2016 MBL
Undergraduate Research Symposium

Thursday, August 18, 2016
Speck Auditorium
8:00 am to 5:30 pm

Program Schedule and Abstracts

Sponsored by:
NSF-REU "Biological Discovery in Woods Hole" program
The Marine Biological Laboratory Office of Research & Programs and Education Office
William D. Cohen Endowed Undergraduate Research Fund
Jane C. Kaltenbach-Townsend Endowment for Undergraduate Research

Program Organizers:

Robert Paul Malchow, Ph.D., University of Illinois at Chicago

Jean Enright – Education Program Coordinator, MBL

2016 MBL Undergraduate Research Symposium
August 18, 2016 – Speck Auditorium

8:00 am **Opening Remarks – Rae Nishi, Director of Education**

Session One - Chair: Jessica Mark Welch

8:05 am **Assessing Microbial Metabolic Function and Circadian Rhythms Over Time And Space In Siders Pond.** **Petra K. Byl**[1,2], Emily Reddington[2], Rika Anderson[3], Joseph J. Vallino[2], Julie A. Huber[2]. [1]*University of Chicago, Chicago, IL;* [2]*Marine Biological Laboratory, Woods Hole, MA*

8:20 am **Identifying autotrophic microbes at deep-sea hydrothermal vents using RNA-SIP and RT-qPCR.** **Paula Pelayo**[1], Caroline S. Fortunato[2], Julie A. Huber[2]
[1]*UCLA, Los Angeles, CA,* [2]*Marine Biological Laboratory, Woods Hole, MA.*

8:35 am **In vitro studies of Penelope-like retroelements in the genome of the bdelloid rotifer Adineta vaga.** **Vishok Srikanth**[1,2], Irina A. Yushenova[1] and Irina R. Arkhipova[1].
[1]*Marine Biological Laboratory, Woods Hole, MA;* [2]*University of Chicago, Chicago, IL.*

8:50 am **De novo detection and annotation of transposable elements in metazoan genomes.** **Brandon M. Lê**[1,2] and Irina R. Arkhipova[1,2]**.** [1]*Brown University, Providence, RI and* [2]*Marine Biological Laboratory, Woods Hole, MA.*

9:05 am **Gene conversion as a possible mechanism behind the asexual thriving of bdelloid rotifers**. **Lawrence Shelven** [1,2], Bette Hecox-Lea [1], Joseph Vineis, MS [1], David Mark Welch, PhD [1]. [1]*Marine Biological Laboratory, Woods Hole, MA;* [2]*Dartmouth College, Hanover, NH*

9:20 am **Species composition of microbial structures in dental plaque**
Alexandra Sjaarda[1,2], Jessica Mark Welch[1], Gary Borisy[3]. [1]*Marine Biological Laboratory;* [2]*University of Chicago;* [3]*The Forsyth Institute*

9:35 am **Tracking biofilm growth of early Plastisphere colonizers**
David Vishny[1], Breaun Meeks[2], Erik Zettler[3], Linda Amaral-Zettler[4,5]. [1]*University of Chicago, Chicago, IL,* [2]*Bowie State University, Bowie, MD,* [3]*Sea Education Association, Woods Hole, MA,* [4]*Marine Biological Laboratory, Woods Hole, MA,* [5]*Brown University, Providence, RI.*

9:50 am **Visualizing the spatial organization of subgingival plaque through spectral imaging fluorescence *in situ* hybridization (FISH).** **Molly Bennett**[1,2], Jessica Mark Welch[2].
[1]*The University of Chicago, Chicago, IL;* [2]*Marine Biological Laboratory, Woods Hole, MA*

10:05 am **Dynamics of *Asterias forbesi* and *A. vulgaris* microbial communities in relation to sea star wasting disease (SSWD) of the Northeast coast of the U.S.**
Isa Alvarez [1], Jessica Mark Welch [2], and David Mark Welch [2]. [1]*University of Chicago, Chicago, IL;* [2]*Marine Biological Laboratory, Woods Hole, MA*

10:20 am BREAK

Session Two – Chair: Lydia Mäthger

10:30 am Using Lipid Biomarkers to Understand Deep Ocean Organic Particle Flux
Leonard Shaw[1], Emily Maness[2], J.C. Weber[3], Maureen Conte[3]. [1]*University of Chicago, Chicago IL;* [2]*University of Tampa, Tampa FL;* [3]*Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA*

