Bigelow Laboratory for Ocean Sciences

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

REU Symposium Program & Abstracts
Thursday, August 11, 2011

Program

1:00 Opening Remarks

1:15 Angel Ruacho - University of California, Irvine
IRON AND LIGAND RELEASE DURING COPEPOD GRAZING
(Twining/Neuster)

1:30 Nicholas Schulte- Tennessee Technological University
SINGLE-CELL GENOMICS UNCOVER DIVERSITY OF *PAULINELLA* AND PICOBILIPHYTA
(Yang/Yoon)

1:45 Alexandra Lopez - Interamerican University of Puerto Rico (San German)
HETEROTROPHIC VS AUTOTROPHIC METABOLISM IN IRON OXIDIZING BACTERIUM *LEPTOTHRIX OCHRACEA*
(Fleming/ Martinez/ Emerson/Stepanauskas)

2:00 Alexander Vermont - Northern Arizona University-Yuma
DIMETHYLSULFONIOPROPRIONATE (DMSP) RELEASE DURING MESOZOOPLANKTON GRAZING: A GRAZING DETERRENT?
(Matrai/Fields/Shema/Rauschenberg)

2:15 Helena Pound - University of Tennessee Knoxville
INVESTIGATION OF LATENT VIRUS INFECTIONS IN MARINE PHYTOPLANKTON
(Floge/Wilson)

2:30 Kate Hamre - Colby College
PREDATOR-PREY DYNAMICS OF *DINOPHYSIS* SPP., ITS PREY *MYRIONECTA RUBRA*, AND CRYPTOPHYTES IN WEST BOOTHBAY HARBOR, MAINE
(Reifel/Sieracki//Haugen/Poulton)

2:45 Break (15 minutes)

3:00 Nicole Messerman – University of Maine Orono
OCEAN ACIDIFICATION AND SPERM MOTILITY IN THE GIANT SEA SCALLOP, *PLACOPECTEN MAGELLANICUS*
(Wahle)

3:15 Lousia Walker - Colby College
DIVERSITY AND GENE EXPRESSION OF *PHAEOCYSTIS*-ASSOCIATED DMSP DEGRADING BACTERIA IN CULTURES & COASTAL GULF OF MAINE WATERS
(Countway)

3:30 Kai Eldredge - University of Chicago
UREA IN CENTRAL MAINE COASTAL WATERS: CONCENTRATIONS AND INTERACTIONS WITH *ALEXANDRIUM FUNDYENSE* IN NATURAL MICROBIAL POPULATIONS.
(Heil)

3:45 Abigail Fuchsman - Bard College
EVOLUTIONARY PRESSURE ON CYTOCHROME *C* OXIDASE I IN COPEPODS RESULTING IN ADAPTATION TO NEW HABITATS
(McClellan)

4:00 Mark Chaffin - Colby College
WHOLE GENOME COMPARISON OF UNCULTURED, AMMONIA-OXIDIZING MARINE GROUP I *ARCHAEA* THROUGH SINGLE-CELL GENOMICS
(Swan/Stepanauskas)

Abstracts

IRON AND LIGAND RELEASE DURING COPEPOD GRAZING

Angel Ruacho[1] Jochen Nuester[2] Ben Twining[2]
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Iron is a key nutrient for many processes in phytoplankton. In high-nutrient, low-chlorophyll regions (e.g. Southern Ocean and Equatorial Pacific) the growth of diatoms is typically limited by Fe. Although most external Fe inputs are Aeolian, Fe is largely produced by recycling within the marine food web. Over 90% of Fe in the ocean is bound to ligands. Grazing is thought to be a major process for the release of both Fe and ligands. In order to test the influence of grazing on Fe and ligand release, 24-hour copepod grazing experiments (*Arcatia tonsa*; density: 0.7 animals/mL) were performed with the diatom *Thalassiosira pseudonana* as prey (initial density: 100,000 cells/mL) and analyzed by competitive ligand equilibration-cathodic stripping voltammetry using trace-metal clean techniques. We calculated an ingestion rate of 81,447 cells/animal/day. Grazing contributed to a 3.41 nM increase of dissolved Fe in comparison to a control treatment with no copepods. Surprisingly, excess ligands were either too weak to be detected or not produced during grazing in our experiments. If produced ligands were fully bound to Fe, then they also couldn't be detected.

