Bigelow Laboratory for Ocean Sciences

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

REU Symposium Program & Abstracts
Thursday, August 9, 2012

Program

1:00 Opening Remarks

1:15 Marie Neidig- University of Southern Maine
PRODUCTION AND ASSIMILATION OF DIMETHYLSULFONIONPROPIONATE (DMSP) BY MARINE PHYTOPLANKTON
(Matrai – Archer)

1:30 James (Nick) Hunt- Texas A&M University – Corpus Christi.
ENHANCEMENT OF LIPID PRODUCTION IN MICROALGAE BY DODECANE ADDITION AND NITRATE LIMITATION
(Wilson- Countway)

1:45 Cynthia Liles- University of California, Riverside
THE RELATIONSHIP BETWEEN MICROALGAE GROWTH PHASE AND LIPID CONTENT
(Countway-Wison)

2:00 Emily Bell-Hoerth- Earlham College
VARIATIONS IN PHYTOPLANKTON COMMUNITIES ASSOCIATED WITH BIVALVE AQUACULTURE IN COASTAL MAINE
(Poulton - Heil)

2:15 Zakary Jaques- Colby College
EFFECTS OF DISSOLVED HUMIC COMPOUNDS FROM THE DAMARISCOTTA AND KENNEBEC RIVERS ON MARINE MICROBIAL COMMUNITIES.
(Poulton - Heil)

2:30 Andrew Burchill – University of Chicago
DETECTING EVOLUTIONARY SELECTION IN HIGHLY CONSERVED PROTEINS: PHYSIOCHEMICAL SHIFTS IN THE MAMMALIAN CYTOCHROME B
(McClellan)

2:45 Break (15 minutes)

3:00 Ashley Poehls – Lake Superior State University
EFFECTS OF OCEAN ACIDIFICATION ON GROWTH RATE, BIOMASS, AND COCCOLITH MORPHOLOGY OF THE COCCOLITHOPHORE *PLEUROCHRYSIS*
(Balch - Fields)

3:15 Melanie Ross - Colby College
INDIRECT EFFECTS OF OCEAN ACIDIFICATION ON ACARTIA TONSA
(Balch - Fields)

3:30 Alyson Lowell - University of Maine
EXPERIMENTAL TECHNIQUES FOR CHARACTERIZING SHEATH-FORMING *ZETAPROTEOBACTERIA:* A METHODOLOGICAL APPROACH
(Field-Emerson)

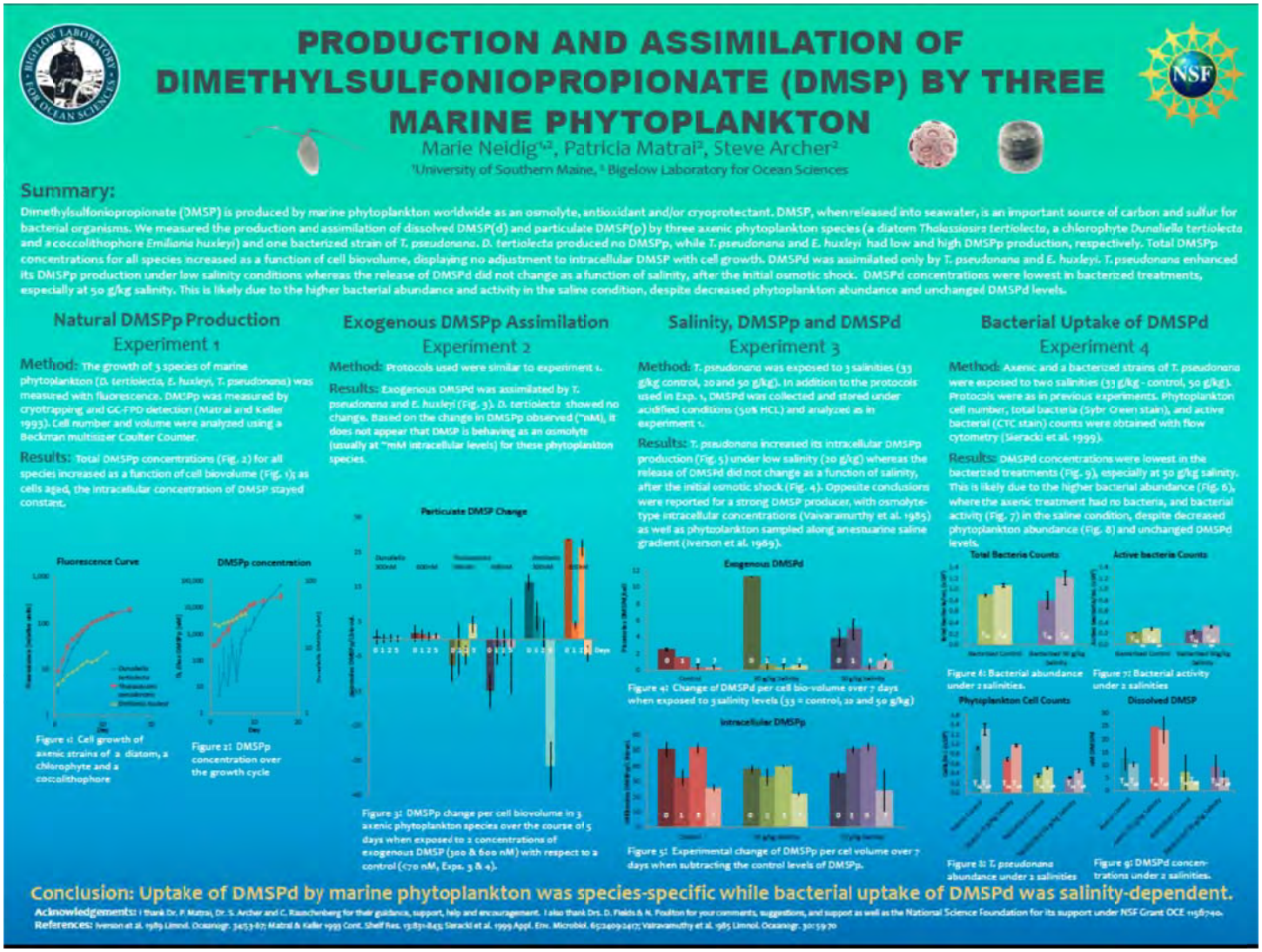
3:45 Kaitlyn Pritchard – Northeastern University
EXAMINING THE GRAZING PREFERENCES AND DIGESTIVE PROCESSES OF OXYRRHIS MARINA USING A RATIOMETRIC FLUORESCENT DYE
(Neuster-Twinning)

Abstracts

PRODUCTION AND ASSIMILATION OF DIMETHYLSULFONIONPROPIONATE (DMSP) BY MARINE PHYTOPLANKTON

Marie Neidig[1,2], Dr. Patricia Matrai[2] & Dr. Steve Archer[2]
[1]University of Southern Maine, [2] Bigelow Laboratory for Ocean Sciences

