2012 Undergraduate Research Symposium

Thursday, August 16, 2012

Lillie Auditorium

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Marine Biological Laboratory

NSF-REU “Biological Discovery in Woods Hole” program

Program Schedule and Abstracts

2012 MBL Undergraduate Research Seminar

August 16, 2012

Session 1 Chair Paul Forlano, Brooklyn College, City University of New York

9:00 Welcome and opening remarks, Bill Reznikoff, Director of Education, MBL

9:10 **Microbes of the Mouth: Visualizing the Spacial Organization of Bacteria in Dental Plaque Biofilms:** Carissa McKinney [1,2], Jessica Mark Welch [1], Blair Rossetti [1], Gary Borisy [1]; *[1] Marine Biological Laboratory, [2]University of Delaware*

9:25 **Fluid interactions that enable stealth predation by the upstream foraging hydromedusa *Craspedacusta sowerbyi:*** Lucas K[1], Colin SP[1,2], Costello JH[2,3], Katija K[4], Klos E[5]; *[1]Roger Williams University, [2]Marine Biological Laboratories, [3]Providence College, [4]Woods Hole Oceanographic Institute, [5]University of Rhode Island*

9:40 **Central Projection of Fish Inner Ear End Organs:** Camillia Monestime[1], Joseph Sisneros [2], Paul Forlano[1]; *Brooklyn College, City University of New York, [2]University of Washington*

9:55 **Effects of Glutamate on Extracellular H^+ Fluxes of Internal Horizontal Cells Isolated from the Retina of the Skate (*Raja erinacea/ R. ocellata*):** Ethan Naylor[1] and Robert Paul Malchow[2]; *[1] Indiana Wesleyan University, [2] University of Illinois at Chicago*

10:10 **The Synergistic Nature of the Behaviors and Mechanisms that Support Effective Burrowing in the Mantis Shrimp *Squilla empusa*** Paul Bump[1] and Kristina Mead Vetter[2] *[1] University of Hawaii at Mānoa, [2] Denison University*

10:25 **Break**

***Session 2 Chair* – Paul Malchow, University of Illinois at Chicago**

10:45 **Morphological examination of the anterior cirri and lateral line papillae in the oyster toadfish, *Opsanus tau*** Ashley N Marranzino[1], Jacqueline F Webb [2,4], Allen F Mensinger [3,4]; *[1]Regis University, [2]University of Rhode Island, [3]University of Minnesota Duluth [4]Marine Biological Laboratory*

11:00 **Measuring ATP levels in hippocampal neurons after long-term potentiation:** Jamila Jamal[1,2], Noah Eisen[2], Edward J. O'Rourke[2], Silvio Sacchetti[3,] Hongmei Li[3], Kambiz N. Alavian[3], Elizabeth A. Jonas[2,3]; *[1]Oberlin College, [2]Marine Biological Laboratory [3]Yale University School of Medicine*

11:15 **Isolation and Identification of a Centrosomal Protein from *Spisula* Oocyte Nucleolini:** Casandra Newkirk[1], Mary Anne Alliegro[2], Mark C. Alliegro[2]; *[1]Claflin University [2], Marine Biological Laboratory*

11:30 **Thymoquinone, a bioactive component of *Nigella Sativa*, modulates β-cell redox status and insulin secretion** Sarah Carter[1], Joshua P. Gray[2], Navindra Seeram[3], and Emma Heart[4]
[1]Carleton College, [2]US Coast Guard Academy, [3]University of Rhode Island, [4]Marine Biological Laboratory

11:45 **RNA-binding protein BrunoL1 mediates translational stimulation of specific mRNAs including *cenp-a* and *cyclinA2* via interactions with eIF3:** Jacqueline Figueredo[1,2], Cristy Lewis[1], Lori Horb[1] and Marko Horb[1]
[1]Marine Biological Laboratory, [2] Boston College

12:00 **Lunch Break**

Session 3: Chair - Allen Mensinger, Univ. Minnesota Duluth

1:00 **Searching for Meiotic Recombinant Gene SPO11 in Asexual Bdelloid Rotifers:** Justin Waraniak[1], Bette Hecox-Lea[2], David Mark Welch[2]; *[1]University of Michigan,[2]Marine Biological Laboratory*

1:15 **No Sex, No Problem. Gene Conversion May Substitute For Sex in Bdelloid Rotifers.** Norian Caporale-Berkowitz[1], Bette Hecox-Lea[2,3], David Mark Welch[1,3] ; *[1]Brown University, [2] Northeastern University, [3] Marine Biological Laboratory*

1:30 **Better color through motor proteins:** Eric Appeldoorn[1], Rachel Zimmerman[2], George Bell[3], Alan Kuzirian[3], Roger Hanlon[3]; *[1]University of Puerto Rico Rio Piedras [2]University of Wisconsin La Crosse [3]Marine Biological Laboratory*

1:45 **Predatory Behaviors of Black Sea Bass, *Centropristis striata*, in Captivity:** Katharine L. Dickson[1], Robyn J. Crook[2,3], Edgar T. Walters[2], Roger T. Hanlon[3] ; [1]George Mason University, [2]University of Texas-Houston Medical School, [3]Marine Biological Laboratory

2:00 **Science and Art Toward a Common Goal: a proposed realignment of contemporary methodology in the study and understanding of nature:** Lizzie Kripke; *Brown University, Rhode Island School of Design*

2:15 **Ecology of Haptophyte Blooms:** Mara Freilich[1], Susanna Theroux [1,2], and Linda Amaral Zettler [1,2]
[1]Brown University, [2]Marine Biological Lab

2:30 **Bacterial communication in deep sea hydrothermal vents:** Sarah Nalven; *Marine Biological Laboratory*

2:45 Break

Session 4 Chair: Anne Giblin, Ecosystem Center, MBL

3:00 **Temporal Carbon Flux Dynamics in New England Salt Marsh Ponds:** Ana Gordon[1], Inke Forbrich[2], Anne Giblin[2]
[1]Syracuse University, [2] Marine Biological Laboratory

