



Bigelow Laboratory for Ocean Sciences

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

**REU Symposium Program & Abstracts
Thursday, August 15, 2013**



Program

10:00 Opening Remarks

10:15 **Yesmalie Alemán Resto** - Universidad Metropolitana, San Juan, Puerto Rico
SCREENING OF THE MMV CHEMICAL LIBRARY FOR COMPOUNDS INHIBITING THE OYSTER PROTOZOAN PARASITE *PERKINS MARINUS*.
Mentor: Dr. José Antonio Fernández Robledo

10:30 **Elizabeth M. Johnsey** - University of South Florida
QUANTIFYING DNA, RNA, AND PROTEIN CONTENT IN PHYTOPLANKTON BY FLOW CYTOMETRY
Mentor: LeAnn Whitney, Michael Lomas

10:45 **Mary E. Mathyer** - Kalamazoo College,
CARBON AND NITROGEN CYCLING BY *NITROSPINA* IN THE DARK OCEAN
Mentors: Brandon K. Swan, Ramunas Stepanauskas

11:00 **Charlotte A. Francisco** – Lewis and Clark College
THE RESPIRATION RATES OF ACARTIA TONSA IN RESPONSE TO CHANGING TEMPERATURE AND SALINITY
Mentors: David Fields, Alex Vermont, Jesica Waller

11:15 **Wilton Burns** - University of North Carolina at Chapel Hill
GROWTH AND GRAZING DYNAMICS OF PHOTOTROPHIC PROTISTS IN BOOTH BAY, MAINE
Mentor Mike Sieracki, Nicole Poulton

11:30 **Ryan Yan** - College of William and Mary
DO HUMIC COMPOUNDS INHIBIT THE AEROSOLIZATION OF CHLOROPHYLL *A* AND BREVETOXINS?
Mentors: Cynthia Heil, Steve Archer

11:45 **Break (45 minutes) *******

12:30 **Victoria Abel** – Colby College
LIGHT DEPENDENCY OF CALCIFICATION AND IMPACTS OF VARYING PH LEVELS IN THE COCCOLITHOPHORID *PLEUROCHRYSID CARTERAE*
Mentors: William Balch, Merideth White.

12:45 **Kara Voss** - RSMAS University of Miami
EFFECTS OF OCEAN ACIDIFICATION ON DMS AND DMSP PRODUCTION BY THE DINOFLAGELLATE *H. TRIQUETRIA*
Mentors: Patricia Matrai, Steve Archer

13:00 **Sean Anderson** – Bigelow Laboratory
GROWTH AND GRAZING OF *SYNECHOCOCCUS* IN BOOTH BAY, MAINE
Mentors: Mike Sieracki, Nicole Poulton

- 13:15 **Kayla Erf** – Colby College
ANALYZING THE POPULATION DIVERSITY OF *SYNECHOCOCCUS* DURING THE 2013 SUMMER BLOOM PERIOD IN THE WEST BOOTH BAY OF MAINE
 Mentors: Pete Countway, Mike Sieracki, Nicole Poulton
- 13:30 **Audrey E. Lyman** - Colby College
BIOINFORMATIC ANALYSES OF EPSILONPROTEOBACTERIA AND THE POTENTIAL INTERACTIONS WITH ZETAPROTEOBACTERIA
 Mentors: Erin K. Field, Dave Emerson
- 13:45 **Break (15 minutes) *******
- 14:00 **Campbell Belisle Haley** - University of Maine
WEAVING THE MICROBIAL TAPESTRY: THE IDENTIFICATION OF A CORE MICROBIOME IN FRESHWATER IRON SEEPS
 Mentors: Jarrod Scott, Emily Fleming, David Emerson
- 14:15 **Kim Dempsey** - Bowdoin College
GROWTH & DEVELOPMENT OF *ZETAPROTEOBACTERIA* SP. DIS-1 BIOFILM ON MILD STEEL
 Mentors: Adam Mumford and David Emerson
- 14:30 **Alice Chapman** – Williams College
REGENERATION OF FE(II) BY PROTIST GRAZING IN THE OCEAN
 Mentors: Ben Twining, Jochen Nuester
- 14:45 **Amy Duarte** - Humboldt State University
DIVERSITY OF LARGE MARINE VIRUSES IN THE GULF OF MAINE
 Mentors: Ilana Gilg, Willie Wilson

Abstracts

Light dependency of calcification and impacts of varying pH levels in the coccolithophorid *Pleurochrysis carterae*

Victoria E. Abel ^{1,2}, William M. Balch², Meredith White²¹Colby College, Waterville, ME; ²Bigelow Laboratory for Ocean Sciences, East Boothbay, ME

Coccolithophores are a group of marine phytoplankton that make calcium carbonate plates called coccoliths. The environmental factors that influence coccolith production are poorly understood; studies investigating the impact of altered pH on coccolith formation have produced conflicting results, though a link between light and calcification has been demonstrated. Here, we study the combined effects of varying light and pH levels on the species *Pleurochrysis carterae*. A plating strain of *P. carterae* grown under three different $p\text{CO}_2$ levels was bottled for 24 hours in light and dark treatments and filtered for SEM comparison of coccolith formation. A t-test was used to determine significance of light-dark differences in calcification rate and a two-factor analysis of variance was performed to analyze the combined impact of light and $p\text{CO}_2$. Our analyses revealed that coccolith production is significantly greater when the phytoplankton are exposed to light ($p < 0.001$) and when grown under $p\text{CO}_2$ ($p = 0.05$) levels of 750 ppm. No significant interactive effects between light and $p\text{CO}_2$ were found ($p = 0.05$). These results demonstrate that light and, to a lesser extent, $p\text{CO}_2$ concentration are involved in coccolithogenesis of *P. carterae*.



Light dependency of calcification and impacts of varying pH levels in the coccolithophorid *Pleurochrysis carterae*

Victoria E. Abel¹, William M. Balch², Meredith White³¹Colby College, Waterville, ME; ²Bigelow Laboratory for Ocean Sciences, East Boothbay, ME

Abstract

Coccolithophores are a group of marine phytoplankton that make calcium carbonate plates called coccoliths¹. The environmental factors that influence coccolith production are poorly understood. Studies investigating the impact of altered pH on coccolith calcification have produced conflicting results²⁻⁴, though a link between light and calcification has been demonstrated^{5,6}. Here, we study the combined effects of varying light and pH levels on the species *Pleurochrysis carterii*. Our analyses revealed that coccolith production is significantly greater when the phytoplankton are exposed to light ($p < 0.001$) and when grown under pCO₂ levels of 750 ppm. No significant interactive effects between light and pCO₂ were found ($p > 0.05$). These results demonstrate that light and to a lesser extent CO₂ concentration are involved in coccolithogenesis of *P. carterii*.

Introduction

- Ocean acidification: drop in seawater pH due to absorption of anthropogenic CO_2 from the atmosphere.
 - Poses a threat to calcifying marine organisms (i.e. coccolithophores).
- Important to understand the energy source for coccolithogenesis and stressors to coccolith formation
- This study investigates the impact of the potential stressors of light limitation and varying pH levels on coccolith calcification

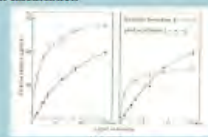


Fig. 1: Coccolith formation increased with light in two weeks old (left) and one week old (right) cultures of *Coccolithus pelagicus*.

Objectives & Hypotheses

- 1) Compare number of coccoliths produced by *P. carterae* cells growing in light and dark.
- 2) Observe coccolith production in light and dark treatments under three pCO₂ concentrations.

- H_0 : No effect of light on *P. carterae* coccolith formation.
- H_1 : There is an effect of light on coccolith formation.
- H_0 : No effect of pH on calcification rate.
- H_1 : Coccolith production will be lower under decreased pH.

Methods

- Aseptic *P. culture* (strain 645A) grown in three semicontinuous batch cultures under a 14:10 L/D cycle.
- pCO₂ adjusted to 280, 380, and 750 ppm by bubbling air/pCO₂ mixtures through CO₂-gas stores into the carboys.
- Air first passed through NaOH scrubbers to remove CO₂.
- Incubation in a temperature-controlled growth chamber with measured pH and alkalinity levels.
- Temperature controlled at 16°C
- Student's t-test used to test significance of light-dark differences.
- Two-factor analysis of variance used to analyze impact of light and CO₂ on calcification.



Fig. 2: Experiment 1 procedure. Repeated for 2000, 3000, and 5000 cases. Supplemental file for Experiment 2.

Results – Experiment 1

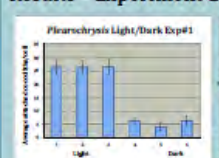


Fig. 3: A significantly higher attached coccolith count was found in in coccolithophores exposed to light (Student's *t*-test, *df*=4, *t*=27.143, *p*=0.001). The average number of attached coccoliths formed per cell in the light treatment was 26.4, whereas the average number formed in the dark was 5.8.

	Light	Dark
α_{off}	3.52	α_{off} = 3.50
α_{on}	3.58	α_{on} = 2.80
β_{off}	24.41	β_{off} = 5.82
β_{on}	5.56	β_{on} = 3.43
$\beta_{\text{off}} - \beta_{\text{on}}$	0.84	
$\alpha_{\text{off}} - \alpha_{\text{on}}$	0.76	

Results – Experiment 2

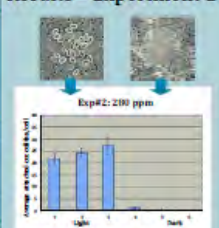


Fig. 4: An average of 24.5 attached coccoliths per cell were formed in the light treatment, and an average of 0.5 were formed in the dark.

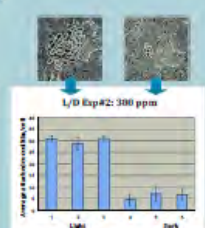


Fig. 5: An average of 30.3 attached caecotrophs per cell formed in the light treatment and an average of 5.1 were formed in the dark.

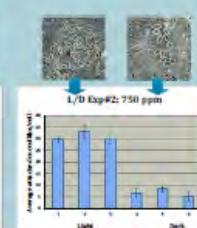


Fig. 5c An average of 30.9 attached cocoon per cell formed in the light treatment and average of 6.7 were formed in the dark.

Discussion

- Coccolith formation was previously found to occur primarily in the light in *Emiliania huxleyi* and also in *Coccolithus* spp.
- Our results demonstrate light-dependency of coccolith formation in *P. peruvianus* for the first time.
 - Some coccoliths still form in the dark, suggesting that they use some other forms of metabolic energy than photosynthesis to do this.
- Further investigation on how formation varies with changing light intensities and identification of the light threshold at which *Pleurochrysis* is no longer capable of producing plates will provide a more complete understanding of calcification bioenergetics.
- Future research on the function of coccolith plates in coccolithophores will be required to explain the trend of higher calcification in cultures raised under 750 ppm.

Conclusions

- From Exp. 1, we observed significantly higher cocolith formation in *P. ortense* cultures incubated in light as opposed to dark treatments (Fig. 3; Table 1).
- From Exp. 2, significantly higher cocolith formation was observed in cultures exposed to light and when grown under pCO₂ levels of 750 ppm (Fig. 4-6; Table 2).
 - This is opposite to what we originally hypothesized, suggesting that the cells may be compensating for high pCO₂ conditions by increasing cocolith production accordingly.
- No significant interactive effects between light and pCO₂ were found (Table 2).

References

- [illegible]

Acknowledgements

- * Thanks to the National Science Foundation for its support under NSF Grant OCE 1156740 - RIBI Site: Bigelow Laboratory for Ocean Sciences - Undergraduate Research Experience in the Gulf of Maine and the World Ocean Awarded to DMF.
- * Thanks also to NSF Grant OCE1220068 and NOAA grant #NA11OAR4310055 to D. Rheda and W. Balch.