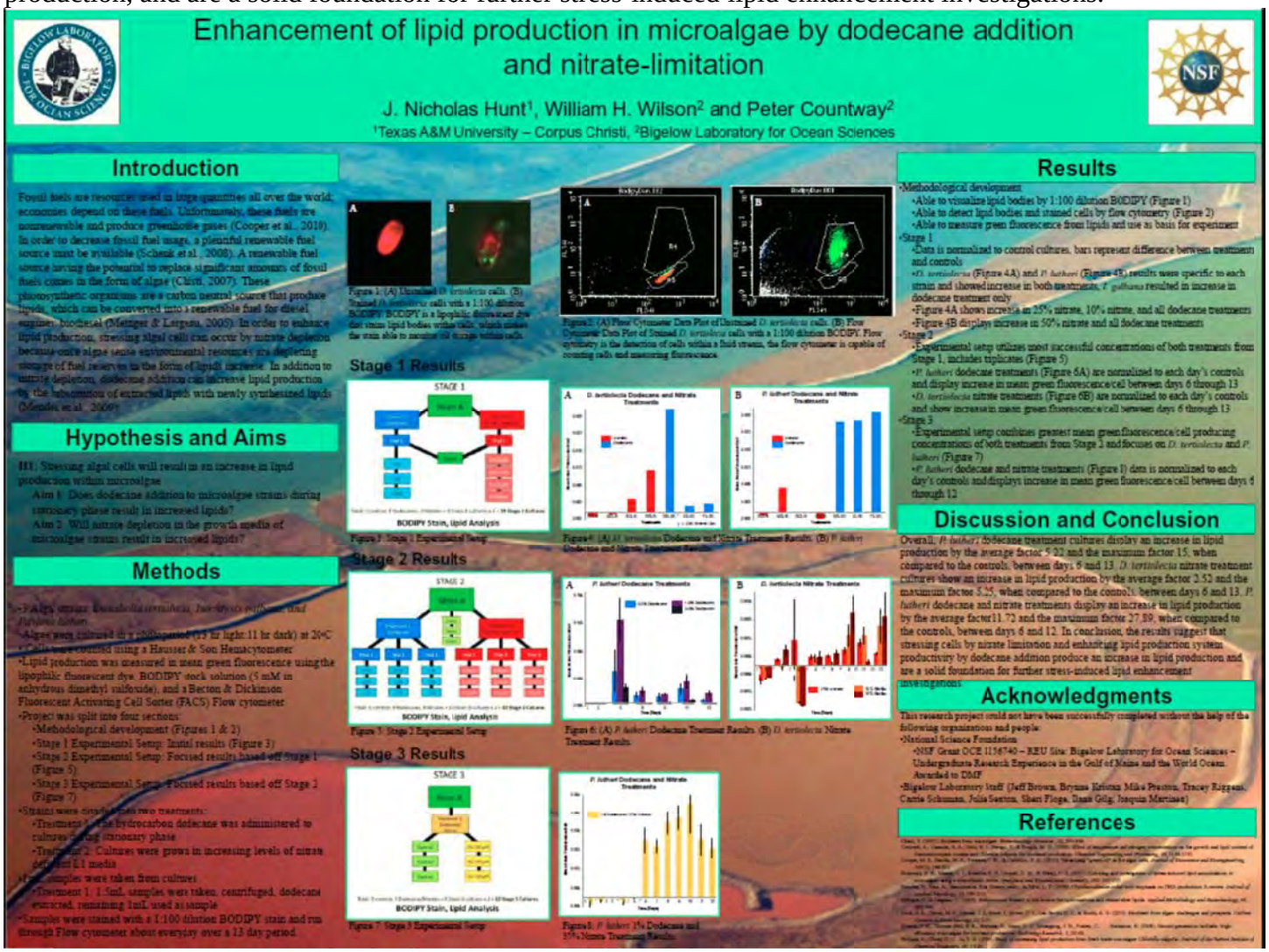
Dimethylsulfoniopropionate (DMSP) is produced by marine phytoplankton worldwide as an osmolyte, antioxidant and/or cryoprotectant. DMSP, when released into seawater, is an important source of carbon and sulfur for bacterial organisms. We measured the production and assimilation of dissolved DMSP(d) and particulate DMSP(p) by three axenic phytoplankton species (a diatom *Thalassiosira tertiolecta*, a chlorophyte *Dunaliella tertiolecta* and a coccolithophore Emiliania huxleyi) and one bacterized strain of *T. pseudonana. D. tertiolecta* produced no DMSPp, while *T. pseudonana* and *E. huxleyi* had low and high DMSPp production, respectively. Total DMSPp concentrations for all species increased as a function of cell biovolume displaying no adjustment to intracellular DMSP with cell growth. DMSPd was assimilated only by *T. pseudonana* and *E. huxleyi. T. pseudonana* enhanced its DMSPp production under low salinity conditions whereas the release of DMSPd did not change as a function of salinity, after the initial osmotic shock. DMSPd concentrations were lowest in bacterized treatments, especially at 50 g/kg salinity. This is likely due to the higher bacterial abundance and activity in the saline condition, despite decreased phytoplankton abundance and unchanged DMSPd levels.



ENHANCEMENT OF LIPID PRODUCTION IN MICROALGAE BY DODECANE ADDITION AND NITRATE LIMITATION

J. Nicholas Hunt[1], William H. Wilson[2] and Peter Countway[2]
[1]Texas A&M University – Corpus Christi, [2]Bigelow Laboratory for Ocean Sciences