3:15 **Does chronic nutrient enrichment result in a trophic bottleneck in a salt marsh?:**
Harriet S. Booth[1], David S. Johnson[2], and Linda Deegan[2]
[1]Brown University, [2]Marine Biological Laboratory

3:30 **The Effect of Eutrophication on the Growth and Productivity of *Fundulus heteroclitus***
Timothy J. Krikorian[1], James Nelson[2], Linda Deegan[2]
[1]Fitchburg State University; [2]Marine Biological Laboratory

3:45 **Changes in lipid biomarker composition of sinking particles during the passage of three distinct eddy types through the deep Sargasso Sea:** Anika Aarons[1,2], Maureen H. Conte[1], J.C. Weber[1] ; *[1] Marine Biological Laboratory,[2]Mount Holyoke College*

4:00 **The effects of eutrophication on the geomorphology of the Plum Island salt marshes**
Katherine M. Stout[1], Christopher Haight[2], Linda Deegan[2] ; [1]Clarkson University, [2]Marine Biological Laboratory

4:15 **Evaluating Toxicity of Mutant SOD1 Forms:** Izrail Abdurakhmanov[1,3], Scott T. Brady[2,3], Weiming Ni[4,5], Arthur L. Horwitch[4,5], Yuyu Song[2,3] ; *[1] CUNY Hunter College[2] University of Illinois at Chicago, [3] Marine Biological Laboratory*

4:30 **Evaluating the effects of the mutant FUS in axonal transport:** Saul Penaranda[1], Scott T. Brady[2], Gerardo Morfini[3], Reddy Sam[3], Daryl A. Bosco[4] ; *[1]CUNY Hunter College [2]University of Illinois at Chicago, [3]University of Massachusetts Medical Center, [4]Marine Biological Laboratory*

Microbes of the Mouth: Visualizing the Spacial Organization of Bacteria in Dental Plaque Biofilms

Carissa McKinney [1,2], Jessica Mark Welch [1], Blair Rossetti [1], Gary Borisy [1]

[1] *Marine Biological Laboratory,* [2]*University of Delaware*

Microbes live and thrive in abundance in our mouth. These microbes live in complex communities in biofilms, notably in the plaque on our teeth. Hundreds of species of microbes residing in the communities in dental plaque have been identified using sequencing of the 16S ribosomal RNA gene. Using fluorescence *in situ* hybridization (FISH) bacteria in these biofilms can be imaged and identified, showing that bacteria of different types are all intermixed in the biofilm. Due to the complexity of these communities, the two to three fluorescent probes that can be employed in a standard FISH experiment do not allow us to fully analyze the communities and structures found within. A new technique known as Combinatorial Labeling and Spectral Imaging FISH (CLASI-FISH) allows us to image these communities using seven or more fluorescent probes simultaneously. CLASI-FISH using multiple probes targeting the same taxon allows for more confidence in the identification of individual ltaxa, while CLASI-FISH using probes targeting different taxa permits visualization of the micron-scale interactions of cells from multiple genera and families of bacteria. Using CLASI-FISH, we have found complex and organized communities of bacteria in plaque including close associations among bacteria identified as Streptococcus, Corynebacterium, Pasteurellaceae, and Porphyromonas. Using CLASI-FISH probes targeting sub-genus-level groups and species we have provisionally identified the taxa in these structures as *Corynebacterium matruchotii*, *Streptococcus cristatus* or *S. mitis*, *Aggregatibacter* sp., and *Porphyromonas* sp.

Fluid interactions that enable stealth predation by the upstream foraging hydromedusa *Craspedacusta sowerbyi*

Lucas K[1], Colin SP[1,2], Costello JH[2,3], Katija K[4], Klos E[5]

1. *Biology, Roger Williams University, Bristol, RI 02809*
2. *Whitman Center, Marine Biological Laboratories, Woods Hole, MA 02543*
3. *Biology Department, Providence College, Providence, RI 02918*
4. *Applied Ocean Physics and Engineering, Woods Hole Oceanographic Institute, Woods Hole, MA 02543*
5. *Graduate School of Oceanography, University of Rhode Island, Narragansett, RI 02882*

Unlike most medusae which forage with tentacles trailing behind their bells, several species forage upstream of their bells using aborally located tentacles. It has been hypothesized that these medusae forage as stealth predators by placing their tentacles in more quiescent regions of flow around their bells. Consequently, they are able to capture highly mobile, sensitive prey. In this study, we used digital particle image velocimetry (DPIV) to quantitatively characterize the flow field around *Craspedacusta sowerbyi*, a freshwater upstream foraging hydromedusa, to evaluate the mechanics of its stealth predation. We found that fluid velocities were minimal in front and along the sides of the bell where the tentacles are located. As a result, the deformation rates in the regions where the tentacles are located were low, below the threshold rates required to elicit an escape response in several species of copepods. Estimates of their encounter volume rates were examined based on flow past the tentacles and trade-offs associated with tentacle characteristics were evaluated.

Central Projection of Fish Inner Ear End Organs

Camillia Monestime[1], Joseph Sisneros [2], Paul Forlano[1]

[1]*Department of Biology, Graduate Studies, Brooklyn College, City University of New York*
[2]*Department of Psychology and Biology, University of Washington*

The auditory system of the plainfin midshipman fish, *Porichthys notatus*, has become a good model for investigating mechanisms of auditory reception and acoustic function, in part because sound detection and localization are essential to the reproductive success of this species. The inner ear of the midshipman and other teleost fishes includes three otolithic end organs (saccule, lagena and utricle) that are proposed to be either auditory or vestibular in function. Although the saccule is the primary auditory end organ and is considered to provide the major auditory input from the inner ear to the medulla, the position and extent of auditory input from the lagena and utricle remain undetermined. We hypothesized that the utricle and lagena would project to known auditory regions in the medulla, similar to the saccule. End organ central projections were examined using neurobiotin and dextran amines (488 and 568). These tracers were placed on the sensory hair cell macula of the individual end organs. The fish were then allowed to survive for 2-3 days which permitted the tracer to transport from the hair cells to the medulla. Animals were transcardially perfused with cold teleost ringers followed by 4% paraformaldehyde; brains were dissected out, postfixed 1 hr., and sectioned on a cryostat at 25 microns. Neurobiotin was visualized by neutravidin conjugated to Texas red, sections were counterstained with fluorescent Nissl and imaged using an epifluorescent microscope. The findings in these experiments show that all three end organs project to: the intermediate and rostral intermediate portion of the descending octaval nucleus, anterior and magnocellular octaval nuclei. The utricle showed distinct projections that converged in the tangential octaval nucleus, a studied vestibular region of the brain. The data supports our hypothesis that the lagena and utricle project to auditory regions in the medulla; this suggests that these end organs function in the auditory system. The projection of the utricle in the tangential octaval nucleus suggests that the utricle has a dual role in both the auditory and vestibular systems.

