



Bigelow Laboratory for Ocean Sciences

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

REU Symposium Program & Abstracts
Thursday, August 13, 2010



Program

1:00 Opening Remarks

- 1:15 Ileana M. Freytes Ortiz
COBBLE-DWELLING FAUNA: INTER-OCEANIC DIFFERENCES IN TROPHIC STRUCTURE?
- 1:30 Jasper Nutt
PHAGE THERAPY AS A VACCINE FOR *AEROMONAS SALMONICIDA*.
- 1:45 Meaghan Daley
LINKING GROWTH, FE UPTAKE AND FE DISTRIBUTION IN *TRICHODESMIUM* TO FE AVAILABILITY.
- 2:00 Macon “Joey” Brown
DEFINING THE MAJOR LINEAGES OF RED ALGAE (RHODOPHYTA).
- 2:15 April Klein
MARINE VIRUSES: A STUDY ON HOST-VIRUS RESPONSE TO ELEVATED LEVELS OF CO₂.
- 2:30 Brittney Honisch
IRON REQUIREMENTS FOR GROWTH IN COASTAL AND OCEANIC STRAINS OF *EMILIANA HUXLEYI*.
- 2:45 Break (15 minutes)**
- 3:00 Brandon Walus
EFFECTS OF SUB-LETHAL CONCENTRATIONS OF CRUDE OIL ON COPEPOD BEHAVIOR: *ACARTIA TONSA*.
- 3:15 Angel Mojarro
MICROGELS AND DMSP IN MARINE PHYTOPLANKTON.
- 3:30 Rachelle R. Campbell
WHAT FREED WILLY: ANCIENT RESPIRATORY ADAPTATIONS IN WHALES
- 3:45 Whitney M. Westman
BARNACLES: FANS OF VISCOSITY. THE EFFECTS OF KINEMATIC VISCOSITY ON FEEDING BEHAVIORS IN *BALANUS BALANOIDES*.
- 4:00 Jennifer R. Fownes
NOVEL METHODS FOR VISUALIZING IRON-OXIDIZING BACTERIA ON MILD STEEL.

Abstracts

COBBLE-DWELLING FAUNA: INTER-OCEANIC DIFFERENCES IN TROPHIC STRUCTURE?

Ileana M. Freytes Ortiz^{1,2}, Richard Wahle³, Álvaro Palma⁴

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Cobble-boulder habitats are common along coastlines worldwide and serve as nurseries for commercially important marine species. An NSF-funded project studying recent tsunami impacts on Robinson Crusoe Island (RCI), Chile, permitted a comparison of the cobble-dwelling fauna of this relatively pristine Southeast Pacific Island and the Gulf of Maine (GOM), where the demersal fish populations, the principal predators of cobble-dwelling fauna, have been decimated by overfishing. Diver-based suction sampling was used to quantify the interstitial invertebrate fauna. Video surveillance was used to quantify resident demersal fish, and their response to an experimental disturbance that exposed the infauna to predation. Fish density, diversity and their response to disturbance were significantly higher in RCI than in GOM, denoting a rapid trophic response by the top consumers. The diversity and density of benthic infauna in RCI, however, were on a par with GOM. Crustaceans were more cryptic and diminutive in body size at RCI than in GOM. Taken together, our results suggest predatory fish play a more important role at RCI than the GOM in shaping the characteristics of the cobble-dwelling fauna.

PHAGE THERAPY AS A VACCINE FOR *AEROMONAS SALMONICIDA*

Jasper Nutt, University of Maine, Orono

Bigelow Mentor: Ilana Gilg, and Willie Wilson

The bacterium *Aeromonas salmonicida* causes the disease furunculosis in fish. As antibiotic-resistant strains become more prevalent it is increasingly important to find an alternative treatment. One such treatment is phage therapy, which uses naturally occurring phage to target bacterial pathogens. We hypothesized that a cocktail of phage isolates could provide an effective alternative treatment for *A. salmonicida*. We identified 105 unique phage isolates and tested phage combinations to find an effective treatment. Growth curves of host infected with phage were observed spectrophotometrically. Varying degrees of resistance were observed. Chinook salmon embryonic cells were infected with 10 strains of *A. salmonicida*; each of these was treated with 4 concentrations of phage (number of infectious phage per bacterium: 10, 1, 0.1, and 0.01). A higher phage concentration resulted in an observable increase in cell health. Salmon cells with *A. salmonicida* and no phage were killed. Salmon cells with phage and no *A. salmonicida* showed no negative effects. Our data reveals that phage therapy is a promising approach to treat salmon furunculosis.