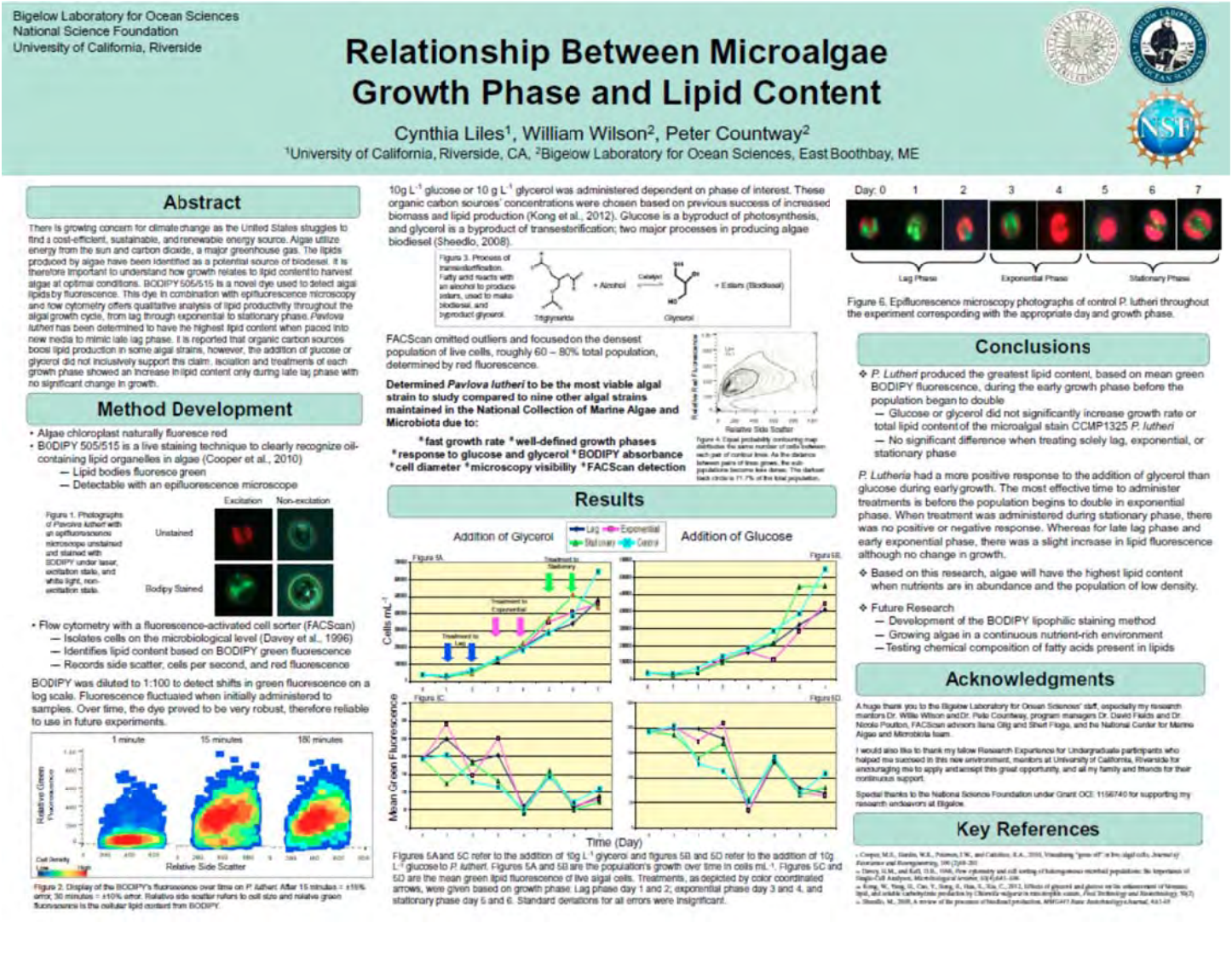
Algae, a renewable fuel source, can potentially replace significant fossil fuel consumption levels. These photosynthetic organisms produce lipids that can be converted into renewable fuel for diesel engines: biodiesel. This research focused on enhancing lipid production by stressing *Dunaliella tertiolecta, Isochrysis galbana,* and *Pavlova lutheri* cultures with the hydrocarbon dodecane and depleting nitrate amounts in the growth medium. Lipid concentrations were monitored using the fluorescent dye, BODIPY 505/515, and flow cytometry. Strains were divided into two treatments: dodecane-administered cultures during stationary phase and nitrate-limited cultures. Samples were stained with a 1:100 dilution BODIPY, and analyzed for green fluorescence by flow cytometry. The main findings display a lipid production increase by the average factor 5.22 and maximum factor 15 for the *P. lutheri* dodecane treatments between days 6 and 13, after dodecane was administered, and an increase by the average factor 2.52 and maximum factor 5.25 for *D. tertiolecta* nitrate-limited treatments between days 6 and 13 compared to the controls. Stressing cells via these methods result in increased lipid production, and are a solid foundation for further stress-induced lipid enhancement investigations.



THE RELATIONSHIP BETWEEN MICROALGAE GROWTH PHASE AND LIPID CONTENT

Cynthia Liles, Environmental Sciences, University of California, Riverside; Dr. Pete Countway and Dr. Willie Wilson, National Center for Marine Algae and Microbiota, Bigelow Laboratory for Ocean Sciences

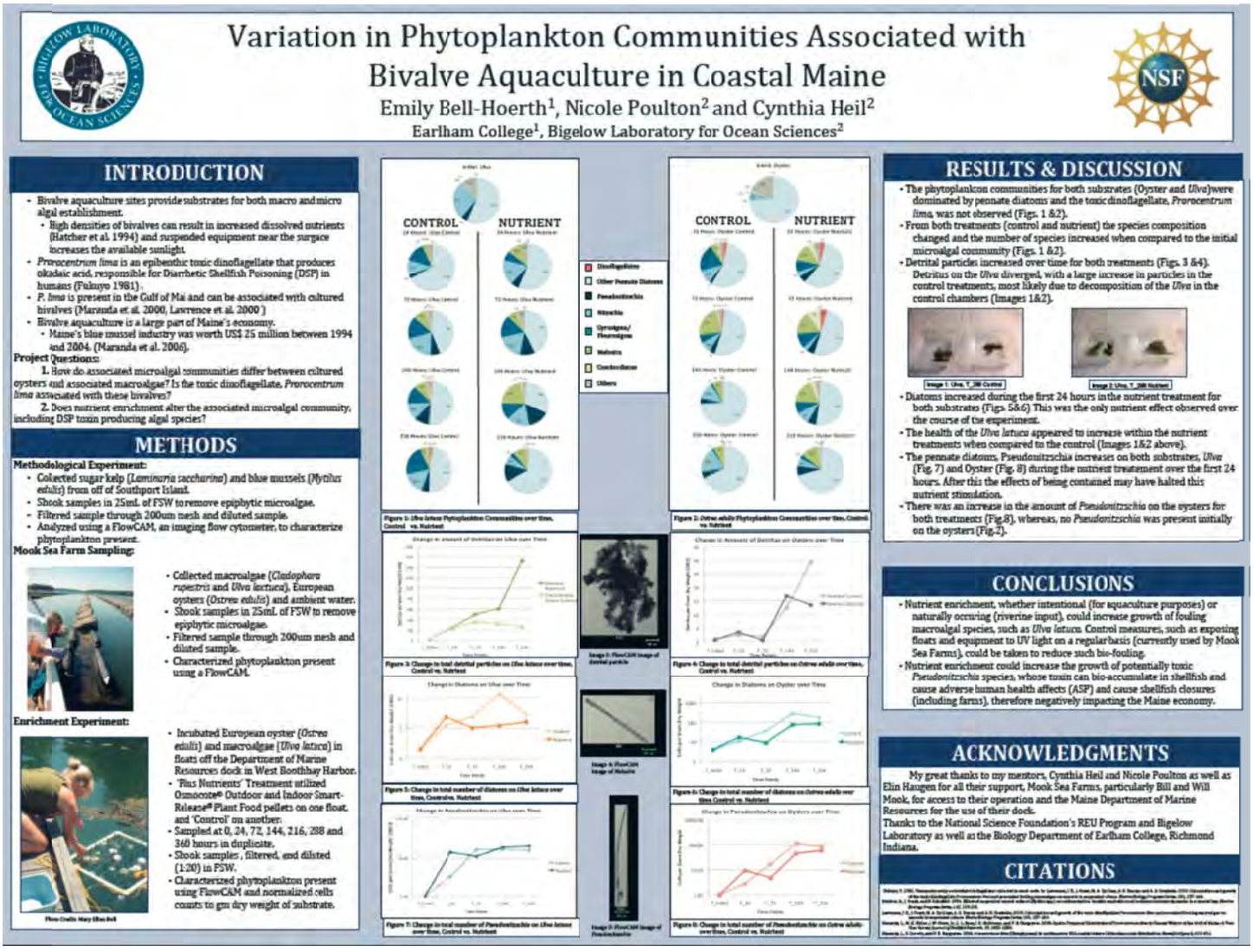
There is growing concern for climate change as the United States struggles to find a cost-efficient, sustainable, and renewable energy source. Algae utilize energy from the sun and carbon dioxide, a major greenhouse gas. The lipids produced by algae have been identified as a potential source of biodiesel. It is therefore important to understand how growth relates to lipid content to harvest algae at optimal conditions. BODIPY 505/515 is a novel dye used to detect algal lipids by fluorescence. This dye in combination with epifluorescence microscopy and flow cytometry offers qualitative analysis of lipid productivity throughout the algal growth cycle, from lag through exponential to stationary phase. *Pavlova lutheri* has been determined to have the highest lipid content when placed into new media to mimic late lag phase. It is reported that organic carbon sources boost lipid production in some algal strains, however, the addition of glucose or glycerol did not inclusively support this claim. Isolation and treatments of each growth phase showed an increase in lipid content only during late lag phase with no significant change in growth.