This work was funded by NSF DBI-1005378; “REU Site: Biological Discovery in Woods Hole”

Effects of Glutamate on Extracellular H^+ Fluxes of Internal Horizontal Cells Isolated from the Retina of the Skate (*Raja erinacea/ R. ocellata*)

Ethan Naylor[1] and Robert Paul Malchow[2]

[1] *Dept. Biological Sciences, Indiana Wesleyan University, Marion IN*

[2] *Depts. Biological Science and Ophthalmology & Visual Science, University of Illinois at Chicago, Chicago IL*

Extracellular H+ has been hypothesized to mediate feedback inhibition from retinal horizontal retinal cells onto vertebrate photoreceptors. This hypothesis predicts that depolarization of horizontal cells should induce an extracellular acidification of the environment adjacent to the cell membrane. Molina et al. (2004) reported that depolarization of isolated external horizontal cells of skate induced an extracellular alkalinization, opposite to the effect predicted by the H+ hypothesis of lateral inhibition. Skate possess two classes of horizontal cells, external and internal horizontal cells, which have been reported to have different physiological properties (Malchow et al. 1990). We sought to examine whether proton flux from internal horizontal cells of the skate differed significantly from that of external horizontal cells. Self-referencing H+-selective electrodes were used to monitor extracellular H+ fluxes from internal horizontal cells isolated using a papain dissociation protocol. We found that unstimulated cells typically displayed a standing proton flux similar to that observed in external horizontal cells, indicating a higher standing concentration of protons adjacent to the membrane as compared to the point 30 µm distant in the surrounding solution. Stimulation of the internal horizontal cells with 1 mM glutamate induced a decrease in proton flux, lowering the concentration of free hydrogen ions around the cell, again similar to that observed in external horizontal cells. These data suggest that both internal and external horizontal cells respond to the presumed photoreceptor neurotransmitter glutamate by reducing the overall pH of the external solution, opposite to the prediction of H+ hypothesis for lateral inhibition.

This work was supported by NSF grants IOS-0924372 and DBI-1005378 “REU Site: Biological Discovery in Woods Hole”

The Synergistic Nature of the Behaviors and Mechanisms that Support Effective Burrowing in the Mantis Shrimp *Squilla empusa*

Paul Bump[1] and Kristina Mead Vetter[2]

[1] *Dept. of Biology, University of Hawaii at Mānoa, Honolulu, HI*

[2] *Dept. of Biology, Denison University, Granville, OH*

The mantis shrimp *Squilla empusa* is a charismatic marine crustacean known for its powerful strike, keen sense of vision, and chemosensory abilities. These benthic creatures create extensive burrows that are important in feeding, reproduction, and protection from predation. Through field observations of a population located in Great Harbor in Woods Hole, MA this species of mantis shrimp has been observed to construct burrows faster and makes more alterations than previously recorded. To understand the mechanics of these burrowing behaviors, mantis shrimp were filmed making burrows in the lab using high-speed videography. *S. empusa* used two markedly distinct methods of burrowing: pleopod fanning and maxilliped bulldozing. Pleopod fanning consists of a swift posterior power stroke, followed by a slower recovery motion towards the anterior. During the power stroke the pleopods are fully extended, while during the recovery phase the pleopods curl up, reducing drag. The pleopods also angle medially during the power stroke to compress the fluid into a narrower jet, creating a more directed flow. In the other form of burrowing, the maxillipeds dig into the substrate, rotate to hold the sediment in a basket, and then deposit the contents outside of the burrow. To understand the fine structure of the mantis shrimp's pleopods and maxillipeds, analysis of the appendages was performed using a Zeiss dissecting microscope. Through this series of observations and analyses we are starting to understand how pleopod anatomy and kinematics work synergistically to create an effective burrowing system.

This work was supported by NSF DBI-1005378 "REU Site:

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Morphological examination of the anterior cirri and lateral line papillae in the oyster toadfish, *Opsanus tau*

Ashley N Marranzino [1,2] Jacqueline F Webb [2,3], Allen F Mensinger [2,4]

1. *Regis University, Denver, CO*
2. *Marine Biological Laboratory, Woods Hole, MA*
3. *University of Rhode Island, Kingston, RI*
4. *University of Minnesota Duluth, Duluth, MN*

The oyster toadfish, *Opsanus tau*, has been an important model organism for biomedical research at the Marine Biological Laboratory since 1888. However, the function of large, fleshy multilobed protuberances, called cirri, which project from the mandible and above the eyes, and paired papillae which bracket superficial neuromasts of the lateral line remain unknown. Scanning electron microscopy was used to examine both of these morphological features. Although mechanosensory hair cells were lacking on the cirri, bulbiformous cells with numerous short microvilli were evident throughout the surface of the cirri. These projections appear to be possible type II extra-oral taste buds, a finding which is consistent with a previous histological study that revealed putative taste buds using light microscopy and provides further evidence that the cirri are used for gustatory detection. The toadfish cephalic region is characterized by a relative absence of canal neuromasts, however many of the superficial neuromasts are flanked by paired papillae. The function of the papillae is unknown, however they may serve to protect the neuromasts from becoming clogged with sediment in the estuarine habitat. They also may serve to channel water over the superficial neuromasts, allowing these superficial neuromasts to respond as canal neuromasts. SEM revealed that hair cell orientation was perpendicular to the axis of the papillae which is consistent with this function.

