within gyres, GS, Norwegian Sea, Iceland Sea, and Lofoten Basin) and lateral exchange (between interior and boundary currents)¹.



Figure 1. Map of the Nordic Seas region with major gyres and boundary currents. East Greenland Current (EGC), Deep Western Boundary Current (DWBC), Norwegian Atlantic Current (NWAC), Icelandic Inflowing Current (IIC), Blue arrows indicate cold water coming from the Arctic Ocean, Red arrows indicate warm water coming from the N. Atlantic Ocean. (Created by WHOI)

The East Greenland Current carries polar surface water from the Arctic along with drift ice into the GS. This drift ice, along with local ice melt, makes up a major source of freshwater for this area. Position of the ice edge influences wind-driven upwelling and downwelling, processes crucial to the movement of nutrients in the water column, the water column stability² and phytoplankton growth. Phytoplankton phenology (i.e. temporal variability of primary producer biomass) is thus ultimately controlled.

This study aims to characterize the patterns of phytoplankton phenology in the Nordic Seas, and present hypotheses for environmental factors that structure them in the GS, for now, by using remotely-sensed [Chl-a] data as a proxy for phytoplankton biomass.

Approach & Data

Data

- Chlorophyll-a Concentration ([Chl-a]), Sea Surface Temperature (SST), Sea Ice Extent (SIE)
- 25 km resolution, 8day bins, 1998-2017

Building Datasets

- Data Interpolating Empirical Orthogonal Functions (DINEOF)
- 20-Year Climatological dataset
- Annual dataset (x20)

Clustering Methods

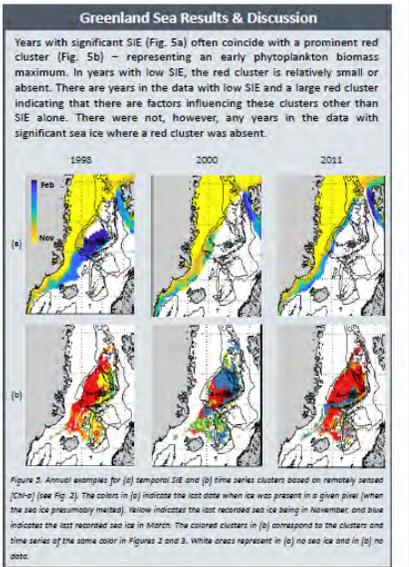
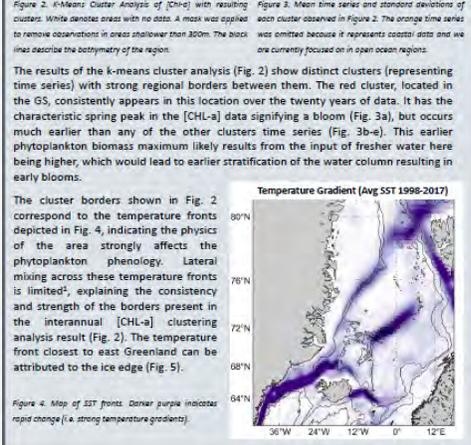
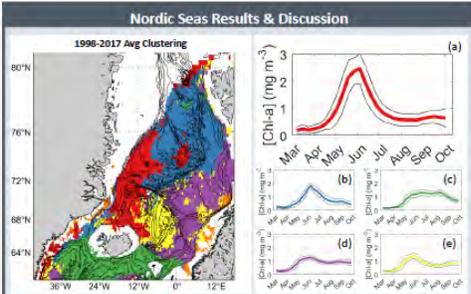
- K-Means Clustering Analysis
- Fuzzy C-Means Analysis

[Chl-a] data from satellite sensors (GloBColour's merged ocean color data) was processed using the DINEOF method to reconstruct missing data (from cloud cover) in the satellite images³. Due to the polar night this region experiences, the data is limited to between March and October.

The SST data was used to find temperature fronts in the region and the sea ice concentration data was used to quantify the regional SIE.

The first analysis used a k-means cluster method⁴ with the climatological 20-year dataset to characterize the spatio-temporal variability of [Chl-a] into clusters representing patterns of phytoplankton phenology.

A second analysis was done using the Fuzzy C-means⁵ method to study the interannual variability of the clusters. The Fuzzy C-means method assigns each pixel a membership value (the likelihood that the pixel belongs to a cluster) for each of the clusters.



Conclusions

From these preliminary results, we can conclude that there are well defined patterns of phytoplankton phenology as quantified by [Chl-a], in time and space over the past 20 years (1998-2017) in the Nordic Seas (Fig. 2). SIE may have an influence on the size, location, and timing of the observed pattern of the early phytoplankton bloom in the GS (Fig. 5). Other factors affecting the stratification of the water column are also likely contributing to the appearance of this early-bloom pattern in the GS. Future analyses should include, for example, regional wind stress, effects and timing of Arctic SIE changes, and planetary atmospheric forcing. Moving forward, we will combine in situ observations of Argo floats (e.g. mixed layer depth, nutrients, etc.) in the region of interest to reveal patterns in the phytoplankton phenology not readily visible with remotely sensed [Chl-a] data.

Acknowledgements

- Thanks to the National Science Foundation for its support under NSF Grant 1460061
- Thanks to Bigelow Laboratory for Ocean Sciences for hosting this Undergraduate Research Experience in the Gulf of Maine and the World Ocean
- Thanks to GHRST, the US National Oceanographic Data Center and NOAA Climate Data Record (CDR) Program for the sea surface temperature data⁷.
- Thanks to National Snow & Ice Data Center and NASA for use of the NSIDC-0051 dataset.

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

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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.

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Unsuspecting Players in the Dissolved Organic Phosphorus Pool: Phosphonates and Eukaryotic Phytoplankton

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Bigelow Laboratory for Ocean Sciences

Introduction

Phosphorus (P) is an essential macronutrient

Phosphate (Pi) is the preferred form of P, however, in the oligotrophic North Atlantic (NA), Pi levels are chronically low (Fig. 1)

In the oligotrophic NA, dissolved organic phosphorus (DOP) fuels primary production (PP) (Fig. 2)

- Major DOP pools³: Phosphorus esters (80%)
- Polyphosphates (10%)
- Phosphonates (PHNs) (10%)

Few studies have investigated the ability of eukaryotic phytoplankton to use PHNs as their sole P source

- Isochrysis galbana* was found to grow on glyphosate, a chemically synthesized PHN⁴
- Alexandrium pacificum* uses hydrolyzed Pi in the presence of bacteria utilizing PHN⁵

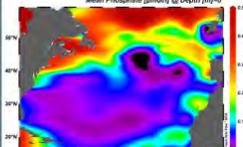


Fig. 1. Average Sept. surface phosphate concentrations (data from WOCE).