10:45 am Decadal shifts in the vegetation structure Great Sippewissett Marsh in response to sea level rise.
Tynan Bowyer[1,2], Ivan Valiela[2], Javier Lloret[2], David Remsen[2], Simon Miner[2]. [1]*University of Chicago, Chicago, IL;* [2]*Marine Biological Lab, Woods Hole, MA.*

11:00 am Long term responses to high N loading in West Falmouth Harbor: the elusive role of eelgrass meadows in determining water quality. **Toby SantaMaria**[1], Anne Giblin[2], and Melanie Hayn[2, 3]. [1]*Kenyon College Dept of Biology, Gambier OH 43022;* [2]*The Ecosystems Center at Marine Biologic Labs, Wood's Hole MA 02543;* [3]*Cornell University, Department of Ecology and Evolutionary Biology, Ithaca NY 49818*

11:15 am Spatial-Temporal Responses of Estuarine Phytoplankton to Changes in Nitrogen Loads from Watersheds.
Clara Maynard[1,2], Lindsay Levine[1,2], Ivan Valiela[2], Elizabeth Elmstrom[2], Javier Lloret[2], Emily DeFries[2,3]. [1]*Brown University, Providence, RI;* [2]*Marine Biological Laboratory, Woods Hole, MA;* [3]*Purdue University, West Lafayette, IN.*

11:30 am Understanding changes in estuarine food webs under the influence of increased nitrogen loads: An analysis of carbon and nitrogen isotopic signatures
Emily DeFries[1,2], Elizabeth Elmstrom[2], Javier Lloret[2], Lindsay Levine[3], Clara Maynard[3]
Linda Deegan[4], Ivan Valiela[2] . [1]*Purdue University,* [2]*Marine Biological Laboratory,* [3]*Brown University,* [4]*Woods Hole Research Center.*

11:45 am Can in situ aeration reduce eutrophication in coastal ecosystems?
Christian M. Bruce[1,2] and Anne E. Giblin[2]. [1]*University of Richmond, Richmond, VA; and* [2]*Marine Biological Laboratory, Woods Hole, MA.*

12:00 pm Lunch Break

Session Three – Chair: Beth Giuffrida

1:00 pm Fight or Flight: analyzing the metrics and behavioral context of rapid neural polyphenism in cephalopod mollusks. **Olivia Cattau**[1,2] and Roger T. Hanlon[2]. [1]*University of Chicago, Chicago, IL;* [2]*Marine Biological Laboratory, Woods Hole, MA*

1:15 pm Comparing Scanning Electron Microscopy and Micro-CT Imaging Techniques for the Mechanosensory Lateral Line. **Loranzie S. Rogers**[1,2], Beth Giuffrida[2,3], Veronique Le Roux[4], Allen F. Mensinger[1,2]. [1]*University of Minnesota Duluth, Duluth, MN;* [2] *Marine Biological Laboratory, Woods Hole, MA,* [3]*Wareham Middle School, Wareham, MA,* [4]*Woods Hole Oceanographic Institute, Woods Hole, MA*

1:30 pm **Molecular identification of neuron types in the *Xenopus Laevis* vocal circuit**
Claire P. Everett[1,3], Darcy B. Kelley[2,3]. *[1]Barnard College, New York, NY; [2]Columbia University, New York, NY; [3]Marine Biological Laboratory, Woods Hole, MA.*

1:45 pm **Measurement of extracellular changes in acidity mediated by radial glial cells (Müller cells) of the vertebrate retina.** **Chad Heer[1]**, Marcin Wlizla[2], Marko Horb[2] & Robert Paul Malchow[3]. *[1]Indiana Wesleyan University, Marion, IN, [2]Marine Biological Laboratory, Woods Hole MA, & [3]University of Illinois at Chicago, Chicago, IL.*

2:00 pm **FMRP localizes adjacent to mitochondria in synaptic spines of rat hippocampal neurons.** **Nicole M. Cruz-Reyes[1,4]**, Nikita Mehta[2,4], Han-A Park[3], Paige Miranda[3], Nelli Mnatsakanyan[3], Elizabeth Jonas[3,4]. *[1]University of Puerto Rico, Cayey, PR, [2]University of Chicago, Chicago, IL, [3]Yale University, New Haven, CT, [4]Marine Biological Laboratory, Woods Hole, MA*