This work was supported by grant NSF DBI-1005378

Measuring ATP levels in hippocampal neurons after long-term potentiation

Jamila Jamal[1,2], Noah Eisen[2], Edward J. O'Rourke[2], Silvio Sacchetti[3,] Hongmei Li[3], Kambiz N. Alavian[3], Elizabeth A. Jonas[2,3]

[1]Departments of Neuroscience and Biology, Oberlin College, Oberlin, OH, [2]Marine Biological Laboratory, Woods Hole, MA, [3]Intrnl. Med. Endocrinology Yale University School of Medicine, New Haven, CT

Previous studies have shown that Bcl-xL, an anti-cell death protein, causes cells to produce ATP at a higher efficiency. Long term increases in synaptic transmission could require an increase in ATP production, but if this were the case, then substrate and oxygen use might eventually become limiting. Hippocampal neurons undergo long-term potentiation (LTP) of synaptic responses after high-frequency stimulation, consequently requiring less stimulation for the same postsynaptic response, or the same amount of stimulation to produce a stronger response. We hypothesized that if mitochondria become more efficient at ATP production, they may assist in readying the neuron for stronger synaptic responses. Using a calcium phosphate transfection technique, we transfected cultured hippocampal neurons from rats (E18) with AT1.03 DNA. AT1.03 DNA codes for a protein that detects ATP levels in live neurons using FRET (Fluorescence Resonance Energy Transfer). After a control period, which indicated that the cell was healthy and at rest, LTP was stimulated by adding glycine, a co-activator of the NMDA receptor, in a medium lacking Mg^{2+} for 3 minutes and FRET signals were measured before, during and after stimulation. We found after glycine an increase in FRET signal, signifying an increase in ATP levels, perhaps indicating a change in mitochondrial metabolism. In future plans, we will measure oxygen uptake in single cultured neurons to determine if there is an increase in metabolic efficiency.

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Isolation and Identification of a Centrosomal Protein from *Spisula* Oocyte Nucleolini

Casandra Newkirk[1], Mary Anne Alliegro[2], Mark C. Alliegro[2]

[1]Claflin University [2] Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory

The nucleolinus is a cellular structure within the nucleolus that has a long but nearly forgotten history. It has since been determined that while these two structures may be related morphologically, there are specific molecules that can clearly distinguish between them. The nucleolinus itself has been thought to play a role in cell division, and evidence in support of this hypothesis has recently been provided in experiments using surf clam (*Spisula)* oocytes. One antigen in particular has been localized in the nucleolinus of *Spisula* oocytes and can be recognized by mcAb23. This antigen is also expressed in the nucleus of human, frog, and yeast cells. Because there is little sequence and protein data currently available for the surf clam, the nucleoli of HeLa cells were used in conjunction with *Spisula* oocyte nucleolini in efforts to isolate and identify this antigen. The use of the HeLa cells alongside protein identification in surf clam nucleolini will allow us to determine if the antigen isolated in the surf clam oocytes is homologous, and provide an extensive database to help identify the protein and address questions of function. To purify this nucleolinar antigen in preparation for mass spectrometry, an immunoaffinity column was constructed with mcAb23 IgG isolated on a protein G column. Isolated HeLa cell nucleoli were solubilized and applied to the column overnight at 4°C. Column fractions were assayed for protein content by absorbance at 280nm and polyacrylamide gel electrophoresis. Our results are unclear and this time and analysis of the data is currently ongoing and will continue into the fall.

Thymoquinone, a bioactive component of *Nigella Sativa*, modulates β-cell redox status and insulin secretion

Sarah Carter[1], Joshua P. Gray[2], Navindra Seeram[3], and Emma Heart[4]

[1]Carleton College, Northfield, MN; [2]US Coast Guard Academy, New London, CT; [3]University of Rhode Island, Kingston, RI; [4]Marine Biological Laboratory, Woods Hole, MA

Insulin secretion from pancreatic β-cells is dependent upon glucose metabolism inside these cells. Glycolysis is central to the metabolic signaling pathways which lead to glucose-stimulated insulin secretion (GSIS), and requires continuous re-oxidation of glycolytically-derived NADH back to NAD^+. Thymoquinone, a main active ingredient of plant *Nigella sativa* can regenerate NAD^+ in the β-cell cytosol via glucose-dependent redox cycling, a process which utilizes NAD(P)H and generates H_2O_2.

In the rat β-cell line, INS-1 832/13 cells, thymoquinone, applied at low micromolar levels, was found to decrease NAD(P)H/$NAD(P)^+$ ratio and generate low levels of H_2O_2, a novel coupling factor required for GSIS. In parallel, same doses of thymoquinone enhanced GSIS. This supports the hypothesis that thymoquinone amplifies GSIS via 1) generation of low levels of H_2O_2 and 2) enhancement of glycolytic flux via regeneration of NAD^+.

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RNA-binding protein BrunoL1 mediates translational stimulation of specific mRNAs including *cenp-a* and *cyclinA2* via interactions with eIF3

Jacqueline Figueredo[1,2], Cristy Lewis[1], Lori Horb[1] and Marko Horb[1]

[1] Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory, Woods Hole, MA [2] Boston College, Chestnut Hill, MA