Screening of the MMV Chemical Library for Compounds Inhibiting the Proliferation of the Oyster Protozoan Parasite *Perkinsus marinus*

Yesmalie Alemán Resto, Universidad Metropolitana, San Juan, Puerto Rico
Mentor: Dr. José Antonio Fernández Robledo
Bigelow Laboratory for Ocean Sciences, REU Program

Abstract

Perkinsus marinus is a protozoan parasite that causes mass mortality in eastern oyster (*Crassostrea virginica*) and clam populations. *Plasmodium falciparum*, the agent of malaria, is a close relative of *Perkinsus*. Based on this phylogenetic relationship, it was hypothesized that compounds that are active against *Plasmodium* might also be active against *Perkinsus*. We exposed *Perkinsus trophozoites* to the 400 compounds in the MMV library and measured the viability using ATP-based luminescence assay at days 4 and 8. We found that 77% the compounds active against *Plasmodium* are also active against *Perkinsus*. To establish the toxicity to the bivalves of the hit compounds, we attempted to establish a cell line derived from *Mya arenaria* affected by disseminated neoplasia (DN) a tumor that affect clam hemocytes. We are now expanding the cultured cells derived from clam hemocytes in order to establish their origin. In addition we are characterizing the best *Perkinsus* inhibitors by determining the EC50 of selected hit compounds. With this study we have expanded the range of compounds available for inhibiting *Perkinsus* opening the door to future treatments for diseased oysters.

Screening of the MMV chemical library for compounds inhibiting the proliferation of the oyster parasite *Perkinsus marinus*
Yesmalie Alemán Resto
Universidad Metropolitana, Bigelow Laboratory for Ocean Science
Mentor: José Antonio Fernández Robledo

Abstract

Perkinsus marinus is a protozoan parasite that causes mass mortality in eastern oyster (*Crassostrea virginica*) and clam populations. *Plasmodium falciparum*, the agent of malaria, is a close relative of *Perkinsus*. Based on this phylogenetic relationship, we hypothesized that compounds that are active against *Plasmodium* might also be active against *Perkinsus*. We exposed *Perkinsus trophozoites* to the 400 compounds in the MMV library and measured the viability using ATP-based luminescence assay at days 4 and 8. We found that 77% the compounds active against *Plasmodium* are also active against *Perkinsus*. To establish the toxicity to the bivalves of the hit compounds, we attempted to establish a cell line derived from *Mya arenaria* affected by disseminated neoplasia (DN), a tumor-like that affect clam hemocytes. We are now expanding the cultured cells derived from clam hemocytes in order to establish their origin. In addition we are characterizing the best *Perkinsus* inhibitors by determining the EC50 of selected hit compounds. With this study we have expanded the range of compounds available for inhibiting *Perkinsus* opening the door to future treatments for diseased oysters.

Introduction

Perkinsus marinus is the causative agent of "Dermo" disease in oysters and clams. Described in the early 1950s as associated with mass mortalities of the eastern oyster (*Crassostrea virginica*) on the Gulf Coast, the expansion of the *P. marinus* distribution range in the USA has been associated with global warming and the shellfish trade (Parker 1985). Recently, *P. marinus* has been identified in the Pacific coast of North America. *Perkinsus* species damage shellfish and the environment worldwide. Currently, there is no effective treatment for Dermo disease, and the alternative strategies for management of the disease, such as restricting transport of oyster stocks, have had limited success.

To address the need for identification of novel anti-*Perkinsus* drugs for both prophylaxis and therapeutic use, we tested the MMV compound library for inhibitors of metabolic pathways likely to be unique to the parasite. This rational target selection was based on the ChemoGenetics hypothesis, under which *Perkinsus* and other non-photosynthetic relatives of both drug-resistant and apicomplexans, share a phylogenetic ancestor via a secondary endosymbiosis with a red alga, raising the possibility that these lineage retain cryptic plastids. Genes involved in plastid pathways in apicomplexans have been implicated as promising drug targets (Fernandez Robledo et al. 2013).

Hypothesis and Objectives

Hypothesis:
Based on the close phylogenetic relationship between the Apicomplexans and the Perkinsus and the fact that organisms in both group evolved to become parasites, we hypothesized that compounds that are active against Plasmodium (Apicomplexans) might also be active against Perkinsus (Perkinsus).

Objectives:
• Validate the viability assay (metabolic cell number) and using a Perkinsus-resistant (Pm PRA393) that express green fluorescent protein (GFP).
• Screen the MMV compound library using a single concentration of the compound (10 µM) at days 4 and 8.
• Calculate the EC50 (concentration of the compound to produce 50% inhibition) for selected hits.
• Establish a cell line from clams (Perez et al. 1994, Hesse et al. 1995) to study the toxicity on bivalves of the compound active against Perkinsus.

Methods

Perkinsus marinus culture:
P. marinus PRA240 (white type) and PRA393 (mutant expressing GFP) were maintained in DMEM: HAM'S 1:2:5% FBS at 28°C. The culture was refreshed every other week by substituting 90% of the culture with fresh medium. For the assay cells were counted using a hemocytometer.

Disseminated Neoplasia cell culture:
The Malena Box is a resource assembled by MMV to catalyze malaria and neglected disease drug discovery and research. All of the compounds have confirmed activity against *P. falciparum*. It includes 400 diverse drug-like and probe-like compounds for use as biological tools in malaria research.

Methods

MMV screening:
• *P. marinus* trophozoites were seeded in 96-well plates at a density of 2×10^5 (90 µl).
• Ten µl of a 100 µM dilution of each compound was added to each well. A total of 5 plates (x2 replicates) (x2 sampling days) were prepared.
• Plates were incubated at 26°C.
• Plates were read at days 4 and 8.

ATPLife Assay®:
• We applied the assay following the manufacturer's recommendations (Stratagene et al. 2013).
• The viability assay based on measuring the fluorescence of *P. marinus* PRA393 was not used since it needs further optimization.

Perkinsus marinus viability assay (luminescence Assay)
Microplate Reader (Molecular Device)
Microplate Reader output

Disseminated Neoplasia cell culture
• Clam sampling site: Damariscotta (n=60) and Hapswell Cove (n=90).
• Hemolymph was withdrawn from posterior adductor muscle and examined under the microscopy for transformed cells.
• Cells from positive samples were seeded in culture medium (DMEM culture medium based on Walker et al. 2009) and incubated at 16°C.
• Cultures were examined over time for cell growth.

Results

MMV screening
Figure 1: Bar chart showing the number of compounds that inhibited Perkinsus proliferation at Day 4 and Day 8. The x-axis represents the number of compounds (1 to 400) and the y-axis represents the number of compounds that inhibited Perkinsus proliferation. The legend indicates that the blue bars represent Day 4 and the red bars represent Day 8.

Disseminated Neoplasia cell culture
Figure 2: Micrographs showing the growth of cells in the wells after the hemolymph was seeded in the culture medium. The images are labeled MA_D_10C, MAD_10C, and MAD_10B. The caption states: "Cells growing in the wells after the hemolymph was seeded in the culture medium. MAD_10C: primary culture resembles DN cells. MAD_10B cells although originated from clams appears to be neither DN cells nor cells from other tissue from the clam (magnification 200x)."

Conclusions

- Compounds active against *Plasmodium* are also active against *Perkinsus* when tested at the concentration 10 µM.
- The repertoire of compounds against *Perkinsus* was increased by more than 14 folds (203 new drug-like and probe-like compounds).
- Preliminary cultures derived from clams *Mya arenaria* were established; origin and identity of the cells remain to be determined.

Acknowledgments

Dr. José A. Fernández Robledo and Dr. David M. Fields for their continued support and efforts. Thank you for your guidance and friendship. Thanks to the National Science Foundation for its support under NSF Grant OCE 1156743 - REU: The Bigelow Laboratory for Ocean Sciences - Undergraduate Research Experience in the Gulf of Maine and the World Ocean Awarded to DMR.

References

Fernandez Robledo et al. 2013. The search for the missing link: A new paradigm in Perkinsus. *International Journal of Parasitology* 43: 1217-1228.
Hesse et al. 1995. The development of the oyster parasite Perkinsus marinus: a review. *Journal of the World Aquaculture Society* 24: 1-14.
Parker 1985. The oyster parasite Perkinsus marinus. *Journal of the World Aquaculture Society* 14: 227-237.
Parker et al. 1984. The oyster parasite Perkinsus marinus: a review. *Journal of the World Aquaculture Society* 13: 1-14.
Walker et al. 2009. Quantitative assessment of the proliferation of the peritrophic matrix (PM) in the oyster parasite Perkinsus marinus. *Journal of the World Aquaculture Society* 38: 1-14.
Walker et al. 2009. Quantitative assessment of the proliferation of the peritrophic matrix (PM) in the oyster parasite Perkinsus marinus. *Journal of the World Aquaculture Society* 38: 1-14.
Walker et al. 2009. Quantitative assessment of the proliferation of the peritrophic matrix (PM) in the oyster parasite Perkinsus marinus. *Journal of the World Aquaculture Society* 38: 1-14.

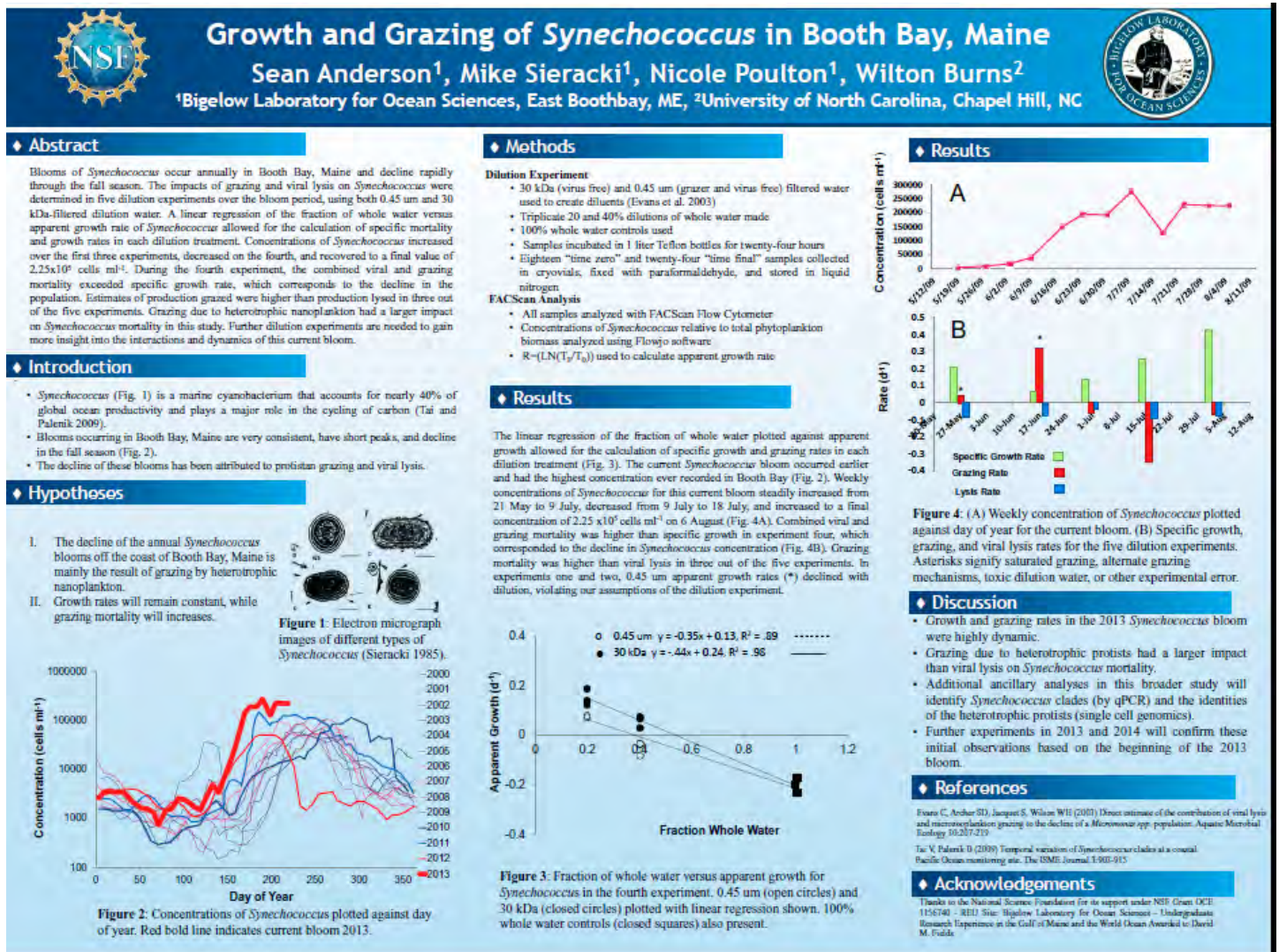
GROWTH AND GRAZING OF *SYNECHOCOCCUS* IN BOOTH BAY, MAINE

Sean Anderson¹, Mike Sieracki¹, Nicole Poulton¹, Wilton Burns²

¹Bigelow Laboratory for Ocean Sciences, East Boothbay, ME

²University of North Carolina, Chapel Hill, NC

Blooms of *Synechococcus* occur annually in Booth Bay, Maine and decline rapidly through the fall season. The impacts of grazing and viral lysis on *Synechococcus* were determined in five dilution experiments over the bloom period, using both 0.45 μm and 30 kDa-filtered dilution water. A linear regression of the fraction of whole water versus apparent growth rate of *Synechococcus* allowed for the calculation of specific mortality and growth rates in each dilution treatment. Concentrations of *Synechococcus* increased over the first three experiments, decreased on the fourth, and recovered to a final value of 2.25×10^5 cells mL^{-1} . During the fourth experiment, the combined viral and grazing mortality exceeded specific growth rate, which corresponds to the decline in the population. Estimates of production grazed were higher than production lysed in three out of the five experiments. Grazing due to heterotrophic nanoplankton had a larger impact on *Synechococcus* mortality in this study. Further dilution experiments are needed to gain more insight into the interactions and dynamics of this current bloom.