LINKING GROWTH, FE UPTAKE AND FE DISTRIBUTION IN *TRICHODESMIUM* TO FE AVAILABILITY

Meaghan Daley^{1,2}, Jochen Nuester², Ben Twining²

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Cyanobacteria of the genus *Trichodesmium* are considered the dominant Nitrogen fixing organisms in subtropical and tropical regions. Nitrogen fixation rates are often limited by iron (Fe) availability, yet cellular response to Fe availability is unknown. *Trichodesmium* growth, Fe uptake and Fe distribution were studied. Growth and Fe uptake rates were monitored at 2, 20, 200, and 2000 nM dissolved Fe. Light microscopy was used to count and measure trichomes, and ⁵⁵Fe was used to determine Fe uptake rates. Cultures grown at 20 and 200 nM Fe exhibited maximum growth rates, while Fe uptake rates decreased between cultures grown at 2 and 2000 nM Fe. This suggests that cells in Fe depleted cultures may be up-regulating Fe transport systems. Synchrotron X-ray fluorescence microscopy was used to study elemental distribution in trichomes. Mapping revealed that Fe was relatively evenly distributed in some trichomes, while other trichomes showed Fe localization. Fe distributions were often decoupled from phosphorous (P) distributions. The cause of these distribution patterns is currently unknown.

DEFINING THE MAJOR LINEAGES OF RED ALGAE (RHODOPHYTA)

Macon Brown^{1,2}, Eun Chan Yang¹, Hwan Su Yoon¹

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The two main goals that were set at the start of this internship and accomplished by the end were to learn proper molecular techniques in terms of DNA sequencing as well as to understand the phylogeny by experience of phylogenetic methods. DNA was successfully extracted, purified and sequenced, and set in its rightful position on the red algae phylogenetic tree for 28 strains during this summer. This is only a small part of a RedTol project (<http://dmlab.rutgers.edu/redtol>) which includes 400 sample. Dr. Yoon has turned his research attention to building a “Red Algae Tree of Life” because it has been astonishingly understudied and its important evolutionary role of primary endosymbiosis and as a donor of secondary endosymbiosis giving rise to cryptophytes, haptophytes, stramenopiles, and dinophyta.

MARINE VIRUSES: A STUDY ON HOST-VIRUS RESPONSE TO ELEVATED LEVELS OF CO₂

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2. University of Texas at Arlington, Arlington, TX, USA

Rising concentration of atmospheric CO₂ causes the ocean to become more acidic as CO₂ dissolves into the ocean and forms carbonic acid. This is termed ocean acidification. *Emiliana huxleyi* is a globally significant coccolithophore. The coccolithovirus EhV can infect and cause large scale mortality of *E. huxleyi* blooms. We tested the hypothesis that elevating the CO₂ concentration of natural seawater samples collected during a coccolithophore-dominated bloom would result in a detectable change in the genetic composition of the host (*E. huxleyi*) and virus (EhV) populations. Seawater samples were collected during a bloom off the coast of Argentina and incubated with various CO₂ levels representing present-day through predicted future scenario concentrations. We used PCR and denaturation gradient gel electrophoresis (DGGE) to identify CO₂ induced changes in the genetic structure of the virus-host populations. CO₂ had an affect on the genetic composition as some genotypes were present only at low CO₂ and others were present only at high CO₂. These results have implications for the role of ocean acidification on the dynamics of a globally important primary producer.

**IRON REQUIREMENTS FOR GROWTH IN COASTAL AND OCEANIC
STRAINS OF *EMILIANA HUXLEYI***

Brittney Honisch, Western Washington University

Bigelow mentors: Ms. Sara Rauschenberg, Dr. Benjamin Twining

Dissolved Fe concentrations in open ocean regions are up to 1,000-fold lower than in coastal waters. A previous comparison of diatom species isolated from these two habitats found lower Fe requirements in the oceanic species. We investigated whether a similar trend could be observed in the coccolithophore *Emiliana huxleyi*, a calcareous phytoplankton that plays a globally-significant role in carbon cycling. Three coastal and three oceanic *E. huxleyi* strains were grown in triplicate at 0 nM and 100 nM dissolved Fe. Additionally, two oceanic strains were grown at 0, 2, 5, 10, 100, 500 nM Fe concentrations to generate Monod curves. Cell concentration was measured regularly with a Coulter Counter or flow cytometer and growth rates determined. Growth rates of oceanic strains decreased 26 – 34% under low Fe conditions. By comparison, more substantial decreases were observed in the coastal strains (72 – 100%) under low Fe. Monod curve fits estimated half-saturation constants <1 nM Fe for both oceanic strains. Oceanic strains required less Fe for growth than coastal strains.

**EFFECTS OF SUB-LETHAL CONCENTRATIONS OF CRUDE OIL ON COPEPOD
BEHAVIOR: ACARTIA TONSA**

Brandon Walus, University of Maine, Orono

Mentors: David Fields and Steven Shema

Deep sea and offshore drilling is becoming the preferred method of accessing crude oil. Oil spilled during offshore operations rises through the water column releasing the water-soluble fraction (WSF). While the hydrophobic portion of crude oil floats on the sea surface, the WSF remains in solution throughout the water column. Little is known about the lethal concentration of WSF or the effects of the sub-lethal concentrations on the behavior of planktonic organisms. In this study we quantified the lethal concentration of the WSF of crude oil on *Acartia tonsa*, a planktonic copepod. We used three behavioral proxies to assess the effects of the sub-lethal WSF on copepod populations. Our data shows increasing mortality at concentrations of the WSF greater than 1%. We also found a decrease in the escape jump capabilities at concentrations as low as 0.03%. These results suggest that *A. tonsa* has high sensitivity to low concentrations of the WSF that could negatively impact recruitment rates.