Protein synthesis occurs when mRNA is translated into amino acids. RNA-binding proteins interact with specific mRNAs in the 5' or 3' Untranslated Region (UTR) in order to enhance or repress translation of those mRNAs. Our lab has found that *Xenopus* BrunoL1 is expressed in endoderm progenitor cells and is required for proliferation and differentiation of these cells. Surprisingly, unlike its other family members, BrunoL1 enhances translation of specific mRNAs including *cyclin A2*. Coimmunoprecipitation of BrunoL1 followed by reverse transcription PCR was performed to test whether BrunoL1 bound other mRNAs. One such mRNA found codes for Centromere Protein A (Cenp-A), a protein involved in cell cycle that replaces the histone H3 in the centromeres of chromosomes. To test for translational upregulation of this mRNA, embryos were injected with the 3' UTR of *cenpA* fused to the open reading frame of *green fluorescent protein (gfp-cenpA)* either alone or with *brunol1.* A stronger GFP signal in those embryos injected with *brunol1* than those that were not indicated that BrunoL1 was sufficient to enhance translation of *gfp-cenpA.* Yet it is unclear how BrunoL1 does this. To identify the BrunoL1 complex that mediates translational activation we performed a co-immunoprecipitation/mass spectrometry experiment to identify BrunoL1 partner proteins that are involved in translational stimulation. From this experiment five subunits of eukaryotic initiation factor 3 (eIF3) were identified. eIF3 is the largest initiation complex involved in translation and acts as a scaffold for the preinitiation complex for translation by binding to the small ribosomal unit and to eIF4. Through the use of cloning and in situ hybridization, it was found that regions that actively expressed BrunoL1 also actively expressed the eIF3 subunits b, i, and m during various developmental stages. This suggests that BrunoL1 interacts with these subunits to enhance translation of specific mRNAs at these stages. Our findings may be a model for how other RNA-binding proteins enhance translation.

This work was supported by NSF grant DBI-1005378 "REU Site: Biological Discovery in Woods Hole"

Searching for Meiotic Recombinant Gene SPO11 in Asexual Bdelloid Rotifers

Justin Waraniak[1], Bette Hecox-Lea[2], David Mark Welch[2]

[1]Dept. of Ecology and Evolutionary Biology, University of Michigan, Ann Arbor MI

[2]Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory, Woods Hole, MA

Bdelloid rotifers are one of the few groups of multicellular eukaryotes that had avoided extinction for millions of years despite reproducing exclusively asexually. Without genetic recombination through sex, it is not fully understood how bdelloids maintain genetic variation, and discovering how bdelloids are able to survive as a lineage could reveal why there is such strong evolutionary selection for animals to maintain sex. Possible mechanisms through which these rotifers maintain this variation is an area of interest we have been exploring in the Mark Welch lab. This summer, we have focused on one gene in particular, spo11. In most sexually reproducing eukaryotes, the main function of spo11 is to cause double stranded breaks in DNA during meiosis so recombination between homologous chromosomes can occur. The Mark Welch Lab had mapped a small number of expressed sequence tags to spo11 in two species of bdelloid rotifers, but no test had shown that spo11 was being expressed at any meaningful level.

Another interesting part about bdelloid biology is the fact that they can survive complete desiccation, a process that usually inflicts a large amount of damage to DNA. We hypothesized that spo11 could be involved in preventing or mediating DNA damage when rotifers are desiccated. In order to test this, we put rotifers through desiccation and collected them at various points throughout the desiccation process, including before they were completely dehydrated and after they had been rehydrated.

In order to determine if spo11 was being expressed, we designed gene-specific primers to use in PCR on both genomic DNA and cDNA. For experiments in which spo11 appeared to be amplified, we cleaned the amplification products that looked like spo11 and cloned them into E. coli vectors to amplify them for sequencing. Once sequenced, we compared the amplicons and their possible polypeptide sequences to known sequences of spo11 from monogononts (sexually reproducing rotifers) and the sequences from bdelloids that had previously appeared to be spo11. We also compared collected sequences to spo11 from yeast in order to determine if the rotifer genes retained the coding sequence for amino acids important for the protein's function. This work was supported by NSF grants MCB-0923676 and DBI-1005378 "REU Site: Biological Discovery in Woods Hole"

No Sex, No Problem. Gene Conversion May Substitute For Sex in Bdelloid Rotifers.

Norian Caporale-Berkowitz[1], Bette Hecox-Lea[2,3], David Mark Welch[1,3]

1 *Department of Ecology and Evolutionary Biology, Brown University, Providence, Rhode Island, United States of America,* ***2*** *Department of Biology, Northeastern University, Boston, Massachusetts, United States of America,* ***3****Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory, Woods Hole, Massachusetts, United States of America*

Having diversified into nearly 500 species during 40-million years of strict asexuality, bdelloid rotifers seem to challenge the general rule that sex is necessary for long-term animal survival. Bdelloids are also able to withstand full dehydration at any life stage without significant impact on fecundity or longevity. After examining transcriptomes of clonal lineages taken through multiple rounds of desiccation and rehydration, we present evidence that non-GC-biased gene conversion is a direct effect of desiccation conserved across Bdelloidea. These findings suggest that although no bdelloids have sex, all bdelloids may have an alternate strategy for eliminating deleterious alleles and creating the genomic variation that acts as the substrate for natural selection.

Better color through motor proteins

Eric Appeldoorn[1], Rachel Zimmerman[2], George Bell[3], Alan Kuzirian[4], Roger Hanlon[5]

[1]*University of Puerto Rico Rio Piedras* [2]*University of Wisconsin La Crosse* [3,4,5]*Marine Biological Laboratory in Woods Hole*

Pigmented chromatophores allow rapid skin color change in cephalopods. Little is known regarding pigment granule distribution within the chromatophore pigment cell, but preliminary findings suggest fibers control the dispersion of the granules during chromatophore expansion and retraction. Composition of the fibers and the identity of the structures that link the granules to the fibers are unknown. Skin tissue was dissected from different parts of the mantle of the squid *Doryteuthis pealeii* and preserved. Immunohistochemical labeling of histologic sections was employed to test whether motor proteins may be involved in an association between pigment granules and the fibers. Samples were examined using brightfield, laser scanning confocal and transmission electron microscopy. Of the various motor proteins tested, the most effective antibody labeling occurred with Myosin VI. Its labeling pattern suggested that this motor protein is involved in an association between pigment granules and the tethering fibers. The exact nature of this association is ongoing.