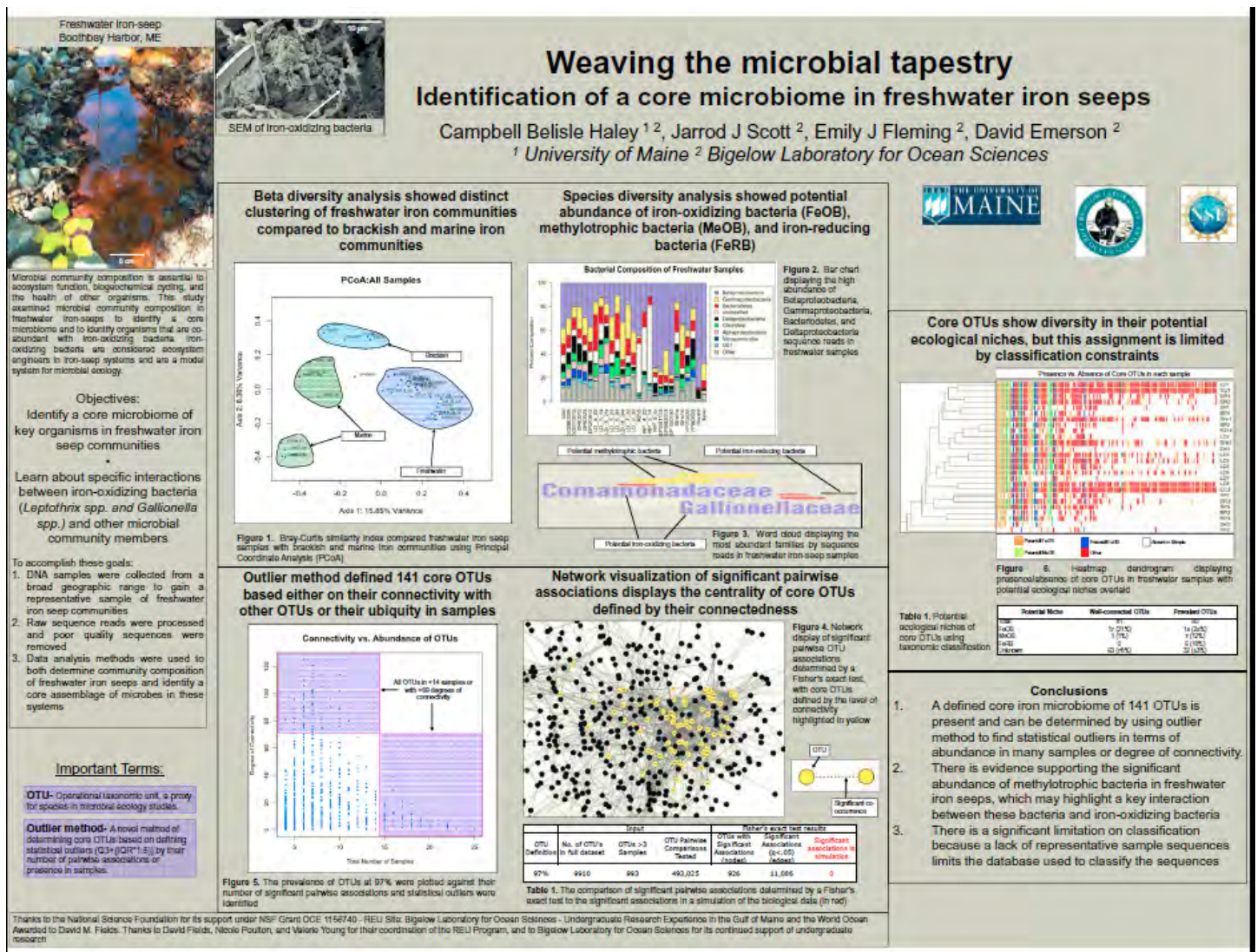


WEAVING THE MICROBIAL TAPESTRY: THE IDENTIFICATION OF A CORE MICROBIOME IN FRESHWATER IRON SEEPS

Campbell Belisle Haley^{1,2}, Dr. Jarrod Scott², Dr. Emily Fleming², Dr. David Emerson²

¹University of Maine, ²Bigelow Laboratory for Ocean Sciences

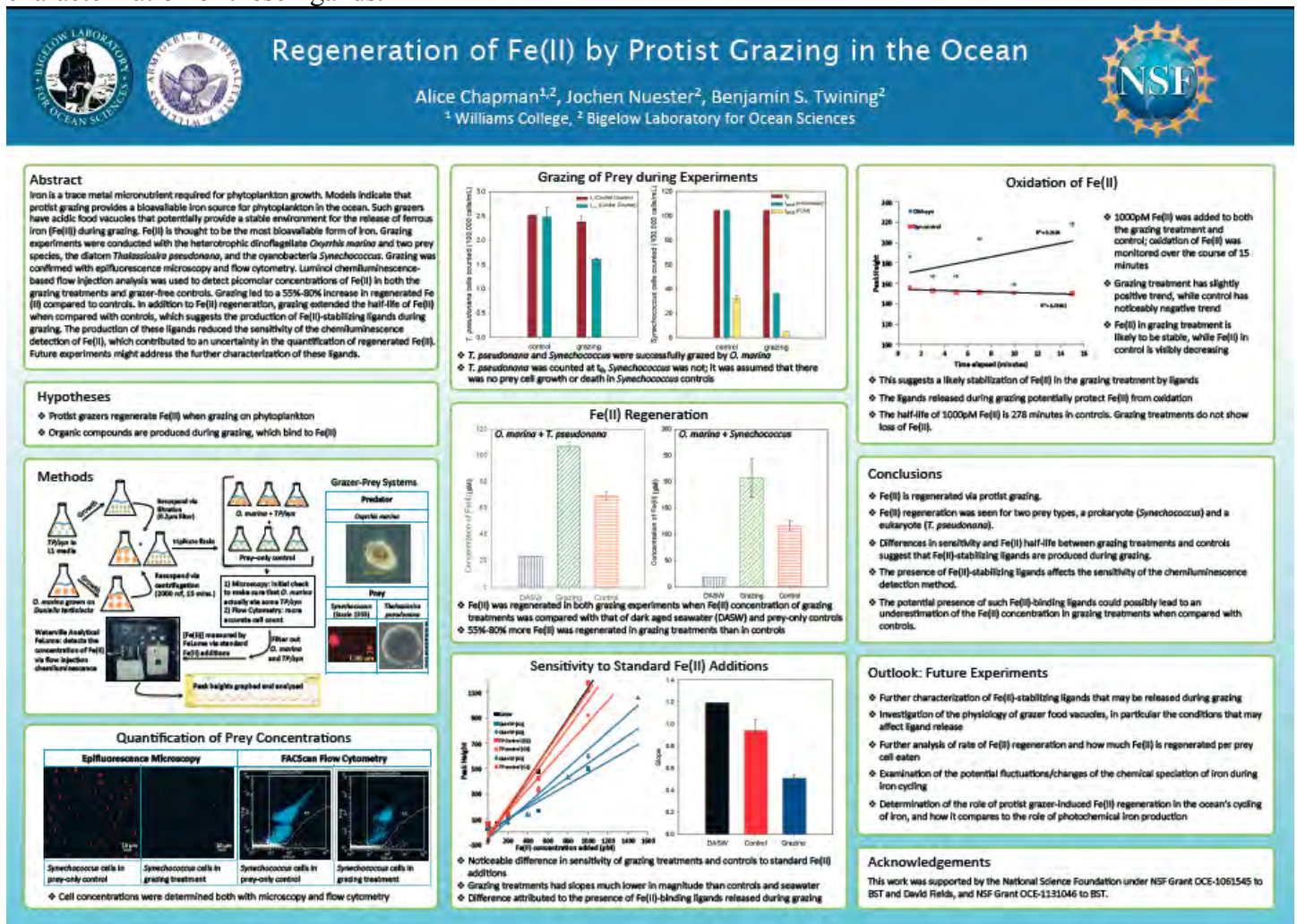
Microbial community composition is essential to ecosystem function, biogeochemical cycling, and the health of local organisms. This study examined microbial community composition in freshwater iron-seeps to identify a core microbiome that represents an assemblage of specific microbes and their potential ecological niches. 32 DNA samples were collected from a broad geographic range to gain a representative sampling of microbial communities in these systems. After targeted 16S rRNA gene pyrosequencing of the V4-V6 region, samples were processed and subsequently analyzed for species diversity. Comamonadaceae, Gallionellaceae, Methylococcaceae, and Crenotrichaceae were the most abundant groups identified in freshwater samples. These families contain most studied freshwater iron-oxidizing bacteria and methylotrophic bacteria, and this co-abundance suggests a potentially important relationship. Also, a Fisher's exact test identified 11086 significant pairwise co-occurrence associations ($q < 0.05$) between 926 operational taxonomic units (OTUs) in freshwater samples. 141 OTUs were statistical outliers ($Q3 + (IQR * 1.5)$) in either their ubiquity across samples or total associations from the co-occurrence analysis. This outlier method identified an assemblage of potential core microbes in freshwater iron-seeps and elucidated methods for defining the key members of microbial communities.



REGENERATION OF Fe(II) BY PROTIST GRAZING IN THE OCEAN

Alice Chapman^{1,2}, Dr. Ben Twining² & Dr. Jochen Neuster²
¹Williams College, ²Bigelow Laboratory for Ocean Sciences

Iron is a micronutrient required for phytoplankton growth. Models indicate that protist grazing provides a bioavailable iron source for phytoplankton in the ocean. Such grazers have acidic food vacuoles that potentially provide a stable environment for the release of ferrous iron (Fe(II)) during grazing. Fe(II) is thought to be the most bioavailable form of iron. Grazing experiments were conducted with the heterotrophic dinoflagellate *Oxyrrhis marina* and two prey species, the diatom *Thalassiosira pseudonana*, and the cyanobacteria *Synechococcus*. Grazing was confirmed with epifluorescence microscopy and flow cytometry. Luminol chemiluminescence-based flow injection analysis was used to detect picomolar concentrations of Fe(II) in both the grazing treatments and grazer-free controls. Grazing led to a 55% - 80% increase in regenerated Fe(II) compared to controls. In addition to Fe(II) regeneration, grazing extended the half-life of Fe(II) when compared with controls, which suggests the production of Fe(II)-stabilizing ligands during grazing. The production of these ligands reduced the sensitivity of the chemiluminescence detection of Fe(II), which contributed to an uncertainty in the quantification of regenerated Fe(II). Future experiments might address the further characterization of these ligands.