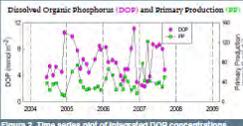


Figure 2. Time series plot of integrated DOP concentrations and primary production⁶.

We investigated the growth and physiological response of eukaryotic phytoplankton using phosphonates as the sole phosphorus source

Can eukaryotic phytoplankton use natural phosphonates as a P source?

CC(=O)OC(=O)C

Methylphosphonate (MPN)

NC(=O)OC(=O)C

Aminoethylphosphonate (AEPN)

Figure 3. PHN compounds found in the marine environment and used in this study.

PHN utilization is shown by increased growth compared to Pi limited

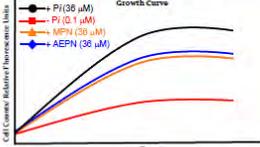


Figure 3. Growth Curve showing cell counts for Pi (36 μM), Pi (0.1 μM), MPN (36 μM), and AEPN (36 μM).

How does cellular phosphorus change in *Isochrysis galbana* utilizing phosphonates?

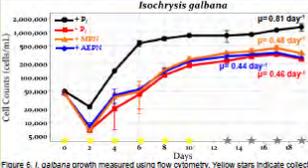


Figure 6. *I. galbana* growth measured using flow cytometry. Yellow stars indicate collection for cellular P. Green boxes and gray stars indicate measurements taken but results are not shown.

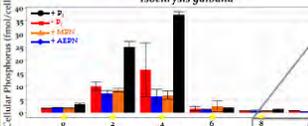


Figure 7. Changes in *I. galbana* cellular P over time. Days 8 and 10 are displayed in inset for clarity.

Utilization of PHNs widespread among eukaryotic phytoplankton, but not universal

PHNs also support growth of *Aureococcus anophagefferens*, *Emiliania huxleyi*, *Micromonas pusilla*, *Ostreococcus lucimarinus*, *Thalassiosira pseudonana*

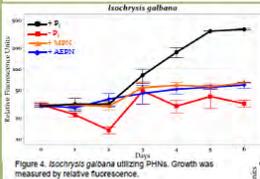


Figure 4. *Isochrysis galbana* utilizing PHNs. Growth was measured by relative fluorescence.

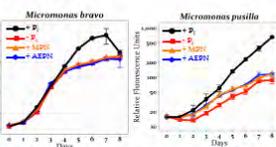


Figure 5. *M. bravo* unable to utilize phosphonates while *M. pusilla* shows enhanced growth under PHN conditions. Growth was measured by relative fluorescence.

Does *E. huxleyi* benefit from bacteria utilizing phosphonates?

Emiliania huxleyi (Axenic)

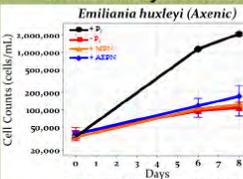


Figure 8. Axenic *E. huxleyi* growth measured using flow cytometry.

Emiliania huxleyi (With Bacteria)

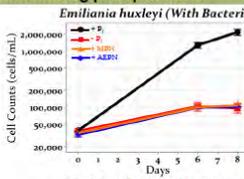


Figure 9. Axenic *E. huxleyi* growth measured using flow cytometry.

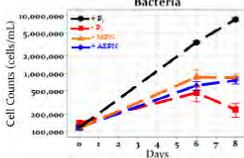


Figure 10. Growth of bacteria population in *E. huxleyi* measured using flow cytometry. Bacteria were counted with nucleic acid stain.

Conclusions and Implications

Growth studies suggest some eukaryotic phytoplankton are able to use PHNs as their sole P source

Growth on PHNs reduced cellular P in *I. galbana*

- Cells may be decreasing polyP pools; future experiments will explore this

E. huxleyi does not benefit from bacteria utilizing PHNs; different results seen in dinoflagellates⁵

This study expands our understanding of P cycling and availability

- As oceans warm and oligotrophic regions expand⁸, the impact of PHNs may increase
- Identifying all of the players able to use PHNs may inform the make-up of future oceans

References and Acknowledgements:

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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.



Hamilton

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

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Bigelow | Laboratory for Ocean Sciences

Abstract

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. It has been found that crude oil, composed of alkanes and PAHs, can be biodegraded by marine microbes. This naturally-occurring process has been utilized as a method for oil spill remediation. This study focused on whether the oxidized hydrocarbon photo-products can also be biodegraded by marine microbes. Microcosms were created containing marine bacteria and oil in artificial seawater. These samples were incubated for 0, 7, and 14 days. Oxygen sensing was done in order 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. 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 will be done to determine to what extent the oxidized hydrocarbons can be biodegraded and which bacteria populations are biodegrading the oil.



Background

There have been 592 oil spills since 1990 resulting in 1,377,000 tons of oil in the marine environment¹. The presence of this oil in the environment can have huge ecological effects, which can then damage the economy and human health.

Biodegradation of Oil
Crude oil is made up of alkanes and polycyclic aromatic hydrocarbons (PAHs). It has been found that marine microbes can biodegrade this oil when the oil is in a marine environment. This process has been utilized as a method for cleaning up oil spills.²

Photo-Oxidation of Oil
One of the major discoveries from the 2010 Deepwater Horizon Oil Spill was that crude oil underwent photo-oxidation. During this process, over 50% of the oil that formed slicks on the surfaces was being converted from alkanes and PAHs to oxidized hydrocarbons (OxHC).³⁻⁵



Will oxidized hydrocarbons from oil spills be biodegraded by marine microbes?

Methods

Experimental Design

A. Marine Bacteria
A. Marine Bacteria was collected from seawater from the Democratic Rives Estuary on a 0.2 µm filter. This filter was added to the microcosm.

C. Artificial Seawater
Artificial seawater was used in order to limit background dissolved organic carbon (DOC) present in the water. This made the oil samples the only source of carbon within the microcosms.

1. Unfractionated Oil
Microcosms were created in 10 mL airtight vials. These samples were incubated in these microcosms for 0 days, 7 days and 14 days.