SINGLE-CELL GENOMICS UNCOVER DIVERSITY OF *PAULINELLA* AND PICOBILIPHYTA

Nick Schulte[1,2], Eun Chan Yang[2], Hwan Su Yoon[2]
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The Rhizarian genus *Paulinella* exhibits evidence of plastid formation through recent primary endosymbiosis. Picobiliphyta is a novel Hacrobian division of heterotrophic algae that is understudied and scattered in distribution. The diversities of *Paulinella* and Picobiliphyta microprotist clades were investigated in this study using single-cell genomics. From 6 collection sites in ME, RI, NJ, MD, NC, and LA (United States east coast) 585 unicellular protists were isolated by cell size (10-20 μm) and trophism, whole genomes were amplified, and 18S rRNA was sequenced. Sixty-two Rhizaria were discovered and identified into 20 clades in a ML phylogenetic tree and BLAST. Four species of heterotrophic *Paulinella* were detected from RI, MD, NC, and LA, supporting a monophyly of *P. ovalis* and *P. chromatophora*. The results support photoautrophic *Paulinella* divergence from heterotrophic *Paulinella*. Picobiliphytes were found exclusively through heterotrophic cell sorting in ME, NJ, NC, and LA. Thirty picobiliphytes were resolved in 2 assemblages within the BP1 clade, elucidating unprecedented picobiliphyte lineages and a potential and inexplicable correlation between distribution and diversity. These results augment the deficient species diversity knowledge among microprotists.

HETEROTROPHIC VS AUTOTROPHIC METABOLISM IN IRON OXIDIZING BACTERIUM *LEPTOTHRIX OCHRACEA*

Alexandra Lopez[1,2] Emily Fleming[1], Manuel Martinez[1], David Emerson[1] and Ramunas Stepanauskas[1]

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Freshwater microbial iron seeps are communities denominated by lithotrophic iron oxidizing bacteria (FeOB) that make brown-orange mats causing: biocorrosion, biofouling, and reducing water flow. The most active FeOB is *Leptothrix ochracea*, which grows filamentously in a tubular iron sheath. *L. ochracea* requires high concentration of iron for the growth of only few cells (similar to autotrophic FeOB). It is also phylogenetically related to other cultured, filamentous, heterotrophic *Leptothrix* species. Previous attempts to isolate *L. ochracea* in heterotrophic and autotrophic media, have failed and its carbon metabolism remains unclear. Therefore to establish the carbon metabolism of *L. ochracea* two approaches were used. First, the presence of RubisCO and Lactate dehydrogenase (LDHase) genes in *L. ochracea* were established by amplification of both of these genes using gene-specific primers and PCR on a single amplified genome of *L. ochracea*. This result indicates that *L. ochracea* has the capacity for autotrophic and heterotrophic metabolism. Secondly, RNA was extracted from *in vitro* incubations of *L.ochracea*- rich mats and successfully converted to cDNA (as confirmed by positive PCR using 16S rRNA specific primers. Future work will focus on demonstrating the expression of LDHase and/or RubisCO genes by PCR on this cDNA preparation. Positive PCR products of the correct size will be sequenced to confirm their identity as RubisCO or LDHase.

DIMETHYLSULFONIOPROPRIONATE (DMSP) RELEASE DURING MESOZOOPLANKTON GRAZING: A GRAZING DETERRENT?

Alexander Vermont[1], Patricia Matrai[2], David Fields[2], Steven Shema[2], Carlton Rauschenberg[2]
[1]Northern Arizona University-Yuma, [2]Bigelow Laboratory for Ocean Sciences

The effect of copepod grazing on algal DMSP production was investigated using *A. tonsa* and two algal species that produce high and low concentrations of DMSP (*Emiliania huxleyi* and *Thalassiosira weissflogii*, respectively). Results suggest that grazed cells released a significant proportion of the particulate DMSP (DMSPp) into the dissolved state (DMSPd), whereas controls remained constant. Additionally, the average particulate cell quotas of remaining cells decreased in cultures exposed to grazers, suggesting that DMSP release is not solely a product of cell lysis. Grazing experiments with added DMSP suggest that increased extracellular DMSP results in decreased ingestion rates by copepods. This suggests a negative feedback loop between metazoan grazing pressure and algal DMSP, where grazing pressure results in higher proportions of dissolved DMSP, which in turn deters further grazing.