2:15 pm **The effects of amyloid beta (Aβ) on mitochondrial function in hippocampal neurons.** **Nikita Mehta[1,4]**, Nicole Cruz-Reyes[3,4], Han-A Park[2], Nelli Mnatsakayan[2], Paige Miranda[2], Elizabeth Jonas[2,4]. *[1]University of Chicago, Chicago, IL; [2]Yale University, New Haven, CT; [3]University of Puerto Rico, Cayey, PR; [4]Marine Biological Laboratory, Woods Hole, MA.*

2:30 pm **Isolated squid axoplasm: An experimental system for the study of axon-autonomous events relevant to human neurodegenerative diseases.** **Henry Thomsett[1,3]**, Minsu Kang[2,3], Scott T. Brady[2,3], Gerardo Morfini[2,3]. *[1]Department of Chemistry, Skidmore College, Saratoga Springs, NY, USA. [2]Department of Anatomy and Cell Biology, University of Chicago at Illinois, Chicago, IL, USA. [3]Marine Biological Laboratory, Woods Hole, MA, USA.*

2:45 pm **Inhibition of fast axonal transport in neurons is mediated by mutant huntingtin protein.** **Karen G. Ebenezer[1,3]**, Minsu Kang[2,3], Gerardo Morfini[2,3], Scott T. Brady[2,3]. *[1]CUNY Hunter College, New York City, NY; [2]University of Illinois at Chicago, Chicago, IL; and [3]Marine Biological Laboratory, Woods Hole, MA.*

3:00 pm **Mutant huntingtin-mediated inhibition of Fast Axonal Transport involves activation of Mixed Lineage Kinases.** **Alison Klein[1,3]**, Minsu Kang[2,3], Gerardo Morfini[2,3], Scott Brady[2,3]. *[1]CUNY Hunter College, New York, NY; [2]University of Illinois at Chicago, Chicago, IL; [3]Marine Biological Laboratory, Woods Hole, MA*

3:15 pm **BREAK**

Session Four – Chair: Stephen Senft

3:30 pm **The mechanism of pupillary movement in the little skate *Leucoraja erinacea.***
Lucia Sicius[1,2], Shane Jinson[2], Justine J. Allen[3], Lydia M. Mäthger[2]. *[1]Florida State University, Tallahassee, FL; [2]Eugene Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory, Woods Hole, MA; [3]Department of Ecology and Evolutionary Biology, Brown University, Providence, RI*

3:45 pm **The effects of different visual background cues on dynamic expression of cuttlefish 3D skin papillae for camouflage.** **Marcus Van Ginkel[1]**, Deanna R. Panetta[2], Roger T. Hanlon[2]. *[1]Carleton College, Northfield, MN; [2]Marine Biological Laboratory, Woods Hole, MA.*

4:00 pm **Morphological characterization of chromatophore granules in the squid *Doryteuthis pealeii* and implications for biophotonics.** **James F. Peyla[1]**, Stephen L. Senft[2], and Roger T. Hanlon[2]. *[1]College of Charleston, Charleston, S.C.; [2]Marine Biological Laboratory, Woods Hole, M.A.*

4:15 pm **Effects of Flow and Hypoxia on Developing Squid (*Doryteuthis pealeii*) Egg Capsules.** **Lucy M. Fitzgerald[1.]**, Matthew H. Long[2.], T. Aran Mooney[3]. *[1.]Eckerd College, St. Petersburg, FL; [2.]Department of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, MA; [3.] Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA.*

4:30 pm **Exploring the light environment for larval culturing of MBL squid.** **Kirsten Peramba[1,2]**, Scott Bennett[2] and Eric Edsinger[2]. *[1]Bridgewater State University, [2]Marine Biological Laboratory.*

4:45 pm **Intravital imaging of hepatocyte response to tunicamycin induced ER-stress in zebrafish.** **Leonore Wünsche**[1] and Kirsten C. Sadler[2]. *[1]Sadler Lab, New York University, [2]Abu Dhabi, Saadiyat Island, PO 129188, Abu Dhabi, United Arab Emirates.*