B. Oil Samples were fractionated on silica gel. The oil samples added to the microcosms were:
1. Unfractionated Oil
2. Hydrocarbon Fraction (Alkanes and PAHs)
3. Oxidized Fraction

Data Collection

Oxygen Sensing:
• Measured oxygen levels → indicates level of cellular respiration
• Oxygen replenished when samples showed low levels of O₂

Flow Cytometry:
• Measured bacteria concentrations → indicates bacterial growth

Carboxylic Acid GC/MS:

• Measured the change in abundance of specific compounds within the complex oil mixture
• Carboxylic acids used as a proxy for oxidized hydrocarbons

Results

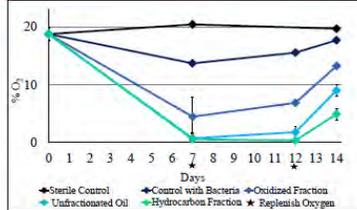


Figure 1: The mean of the % O₂ for samples with oil and controls over 14 days. The stars represent times that the oxygen was replenished within the microcosms. The results show that O₂ was consumed at a higher level in the oxidized fraction samples compared to the control with bacteria, however it was consumed at a lower level compared to the hydrocarbon and unfractionated samples.

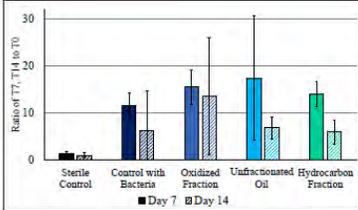


Figure 2: The mean of the amplification of the bacteria concentration compared to the Day 0 bacteria concentrations within the samples with oil and control. There is no significant difference between the control with bacteria and experimental values; however, the oxidized fraction consistently has a high level of mean amplification.

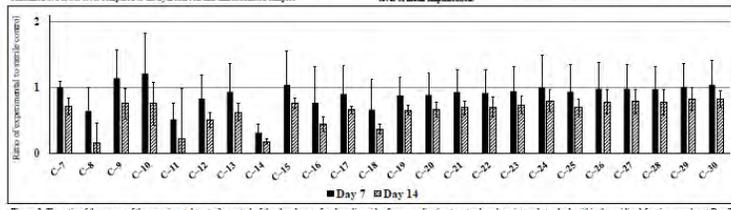


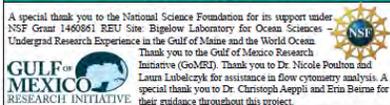
Figure 3: The ratio of the means of the experimental to sterile control of the abundance of carboxylic acids after normalization to n-octadecanoic acid, an internal standard, within the oxidized fraction samples at Day 7 and Day 14. Each pair of peaks represents the abundance of carboxylic acids with the given numbers of carbons. When this ratio is < 1.0, this indicates a drop in the abundance of that compound within the sample. The values above one (C-9, C-10, C-15, C-30) all have relatively low concentrations within the OxHC, which can account for the apparent increase in the level of these compounds.

Conclusions

- Shows preliminary evidence that OxHC can be biodegraded by marine microbes
- The decrease in oxygen was greater for the OxHC samples than it was for the control with bacteria
- Although there was no significant difference in the amplification of cell concentrations, the OxHC samples had a higher mean amplification than the control with bacteria at each timepoint
- The GC/MS data for carboxylic acid shows a clear decrease for the compounds that are most bioavailable (fewer carbons with a high abundance), while larger compounds show little decrease

Acknowledgments

A special thank you to the National Science Foundation for its support under NSF Grant 1460861 REU Site: Bigelow Laboratory for Ocean Sciences Undergrad Research Experience in the Gulf of Maine and the World Ocean. Thank you to the Gulf of Mexico Research Initiative (GoMRI). Thank you to Dr. Nicole Poulton and Lauren Lubczyk for assistance in flow cytometry analysis. A special thank you to Dr. Christoph Aeppli and Erin Beirne for their guidance throughout this project.



15

Currently, there are no site-specific forecasts of paralytic shellfish poisoning outbreaks in Maine. Recent mechanistic models operate on larger time and geospatial scales¹. 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 costs², 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.



Shellfish Toxicity Forecast in the Gulf of Maine using Neural Networks

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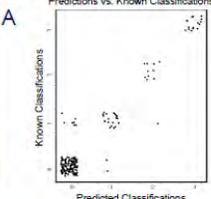


Abstract

Currently, there are no site-specific forecasts of paralytic shellfish poisoning outbreaks in Maine. Current models operate on larger time and geospatial scales¹. Given the impacts of paralytic shellfish poisoning and harmful algal blooms on Maine's economy in our tourism, restaurant, and aquaculture industries, not to mention the public health impacts², site-specific forecasting tools such as neural networks could aid management and monitoring. Machine learning and other powerful technological tools are being increasingly utilized across many industries such as government organizations, marketing teams, and in science as well. Neural Networks, a machine learning tool, is an extremely powerful predictive 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 the Gulf of 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 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. Compared with simpler methods, the neural networks was a superior predictive tool, suggesting this algorithm is worth analyzing for other ecological forecasts.

Forecast Accuracy and Length

Predictions vs. Known Classifications



Weeks Ahead vs. Accuracy

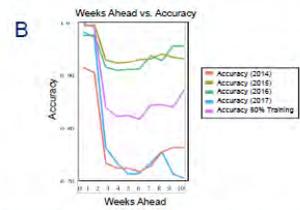


Figure 3: Predicted classifications compared to given classifications using 2014-2016 toxicity data to train the neural network and make predictions of 2017 toxicity levels. There are no errors at the closure level, with much of the variability in the lower classifications (A). The data were split up in a variety of ways to test how many weeks in advance a forecast could be made. *Accuracy (2014)* means that the rest of the data was used to train the algorithm to predict 2014 closures. Accuracy dips abruptly after two weeks for each data split (B).

Approach & Data

We utilized data collected by the Department of Marine Resources at various harvesting sites on the Maine coastline and screened for twelve different toxins. We then used the Keras neural networks algorithm to build our forecast using image recognition. This forecast predicts the magnitude of toxicity as well as timing of closure of the tested harvesting sites on Maine's coastline. Both toxicity data and sea surface temperature were fed to the neural network. These methods were then measured on several metrics and compared to simpler statistical methods of forecasting.