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Predatory Behaviors of Black Sea Bass, *Centropristis striata*, in Captivity

Katharine L. Dickson[1], Robyn J. Crook[2,3], Edgar T. Walters[2], Roger T. Hanlon[3]

[1]*Department of Biology, George Mason University, Fairfax, VA, USA*

[2]*Department of Integrative Biology and Pharmacology, University of Texas-Houston Medical School, Houston, TX, USA*

[3]*Marine Resources Center, Marine Biological Laboratory, Woods Hole, MA, USA*

The black sea bass, *Centropristis striata* Linnaeus (1758), is a serranid fish found in the northwest Atlantic Ocean. This species, which supports a significant fishery of some economic importance, has been well described in terms of population, life history, habitat characteristics and the species distribution of its diet, but its predatory behavior has received little study. *C. striata* employs a hybrid predation strategy that includes cruising, pursuit hunting and ambush attack. The success rate of this mixed strategy and the anti-predation tactics used by prey in response to these variable tactics are poorly quantified. Here we aimed to describe the predatory behavior of the black sea bass on the scale of individual encounters between a fish and its prey. Black sea bass were housed in a round tank with a cylindrical divider suspended above it. Before each trial, the divider was lowered, confining the fish to the outside of the tank, and then squid – the prey animals – were introduced into the center of the divider. The divider was lifted at the start of each trial to permit interaction between predators and prey, and the trial was recorded for a maximum of 30 minutes (less if all squid were verified to have been eaten). We quantify a range of behaviors, including duration of each stage of fish-squid encounters (detection, pursuit, attack, consumption), distance at initiation of each stage of fish-squid encounters, pursuit and attack angles, and stereotypic behavior observed following each predation event. Our data suggest a mixed hunting strategy is highly efficient over short spatial scales. Results from this study may inform population-scale studies of black sea bass and aid in management of bass stocks.

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Science and Art Toward a Common Goal: a proposed realignment of contemporary methodology in the study and understanding of nature

Lizzie Kripke

Roger T Hanlon Lab, Brown University, Rhode Island School of Design

Adaptive discovery is a cyclic system of stimulation and simulation – observation prompts questioning, questioning prompts experimentation, experimentation prompts further observation, which prompts further questioning, and so on, until somewhere along the way, conclusions will be drawn to not only make sense of these observations but also to pinpoint what remains to be discovered. In this way, adaptive discovery is self-perpetuating. Collaboration, creativity, failure, chance, obsession, and critique underpin this process as it is practiced today. There are two particular fields of work that make exhaustive use of this system - science and art. Paradoxically, despite innumerable commonalities, including a shared goal to better understand and describe nature, contemporary society classifies these two fields as opposite. Over the past few centuries, this outlook has progressed in tandem with the development of distinct, independent institutional frameworks for each discipline. Such institutional disparity has further encumbered any sort of practical collaboration between the two fields. There is, however, emerging interest in realigning the practices of art and science in order to more thoroughly advance the study of nature.

As an artist-in-residence at the Hanlon Lab, I have begun to explore the potential realities of this "re-alignment." One major project has been to develop three-dimensional computer models of the biological mechanisms of camouflage in cephalopods. Another project has been to develop a body of fine art work based on current research occurring in the lab. While the tangible outcome (animations and art work) of these projects has proven worthwhile, it is the collaborative process of generating this output that proves particularly compelling for future investigation. It is the interaction between artists and scientists that promises to most meaningfully enrich the common pursuit of natural discovery.

Ecology of Haptophyte Blooms

Mara Freilich, Brown University, Susanna Theroux, Brown University, Marine Biological Lab, Linda Amaral Zettler, Brown University, Marine Biological Lab

Alkenones produced by lacustrine haptophyte algae have the potential to be valuable temperature proxies for continental paleoclimate studies using the alkenone unsaturation index (Uk37). Past studies in Lake George, North Dakota have shown that at least three different alkenone-producing haptophytes are present during the spring bloom period, each with a unique alkenone signature. In this study, we recreated a mock-bloom event in an enrichment culture and collected DNA and alkenone samples throughout to determine the timing of peak alkenone production during a bloom. We sampled throughout the mock-bloom and analyzed these samples for DNA, quantitative PCR (qPCR) and alkenone lipids. Our sequencing analyses revealed the presence of a single haptophyte species, and our alkenone lipid signature remained consistent throughout the mock-bloom. For the first time, our qPCR data provides an estimation of alkenone concentration per cell. Finally, the Uk37 values from the enrichment culture matched the Uk37 calibration determined from the Lake George 2011 bloom event. These results give us new insights into haptophyte ecology during bloom events.

Bacterial communication in deep sea hydrothermal vents

Sarah Nalven

Josephine Bay Paul Center, Marine Biological Laboratory

Hydrothermal vents are recently discovered ecosystems in the deep sea that foster a wide diversity of life in dynamic and “extreme” conditions. A wide array of microorganisms are adapted to life in these vents and comprise the basis of vent food webs through the chemosynthetic fixation of carbon. Many aggregate on vent chimneys or other surfaces to form biofilms, a mode of living in which cells can minimize exposure to environmental fluctuations through maintenance of a constant location and an extracellular protective layer. In previously studied biofilms, cell-to-cell communication has been shown to be an important factor in establishing these layers. Vent microorganisms may engage in similar behaviors called quorum sensing, a process in which cells produce and detect signaling molecules that allow them to monitor their own community density and coordinate gene expression and other life cycle events. An assay was adopted and optimized to detect these molecules in hydrothermal vent monocultures in order to investigate if microorganisms from hydrothermal vents are engaging in quorum sensing like microorganisms from other systems. This assay takes advantage of the luminescent, gram-negative bacterium, Vibrio harveyi, whose quorum sensing system is well established. Using different strains of V. harveyi that luminesce in the presence of different quorum sensing molecules, hydrothermal vent monocultures can be screened for these molecules through the addition of their culture fluid to V. harveyi cultures.