5:00 pm **Investigation of choanocyte-like structures on the ovary epithelium in the bat star *Patiria miniata.*** **Kyle R. Ford[1]**, Mark Terasaki [2]. *Marine Biological Laboratory, Woods Hole, MA.*

5:15 pm **Microfluidic Droplets as Substrates for Custom Photolithographic Patterning.** **Michael J. Sloyan[1,5]**, Taylor Sulerud[2,5], Ben Noren[2,5], James Pelletier[3,5], Jesse Gatlin[2,5], Christine Field[4,5], Timothy Mitchinson[4,5], John Oakey[2,5]. *[1]University of Chicago, Chicago, IL; [2]University of Wyoming, Laramie, WY; [3]Massachusetts Institute of Technology, Cambridge, MA; [4]Harvard Medical School, Boston, MA; [5]Marine Biological Laboratory, Woods Hole, MA.*

Dynamics of *Asterias forbesi* and *A. vulgaris* microbial communities in relation to sea star wasting disease (SSWD) of the Northeast coast of the U.S.

Isa Alvarez [1], Jessica Mark Welch [2], and David Mark Welch [2].
[1]*University of Chicago, Chicago, IL;* [2]*Marine Biological Laboratory, Woods Hole, MA*

Sea stars play a crucial role in coastal marine ecosystems as keystone predators. Recently, a new disease has caused thousands of stars to wash up on the west and east coast shores of the U.S., puzzling researchers everywhere. Previous works have established a connection between the sea star associated densovirus (SSaDV) and the wasting disease affecting different species on the west coast. In this study, I closely monitor the effects of sea star wasting disease on *A. forbesi* and *A. vulgaris* on the east coast of the U.S. in regards to the microbiome of sea stars and the effects of the microbiome on susceptibility to SSWD. First, I examined several sea stars using FISH (fluorescence in situ hybridization) to determine what structures, if any, possess a complex microbiome. Only the aboral surface had a microbiome. I then collected DNA samples from the aboral surface of fresh-caught stars and from the same stars after acclimating to the aquarium environment of the MRC. We will examine infected and non-infected stars under the confocal microscope, amplify and sequence DNA, and isolate viral fraction of infected stars for metagenomic sequencing to determine the effect of handling/swabbing stars in regards to passing the infection. Potential findings in this study could help future researchers studying the disease on the west coast and provide information on the potential differences in the disease due to geography.

Funding through the collaboration of the Marine Biological Laboratory and the University of Chicago by the McArthur Family Metcalf Fellow Award, and by a gift from the Vetlesen Foundation to the Josephine Bay Paul Center for Molecular Biology and Evolution.

Visualizing the spatial organization of subgingival plaque through spectral imaging fluorescence *in situ* hybridization (FISH)

Molly Bennett[1,2], Jessica Mark Welch[2]
[1]*The University of Chicago, Chicago, IL;* [2]*Marine Biological Laboratory, Woods Hole, MA*

Because physical proximity between microbes has been shown to dramatically influence their physiology, information about the spatial structure of multispecies biofilms like dental plaque is critical to our understanding of how they function. Currently, a lack of detailed spatial information limits our knowledge of how individual microbes interact within oral microbial communities, but fluorescence *in situ* hybridization (FISH) has made it possible to identify different cell types in the same microscopic field by targeting the 16S rRNA sequences of microbes of interest. Using FISH to visualize the major genera identified by metagenomic sequence analysis in supragingival plaque (SUPP), the Mark Welch laboratory has found distinct microbial consortia like the "hedgehog" structure, which is formed by a core of *Corynebacterium* filaments coated with aero-tolerant cocci like *Streptococcus* at their distal tips that are hypothesized to create a low O_2 and high CO_2 micro-environment ideal for the proliferation of microaerophilic genera in an annulus just below the hedgehog's outer shell. The subgingival plaque (SUBP) microbiome is closely associated with the SUPP microbiome and contains many of the same taxa, but *Corynebacterium* is lower in SUBP while the abundance of *Fusobacterium* is higher, raising the question of whether the hedgehog structure is also prominent in SUBP or if *Fusobacterium* assumes a more central role instead as predicted by earlier models of plaque accumulation. Accordingly, we designed two probe sets for FISH on SUBP and validated them on pure cultures, with each set targeting *Corynebacterium, Fusobacterium,* and additional taxa that showed high abundance in SUBP based on sequencing data. Through the application of these probe sets to SUBP sampled from healthy donors, we are investigating the spatial structure of SUBP with a focus on the relationship of filamentous bacteria like *Corynebacterium* and *Fusobacterium* to other taxa.