VARIATIONS IN PHYTOPLANKTON COMMUNITIES ASSOCIATED WITH BIVALVE AQUACULTURE IN COASTAL MAINE

Emily Bell-Hoerth[1], Cynthia Heil[2] and Nicole Poulton[2]
Earlham College[1], Bigelow Laboratory for Ocean Sciences[2]

Bivalve aquaculture provides substrate for epibenthic microalgae and can alter nutrient dynamics in the surrounding water. Harmful algal species, such as *Prorocentrum lima*, which produces okadaic acid causing Diarrhetic Shellfish Poisoning (DSP), have been associated with bivalve aquaculture in the Gulf of Maine. To understand the relationship between cultured bivalves and their associated macro and microalgae, we examined the effect of nutrient additions on the microalgal communities associated with the European Oyster, *Ostrea edulis* and a bio-fouling macroalgal species, *Ulva latuca*. *Ostrea edulis* and *U. latuca* were collected from the Damariscotta River and incubated in situ with and without nutrients (Osmocote©) in West Boothbay Harbor and sampled on a regular basis. Changes in the phytoplankton community on each substrate over time were characterized using an imaging-flow-cytometer (FlowCAM). All communities were dominated by pennate diatoms. Detrital matter increased on both *Ulva* and oyster incubations over time. *Pseudonitzschia* responded positively to nutrient stimulation within the first 24 hours on both substrates. This potentially toxic species could prove harmful to human health and bivalve farms. *Prorocentrum lima* was not present in significant amounts.

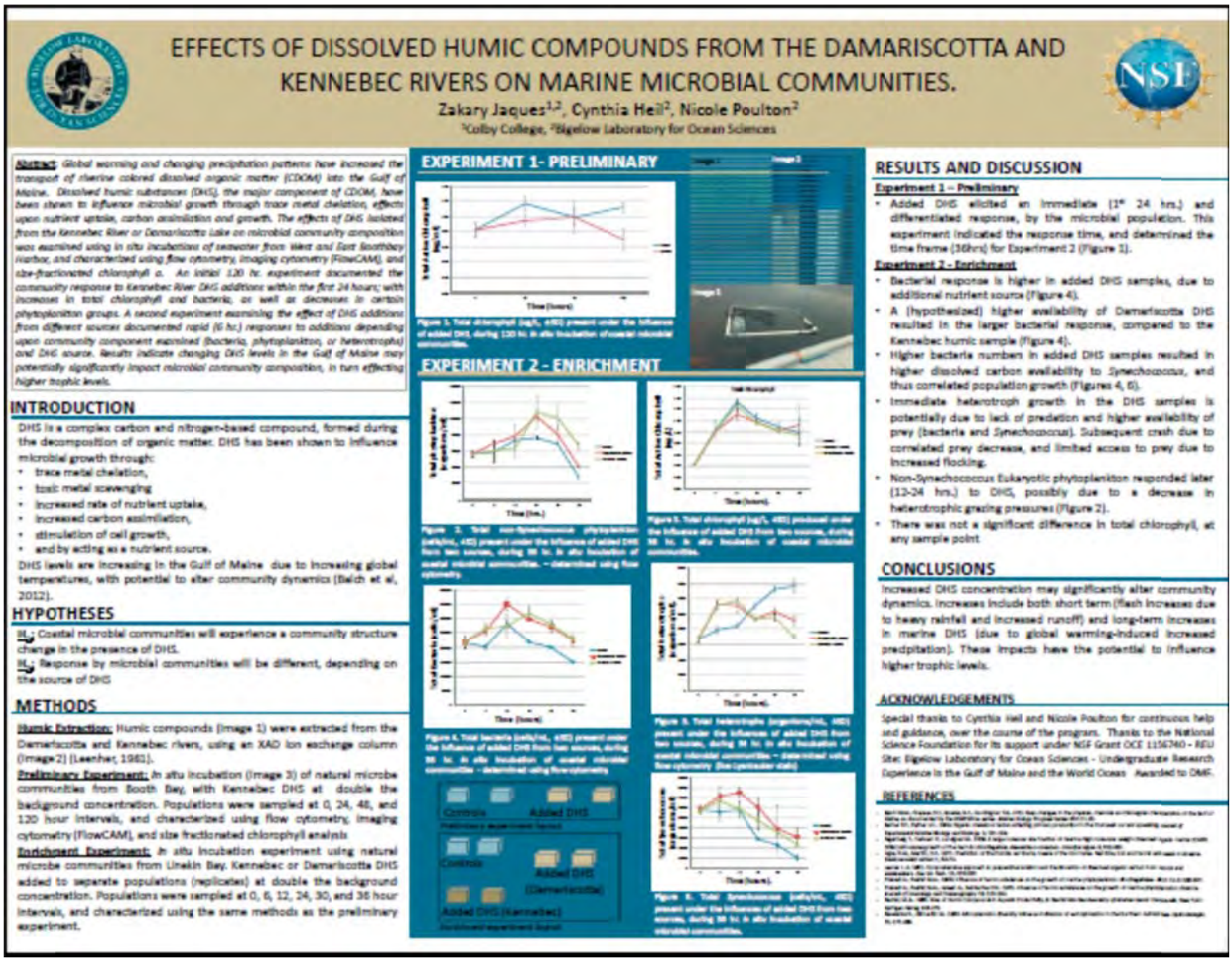


EFFECTS OF DISSOLVED HUMIC COMPOUNDS FROM THE DAMARISCOTTA AND KENNEBEC RIVERS ON MARINE MICROBIAL COMMUNITIES.