Changes in lipid biomarker composition of sinking particles during the passage of three distinct eddy types through the deep Sargasso Sea

Anika Aarons[1,2], Maureen H. Conte[1], J.C. Weber[1]

[1]*Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA*

[2]*Mount Holyoke College, South Hadley, MA*

The Oceanic Flux Program (OFP) is the longest running deep ocean sediment-trap time series (34 years+) in the world, measuring and characterizing particle flux in the deep Sargasso Sea, 75km southeast of Bermuda. Oceanic particle flux is a key component of the global carbon cycle as sinking organic matter supports carbon sequestration and deep ocean productivity. Lipid biomarkers, especially sterols, extracted from the sinking particles have unique biological sources and can be used to elucidate changes in community structure and biogeochemical processes. Transient physical forcing by upper ocean mesoscale features such as eddies can have a dramatic effect on particle flux and productivity. In 2007, three different types of eddies passed over the OFP site; an in-bloom cyclonic (February – mid-April), post-bloom mode-water (late April – May) and anti-cyclonic eddy (late August – October). In this study, organic lipids were extracted from sediment trap samples collected during this year and separated into sterols and other biomarker classes using solid phase extraction. The highest productivity was observed in the cyclonic eddy with phytosterols (63.6 µg/m^2/d) and cholesterol (87.6 µg/m^2/d) maxima at 500m during the middle of the eddy's passage, corresponding with strong nutrient upwelling caused by the cyclonic circulation. The cholesterol:phytosterols ratio steadily increased during the cyclonic eddy and the cholesterol maximum occurred two weeks after the phytosterols' suggesting a zooplankton functional response to a phytoplankton bloom. While both cholesterol and phytosterols decreased with depth, the phytosterols flux maxima at 1500m (11.6 µg/m^2/d) was still higher in the cyclonic eddy compared to the later mode-water and anti-cyclonic eddies. The mode-water eddy had the shortest duration over the site and both phytosterol and cholesterol flux maxima at 1500m (10.4 µg/m^2/d & 50.1 µg/m^2/d, respectively) coincided near the end of its passage. The anticyclonic eddy demonstrated a similar trend to the mode-water eddy with a concurrent increase in phytosterols and cholesterol flux at 1500 and 3200m but in the middle of its passage. Increased secondary zooplankton and bacterial production caused by rapidly sinking labile aggregates during the eddy-induced blooms was observed in biodegradation trends of the stanol:sterol ratios. Lipid biomarkers effectively characterize changes in organic composition of particle flux resulting from eddies, an important factor in global carbon cycling.

Does chronic nutrient enrichment result in a trophic bottleneck in a salt marsh?

Harriet S. Booth[1], David S. Johnson[2], and Linda Deegan[2]

[1]*Brown University, Providence, RI*

[2]*Marine Biological Laboratory, Woods Hole, MA*

Classic bottom-up theory predicts that increased primary production will stimulate growth at higher trophic levels. Increased nutrients, however, may stimulate the abundance of inedible or inaccessible prey, attenuating the flow of energy to higher trophic levels resulting in a trophic bottleneck. In this study, we examine how nutrient enrichment affects the densities and biomass of benthic invertebrates in a salt marsh, including those species that may be responsible for a trophic bottleneck in the Plum Island Estuary, Massachusetts. Nekton production, which is dominated by the mummichog, *Fundulus heteroclitus*, may be impacted by this bottleneck. As part of a long-term ecological project, two tidal creeks were enriched by adding 15 times the reference level of nitrate to flooding tidal waters throughout the past 9 growing seasons, each paired with an unfertilized, reference creek. *Nassarius obsoletus*, an "inedible" mud snail, and *Melampus bidentatus*, an inaccessible high marsh snail, were collected in the beginning of the growing season and density and biomass measurements were carried out in the lab. Benthic cores to determine densities of edible prey (e.g., annelids) will be taken at the end of August. Preliminary results show that there were higher densities and biomass of *N. obsoletus* in the fertilized creeks than the reference creeks, suggesting the presence of a trophic bottleneck. Samples from the current year are still being processed for edible prey, which we hypothesize will be reduced due to negative interactions with the high density of mudsnails.

The Effect of Eutrophication on the Growth and Productivity of *Fundulus heteroclitus*

Timothy J. Krikorian[1], James Nelson[2], Linda Deegan[2]

[1]*Department of Biology, Fitchburg State University, Fitchburg MA*

[2]*Marine Biological Laboratory, Woods Hole, MA*

The accumulation of nutrients such as nitrogen and phosphorus from human activities can disrupt ecosystems and their greater functions. Salt marshes are of particular concern due to their environmental importance as fundamental contributors to coastal food webs. The TIDE Project has mimicked anthropogenic enrichment in two saltmarsh creeks in the Plum Island Estuary, Massachusetts, for the past 9 years. Eutrophication has weakened creek-bank stability resulting in the collapse of this critical habitat. This collapse may impact a key predator in this system, *Fundulus heteroclitus* (mummichogs), by decreasing foraging and spawning habitat. These effects could contribute to the decrease of mummichog productivity which would diminish the export of fish out of the marsh and into the broader coastal food web. To determine how eutrophication affects the productivity of mummichogs in the marsh, flume nets were set up in fertilized and control creeks. The nets encompassed 30m^2 areas of high marsh and tall *Spartina alterniflora* to determine densities and biomass of mummichogs/m^2. Mummichogs were also seined in quantities of roughly 1,000 from each creek (2 reference, 2 fertilized) to assemble a length frequency analysis to determine relative seasonal growth rates. Fishing was done in June and July in order to see the effects of nutrient enrichment on productivity as the season progressed. We determined that the productivity of mummichogs in our fertilized creeks has been negatively impacted by eutrophication. Initially, growth rates were higher in the fertilized creek but growth rates slowed by mid-season while reference growth rates remained constant. Data will continue to be collected through September in order to observe if the growth trend persists.

Temporal Carbon Flux Dynamics in New England Salt Marsh Ponds

Ana Gordon[1], Inke Forbrich[2], Anne Giblin[2]

[1]*Department of Civil and Environmental Engineering, Syracuse University, Syracuse, NY*
[2]*Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA*

The rate of carbon flux in a salt marsh can indicate if marsh production will keep up with rising sea levels. Because salt marshes are dynamic ecosystems, pond formation and development can have significant contributions to the overall net carbon balance of the entire marsh. To further examine this relationship, metabolism rates of four ponds at varying stages of development in the Plum Island, MA estuary were studied. Dissolved oxygen, temperature, salinity, specific conductivity and depth were recorded at ten minute intervals. Assuming a one to one molar ratio, this data was used to calculate the rates of daily production, nightly respiration and net carbon flux of each pond per sampling day. The gas transfer coefficient was also measured for each pond to correct for oxygen diffusion through the air-water interface. Additional water samples were taken to measure alkalinity, dissolved inorganic carbon and ammonia over three 24 hour periods to provide supporting data. Results indicate that pond size is of little importance, with respiration dominating all fluxes suggesting that the ponds are heterotrophic and expanding.