GROWTH & DEVELOPMENT OF *ZETAPROTEOBACTERIA* SP. DIS-1 BIOFILM ON MILD STEEL

Kim Dempsey^{1,2} Adam Mumford² and David Emerson²

¹Bowdoin College, Brunswick ME; ²Bigelow Laboratory for Ocean Sciences, East Boothbay, ME

Microbial induced corrosion (MIC) of steel in marine environments is a serious, documented problem for marine steel structures, such as bridges or naval ships. Recently microaerophilic marine iron-oxidizing *Zetaproteobacterium* DIS-1 was isolated from steel coupons incubated in West Boothbay Harbor. This study characterized the colonization of 1018 mild steel surfaces by stalk-forming DIS-1, by observing biofilm and stalk formation under various concentrations of dissolved iron. DIS-1 cells grew on steel coupons in continuous flowing microcosms. The resulting microbial biofilms were stained essentially *in situ* with Syto13 for DNA (cells) and rhodamine-conjugated *Ricinus communis* Agglutinin 1 for Fe-encrusted stalks, allowing for three-dimensional imaging using a novel confocal microscopy technique. Most cells visualized were associated with stalk structures, not directly in contact with the coupon surface. Three-dimensional images were generated and subjected to automated cell counting using ImageJ, which suggested that high concentrations (>500 & microM) of iron in seawater inhibited DIS-1 growth on coupons. This work provides insight into DIS-1 growth on steel, and introduces novel methods and tools for future investigation into the role of iron-oxidizing bacteria in MIC.



Growth & development of *Zetaproteobacteria* sp. DIS-1 biofilm on mild steel

Kim Dempsey^{1,2}, Adam Mumford², David Emerson²

¹Bowdoin College, Brunswick, ME; ²Bigelow Laboratory for Ocean Sciences, East Boothbay, ME



Abstract

Microbial Induced corrosion (MIC) of steel in marine environments is a serious, documented problem for marine steel structures, such as bridges or naval ships.^{1,2} Adaktylou and McBeth³ isolated microaerophilic marine iron-oxidizing γ -proteobacterium DIS-1 from steel coupons incubated in West Boothbay Harbor. This study characterized the colonization of 1018 mild steel surfaces by stalk-forming DIS-1, by observing biofilm and stalk formation under various concentrations of dissolved iron. DIS-1 cells grew on steel coupons in continuous flowing microcosms. The resulting microbial biofilms were stained essentially *in situ* with Syto13 for DNA (cells) and rhodamine-conjugated *Ricinus communis* Agglutinin 1 for Fe-encrusted stalks, allowing for three-dimensional imaging using a novel confocal microscopy technique. Most cells visualized were associated with stalk structures, not directly in contact with the coupon surface. Three-dimensional images were generated and subjected to automated cell counting using ImageJ, which suggested that high concentrations (>500 μ M) of iron in seawater inhibited DIS-1 growth on coupons. This work provides insight into DIS-1 growth on steel, and introduces novel methods and tools for future investigation into the role of iron-oxidizing bacteria in MIC.

Hypotheses

- Time Course 1: Single culture incubation.**
Hypothesis: biofilm development; cell count will increase over time.
- Time Course 2: Mixed cultured incubation.**
Hypothesis: increased biofilm development; DIS-1 cell growth upon addition of sulfate-reducing bacterium *Desulfosporosinus indanensis* ind-1.
- Time Course 3 - 5: Incubation with supplemented iron (single (3, 4) and mixed culture (5)).**
Hypothesis: DIS-1 will not grow in regions made anoxic by high Fe(II) concentrations. In oxic regions, growth will be accelerated.

Methods

Continuous flowing microcosm⁴ setup

- Seawater ran through reactor tubes at a rate of 1mL/min.
- Once the reactors were filled, 1mL of DIS-1 culture was added to each reactor.
- For mixed culture incubation experiments, 1mL of ind-1 culture was also added to each reactor.
- For experiments without supplemented iron, the Fe(II) packed column was not included in the setup.

Coupon imaging

- One coupon per day was removed for imaging.
- Slides were stained by an agarose solution (0.5% low melt agarose, rhodamine-conjugated lectin (*Ricinus communis* Agglutinin 1 (RCA-1)), and Syto13 green fluorescent DNA stain) immediately upon removal from seawater. A coverslip was placed over a 3.175mm thick plastic frame (25x75mm), and placed over the coupon slide, which was then incubated for one hour at 4°C.
- A confocal laser scanning microscope (Zeiss LSM 700) was used to image the coupon surface and any associated cells/biofilm.

Image Analysis

- Cell count data was generated using the 3D Object Counter plugin of the Fiji implementation of imageJ running on Charlie.

Determination of iron concentration

- A ferrozine assay was performed each day on reactors containing coupons.
- A_{630} was read, and a standard curve was generated.
- From the standard curve, Fe (II), Fe (III), and Fe (total) concentrations in each reactor were determined for both inlet and waste seawater lines.

Results

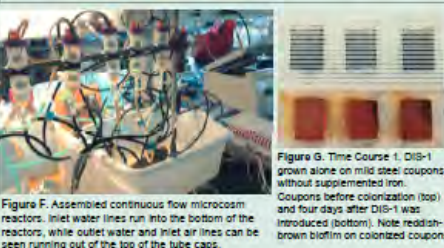
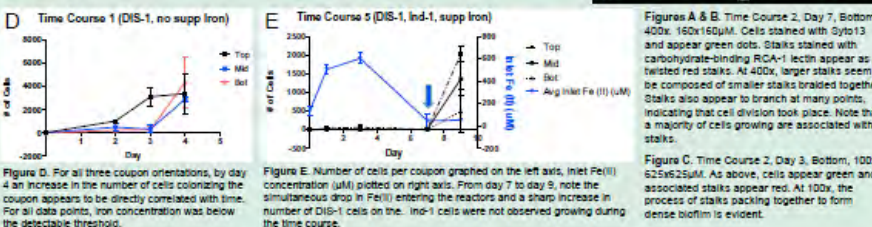
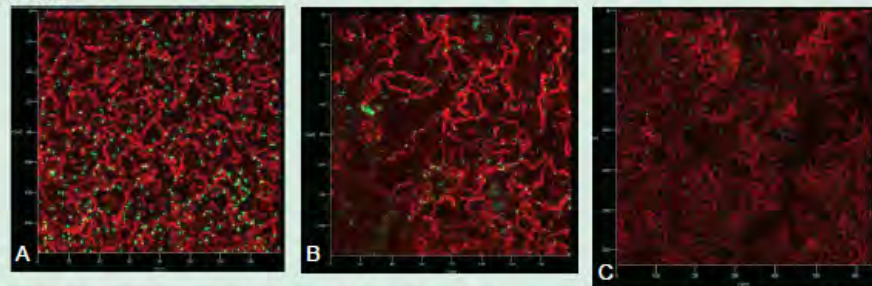


Figure 3. Assembled continuous flow microcosm reactor. Inlet water lines run into the bottom of the reactors, while outlet water and inlet air lines can be seen running out of the top of the tube caps.

Conclusions

- Successfully tested and refined new tools and techniques for growing and imaging Fe-oxidizing bacteria growing on solid steel surfaces.
- 3-D images generated allow for thorough examination of DIS-1 cell/stalk structures, and stalk growth from coupon surface (Fig A-C).
- Quantitative results from Time Course 5 (Fig E) suggest an inverse relationship between DIS-1 growth on steel and Fe(II) concentration in the environment. This relationship should be further tested, as it could be valuable in anti-corrosion projects and provides valuable insight into the physiology of DIS-1.
- Ind-1 did not appear to grow in any of the multi-species experiments, perhaps because ind-1 takes longer than 4-9 days to begin to colonize steel.

Acknowledgments

- Dr. David Emerson and Dr. Adam Mumford for their support and guidance.
- The National Science Foundation for its support under NSF Grant OCE 1156740 - REU Site: Bigelow Laboratory for Ocean Sciences - Undergraduate Research Experience in the Gulf of Maine and the World Ocean Awarded to DMF.
- Office of Naval Research grant N00014-08-1-0334.

References

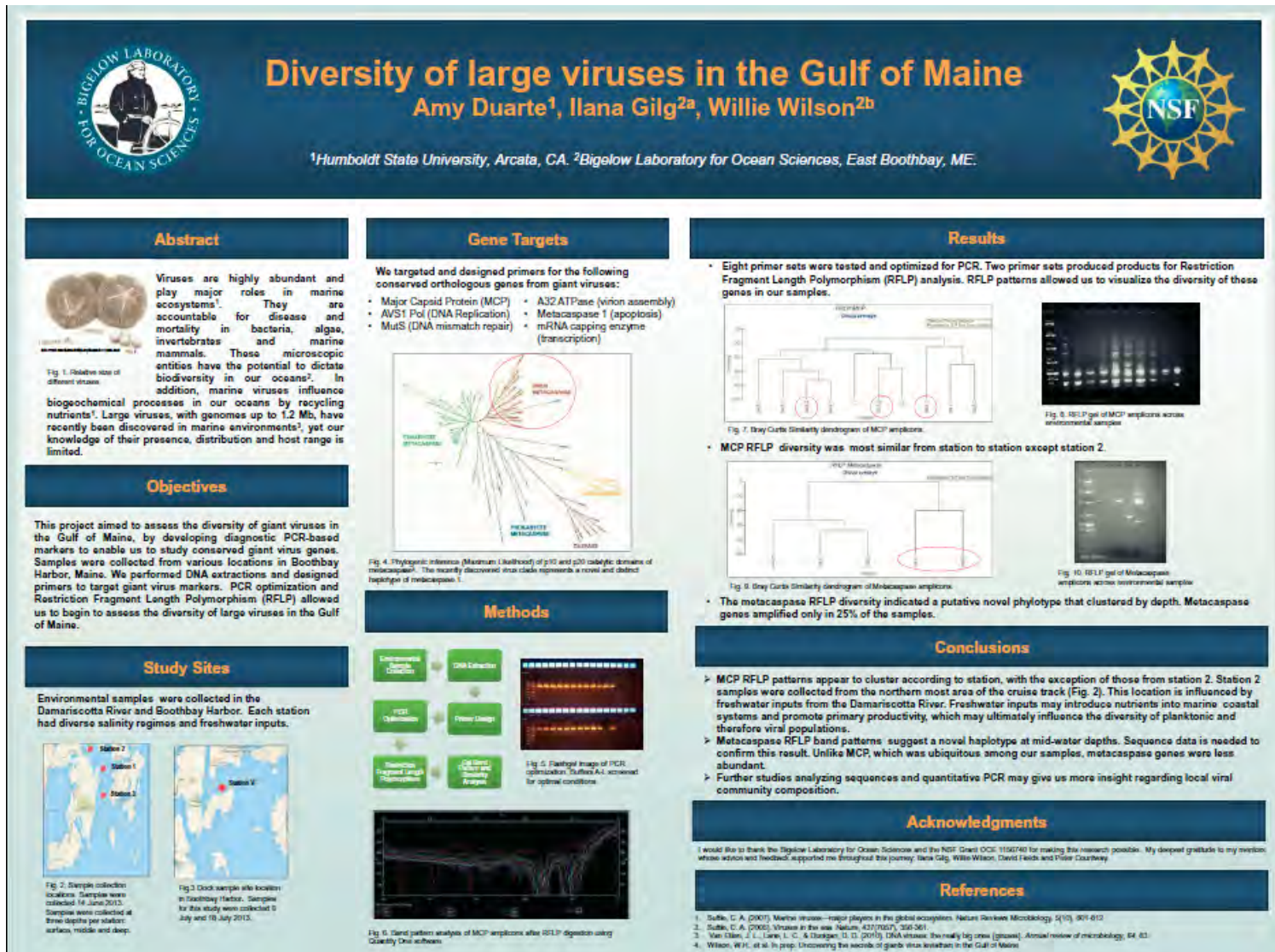
1. Odom, J.M. 1980. Industrial and environmental concerns with sulfate-reducing bacteria. *ADM News* 60: 475-476.
2. Little, B.J. and J.S. Lee. 2007. Microbiologically influenced corrosion. Wiley-Interscience, Hoboken, NJ.
3. Adaktylou, I. and J. McBeth. 2012. Growth and study of thermophilic Fe-oxidizing bacteria at the Bigelow Laboratory for Ocean Sciences (JOS) within the framework of the IOP activity entitled: "The Functionality of Iron Microbes in Environmental Processes." Scientific Report of the Exchange Grant.
4. Setup developed by Dr. Adam Mumford (Bigelow Laboratory for Ocean Sciences, East Boothbay, ME).