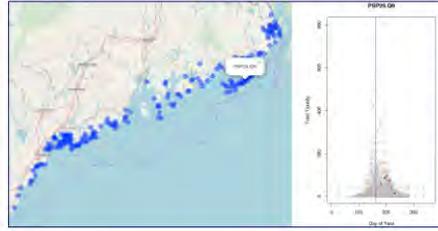
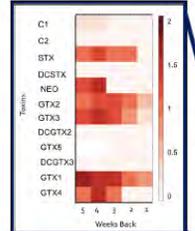


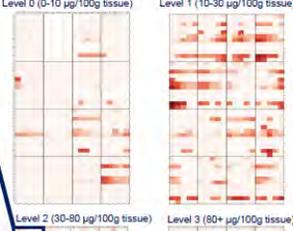
Figure 1: Shellfish toxin data collected by Maine Department of Marine Resources, analyzed by Bigelow Laboratory (2014-2016), visualization from an interactive web tool. The blue dots represent sampling stations. The blue line in the figure to the right represents the current day of the year; the red dots represent the present data for that location, and the gray dots are all data for that location.

Neural Networks for Image Recognition

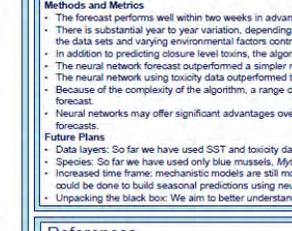
The data was structured as a list of images against their classifications. We created four classifications based on four levels of toxicity with the highest being the cut off for closure, 80 µg per 100g of shellfish tissue. Each sample at each site represented one image, containing the toxicity levels of the twelve toxins for the past five weeks, and matched up with a classification for the current sample. The 2014-2016 data was used to train the algorithm to predict the 2017 data.



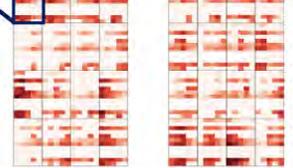
Level 0 (0-10 µg/100g tissue)



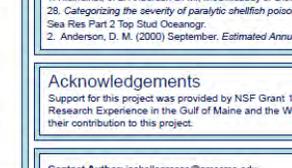
Level 1 (10-30 µg/100g tissue)



Level 2 (30-80 µg/100g tissue)

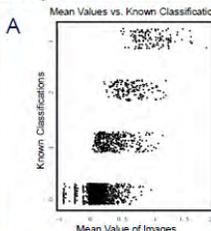


Level 3 (80+ µg/100g tissue)



Comparative Methods

Mean Values vs. Known Classifications



SST (Predictions vs. Known Classifications)

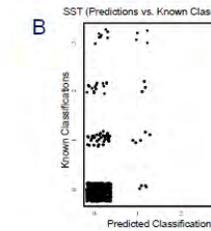


Figure 4: Mean value of images, log scaled, vs. the classifications of those images. There lacks distinct cut offs for classification based on the mean toxicity value of the image associated with that classification (A). Predicted classifications compared to given classifications using 2014-2016 sea surface temperature data to train the neural network and make predictions of 2017 toxicity levels (B).

Discussion & Future Work

Methods and Metrics

- The forecast performs well within two weeks in advance (Fig 3B).
- There is substantial year to year variation, depending on how the data is split (Fig 3B). We hypothesize that the varying sizes of the data sets and varying environmental factors contributes to this.
- In addition to predicting closure level toxins, the algorithm accurately predicts when toxic events end.
- The neural network forecast outperformed a simpler method of prediction (i.e. mean toxicity, Fig 4A).
- The neural network using toxicity data outperformed the same method using sea surface temperature data (Fig 4B).
- Because of the complexity of the algorithm, a range of configurations and metrics must be tested to determine the validity of the forecast.
- Neural networks may offer significant advantages over traditional mechanistic forecasting, particularly for short term site-specific forecasts.

Future Plans

- Data layers: So far we have used SST and toxicity data. We plan to test more data layers and combinations of data layers.
- Species: So far we have used only blue mussels, *Mytilus*, for the forecast. We plan on forecasting other species.
- Increased time frame: mechanistic models are still more useful in larger time frame and seasonal predictions. Further research could be done to build seasonal predictions using neural networks.
- Unpacking the black box: We aim to better understand the patterns the algorithm is picking up on to predict closures.

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2. Anderson, D. M. (2000) September. *Estimated Annual Economic Impacts from Harmful Algal Blooms (HABs) in the United States.*

Acknowledgements

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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 pCO₂ (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 1st 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 pCO₂) 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 pCO₂ while RI larvae showed no change. MC larvae experienced the fastest rate of weight loss at high temperature and high pCO₂. RI larvae showed no treatment effects of elevated temperature and pCO₂ 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.

Effects of Ocean Acidification and High Temperature on Metabolic Rates of Lobster Larvae

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Introduction

Abstract

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 pCO₂ (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 1st 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 pCO₂) 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 pCO₂ while RI larvae showed no change. MC larvae experienced the fastest rate of weight loss at high temperature and high pCO₂. RI larvae showed no treatment effects of elevated temperature and pCO₂ 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.

Methods

- Larvae came from Mid-Coast and Rhode Island mothers kept at the Darling Center
- For each population 200 stage I larvae were divided into color-coded pint sized jars to keep track of the mother
- Larvae were sampled every 2 days for carbon, nitrogen, lipid, OCR and measured dry weights
- Throughout this 6-day experiment we starved the larvae
- All treatments were housed in environmentally controlled chambers at 16°C and 19°C
- Ambient air at 400ppm and 1200ppm of CO₂ were pumped into their respective tanks
- Spec pH and alkalinity were done weekly
- Temperature, pH, and salinity were measured daily

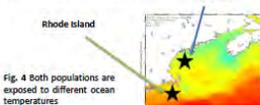


Fig. 4 Both populations are exposed to different ocean temperatures

Results

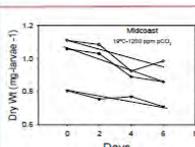


Fig. 12 The dry weights for each larvae diminished over the course of the experiment. Larvae from each mother was monitored independently. Rate of weight loss was calculated for each larvae and used to compare treatments and populations (Fig. 13)

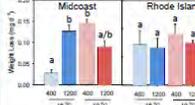


Fig. 13 Mid-Coast Larvae had a faster rate of weight loss than Rhode Island Larvae. Interactive effect of pCO₂ (400ppm) and at 19°C.