The effects of eutrophication on the geomorphology of the Plum Island salt marshes

Katherine M. Stout[1], Christopher Haight[2], Linda Deegan[2]

[1]Clarkson University, Potsdam, NY

[2]Marine Biological Laboratory, Woods Hole, MA

The Plum Island Estuary salt marshes have been studied by the TIDE project, where one creek has been fertilized for nine years and another for four years, with excess nitrate to study the effects of eutrophication. After four years, TIDE scientists began noticing significant changes in the geomorphology of the fertilized creeks such as creek-bank slumping. These changes could have detrimental effects on the salt marsh habitat that many species depend as vegetated marsh is lost. We measured the areas where the *Spartina patens* that dominates the high marsh is cracking and breaking off into the creek, leaving a sheer edge with a disturbed low marsh habitat. Additionally, we measured the peat islands that form when pieces of the creek bank fall into the channel and the width of the *Spartina alterniflora* and mud flat habitats. The data shows a significant difference in the number of cracks in the fertilized and reference creeks ($p<0.05$). These data suggests that eutrophication may have an impact on the stability of the creek banks. There were significantly more islands in chronically fertilized creeks ($p<0.05$). As a result, the fertilized creeks have a lower percent of *Spartina alterniflora* habitat, and a higher percent of mud flat habitat when compared to the reference creeks. These data implies that the eutrophication may be causing a decrease in extent of the low marsh, which is a critical breeding ground for *Fundulus heteroclitus*. These changes in geomorphology could have serious effects on the future of the salt marsh habitat, and may show an unexpected effect of eutrophication.

Evaluating Toxicity of Mutant SOD1 Forms

Izrail Abdurakhmanov[1,3], Scott T. Brady[2,3], Weiming Ni[4,5], Arthur L. Horwitch[4,5], Yuyu Song[2,3]

[1] *Department of Biological Sciences, CUNY Hunter College, New York, NY*

[2] *Department of Anatomy and Cell Biology, University of Illinois at Chicago, IL*

[3] *Marine Biological Laboratory, Woods Hole, MA*

[4] *Department of Genetics, Yale University, New Haven, CT*

[5] *Howard Hughes Medical Institute*

Amyotrophic Lateral Sclerosis (ALS), a.k.a Lou Gehrig's disease, is a fatal adult onset neurodegenerative disease affecting upper and lower motor neurons. Five to ten percent of ALS cases result from autosomal dominant inheritance of a disease gene (Familial ALS), whereas the majority of cases have no known familial history and are thus called Sporadic ALS. Mutations in the gene that codes for the enzyme superoxide dismutase 1 (SOD1) have been discovered to cause twenty percent of familial ALS cases. Wild type SOD1 has been proposed to play a role in reducing free oxygen radicals in the cytosol and is involved in redox signaling. It has been demonstrated that pathological SOD1 mutations give rise to a gain of toxic function as opposed to loss of dismutase activity. Mutant SOD1 is thought to cause toxicity by negatively affecting axonal transport, which leads to motor neuron degeneration. There are different structures that mutant SOD1 may take, however, and some structures may prove to be more toxic than others. Four classes of structure of the G85R SOD1 mutant were studied: the monomeric, dimeric, medium aggregate, and big aggregate forms. By performing vesicle motility assays in squid giant axons with the different structural isolates of SOD1 mutant proteins, we were able to see how fast axonal transport (FAT) was affected by each structure. With wild type SOD1 as the control, no change in rate of FAT was observed but with the monomeric and dimeric mutant forms, the most dramatic decrease in anterograde activity was observed. The medium and big aggregate forms were not as dramatic hinting that the monomeric and dimeric forms of mutant SOD1 are more toxic.

EVALUATING THE EFFECTS OF MUTANT FUS IN AXONAL TRANSPORT

Saul Penaranda[1], Scott T. Brady[2], Gerardo Morfini[3], Reddy Sam[3], Daryl A. Bosco[5],

[1]*Department of Biological Sciences, CUNY Hunter College, New York, NY.*

[2]*Department of Anatomy & Cell Biology, University of Illinois at Chicago, Chicago, IL.*

[3]*Department of Neurology, University of Massachusetts Medical Center, Worcester, MA.*

[4]*Marine Biological Laboratory, Woods Hole, MA.*

Amyotrophic Lateral Sclerosis (ALS) is a disease of motor neurons in the brain and spinal cord that control voluntary muscle movement. Approximately 5-10% of ALS cases are genetic diseases that are transmitted as a dominant trait with variable age of onset. One of the recently discovered genes with mutations that cause ALS is FUS (Fused in Sarcoma), which encodes a nuclear polypeptide. FUS in healthy individuals is found in the nucleus and is involved in DNA repair, RNA splicing, and transcription, while mutant forms are enriched in the cytoplasm. In the cytoplasm, mutant FUS has been shown to be involved in the recruitment of other proteins and aberrant aggregation. Cytoplasmic FUS and FUS aggregates are thought to contribute to this disorder by activating or deregulating different pathways. Here, we investigated the effects of a variety of FUS mutations on fast axonal transport (FAT) using axoplasm extracted from squid giant axon. The effects of different mutations were evaluated in Anterograde and Retrograde directions using differential interference contrast microscopy. It was found that all ALS-related FUS proteins decreased vesicular transport in both directions. Pharmacological approaches were tested using diverse kinases inhibitors, and some of these agents rescued both anterograde and retrograde transport. It is thought that mutant FUS can lead to the direct or secondary activation of protein kinase p38, which phosphorylates kinesin and other motors involved in transport resulting in the detachment of motor proteins carrying vesicles from microtubules.