DIVERSITY OF LARGE MARINE VIRUSES IN THE GULF OF MAINE

Amy Duarte¹, Ilana Gilgi², Willie Wilson²

¹Humboldt State University, ²Bigelow Laboratory for Oceans Sciences

Viruses are highly abundant and play major roles in marine ecosystems. They are accountable for disease and mortality in bacteria, algae, invertebrates and marine mammals. These microscopic entities have the potential to dictate biodiversity in our oceans. In addition, marine viruses influence biogeochemical processes in our oceans by recycling nutrients. Large viruses, with genomes up to 1.2 Mb, have recently been discovered in marine environments, yet our knowledge of their presence, distribution and host range is limited. Nothing is known about the large virus population in the Gulf of Maine. This project aimed to assess the diversity of giant viruses in the Gulf of Maine, by developing diagnostic PCR-based markers to enable us to study conserved giant virus genes. Samples were collected from various regions around Boothbay Harbor, Maine. We performed DNA extractions and designed primers to target giant virus markers. PCR optimization and Restriction Fragment Length Polymorphism (RFLP) allowed us to identify and to begin to assess the diversity of large viruses in the Gulf of Maine.



ANALYZING THE POPULATION DIVERSITY OF *SYNECHOCOCCUS* DURING THE 2013 SUMMER BLOOM PERIOD IN THE WEST BOOTH BAY OF MAINE

Erf, K.M.¹, Countway, P.D.², Sieracki, M.², Poulton, N.²

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

Marine cyanobacteria of the genus *Synechococcus* play a critical role in the global carbon cycle and other marine biogeochemical processes throughout the world's oceans. *Synechococcus* are a highly diverse group of phytoplankton, yet little is currently known about the temporal distribution of various *Synechococcus* clades in the Gulf of Maine or the ecological significance thereof. The present research was conducted to document the seasonal bloom dynamics of *Synechococcus* between June and August 2013. DNA sequencing was completed to identify the clades of *Synechococcus* present at the peak of the bloom period, while quantitative PCR was used to determine the relative concentration of Clade IV across an eight-week time-series of surface samples collected in West Boothbay Harbor. These techniques were optimized over the course of the study and will be used to complete the project over the remaining two years. DNA sequencing of the RNA polymerase gene (*rpoC1*) confirmed that *Synechococcus* clades I and IV were the dominant ecotypes at our coastal study site, accounting for most of the *Synechococcus* abundance as determined by flow cytometry.

Analyzing the Population Diversity of *Synechococcus* in the Booth Bay During the 2013 Summer Bloom Period

Kayla Erf¹, Dr. Peter Countway², Dr. Mike Sieracki², Dr. Nicole Poulton²

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

Abstract

Marine cyanobacteria of the genus *Synechococcus* play a critical role in the global carbon cycle and other marine biogeochemical processes throughout the world's oceans. *Synechococcus* are a highly diverse group of phytoplankton, yet little is currently known about the temporal distribution of various *Synechococcus* clades in the Gulf of Maine or the ecological significance thereof. The present research was conducted to document the seasonal bloom dynamics of *Synechococcus* between June and August 2013. DNA sequencing was completed to identify the clades of *Synechococcus* present at the peak of the bloom period, while quantitative PCR was used to determine the relative concentration of Clade IV across an eight-week time-series of surface samples collected in West Boothbay Harbor. These techniques were optimized over the course of the study and will be used to complete the project over the remaining two years. DNA sequencing of the RNA polymerase gene (*rpoC1*) confirmed that *Synechococcus* clade I was the dominant ecotype at our coastal study site, accounting for most of the *Synechococcus* abundance as determined by flow cytometry. Additionally, several other marine cyanobacterial groups were detected by sequencing and contribute to the diversity of the prokaryotic phytoplankton in Booth Bay. Several cultured *Synechococcus* strains were sequenced in order to generate *rpoC1* DNA standards for qPCR. During the course of this work, two cultured strains of *Synechococcus* were identified as probable new species based on their low sequence similarity to known taxa.

Purpose

The presence or absence of different clades throughout the bloom cycle may speak to their:

- ecological role
- susceptibility to various viruses or grazers
- ability to thrive in temperate waters under changing environmental conditions

These cyclic blooms are also

- the ideal model system for studying the interactions of the microbial food web
- potential indicators of future climate change.

Methods

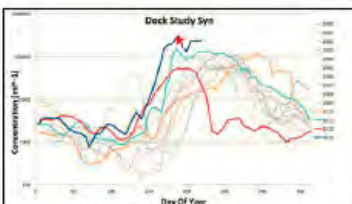


Figure 1: *Synechococcus* cell count as recorded by flow cytometry for weekly deck study (2006–2013).
★ Peak abundance over the past 13 years & date of sequencing analysis

Results

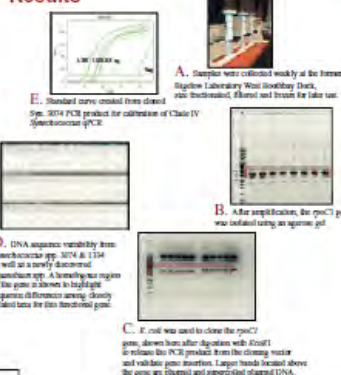


Figure 2: Phylogenetic tree representing relationship between four cyanobacterial strains (1074, 1234, 2515, 8c-14) and various clones from an environmental sample (Syr #144) taken at the height of the *Synechococcus* summer bloom. Sequences were obtained with PCR primers described in Tai and Pálfi (2009).

References:
Tai V, Pálfi B (2009) Temporal variation of *Synechococcus* clades at a coastal Pacific Ocean station: the SMOE Journal 5:93-103

A Newly Discovered Species!

Putative *Synechococcus* isolated by Dr. Maureen Keller at Bigelow Laboratory, but never previously identified by genetic techniques. *rpoC1* gene sequencing revealed only an 89% similarity to *Cyanobium*, likely indicating a new species.

Future Research

DNA sequencing will be completed for two additional environmental samples – one from bloom initiation and a second from bloom termination. The clades of *Synechococcus* identified by this process will be analyzed via qPCR to determine relative concentration over time. Additionally, qPCR will be used to quantify the grazing rates for each individual clade. This information will help determine the ecological roles of individual clades and form the beginning of a microbial food web.

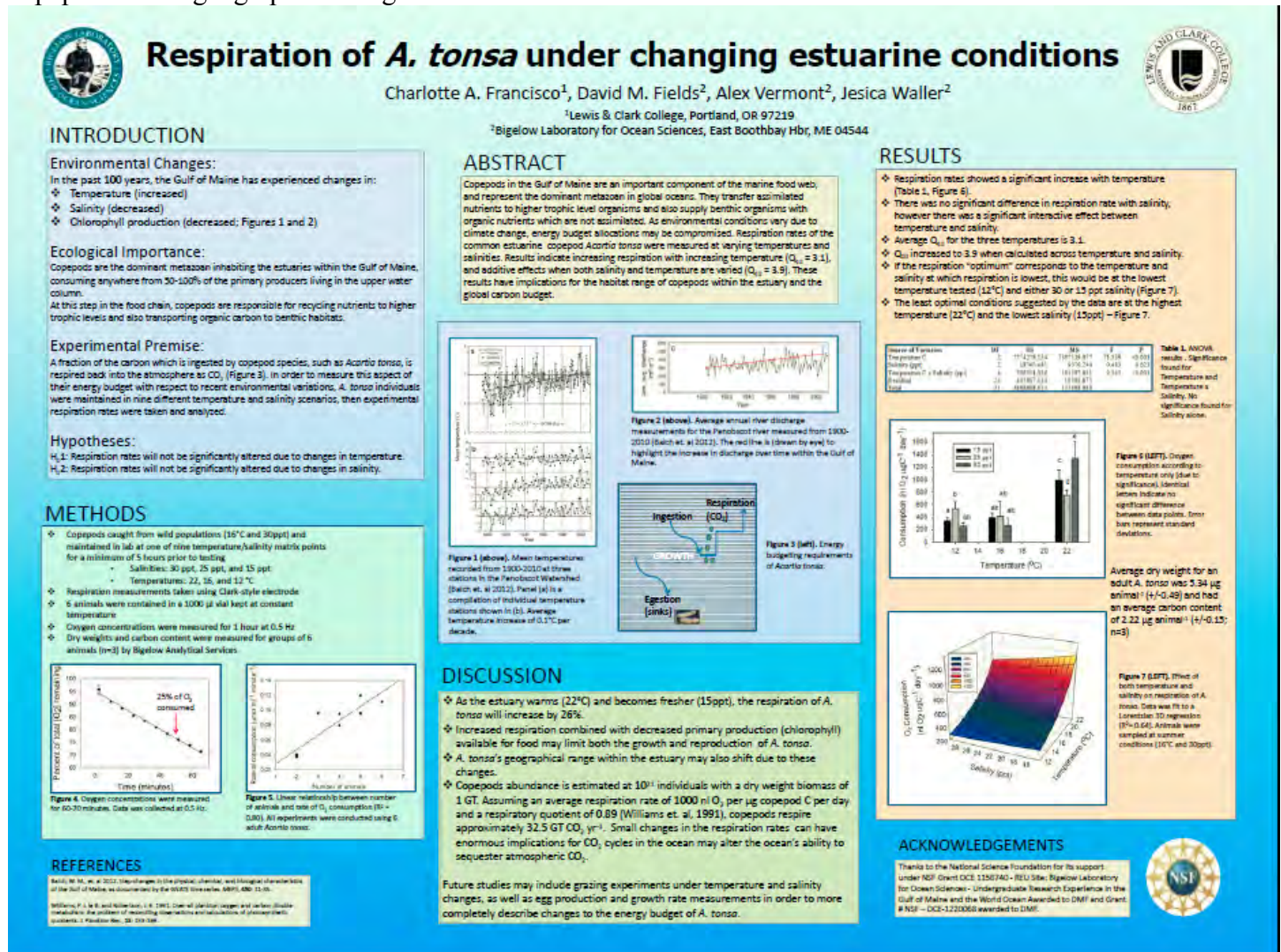
Acknowledgements

Thanks to the National Science Foundation for its support under NSF Grant OCE 1156740 – REU Site: Bigelow Laboratory for Ocean Sciences – Undergraduate Research Experience in the Gulf of Maine and the World Ocean Awarded to DMF

THE RESPIRATION RATES OF ACARTIA TONSA IN RESPONSE TO CHANGING TEMPERATURE AND SALINITY

Charlotte A. Francisco^{1,2}, David M. Fields², Alex Vermont², Jesica Waller²
¹Lewis and Clark, ²Bigelow Laboratory for Ocean Sciences

In the past century, increased temperatures, decreased salinity, and decreased chlorophyll concentrations have occurred within Gulf of Maine estuaries. Copepods, the dominant metazoans on the planet, are an important component of marine ecosystems, bridging the gap between phytoplankton and higher trophic level organisms, recycling nutrients, and transporting carbon to the benthos. The respiration rates of adult *Acartia tonsa* (a principal estuarine copepod) were measured under nine temperature and salinity scenarios using microrespirometry techniques. Oxygen concentrations were measured at 0.5 Hz for one hour in 1000 μl chambers. A two-way ANOVA showed a significant relationship between respiration rates and temperature and a significant interactive effect of temperature and salinity on respiration. The average Q10 for respiration was 3.1 which increased to 3.9 over the salinity range used in this study. These results suggest that as the estuaries warm and become fresher, the respiration rates of *Acartia tonsa* will increase by 26%. The increased respiration rates combined with decreasing rates of primary productivity may limit the growth and reproduction of this copepod and its geographical range.