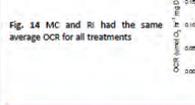


Fig. 14 MC and RI had the same average OCR for all treatments

Rising Temperatures and Ocean Acidification

Stage I lobster larvae hatch with a small lipid reserve that is used for metabolic processes until the animal first feeds. The rate of catabolism of that lipid store dictates how long the larvae will survive until it finds other energy sources



Fig. 1 Lobster larvae go through 4 stages of development. Stage I is the first step in creating adults



Figure 2 End-century predicted temperatures rise from increasing CO₂ emissions (What is Ocean Acidification, 2018)



Figure 3 CO₂ mixes with ocean water causing a more acidic ocean

- OA causes a decrease in available carbonate ions that calcifying organisms require
- Higher temperatures inhibits a lobster's immunity and reproduction (Greenhalgh, 2016)
- Climate change creates ecological and economic impacts which affect fisheries due to lobster populations moving northward (Mills, 2015)

Hypothesis: Lobster larvae under the most extreme conditions will show the most effect on their metabolic rates. However Mid-Coast larvae will have lower dry weights, carbon, nitrogen, and lower lipid content compared to the RI larvae due to the lobster already being exposed to these stressors for decades

Treatments



Fig. 5 Total Number of Tanks: 4
Number of Jars per Tank: 50
Experimental Tanks: 3
Total number of larvae: 200 for each population
Populations: Mid-Coast Maine and Rhode Island

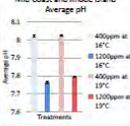
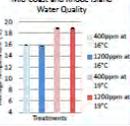


Fig. 6, 7 Show the water quality of all populations throughout the entire 6-day experiment



Measuring Metabolic Response

To measure metabolic performance, we looked at carbon, nitrogen, and oxygen consumption rates. We sampled out larvae every other day for analysis.



Fig. 8 OCR measured for 3 larvae over 75-1h

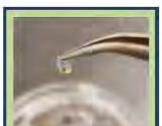


Fig. 10 Larvae were sampled for dry weight, lipid content, CHN, and OCR

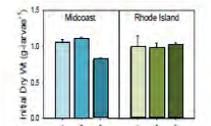


Fig. 11 Initial dry weights of Mid-Coast and Rhode Island larvae. There were no significant differences between dry weights for either population

Discussion

At present day conditions MC lobster larvae lose weight at a significantly slower rate than RI larvae. These results suggest that the MC population is less likely to consume all of their internal energy reserves prior to their first feed. The increased weight loss of MC larvae at the end of century conditions suggest an increased risk of starvation for this early feeding stage. The end of century conditions had no effect on Rhode Island larvae. The RI population may already be at its maximum metabolic rate and may foreshadow the future response of MC larvae to future conditions.

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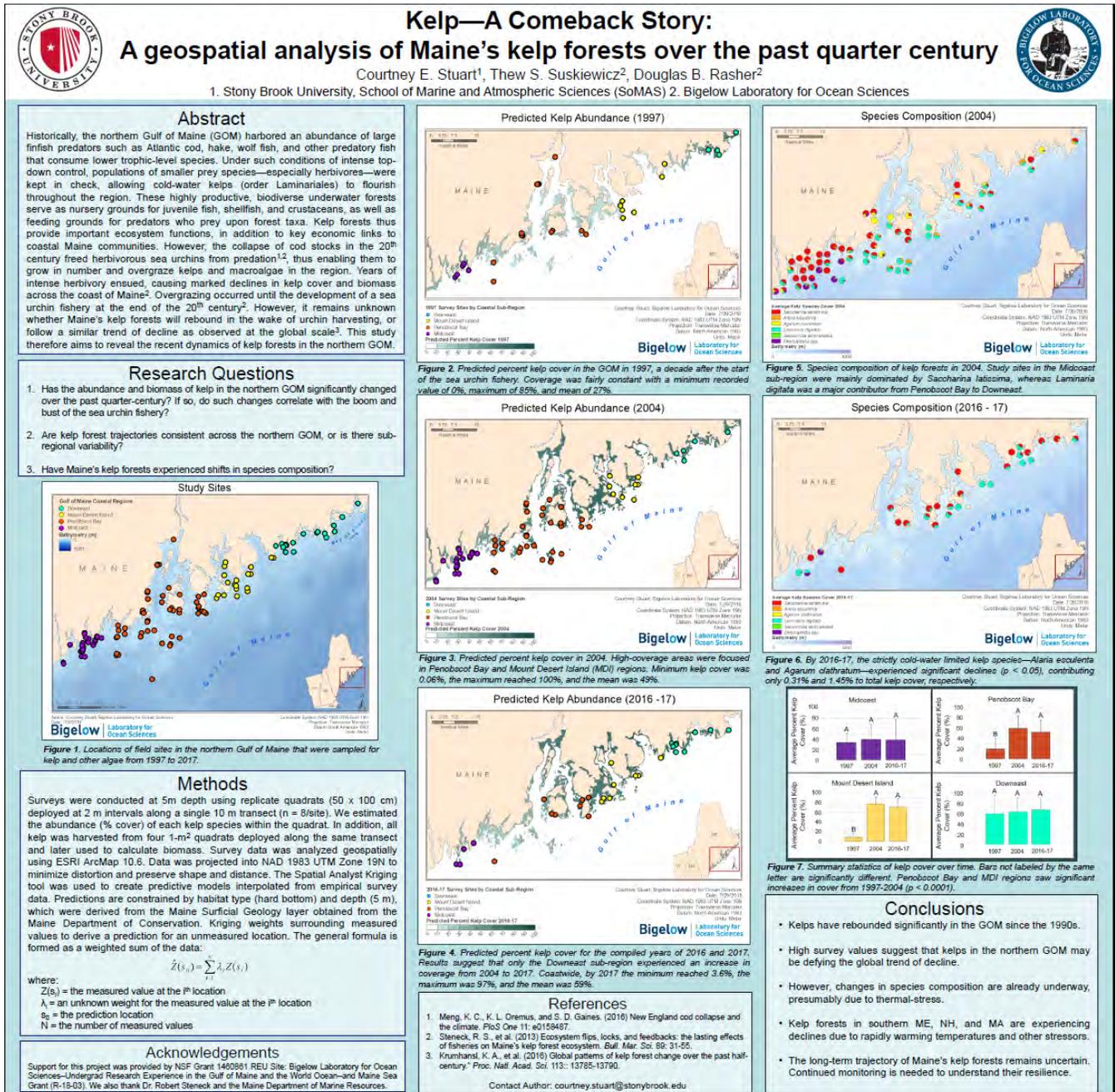
Support for this project was provided by NSF Grant 14600861 (REU Site: Bigelow Laboratory for Ocean Sciences - Undergrad Research Experience in the Gulf of Maine and the World Ocean) and a NOAA-Maine Sea Grant (program development) awarded to DMF.

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

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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 20th century freed herbivorous sea urchins from predation^{1,2}, 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 Maine². Overgrazing occurred until the development of a sea urchin fishery at the end of the 20th century². 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 scale³. 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?