INVESTIGATION OF LATENT VIRUS INFECTIONS IN MARINE PHYTOPLANKTON

Helena Pound[1], Sheri Floge[2], William Wilson[2]
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Viruses are found in massive abundances in marine environments and play a key role in shaping these systems. Viruses have three primary infection strategies, termed lytic, chronic, and latent infections. However, to date most research on marine viruses has focused on lytic infections. Virus-like particles (VLPs) have been observed in apparently healthy cultures of marine phytoplankton for over 40 years, these observations have largely been ignored. We investigated three strains of marine phytoplankton thought to harbor latent virus infections, *Pleurochrysis carterae* (CCMP 645), *Dunaliella tertiolecta* (CCMP 1320), and *Isochrysis sp*. (CCMP 1324), using temperature induction as a mechanism to induce the lytic infection strategy. A combination of flow cytometry and transmission electron microscopy revealed that VLP abundance parallels that of the host phytoplankton abundance in unstressed control cultures of all three strains, suggesting these viruses persist in stable coexistence with their phytoplankton hosts in culture, without any apparent deleterious effect to their growth. These data highlight a new mechanism to consider for virus-host interactions in the open ocean.

PREDATOR-PREY DYNAMICS OF *DINOPHYSIS* SPP., ITS PREY *MYRIONECTA RUBRA*, AND CRYPTOPHYTES IN WEST BOOTHBAY HARBOR, MAINE

[1]**Kate Hamre**, [2]Kristen Reifel, [2]Mike Sieracki, [2]Elin Haugen, [2]Nicole Poulton
[1]Colby College, Waterville, Maine[2]Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine

Dinophysis spp. is a toxic dinoflagellate known to cause Diarrhetic Shellfish Poisoning (DSP) blooms in some coastal regions. In the Gulf of Maine, *Dinophysis* appears on an annual basis, but DSP-producing blooms have not been observed or detected. Changing environmental conditions due to climate destabilization and anthropogenic stressors may cause *Dinophysis* blooms to occur in the Gulf of Maine. In this study, the population dynamics of *Dinophysis*, *Myrionecta rubra*, and single-celled cryptophytes (5-10 um) were examined over a three year period. *Dinophysis* preys on *Myrionecta*, stealing its chloroplasts for photosynthesis. Sea water samples were collected at high tide as part of a long term (10 year) dock study in West Boothbay Harbor, ME, and plankton abundances were analyzed using a FlowCAM and traditional flow cytometry. *Dinophysis*, *Myrionecta*, and cryptophytes showed similar dynamics over the three year period of this study. *Myrionecta* abundance increased in the spring, leading to an increase in *Dinophysis*. Subsequently, levels of *Myrionecta* dropped, indicating that *Dinophysis* potentially consumes *Myrionecta*. A greater understanding of the ecology of *Dinophysis* could allow detection and possible prediction of harmful blooms.

OCEAN ACIDIFICATION AND SPERM MOTILITY IN THE GIANT SEA SCALLOP, *PLACOPECTEN MAGELLANICUS*

Nicole A. Messerman & Richard A. Wahle, University of Maine

The ocean is being acidified by increasing levels of atmospheric CO_2. Ocean pH is predicted to decline from current levels of pH 8.0 to as low as 7.6 by 2100. Few have examined the reproductive consequences of acidification in marine organisms. We suspect that gametes of broadcast spawners that spawn into the water may be vulnerable to changes in environmental pH. The giant sea scallop is one of New England's largest fisheries, and it is a broadcast spawner. We evaluated the effects of varying pH, gamete age and individual differences among scallops on sperm motility that is critical to fertilization. We measured sperm swimming speed and a swimming trajectory index (= actual path/straight line path). Sperm swimming speed ranged from about 90-200 μms^{-1}, but changed little over three pH treatments, ranging from pH 8.0 (normal sea water) to 7.1 (acidified with CO_2). Within 10 minutes of dilution, swimming speed decreased by 25-35% and paths became straighter, but age effects were independent of pH. In short, predicted pH change is not likely to affect sperm motility in the giant sea scallop.

DIVERSITY AND GENE EXPRESSION OF *PHAEOCYSTIS*-ASSOCIATED DMSP DEGRADING BACTERIA IN CULTURES & COASTAL GULF OF MAINE WATERS