Zakary Jaques[1,2], Cynthia Heil[2], Nicole Poulton[2]

[1]Colby College, [2]Bigelow Laboratory for Ocean Sciences

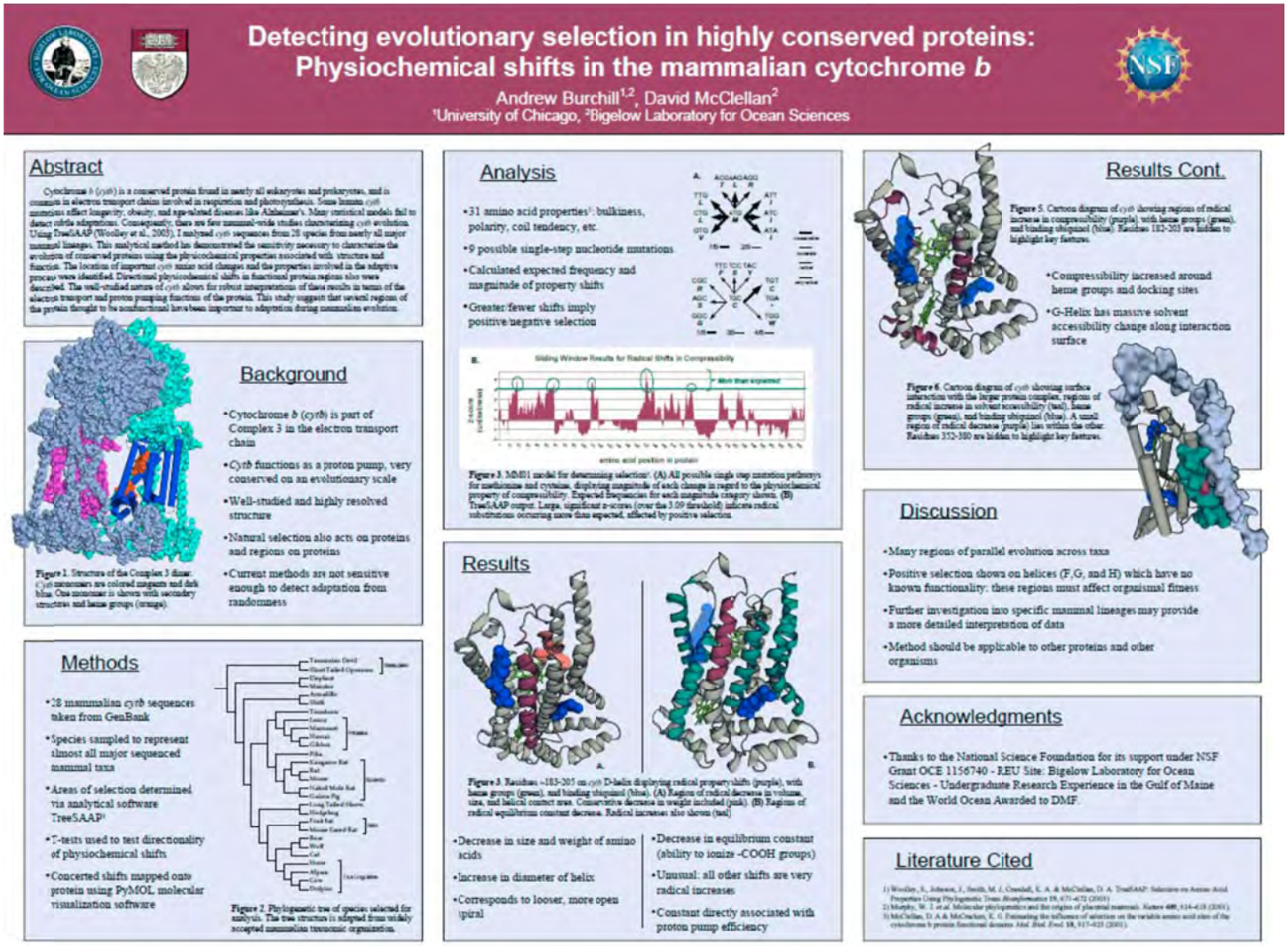
Global warming and changing precipitation patterns have increased the transport of riverine colored dissolved organic matter (CDOM) into the Gulf of Maine. Dissolved humic substances (DHS), the major component of CDOM, have been shown to influence microbial growth through trace metal chelation, effects upon nutrient uptake, carbon assimilation and growth. The effects of DHS isolated from the Kennebec River or Damariscotta Lake on microbial community composition was examined using *in situ* incubations of seawater from West and East Boothbay Harbor, and characterized using flow cytometry, imaging cytometry (FlowCAM), and size-fractionated chlorophyll *a*. An initial 120 hr experiment documented the community response to Kennebec River DHS additions within the first 24 hours; with increases in total chlorophyll and bacteria, as well as decreases in certain phytoplankton groups. A second experiment examining the effect of DHS additions from different sources documented rapid (6 hr) responses to additions depending upon community component examined (bacteria, phytoplankton, or heterotrophs) and DHS source. Results indicate changing DHS levels in the Gulf of Maine may potentially significantly impact microbial community composition, in turn effecting higher trophic levels.



DETECTING EVOLUTIONARY SELECTION IN HIGHLY CONSERVED PROTEINS: PHYSIOCHEMICAL SHIFTS IN THE MAMMALIAN CYTOCHROME B

Andrew Burchill[1], David McClellan[2]
[1]University of Chicago, [2]Bigelow Laboratory for Ocean Sciences