Groves SL^{1,2}, Honisch B¹, Price NN¹

Bigelow Laboratory for Ocean Sciences¹, Mount Holyoke College²

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 CO₂ 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).

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¹Bigelow Laboratory for Ocean Sciences, ²Mount Holyoke College

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

Background

Ocean acidification can impact the US shellfish industry by weakening shells and disrupting larval development in hatcheries. On farm, cultivated seaweed with shellfish could remove sufficient amounts of CO₂ to mitigate acidification at small spatial scales during peak primary production. This study investigates the potential for sugar kelp (*Saccharina latissima*) to create seawater conditions more favorable for blue mussel (*Mytilus edulis*) health, development, recruitment, and growth.

Figure 1. Conceptual diagram of CO₂ transfer and phytoresmediation's localized impact on aquaculture mussel growth.

H1a: Shell Strength

Figure 4. The mean force (p=0.023, F_{3,18}=5.0, ANOVA) and acute pressure (p<0.001, F_{3,18}=37.1, ANOVA) that elicited morphological damage to the shell.

H2: Invertebrate and Parasite Recruitment

Figure 6. The kelp farm provided a more suitable habitat for invertebrate recruitment (p=0.135, F_{3,42}=4.0, ANOVA).

Figure 7. The mean amount of parasites infecting individual mussels. Mussels grown within the kelp farm had a higher number of parasite recruits (Fig. 6) (p=0.0140, F_{3,42}=4.0, ANOVA), but showed no difference in parasite abundance (Fig. 7) (p=0.5100, F_{3,36}=0.8, ANOVA).

Research Question

Does co-cultivation of blue mussels and sugar kelp confer value-added benefits to mussel production, even in today's CO₂ environment?

Hypotheses

If blue mussels are aquacultured alongside kelp, then local photosynthesis will provide a Halo region that will facilitate:

- (1) Increased mussel product quality (e.g., greater shell strength, more meat)
- (2) Increased levels of invertebrate and parasite recruitment

H1b: Mussel Growth

Figure 8. Compositional masses calculated after demineralization, which may represent a material tradeoff between CaCO₃ (p=0.0142, F_{3,42}=3.9, ANOVA) and other composites such as FeO₂ (p=0.0134, F_{3,42}=4.0, ANOVA).

Conclusions

H1a: Co-cultivated mussels exhibited significantly stronger, denser, and thicker shells when grown inside a kelp farm.

- Acute pressure required to break shells and tolerance to shattering force was 75% and 5% higher, respectively, inside the kelp farm as compared to all other sites
- Shell thickness and density were 27% and 58% greater for mussels paired with kelp

H1b: Mussels exhibited compositional differences, but all demonstrated growth.

- Mussels grown at an intermediate distance from the kelp farm (180m) contained significantly less calcium carbonate and more demineralized tissue than those grown elsewhere
- Mussels co-cultivated with kelp yielded 28% more meat than the other sites
- All mussels grew in length, width, and volume compared to initial values, but there was no difference in growth rate across treatments

H2: The kelp farm facilitated higher invertebrate recruitment than the other sites.

- Despite greater invertebrate recruitment inside the kelp farm, parasite loads in mussels were equivalent at each deployment distance
- Mussel recruitment rates were too low during the experiment for analysis

Methods

- 0.5m of 1-yr mussel lines were randomly assigned to 12 cages. Groups of 3 cages were suspended 2m deep at 0, 125, 180, and 395m from the kelp farm for 3 months
- Pressure and force thresholds for shell cracking were determined with a pulley system and pressure paper indicator dye (Fig. 3)
- Mussel morphometrics (meat mass, shell thickness and length, proportion of CaCO₃ per shell, and shell density) and recruitment (of mussels and mussel parasites) were recorded at the end of the experiment

Figure 3. The double-pulley system used to test acute pressure and force thresholds of blue mussel.

H1b: Mussel Growth

Figure 9. The mean mussel meat mass (p<0.001, F_{3,18}=35.0, ANOVA) and length (p=0.3985, F_{3,18}=1.0, ANOVA).

Implications: Good News for Aquaculturists

- The data from this experiment suggests co-cultivated mussels:
 - Can sustain greater crushing forces and larger handling damage during growth and distribution than mussels grown without kelp
 - Produce higher meat yields per mussel
 - Have thick, dense shells with greater CaCO₃ composition that may contribute to lower parasite loads
- Co-cultivation may provide a space and bio-efficient methodology for reducing marine calcifier stress now and in a more acidic ocean
- The underlying mechanism behind co-cultivative benefits – amelioration of acidification or supplemental feed from kelp detritus – has not yet been determined.

Acknowledgements

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

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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 ± 19.3 mg/g). *Alaria esculenta* and *Laminaria digitata* had total arsenic means of 60.8 ± 18.3 and 66.0 ± 31.7, respectively. Lead content was highest in *Alaria esculenta* (0.398 ± 0.281 mg/g), and lowest in sugar kelp (with an average value of 0.196 ± 0.0100 mg/g). Both *Saccharina lattissima* and *Alaria esculenta* had higher values of cadmium (mean= 2.49 ± 1.15 mg/g and 2.44 ± 0.825 mg/g, respectively) compared to *Laminaria digitata* (mean= 0.117 ± 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.

Determination of Arsenic Species in Kelp Marketed for Human Consumption Using HPLC-ICP-MS Techniques

Erika Alvarado¹ Sara Rauschenberg² Benjamin S. Twining²

Questions

- How much total arsenic and harmful inorganic arsenic are present in marketed seaweed?
- How much lead and cadmium are present in marketed seaweed?
- What percentage of total arsenic is inorganic?

Results

	Arsenate	Arsenic	Lead	Cadmium
Kelp 3232 SRM	0.247 ± 0.0190 µg/g	38.3 ± 1.30 µg/g	1.03 ± 0.0390 µg/g	0.426 ± 1.30 µg/g
This Study	0.228 ± 0.00570 µg/g	36.9 ± 4.00 µg/g	1.01 ± 0.144 µg/g	0.406 ± 0.0670 µg/g

Results for the total digestions closely matched the values in the standard reference material.