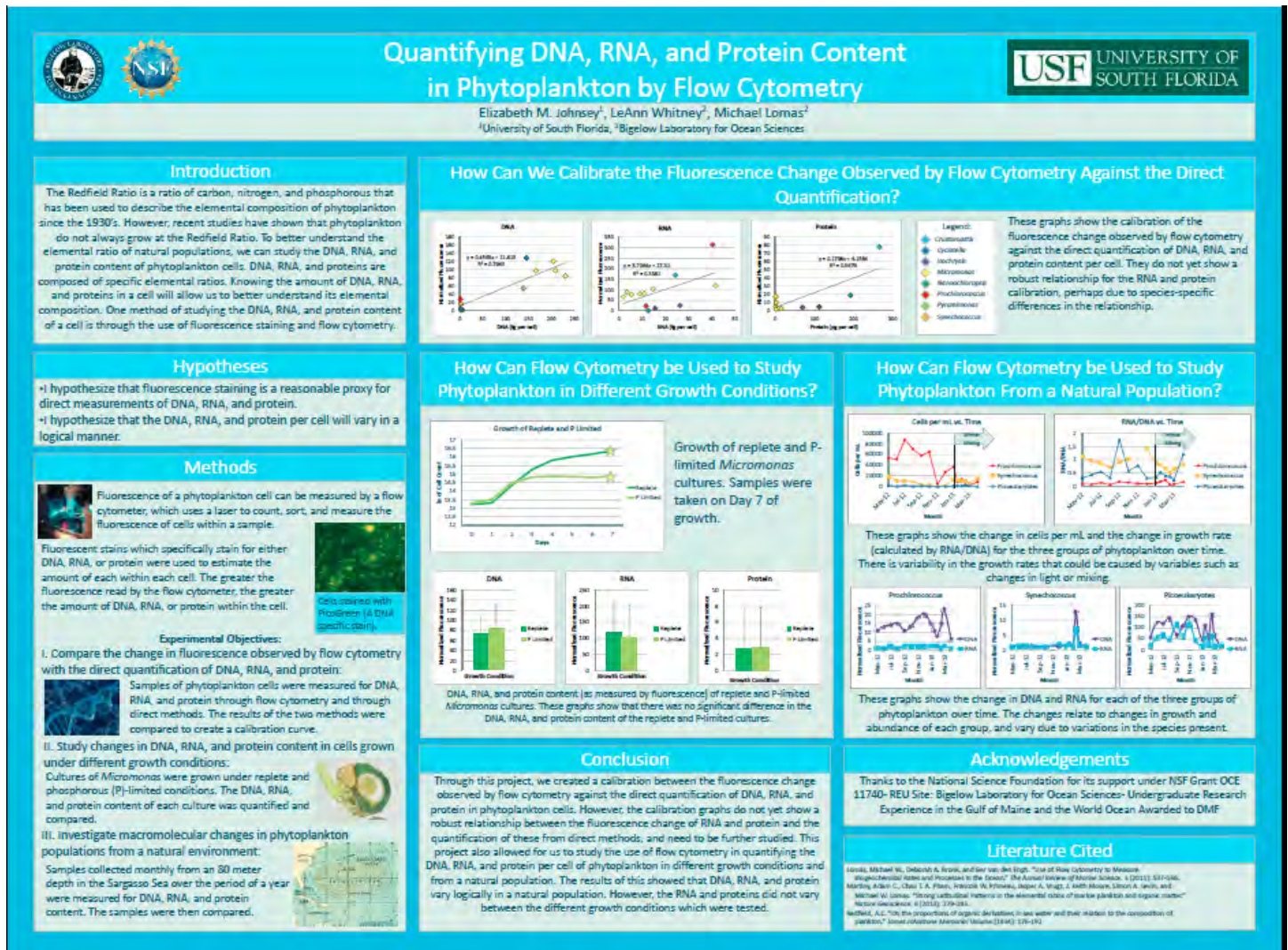


QUANTIFYING DNA, RNA, AND PROTEIN CONTENT IN PHYTOPLANKTON BY FLOW CYTOMETRY

Elizabeth M. Johnsey¹, LeAnn Whitney², Michael Lomas²

¹University of South Florida, ²Bigelow Laboratory for Ocean Sciences

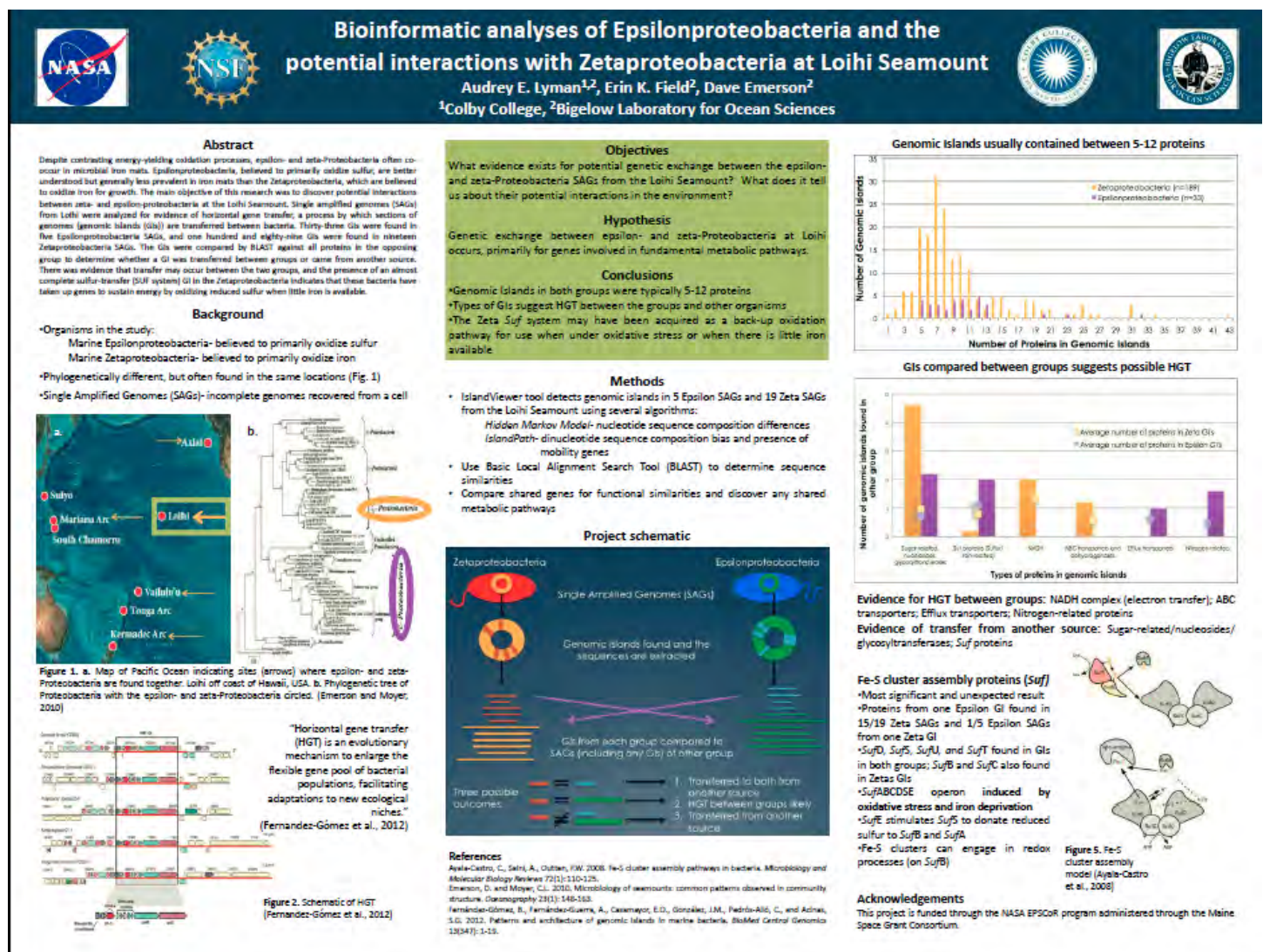
The Redfield ratio has been used to describe the elemental composition of phytoplankton since the 1930s. Recent studies have shown deviations from this ratio, indicating that we don't understand how and why these deviations occur. Studying the DNA, RNA, and protein content of phytoplankton cells will allow us to better understand their elemental composition as these biochemicals have well constrained elemental ratios and comprise the bulk of phytoplankton organic material. We coupled fluorescence staining and flow cytometry to investigate the macromolecular composition of phytoplankton. Our goals were to determine whether fluorescence staining is a reasonable proxy for direct measurements of DNA, RNA, and protein per cell and whether DNA, RNA, and protein per cell varies in a predictable manner. We calibrated the fluorescence observed by flow cytometry against direct quantification of DNA, RNA, and protein per cell. Macromolecular composition was also measured in phytoplankton grown under different growth conditions as well as from a natural sample. Data collected thus far do not yet show a predictable relationship between fluorescence and quantification from direct methods, and need further study.



BIOINFORMATIC ANALYSES OF EPSILONPROTEOBACTERIA AND THE POTENTIAL INTERACTIONS WITH ZETAPROTEOBACTERIA

Audrey E. Lyman^{1,2}, Erin K. Field², Dave Emerson²
¹Colby College, ²Bigelow Laboratory for Ocean Sciences

Despite contrasting energy-yielding oxidation processes, epsilon- and zeta-Proteobacteria often co-occur in microbial iron mats. Epsilonproteobacteria, believed to primarily oxidize sulfur, are better understood but generally less prevalent in iron mats than the Zetaproteobacteria, which are believed to oxidize iron for growth. The main objective of this research was to discover potential interactions between zeta- and epsilon-proteobacteria at the Loihi Seamount. Single amplified genomes (SAGs) from Loihi were analyzed for evidence of horizontal gene transfer, a process by which sections of genomes (genomic islands (GIs)) are transferred between bacteria. Thirty-three GIs were found in five Epsilonproteobacteria SAGs, and one hundred and eighty-nine GIs were found in nineteen Zetaproteobacteria SAGs. The GIs were compared by BLAST against all proteins in the opposing group to determine whether a GI was transferred between groups or came from another source. There was evidence that transfer may occur between the two groups, and the presence of an almost complete sulfur-transfer (SUF system) GI in the Zetaproteobacteria indicates that these bacteria have taken up genes to sustain energy by oxidizing reduced sulfur when little iron is available.

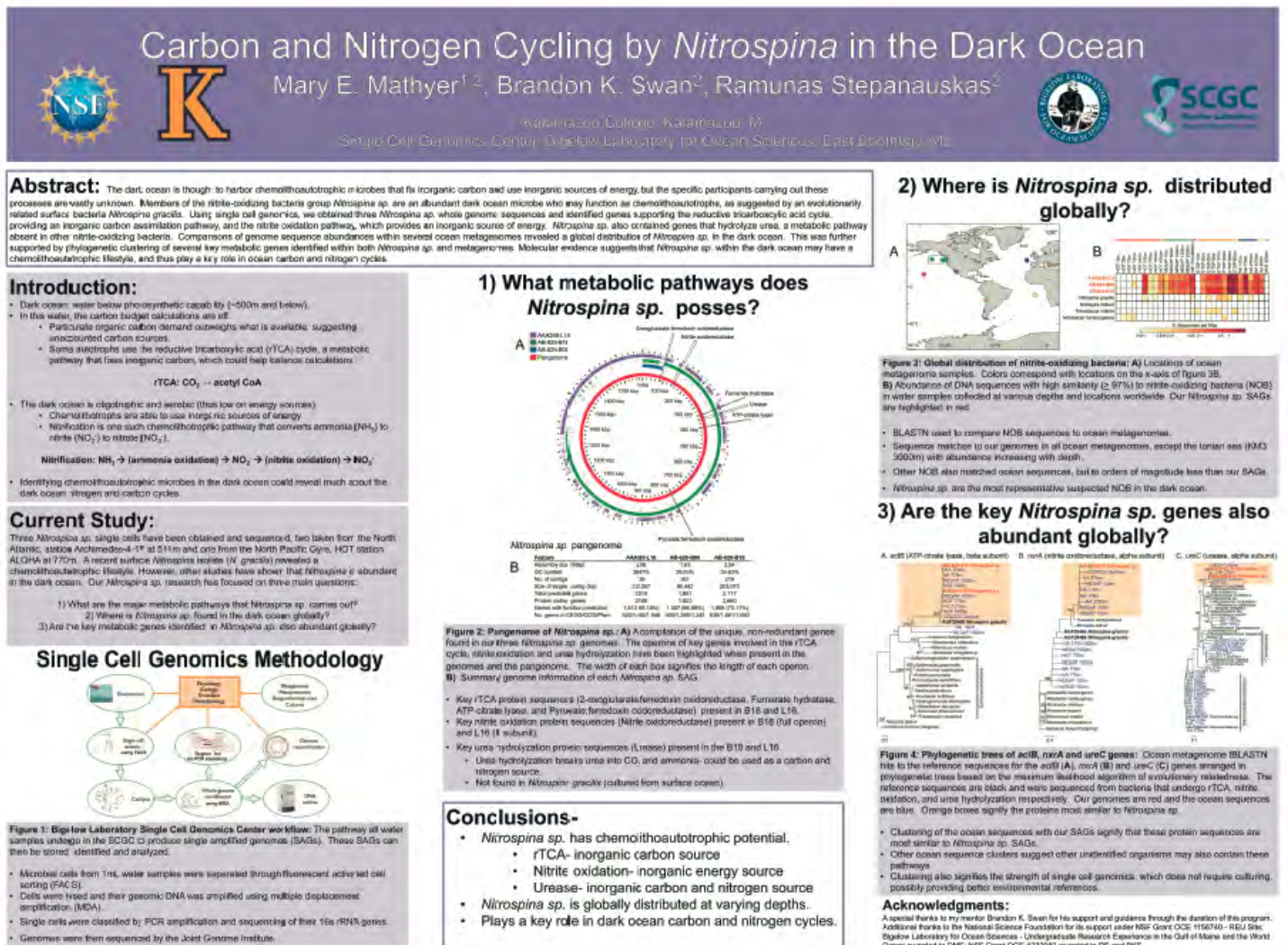


CARBON AND NITROGEN CYCLING BY *NITROSPINA* IN THE DARK OCEAN

Mary E. Mathyer^{1,2}, Brandon K. Swan², Ramunas Stepanauskas²

¹Kalamazoo College, Kalamazoo, MI, ²Bigelow Laboratory for Ocean Sciences, East Boothbay, ME