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Dimethylsulfide (DMS) is a phytoplankton-derived compound that forms cloud condensation nuclei in the atmosphere and is produced as a degradation product of Dimethylsulfonio-propionate (DMSP). Some DMSP-producing phytoplankton release DMS, however DMSP-degradation genes have been identified only in bacteria and fungi. When DMSP is released, it becomes available for use by marine microorganisms, most notably, bacteria that assimilate DMSP as a source of carbon and sulfur. Some bacteria produce DMS as a by-product of these processes, while others possess a pathway that demethylates DMSP and produces methymercaptopropionate (MMPA). The genes that regulate these metabolic pathways (*ddd** and *dmdA* genes respectively) were recently developed as targets for quantitative PCR. Until now, most research has investigated specific DMSP-degrading strains at specific locations. Our project used qPCR to investigate DMSP-degrading bacteria associated with cultures of the DMSP-producing algal genera *Phaeocystis* from the North Atlantic Ocean and Mediterranean Sea as well as environmental samples from Boothbay Harbor. We found a consistent population of DMSP degrading bacteria living among *Phaeocystis* cultures, although it is apparent that certain types of DMSP degrading bacteria may dominate certain locations.

UREA IN CENTRAL MAINE COASTAL WATERS: CONCENTRATIONS AND INTERACTIONS WITH *ALEXANDRIUM FUNDYENSE* IN NATURAL MICROBIAL POPULATIONS.

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Urea use in fertilizers has increased in past decades, suggesting that agriculturally derived urea may be transported into coastal Maine waters where it is a potential nutrient source for blooms of *Alexandrium fundyense*. This study examined 1) urea concentration in the St. George River Estuary before and after a large rainfall event, and 2) the influence of nitrogen form, including urea, on growth of natural coastal microbial populations in bioassays with and without added *A. fundyense*. A significant increase in urea concentration post rainfall was documented at only one site in the lower river, suggesting that inputs may occur only immediately after fertilization, that *in situ* nutrient cycling occurs rapidly, or that urea inputs are minimal. In bioassays, *A. fundyense* growth rate did not vary with nitrogen form, however, *A. fundyense* cell yield increased with all nitrogen additions, suggesting urea was bioavailable to *A. fundyense*. *Alexandrium fundyense* differentially influenced coincident microbial populations in all nitrogen enrichments, stimulating smaller (0.7-3 μm) chlorophyll fractions, and inhibiting larger (> 3.0 μm) fractions, suggesting that allelopathic or competition interactions were enhanced with nitrogen enrichment.

EVOLUTIONARY PRESSURE ON CYTOCHROME *C* OXIDASE I IN COPEPODS RESULTING IN ADAPTATION TO NEW HABITATS

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Copepod zooplankton are exceedingly abundant in most bodies of water. Over evolutionary time, they have adapted to several different saline environments. Mutations in respiratory proteins are likely required to maintain proper cellular function during habitat switching. Cytochrome *c* oxidase, Complex IV of the electron transport chain, pumps protons into the intermembrane mitochondrial space to be used later for chemiosmosis. Radical amino acid replacements within the first subunit of cyctochrome *c* oxidase (COI) were phylogenetically reconstructed and statistically assessed within two orders of copepods, Calanoida and Cylopoida. Species pairs from alternative habitat types were used to identify the [1] location of radical amino acid replacements and [2] associated physicochemical properties that necessary for habitat switching. Results indicate that radical mutations associated with a change in salinity within the proton pump nearest the N-terminus cluster near the input and output regions. These mutations likely affect the affinity for protons within the pump. Despite affecting the same properties in these regions, the overall pattern of amino acid adaptation during habitat switching is quite different in the two orders of copepods.

WHOLE GENOME COMPARISON OF UNCULTURED, AMMONIA-OXIDIZING MARINE GROUP I *ARCHAEA* THROUGH SINGLE-CELL GENOMICS

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Ammonia-oxidizing Marine Group I (MG-I) *Archaea* are thought to constitute up to 40% of meso- and bathypelagic picoplankton suggesting a critical role in both nitrogen and carbon cycling. Currently sequenced representatives of this group, *Nitrosopumilus maritimus* and *Cenarchaeum symbiosum*, have inherent differences from free-living populations as the former was isolated from an aquarium and the latter is a marine sponge symbiont. We employed single cell genomics to investigate the genetic potential of uncultured deep ocean MG-I *Archaea*. Genes supporting both autotrophic and heterotrophic carbon assimilation pathways, as well as a significant number of genes involved in the proposed ammonia oxidation pathway of *N. maritimus* were identified. Gene arrangement and content was more similar to *N. maritimus* than *C. symbiosum* and all three maintained a large, core genome. However, we found our genome harbored horizontally transferred genes or genomic islands, potentially providing additional adaptability to deep ocean life or indicative of geographic differences in MG-I populations. Overall, the single cell genome was more similar to deep ocean sequences than current genomic models, suggesting that our genome better represents a "wild-type" population.