Total Arsenic Present in Four Species of Kelp

Introduction

- Seaweed is increasing in popularity as a health food
- Kelp can accumulate significant amounts of total arsenic
- Primarily inorganic forms of arsenic are carcinogenic
 - inorganic arsenic (arsenate, arsenite)
 - 3 µg/g maximum set by the FDA
- Lead and cadmium are also federally regulated and can cause toxic effects
- Three commercially important species of seaweed studied
 - Sugar Kelp (*Saccharina latissima*), Horsetail Kelp (*Laminaria digitata*), and Winged Kelp (*Alaria esculenta*)

Total As Farmed vs. Wild

Farmed samples of winged kelp had lower amounts of total As compared to wild harvested kelp
p value = .0456

Sugar Kelp on average has more total As compared to the other species of analyzed kelp

During HPLC, arsenate elutes first followed by arsenite

Methods

- Seaweed samples were gathered from the field and donated by growers/harvesters
- Total Arsenic-
 - Samples were digested in 50% HNO₃ and heated
 - Y was used as a recovery monitor
- Speciation-
 - Arsenic forms were extracted using 2% HNO₃ and then analyzed with HPLC-ICP-MS

Arsenite in Sugar Kelp and Horsetail Kelp

Arsenite, on average, is higher in horsetail kelp compared to sugar kelp

Arsenate in Sugar Kelp and Horsetail Kelp

Arsenate, on average, is higher in sugar kelp compared to horsetail kelp

Species	Average total As	Average inorganic As Combined	Percent inorganic As in total As
Horsetail Kelp	70.0 µg/g	0.458 µg/g	0.66%
Sugar Kelp	84.3 µg/g	0.959 µg/g	0.74%

Inorganic As is a small percentage of total As – below federal limits

Species	Lead	Cadmium
Horsetail Kelp	0.398 ± 0.281 µg/g	0.675 ± 0.040 µg/g
Sugar Kelp	0.196 ± 0.010 µg/g	0.287 ± 0.012 µg/g
Winged Kelp	0.381 ± 0.008 µg/g	1.28 ± 0.01 µg/g

Lead and Cadmium levels are below federal limit of 5 µg/g for Pb, and 10 µg/g for heavy metals.

Future Research

- Does location affect arsenic concentrations?
- Why does farmed winged kelp have a lower total arsenic concentration when compared to wild harvested winged kelp?
- By what mechanism does kelp accumulate arsenic?
- What are the concentrations of arsenic in water on the coast on Maine?

Acknowledgements:

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

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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 CO₂ and temperature. Extension rates in CCA were similar underneath healthy and infected host tissue, but were reduced by elevated temperature or CO₂ 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 CO₂ conditions had conceptacles free of fungal hyphae. Photosynthetic potential of the host was unaffected by CO₂ 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.

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

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Abstract

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 CO₂ and temperature. Extension rates in CCA were similar underneath healthy and infected host tissue, but were reduced by elevated temperature or CO₂ 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 CO₂ conditions had conceptacles free of fungal hyphae. Photosynthetic potential of the host was unaffected by CO₂ 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.

Figure 1: All samples used in this project were collected in Palmyra Atoll National Wildlife Refuge, which lies about 1200 miles southeast of Hawaii.

Research Questions

- Q1: How does infection by Coralline Fungal Disease (CFD) impact the ecophysiology of its calcified algal host in the face of reef degradation and rapid oceanic change?
- Q2: Does CFD have one causative agent—as opposed to a microbiome—and if so, do temperature and pH impact the types of agents present?

H1: Fate of Infected Tissue

Figure 6: Conceptual correspondence analysis demonstrating that the removal of herbivorous fish causes fungal lesions to remain infected or become turf algae.

H2: Photosynthetic Potential

Figure 10: The impact of CFD infection on phycoerythrin (PE = 10.0, ANOVA) and carotenoid (C = 0.2028, F_{2,11} = 1.66, ANOVA) concentrations in CCA.

Field Methods: Fate of Infected CCA Tissue

H1: herbivorous fish → increased likelihood of competitive overgrowth of CCA tissue that has recently undergone fungally-induced necrosis

Herbivory Exclusion Experiment

- Caged 6 active CFD lesions in situ, paired with exposed controls (Fig. 2)
- Imaged lesions at beginning and end of 14-day experiment
- **Response variables:** Determined Fate of 5 points (2 cm x 2 cm) of CFD lesion
- Characterized each point by 1 of 7 different substrate types pictured (Fig. 3)

Figure 2: Caged in situ CFD lesion.

Figure 3: Substrate types.

H2: Vertical Linear Extension Rates

Figure 7: The impact of CO₂ and temperature on vertical linear extension rates of CCA (interaction: p < 0.0001, F_{3,18} = 40.11, ANOVA).

H3: Fungal culturing

Figure 11: Fungal culturing plate containing multiple morphotypes (A). Fungal wet mount containing expected fungal hyphae and fungal spores dyed blue with Lactophenol Cotton Blue (B).

Lab Methods: CO₂ x T Effects on CCA host and CFD

H2: ocean acidification + elevated temperature + CFD infection will reduce extension rates, lower fecundity and reduce photosynthetic potential of CCA hosts.

Water Table Manipulation Experiment

- Used wild-gathered CCA fragments partially overgrown by CFD lesions (N=24)
- Subjected each fragment to full factorial CO₂ x temperature design (msd)
- Soaked fragments in calcein stain at experiment start and end
- **Response variables:**

1. Vertical Linear Extension Rates

Figure 4: Took the mean of 3 technical replicates between initial/final Calcein stain for CCA in natural and experimental samples underneath fungal infection and in healthy tissue.

2. Fecundity

Figure 5: Counted all conceptacles (reproductive structures) that fell within vertical growth ranges as a proxy for reproductive output of the CCA. Conceptacles containing fungal hyphae (A), lysed conceptacle (B).

3. Photosynthetic Potential

- Used leather hole punch to take plugs from experimental fragments—one each from healthy pink CCA tissue, active fungal lesion, and bleached CCA tissue (Fig. 1)
- Extracted photosynthetic pigments, and determined pigment concentrations using standard spectrophotometric methods

4. Fungal Culturing

H3: CFD has multiple causative agents who's presence or absence is determined by temperature and pH.

- Used water table fragments to prepare fungal cultures
- Classified different growth forms by morphology after 2-week growth period
- Prepared wet mounts with Lactophenol Cotton Blue to confirm presence of fungus

H2: Fecundity

Figure 8: The impact of CO₂, temperature and CFD infection on the presence of reproductive conceptacles in CCA (fungal presence: p = 0.0164, F_{3,18} = 6.57, two-way ANOVA).

Figure 9: Probability of penetration by fungal hyphae in relation to conceptacle distance from epithelial surface of CCA (x², p < 0.0001, logistic ANCOVA).

Figure 12: CFD is composed of a microbiome as opposed to a single causative agent

Figure 12: CFD is composed of a microbiome as opposed to a single causative agent.