The dark ocean is thought to harbor chemolithoautotrophic microbes that fix inorganic carbon and use inorganic sources of energy, but the specific participants carrying out these processes are vastly unknown. Members of the nitrite-oxidizing bacteria group *Nitrospina* sp. are an abundant dark ocean microbe who may function as chemolithoautotrophs, as suggested by an evolutionarily related surface bacteria *Nitrospina gracilis*. Using single cell genomics, we obtained three *Nitrospina* sp. whole genome sequences and identified genes supporting the reductive tricarboxylic acid cycle, providing an inorganic carbon assimilation pathway, and the nitrite oxidation pathway, which provides an inorganic source of energy. *Nitrospina* sp. also contained genes that hydrolyze urea, a metabolic pathway absent in other nitrite oxidizing bacteria. Comparisons of genome sequence abundances within several ocean metagenomes revealed a global distribution of *Nitrospina* sp. in the dark ocean. This was further supported by phylogenetic clustering of several key metabolic genes identified within both *Nitrospina* sp. and metagenomes. Molecular evidence suggests that *Nitrospina* sp. within the dark ocean may have a chemolithoautotrophic lifestyle, and thus play a key role in ocean carbon and nitrogen cycles.



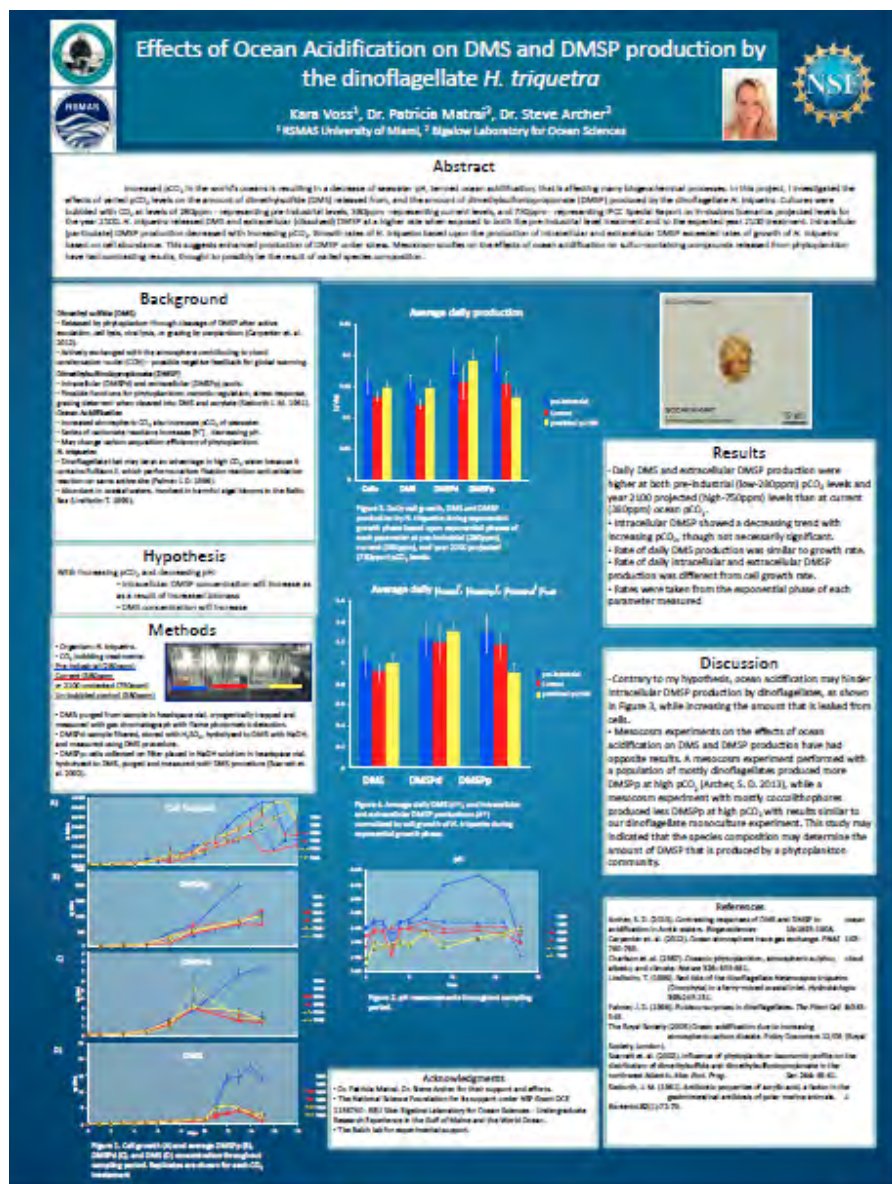
EFFECTS OF OCEAN ACIDIFICATION ON DMS AND DMSP PRODUCTION BY THE DINOFLAGELLATE *H. TRIQUETRIA*

Kara Voss¹, Patricia Matrai², Steve Archer²

¹RSMAS University of Miami, ²Bigelow Laboratory for Ocean Sciences

Increased pCO₂ in the world's oceans is resulting in a decrease of seawater pH, termed ocean

acidification, that is affecting many biogeochemical processes. In this project, I investigated the effects of varied pCO₂ levels on the amount of dimethylsulfide (DMS) released from, and the amount of dimethylsulfoniopropionate (DMSP) produced by the dinoflagellate *Heterocapsa triquetria*. Cultures were bubbled with CO₂ at levels of 280ppm - representing pre-industrial levels, 380ppm - representing current levels, and 750ppm - representing IPCC Special Report on Emissions Scenarios projected levels for the year 2100. *H. triquetria* released DMS and extracellular (dissolved) DMSP at a higher rate when exposed to both the pre-industrial level treatment and to the expected year 2100 treatment. Intracellular (particulate) DMSP production decreased with increasing pCO₂. Growth rates of *H. triquetria* based upon the production of intracellular and extracellular DMSP were higher than growth rates based on cell abundance. This suggests enhanced production of DMSP. Mesocosm studies on the effects of ocean acidification on sulfur containing compounds released from phytoplankton have had contrasting results, thought to possibly be the result of varied species composition (Archer, S. D. 2010).




DO HUMIC COMPOUNDS INHIBIT THE AEROSOLIZATION OF CHLOROPHYLL *a* AND BREVETOXINS?

Ryan Yan^{1,2}, Dr. Cynthia Heil², Dr. Steve Archer²

¹College of William and Mary, ²Bigelow Laboratory for Ocean Sciences

The toxic dinoflagellate *Karenia brevis* blooms annually in the Gulf of Mexico resulting in significant environmental and associated health concerns (Stumpf et al., 2003). These cells are easily broken apart in the surf, releasing brevetoxins which are subsequently incorporated into marine aerosols. Beachgoers inhaling these aerosolized toxins may suffer from acute respiratory problems (Twiner et al., 2007). The influence of dissolved humic substances (DHS), plant degradation products comprised of a mixture of high molecular weight acids, on aerosol formation was examined in a series of laboratory experiments. DHS additions to microbial populations from the lower Damariscotta River resulted in suppression of Chlorophyll *a* in the aerosolized fraction compared with controls. Increasing turbulence also increased the percentage of *K. brevis* cells disrupted, to a maximum of 90% of cells at a flow rate of 500 ml min⁻¹. Addition of DHS reduced *K. brevis* cell loss to 10-60%. Results suggest that DHS inhibit the aerosolization process via the surfactant action of the long-chain acids in DHS, which may decrease the surface tension of associated membranes. Artificial addition of DHS into coastal waters containing *K. brevis* blooms could thus provide a potential mitigation technique for bloom related health issues.



Do Humic Compounds Inhibit the Aerosolization of Chlorophyll *a* and Brevetoxins?

Ryan Yan^{1,2}, Dr. Cynthia Heil², Dr. Steve Archer²

¹College of William and Mary, ²Bigelow Laboratory for Ocean Sciences

Abstract

The toxic dinoflagellate *Karenia brevis* blooms annually in the Gulf of Mexico resulting in significant environmental and associated health concerns (Stumpf et al., 2003). These cells are easily broken apart in the surf, releasing brevetoxins which are subsequently incorporated into marine aerosols. Beachgoers inhaling these aerosolized toxins may suffer from acute respiratory problems (Twiner et al., 2007). The influence of dissolved humic substances (DHS), plant degradation products comprised of a mixture of high molecular weight acids, on aerosol formation was examined in a series of laboratory experiments. DHS additions (1 to 6 mg L⁻¹) to concentrated natural microbial populations from the lower Damariscotta River resulted in suppression of Chlorophyll *a* (Chl *a*) in the aerosolized fraction compared with controls. Increasing turbulence (i.e. bubbling) also increased the percentage of *K. brevis* cells disrupted, to a maximum of 90% of cells at a flow rate of 500 ml min⁻¹. Addition of DHS reduced *K. brevis* cell loss to 10-60%. Results suggest that DHS inhibit the aerosolization process via the surfactant action of the long-chain acids in DHS, which may decrease the surface tension of associated membranes. Artificial addition of DHS into coastal waters containing *K. brevis* blooms could thus provide a potential mitigation technique for *K. brevis* related health issues.

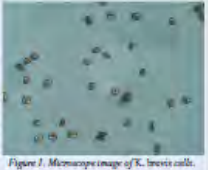


Figure 1. Microscopy image of *K. brevis* cells.

Methodology (cont.)

4. AEROSOLIZATION OF BREVETOXINS FROM *KARENIA BREVIS* CULTURES:

A culture of *K. brevis* (obtained from Dr. Peter Coombs, Clone # 125 of the FWC HAB Culture Collection) was grown in 1.1 medium under a 12:12 L:D cycle at 20°C. Cultures were grown up to high (>5 x 10⁶ *K. brevis* cells L⁻¹) densities. Cultures were diluted by 50% with filtered seawater and 250 ml. samples were used for experimentation. Prior to bubbling, DHS (extracted from the Pease River, FL by standard methods (Laursen, 1993)) were added to the 250 ml of culture and flow rate was adjusted according to the experimental nature (Figure 4). A constant bubbling time of 20 minutes was used for each sample.

Cell concentration (before and after bubbling) and aerosolized and particulate and dissolved brevetoxins were sampled from each aerosolization replicate. Brevetoxins were quantified using liquid chromatography mass spectrometry (LC/MS). Aerosolized brevetoxins were extracted from the GFF filter. Particulate toxins were obtained by filtering the culture medium from the chamber after bubbling and collecting and breaking up the cells (Twiner et al., 2007). Dissolved toxins were extracted from the remaining cell-free medium (Twiner et al., 2007). In addition to these samples, 10 ml. aliquots of the culture were taken from the chamber prior to bubbling and immediately after for determination of cell concentration. These samples were preserved in 7% glutaraldehyde and cell concentration was determined microscopically.