MICROGELS AND DMSP IN MARINE PHYTOPLANKTON

Angel Mojarro, Brown University

Dr. Patricia Matrai, Bigelow Laboratory for Ocean Sciences

Polar algae living near or at the surface of the water are constantly met with prevailing harsh conditions. Low polar temperatures and high ultraviolet (UV) radiation are among the highest risk factors affecting algal growth and death. High UV radiation is extremely oxidizing while below freezing temperatures can cause permanent damage to cell membranes which ultimately leads to cell death. However, high concentrations of dimethylsulphoniopropionate (DMSP) and mucilaginous excretions have been reported amongst many types of polar phytoplankton. Here we report on the production of DMSP and gels by axenic cultures of the prymnesiophyte *Phaeocystis globosa* grown under various settings of temperature and UV radiation. Cell-normalized production for both parameters decreased as temperature and UV levels increased. Studies have suggested that high DMSP production might be a direct result of high UV radiation as an anti-oxidant measure and/or as a cryoprotectant. In addition, high mucilaginous excretion leading to “gloopy” microalgae among polar species is also believed to have similar anti-UV and cryoprotectant measures.

WHAT FREED WILLY: ANCIENT RESPIRATORY ADAPTATIONS IN WHALES

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ABSTRACT. Rapid changes in habitat (terrestrial to aquatic), morphology (e.g., location of nasal openings, presence of hind limbs, development of fusiform body shape), and behavior (e.g., mode of locomotion, extended submersion) in cetacean ancestors over just 30 million years most likely necessitated several molecular adaptations, especially among the proteins integral to cellular respiration. Cytochrome *b* is the central catalytic enzyme of the cytochrome *bc*₁ complex and is fundamental to the electron transfer chain of cellular respiration. 79 cetacean and artiodactyl cytochrome *b* gene sequences were used to estimate the effects of natural selection on the cytochrome *b* protein among ancestral cetacean lineages. Statistically significant results that identify protein sites and affected amino acid properties were mapped onto three-dimensional protein structures in an attempt to associate sites with protein structure and function. Overall conclusions included [1] structural protein adaptations preceded fine-tuned chemical adaptation; [2] adaptive sites cluster around *heme*-groups and proton input sites, suggesting that selection likely affected proton pump function; and [3] protein adaptation paralleled morphological adaptation, with both rates of change reaching a peak in the 55-25 mya range.

BARNACLES: FANS OF VISCOSITY. THE EFFECTS OF KINEMATIC VISCOSITY ON FEEDING BEHAVIORS IN *BALANUS BALANOIDES*.

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Barnacles are filter feeder organisms with global distribution. Ocean currents, ship hulls, and ballast waters all contribute to dispersal of barnacles. At latitudes where barnacles can be found, water temperatures vary, affecting metabolic rates of animals and physical properties of fluids such as viscosity. In this study we explored the effects of fluid viscosity independent of temperature on barnacles' feeding behavior using two prey sizes (~10 μ m; ~150 μ m). Water viscosity was manipulated using polyvinylpyrrolidone. Grazing experiments were conducted at 15^o C over four hours, at low and high viscosity. Despite a 20% increase in viscosity, there was no measurable effect on the ingestion rate of small prey, and an increased ingestion of large prey. To determine if barnacles modify the boundary layer through behavioral mechanisms we measured the feeding fans' beat frequency at a range of temperatures, and then at low and high viscosity. These results show that barnacles alter the boundary layer between cirri to maximize feeding. An increased beat frequency at a higher viscosity allows the ingestion rate to be constant for smaller prey, but to increase for larger prey.

NOVEL METHODS FOR VISUALIZING IRON-OXIDIZING BACTERIA ON MILD STEEL

Jennifer R. Fownes*^{1,2}, Joyce M. McBeth¹, and David Emerson¹

Microbiologically influenced corrosion in marine environments causes large economic damage. Chemolithoautotrophic marine iron-oxidizing bacteria (FeOB) have been observed in biofilms on mild steel surfaces. They produce an easily disturbed flocculent mat of helical iron-oxide encrusted stalks. In this study a new method was developed to assess the relationship between surface topography and biofilm-forming bacteria. Confocal laser scanning microscopy (CLSM) was used to simultaneously image FeOB biofilms and the mild steel surface they colonized. An FeOB pure culture (*Mariprofundus* sp. strain GSB2) was compared to FeOB enrichments and steel samples incubated on marine sediments. We hypothesized that FeOB cells would be associated with scratches on the mild steel surface; however, strain GSB2 and FeOB enrichments were not found on the surface despite the production of a flocculent mat. Natural samples formed a more adhesive biofilm that included both FeOB stalks and other microorganisms, including ones that appeared to contain chlorophyll. In conclusion, FeOB stalks in natural environments are able to adhere to mild steel surfaces, likely due to the presence of a complex biofilm community.

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