Conclusions

- The simulated removal of herbivorous parrotfish and surgeonfish increased the likelihood that fungal lesions would remain infected and that CCA tissue that had recently undergone fungally-induced necrosis would be competitively overgrown by turf algae, indicating that fishing pressure can exacerbate disease severity.
- Vertical linear extension rates in the calcified algal host were not impacted by CFD infection, and responded differentially to treatment condition.
- The number of reproductive conceptacles differed between treatments, and infected CCA tissue saw a significant reduction in the amount of conceptacles.
- There was no penetration of reproductive conceptacles by fungal hyphae in the control, while there was penetration of conceptacles within the elevated CO₂, elevated temperature and combined treatments, suggesting that CFD outbreaks will have negative implications for CCA reproduction in the near-future high-CO₂, high-temperature ocean.
- Picoerythrin and carotenoid pigment concentrations varied only across tissue type, with bleached algal tissue having the lowest concentration, and healthy algal tissue having the highest.
- 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.

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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.

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

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ABSTRACT

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 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 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.

PROJECT GOALS

- To compare the data generated by the Bio-Rad ZE5 and Becton Dickinson FACScan Cell Analyzers
- To compare the preservation cell concentrations using both Glycine Betaine and 10% Paraformaldehyde solutions



The RV Endeavor, the vessel used to reach the offshore sites

RESULTS

- Phytoplankton cell concentrations determined by the BD FACScan and Bio-Rad ZE5 cell analyzers were similar for both on- and offshore samples.
- On average, the Bio-Rad ZE5 provided slightly higher concentrations of phytoplankton, especially cryptophytes in unpreserved samples, as is evident in figure 8.
- The percent cell concentration loss from GB preserved samples on the Bio-Rad ZE5 was lower compared to PFA preserved samples.
- However, the percent cell from GB preserved samples on the BD FACScan was higher compared to PFA preserved samples.
- Cryptophyte populations offshore were very difficult to preserve effectively, samples must be thawed and run immediately, the longer the sample was thawed, the greater the cryptophytes degraded.
- Across all preserved nanoplankton samples (n=38), PFA preserved samples experienced, on average, a significantly greater loss (16.41%) compared to GB preserved samples (14.23%), [p=0.016].

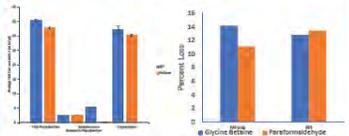


Figure 8: Cell concentrations for samples seen at the West Bluefish Islet. Change bars represent data seen on the FACScan while the blue bars represent the ZE5 Cell Analyzer.

INTRODUCTION

- Flow cytometry is a valuable and widely-used tool for distinguishing populations of microscopic particles and discerning their properties.
- Flow cytometry can quickly enumerate and provide particle properties of plankton populations between 20-2 μm in size.
- Maintaining capable, accurate, and up-to-date instrumentation is important to data continuity and for long-term data generation.
- Greater accuracy, efficiency, and capability are highly desirable, but so is the ability to compare data generated over a long period of time.
- Using a flow cytometer in a long-term timeseries provides data that can be easily compared but, as time passes, the cytometer becomes increasingly obsolete and other more advanced cytometers become available.
- The purpose of this study was to compare the Bio-Rad ZE5 to the older BD FACScan at Bigelow Laboratory.
- It is often necessary to preserve field samples for analysis, as it is difficult to transport a cytometer.
- Preserving cells, as both cell shape and fluorescence must be taken into account (2). The Facility for Aquatic Cytometry in Bigelow Laboratory customarily uses a 10% paraformaldehyde. This preservation works well, but is very toxic.
- Another preservative, glycine betaine, offers a non-toxic alternative. It is an osmoprotectant that has proven to be a competent preserver of prokaryotes during flash freezing and long-term cold storage (1).
- For this reason, the relative success of two different preservatives—paraformaldehyde and glycine betaine—for use in preserving field samples for analysis by flow cytometry were also investigated in this experiment.

Methods

- Samples were collected at a variety of onshore and offshore locations as demonstrated in figure 3 and at a variety of depths, as collected via CTD Niskin rosette as shown in figure 6.
- Sample Treatments:
 - Live and preserved nanoplankton samples
 - Samples were run in triplicate.
- Samples were analyzed on both the Bio-Rad ZE5 and BD FACScan cell analyzer. Note: in some cases both instruments were not always available, therefore, unpreserved sample comparisons across instruments were limited.
- These samples were run unpreserved on the available instrument then preserved and run on both instruments when they were available.

Preservation Techniques:

- Glycine Betaine (GB):
 - 143 μL of 48% glycine betaine added per mL of sample
 - Samples are left to preserve for >10 minutes
 - Samples were then flash frozen in liquid nitrogen and stored at -80 Celsius
- 10% Paraformaldehyde (PFA):
 - 50 μL of 10% paraformaldehyde buffered with sodium hydroxide and filtered seawater added per mL of sample
 - Samples are left to preserve for 30 minutes
 - Samples are flash frozen in liquid nitrogen and stored at -80 Celsius
- All flow cytometric data results were analyzed in FlowJo v. 10 software

ABOUT FLOW CYTOMETRY

Flow cytometry interrogates particles with one or more lasers and using the light scattered or fluorescence they emit to distinguish the different types of particles. Sheath fluid is used to focus the cells into single file. Then, the light is passed through a series of dichroic mirror filters, individual types of fluorescence or scatter are detected with specific photomultiplier tubes. All the data gathered for each particle allows us to determine the type and number of particles present. This data is then displayed as a dot plot.

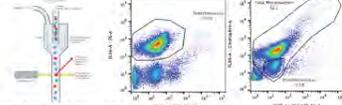


Figure 3: Hydrodynamic focusing is used to push cells into a single file (image credit to source introduction to flow cytometry web page)

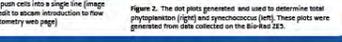


Figure 2: The dot plots generated are used to determine total phytoplankton (left) and synchrochococcus (right). These plots were generated from data collected on the Bio-Rad ZE5.

CONCLUSIONS

- The data generated by the ZE5 Cell Analyzer may be compared to that of the FACScan and be an ideal replacement instrument.
- Furthermore, as a newer instrument, the ZE5 allows for the detection of particles inaccessible to the FACScan and has less electronic "noise" in collection.
- Glycine Betaine is a comparable preservative to paraformaldehyde (the current lab standard)
- Both paraformaldehyde and glycine betaine provide variable results

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