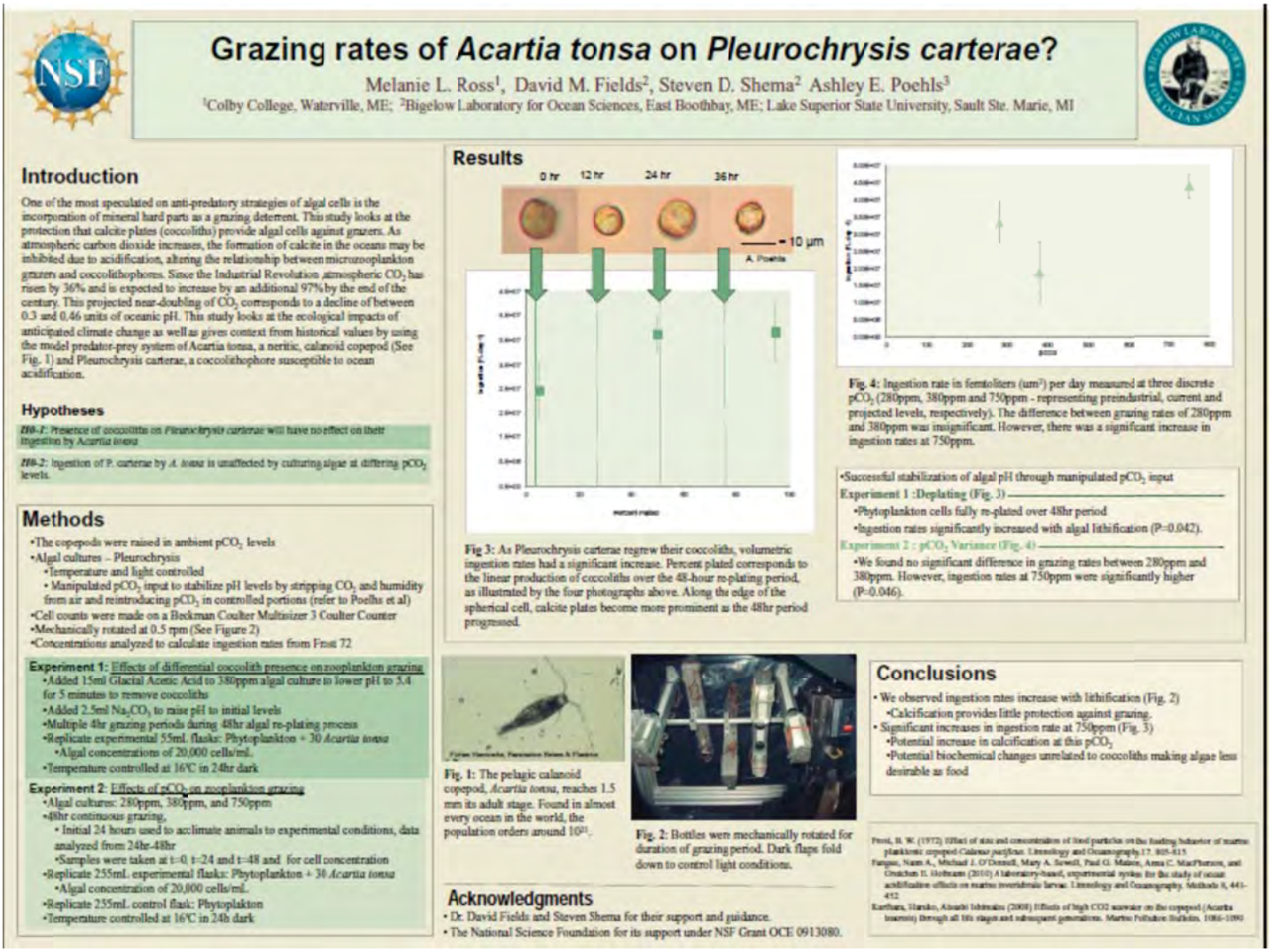
Cytochrome *b* (*cytb*) is a conserved protein found in nearly all eukaryotes and prokaryotes, and is common in electron transport chains involved in respiration and photosynthesis. Some human *cytb* mutations affect longevity, obesity, and age-related diseases like Alzheimer's. Many statistical models fail to detect subtle adaptations. Consequently, there are few mammal-wide studies characterizing *cytb* evolution. Using TreeSAAP (Woolley et al., 2003), I analyzed *cytb* sequences from 28 species from nearly all major mammal lineages. This analytical method has demonstrated the sensitivity necessary to characterize the evolution of conserved proteins using the physicochemical properties associated with structure and function. The location of important *cytb* amino acid changes and the properties involved in the adaptive process were identified. Directional physicochemical shifts in functional protein regions also were described. The well-studied nature of *cytb* allows for robust interpretations of these results in terms of the electron transport and proton pumping functions of the protein. This study suggests that several regions of the protein thought to be nonfunctional have been important to adaptation during mammalian evolution.



INDIRECT EFFECTS OF OCEAN ACIDIFICATION ON ACARTIA TONSA

Melanie Ross[1], David Fields[2], Barney Balch[2], Steve Shema[2], Ashley Poehls[3]
[1]Colby College, [2]Bigelow Laboratory for Ocean Sciences, [3]Lake Superior State University

Atmospheric pCO_2 has risen by 36% over the preindustrial level and is expected to increase by an additional 97% by the end of the century. The oceanic absorption of that influx of atmospheric CO_2 has caused a 0.1 pH unit decrease in the oceans, predicted to decline another 0.3 to 0.46 pH units by 2100 according to the Intergovernmental Panel on Climate Change. This study investigated indirect effects of ocean acidification on the neritic calanoid copepod *Acartia tonsa*. Ingestion rates of *A. tonsa* feeding on the coastal coccolithophore, *Pleurochrysis*, were measured in 24-hour bottle experiments at 16ºC. The copepods were all raised at a pCO_2 level of 380ppm while the algae were grown at three distinct pCO_2 levels (280ppm, 380ppm, and 750ppm, representing pre-industrial, current, and predicted CO_2 inputs). Additionally, de-plated Pleurochrysis from all three pCO_2 levels were fed in three separate 4-hour grazing periods throughout the 48-hour re-plating process to compare ingestion rates with cells' lithe structure. For 280ppm, the grazing rates decreased during the re-plating process, while 380 and 750ppm both saw an increase grazing rates. The highest ingestion rates for regularly plated cells were from 750ppm, followed by 280ppm and 380ppm respectively.



EXPERIMENTAL TECHNIQUES FOR CHARACTERIZING SHEATH-FORMING *ZETAPROTEOBACTERIA:* A METHODOLOGICAL APPROACH

Alyson Lowell[1, 2], Dr. Erin Field[2], Dr. David Emerson[2] and Dr. David McClellan[2]