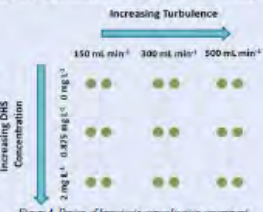
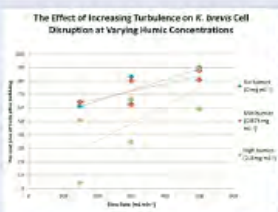


Figure 4. Design of *Karenia brevis* aerosolization experiment. Each large green dot represents a single replicate.

Results (cont.)

The Effect of Increasing Turbulence on *K. brevis* Cell Disruption at Varying Humic Concentrations



The data reveals that as flow rates (i.e. bubbling) increased, the percent loss of intact *K. brevis* cells increased, to a maximum of 90% of the population at the highest flow rate. Humic concentration was shown to have an impact on cell loss at high DHS concentrations, reducing it to 5-60%.

Hypotheses

The purpose of this study was to determine the effects of the addition of DHS on the aerosolization of brevetoxins produced by the Florida red tide dinoflagellate *Karenia brevis*. The hypothesis examined were:

1. The addition of DHS will result in lower production of aerosolized brevetoxins than in a culture with no DHS
2. The greater the concentration of DHS, the lower the concentration, the lower the concentration of aerosolized brevetoxins

Methodology

1. OPTIMIZATION OF FLOW RATE AND RUN TIME:

250 ml. of filtered seawater was added to the bubbling apparatus and bubbled for varying amounts of time and flow rates. A Whatman GFF glass fiber filter on top of the chamber collected aerosolized brevetoxins and was weighed before and after bubbling. The conditions leading to the highest mass difference in the filter was 300 ml/min, 15 min for 20 minutes.

2. AEROSOLIZATION OF AN INERT MOLECULE (PHENOL RED):

A standard solution of phenol red was added to the 250 ml. of filtered seawater in the bubbling apparatus. The solution was bubbled for 15 minutes. A 2nd experiment was performed for 20 minutes, both at the optimized flow rate. Aerosolized dye was collected on filter, which were extracted in acetone and analyzed spectrophotometrically.

3. AEROSOLIZATION OF AN ORGANIC MOLECULE (CHLOROPHYLL *a*) IN A LIVE SAMPLE WITH HUMIC ADDITIONS:

A natural microbial population was collected and concentrated from the lower Damariscotta River using a 10 µm µm. DHS were added to a suspension of the live sample at concentrations of 1, 2, and 4 mg ml⁻¹. DHS, Chl *a* was collected on a GFF filter and analyzed spectrophotometrically.




Figure 2. Aerosolization chamber and for the bubbling experiment. Sample is deposited in long chromatography column with a nitrogen gas inlet connected to the front bottom. Aerosols are collected with a GFF filter resting on top of the column.




Figure 3. Solution of phenol red and filtered seawater producing visible aerosol droplets in bubbling apparatus.

Results

2. AEROSOLIZATION OF AN INERT MOLECULE (PHENOL RED):

The data from the dye experiment (Figure 3) demonstrate that although low, some amount of inert molecules were aerosolized in the bubbling apparatus, and that the longer the duration of bubbling, the greater the amount of aerosols are collected on the filter.

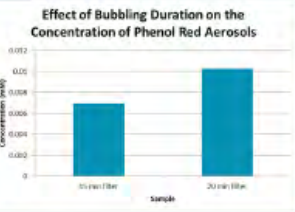


Figure 5. Results of the experiment showing increasing concentration with bubbling time.

3. AEROSOLIZATION OF AN ORGANIC MOLECULE (CHLOROPHYLL *a*) IN A LIVE SAMPLE WITH HUMIC ADDITIONS:

The addition of DHS to concentrated natural microbial communities from the lower Damariscotta River (Figure 6) depressed the aerosolization of Chl *a*. With low concentrations additions of DHS, 1 and 2 mg ml⁻¹, aerosolization was reduced to 10%. However at higher concentrations of DHS (6 mg ml⁻¹), some amount of Chl *a* was captured on the filter, although less than in the no humic control.

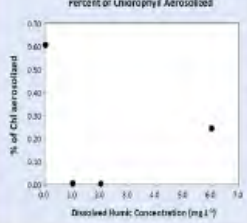


Figure 6. Results of live sample experiment showing a decline of Chl *a* aerosolization with the addition of DHS.

Discussion

- ✓ The results of the aerosolized dye experiment validated the experimental setup and efficacy of the aerosolization apparatus as it demonstrated that bubbling of a solution in the aerosolization chamber did produce aerosol droplets.
- ✓ The results of the Chl *a* experiment had very poor replicability due to the high levels of Chl *a* examined, but demonstrated that the presence of DHS depressed the aerosolization of Chl *a*. No Chl *a* was aerosolized at DHS concentrations of 1 and 2 mg ml⁻¹. However, at very high concentrations, there was a small amount of Chl *a* aerosolized.
- ✓ While the reduction in *K. brevis* cell concentration with bubbling demonstrated that turbulence (such as would occur in beach surf) disrupted and broke apart cells, the *K. brevis* in samples from this experiment have yet to be quantified by LC/MS – so although the effects of DHS have been seen to depress Chl *a* aerosolization, we cannot yet assess the effect of DHS on brevetoxin aerosolization, and whether or not the hypothesis are correct.

Acknowledgements

- Thanks to Dr. Cynthia Heil and Dr. Steve Archer for all of their support and assistance in completing this project.
- Thanks to the National Science Foundation for its support under NSF Grant OCE 1156740.
- Thanks to BUJL for hosting: Bigelow Laboratory for Ocean Sciences - Undergraduate Research Experience in the Gulf of Mexico and the World Ocean Associated to USMEX.

References

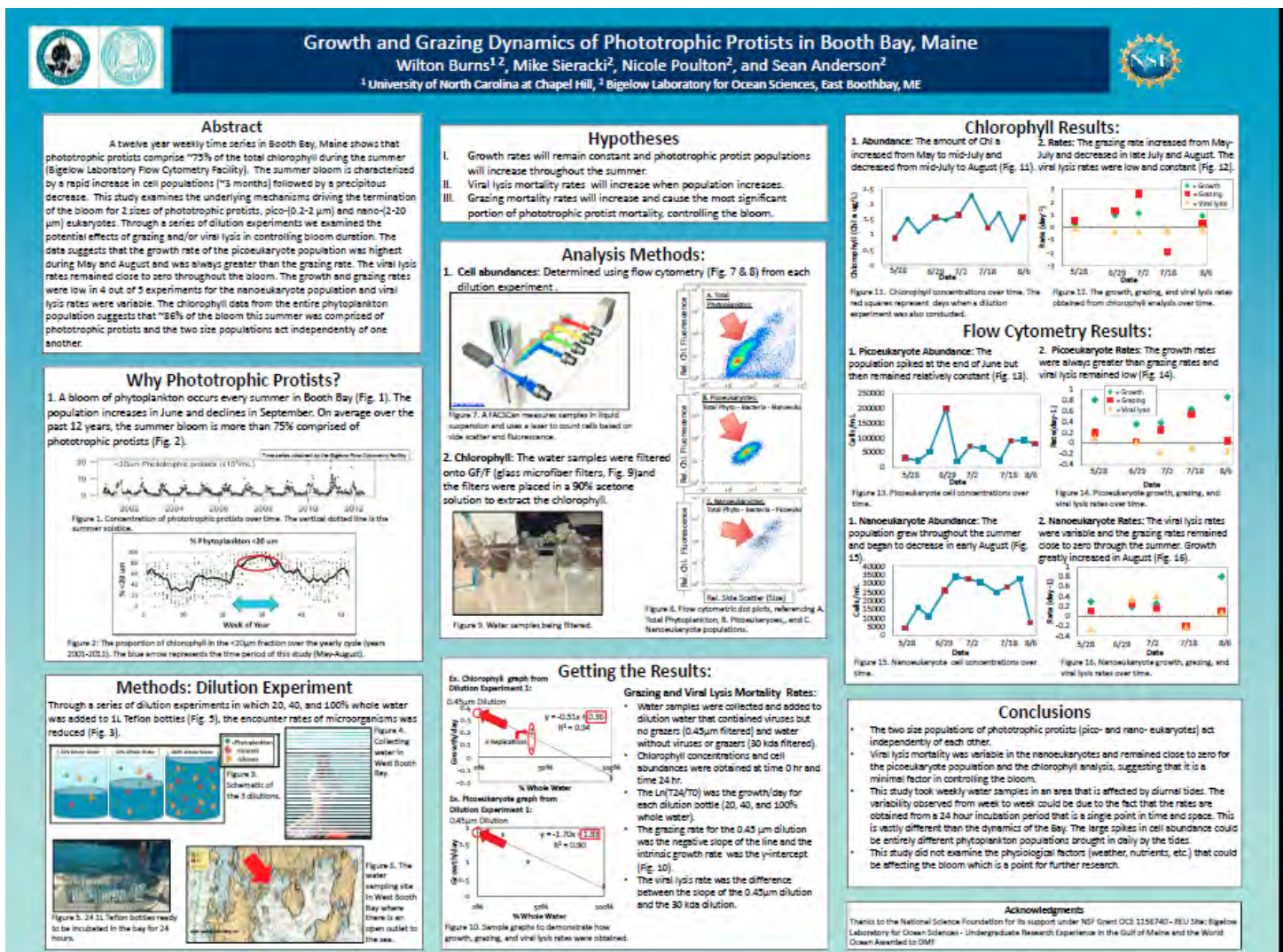
- Chang, Y.-S., McDonald, J. D., Kradin, D., Iwne, C. M., Zhou, Y., Plante, E. L., Henry, M. S., & Brandt, A. N. (2013). Brevetoxins and particle size of aerosolized *Karenia brevis* (dinoflagellate) derived from warm and cold waters. *Marine Science*, 36(1), 345-354. doi: 10.1007/s12237-012-9240-0
- Laursen, J. A. 1993. Comprehensive approach to preservation, isolation and fractionation of dissolved organic matter from natural and wastewater. *Env. Sci. Tech.*, 13, 178-187.
- Stumpf, R. P., Charette, M. H., Twiner, P. A., Truesdale, M., Ruppel, G. J., Pickman, J. A., Traylor, B., Ruckelshausen, V., Swenson, M. (2003). Monitoring *Karenia brevis* blooms in the Gulf of Mexico using satellite remote sensing and other data (Florida Agency, 2003). 147-168.
- Twiner, M. J., Charette, M. H., Wang, Y., Mikulski, C. M., Henry, M. S., Plante, E. L., & Brandt, A. G. (2007). Distribution and analysis of brevetoxins from the red tide dinoflagellate *Karenia brevis*. *Marine Biotechnology*, 19(1), 123-135. doi: 10.1007/s10241-007-0031-1

GROWTH AND GRAZING DYNAMICS OF PHOTOTROPHIC PROTISTS IN BOOTH BAY, MAINE

Wilton Burns^{1,2} Mike Sieracki²

¹University of North Carolina at Chapel Hill, ²Bigelow Laboratory for Ocean Sciences

This study focuses on two sizes of phototrophic protists that bloom every summer in Booth Bay, Maine. Phototrophic protists are unicellular eukaryotes that have compromised over 75% of the total chlorophyll for the past 10 years during the bloom (from a time series done by the Bigelow Flow Cytometry Lab). Every year the bloom is controlled around September and through a series of dilution experiments in which 20, 40, and 100% whole water are added to water containing just viruses and water without viruses and grazers, the encounter rates of the microorganisms are altered and it can be determined whether viral lysis or grazing by predators is more important in the termination of the bloom. The picoeukaryote population declined, the growth and grazing rates increased, and the viral lysis rates remained low. The viral lysis and grazing rates of the nanoeukaryotes stayed relatively constant but the population grew throughout the summer. The chlorophyll data showed that 86% of the total chlorophyll came from phototrophic protists and it did not reflect the individual populations, as they act independently from each other.





Thanks to the National Science Foundation for its support under NSF Grant OCE 1156740 – REU Site: Bigelow Laboratory for Ocean Sciences – Undergraduate Research Experience in the Gulf of Maine and the World Ocean.