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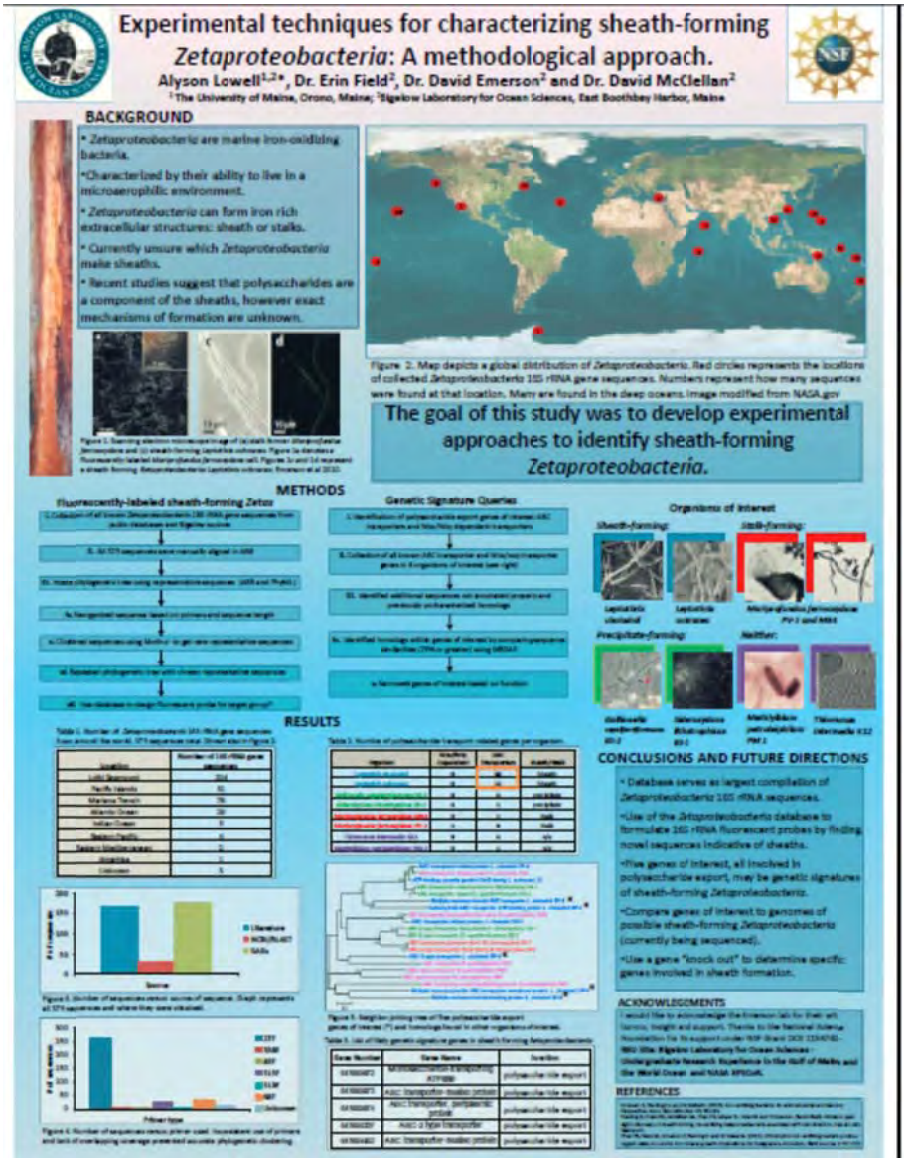
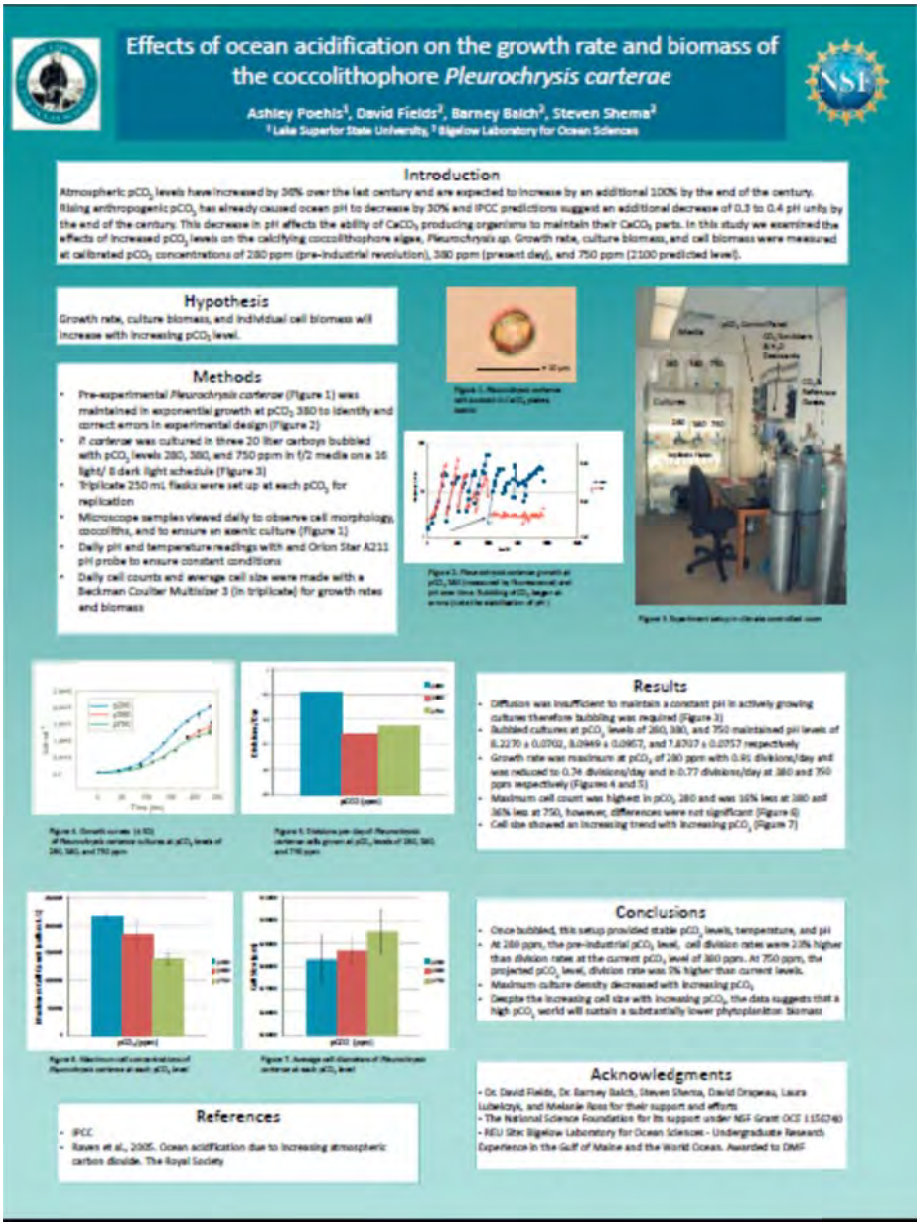
Zetaproteobacteria ("Zetas") are marine microorganisms that oxidize iron in microaerophilic environments. As iron is oxidized, extracellular structures such as helical stalks or tubular sheaths form. How these structures form is largely unknown, however studies have indicated that polysaccharides are an organic component of what is a largely inorganic matrix of Fe-oxides. This research focused on preliminary attempts to identify sheath-forming *Zetaproteobacteria.* Investigations centered on identifying characteristic "*Zeta*" sheath-forming sequences to generate fluorescent *in situ* hybridization (FISH) probes and to identify novel genes as genetic signatures. 375 16S rRNA *Zetaproteobacteria* gene sequences were compiled from both public and in-house sources representing the largest compilation of *Zetaproteobacteria.* Unfortunately, discrepancies in sequence alignment were observed and additional work must be done to increase reproducibility and robustness. Comparative genomics was used to determine possible genetic signatures for sheath-forming *"Zetas."* Eight novel organisms representing stalks, sheaths, precipitates and non-structure forming bacteria were used to determine polysaccharide export genes. Specifically, a large number of ABC transporter genes were identified in sheath-forming *Betaproteobacteria*, a freshwater iron oxidizer. Five genes were targeted as potential genes involved in polysaccharide export.



EFFECTS OF OCEAN ACIDIFICATION ON GROWTH RATE, BIOMASS, AND COCCOLITH MORPHOLOGY OF THE COCCOLITHOPHORE *PLEURochrysis*

Ashley Poehls[1], David Fields[2], Barney Balch[2], Steven Shema[2]
[1] Lake Superior State University, [2] Bigelow Laboratory for Ocean Sciences

Abstract: Atmospheric pCO_2 levels have increased by 36% over the last century and are expected to increase by an additional 100% by the end of the century. Rising pCO_2 has already caused ocean pH to decrease by 0.1 units and IPCC predictions suggest an additional decrease of 0.3 to 0.45 pH units by the end of the century. In this study we examined the effects of increased pCO_2 levels on the calcifying coccolithophore algae, *Pleurochrysis sp.* Growth rate, culture biomass, and cell biomass were measured at calibrated pCO_2 concentrations of 280, 380, and 750 ppm. SEM, birefringence, and light microscope images were used to visualize pCO_2 effects on cell and lith structure. Data shows that with increasing pCO_2 levels, growth rate and culture biomass decreased by X and X respectively, while individual cell biomass increased by X. SEM images revealed a compromised lith structure with increasing pCO_2. These results indicate that *Pleurochrysis* are physiologically and morphologically altered by ocean acidification. Because coccolithophores are major contributors to the ocean's primary productivity, carbon cycle, and optical properties, these effects have many ecological implications.



EXAMINING THE GRAZING PREFERENCES AND DIGESTIVE PROCESSES OF OXYRRHIS MARINA USING A RATIOMETRIC FLUORESCENT DYE

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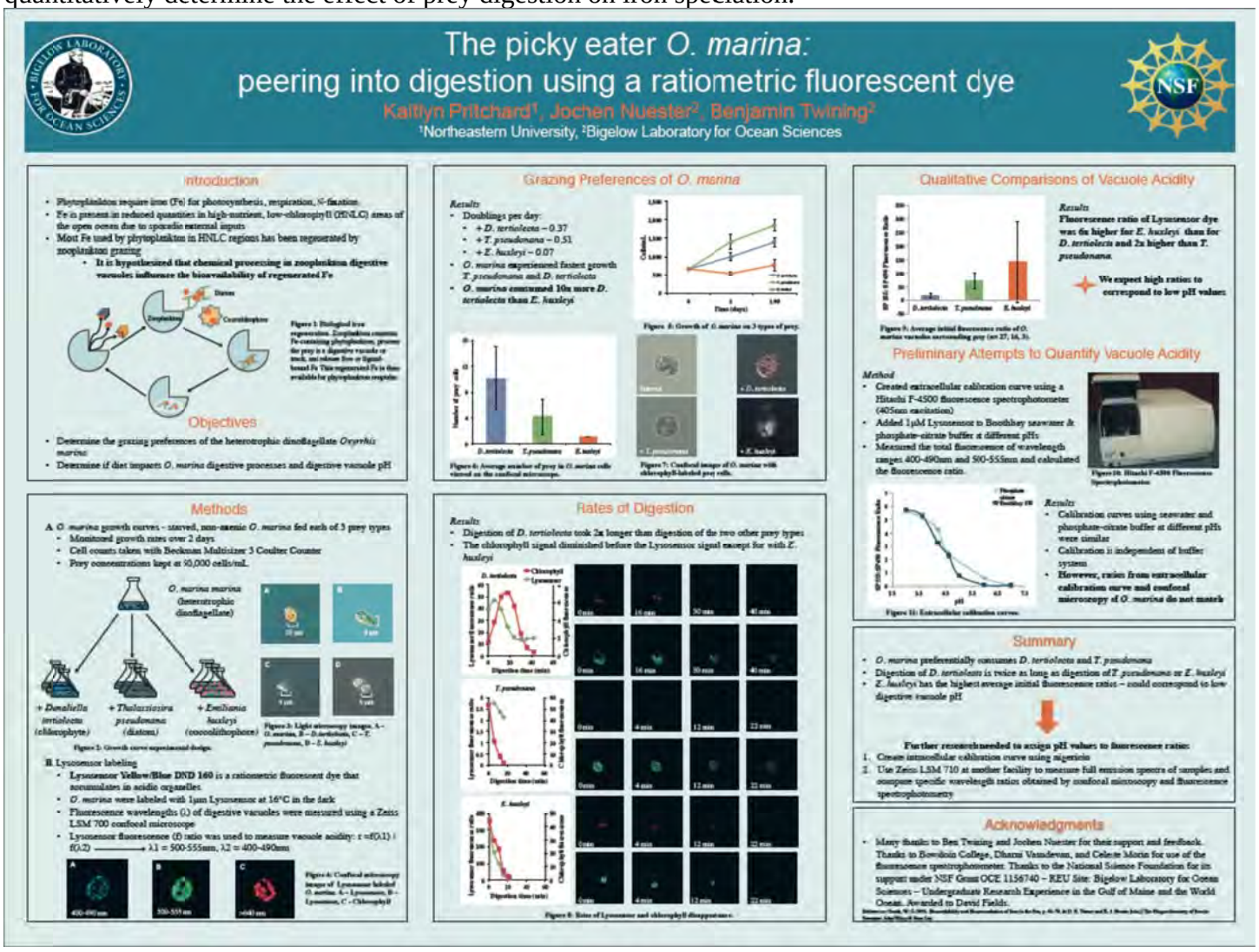
Most of the iron used by phytoplankton in high-nutrient, low-chlorophyll areas comes from remineralization by zooplankton grazers. Understanding chemical processing of iron within grazer digestive systems and the influences of prey-type on this processing is therefore useful to understanding grazer controls on the speciation and bioavailability of regenerated iron. As such, we studied the grazing preferences and digestion of prey within vacuoles of the heterotrophic dinoflagellate *Oxyrrhis marina*. We compared grazing rates of three types of phytoplankton prey (diatom, coccolithophore, chlorophyte) over 24 hours. In addition, we used a ratiometric fluorescent dye to compare digestive vacuole acidity with each prey. Overall, we found that *Oxyrrhis* consumed 10 times more of a chlorophyte than a coccolithophore (the least consumed prey) and took 2-3 times as long to digest these cells. However, initial fluorescence ratios (with high ratios corresponding to low pH) were inversely proportional to prey preference, with vacuole fluorescence ratios approximately 6 times higher with coccolithophores than with dinoflagellates. Additional work will constrain pH from these ratios and quantitatively determine the effect of prey digestion on iron speciation.



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.