Funding by the University of Chicago Jeff Metcalf Fellowship and NIH National Institute of Dental and Craniofacial Research

Decadal shifts in the vegetation structure Great Sippewissett Marsh in response to sea level rise.

Tynan Bowyer[1,2], Ivan Valiela[2], Javier Lloret[2], David Remsen[2], Simon Miner[2]
[1]University of Chicago, Chicago, IL; [2]Marine Biological Lab, Woods Hole, MA

Rates of sea level rise are increasing in much of the world, including New England, and particularly on Cape Cod. The distribution of plant species on salt marshes is sensitive to relative elevation within tidal ranges, so that increased submergence associated with sea level rise must force major shifts on the vegetation of these important coastal environments. To assess decadal vegetation changes forced by recent sea level rise in Great Sippewissett Marsh, a representative Cape Cod salt marsh, we compared a detailed vegetation map, done in 1979, with vegetation distribution in 2015, obtained from high-resolution drone imagery. We used GIS analysis to reveal major shifts in the cover by the different habitats across the tidal range during the 37-year period, all indicating greater submergence. A 14% decrease in area of low-lying creek banks supporting tall form *Spartina alterniflora* made way for the expansion of open water areas by 2015; cover by low marsh—dominated by short form *S. alterniflora*—increased 40%, at the expense of high marsh habitat species—*Spartina patens* and *Distichlis spicata.* High marsh area experienced a dieback of 60%, unable to potentially expand landward in response to rising waters. Changes were also recorded within areas of un-vegetated sediment, with an 82% increase in sand creeks—likely from over wash of sand eroded from the beach and dune that have receded by an average of 22 m since 1979. Salt marshes are well known to play significant roles in coastal dynamics, including provision of a series of important ecological services for which vegetation cover plays a keystone function. The significant structural and vegetation composition changes we report serve notice of substantial shifts in ecosystem function and ecological services as a result of recent sea level rise.

Funding provided by the University of Chicago Jeff Metcalf Research Fellowship. We would like to acknowledge Elizabeth Elmstrom for her support of the project and Emily DeFries for her assistance in fieldwork.

Can in situ aeration reduce eutrophication in coastal ecosystems?

Christian M. Bruce[1,2] and Anne E. Giblin[2]
[1]University of Richmond, Richmond, VA; and [2]Marine Biological Laboratory, Woods Hole, MA.

Hypoxia, occurring when dissolved oxygen (DO) content falls below ≤2 mL of oxygen per liter, is one of the greatest threats to a healthy marine ecosystem. A high DO content is maintained through photosynthesis, and atmospheric exchange. DO is consumed by respiration both in the water column and by the sediments. To prevent hypoxia DO supply must meet or exceed the respiratory demand. Hypoxia is one of the negative effects of eutrophication, an ecosystem's natural response to increased nutrients, which are generally introduced to the ecosystem through the runoff of fertilizers and other human caused pressures. Hypoxia also reduces the ecosystems natural ability to remove nitrogen through coupled nitrification-denitrification. To reduce the effects of eutrophication in a coastal environment, we installed a grid of aerators (bubblers) in Great Pond in Falmouth to mix the water column and to try to increase the amount of dissolved oxygen reaching the sediment. We hypothesized that the in situ aeration would reduce sediment oxygen demand and decrease the flux of nitrogen from the sediments. The in situ aeration system has had no significant effect to reduce hypoxia within the gridded area. One explanation for this is that the water column is too shallow for the aeration to make a significant impact beyond the immediate area of the bubblers. The lack of impact was also confirmed when we compared the respiration rates of sediment cores within the aerated site to the flux rates of cores taken from a control area. To see if short term aeration would have been beneficial if the aerators had worked, we have been aerating cores under laboratory conditions that we will compare to control cores. We are also examining if there are ways to increase the aeration efficiency.

Assessing Microbial Metabolic Function and Circadian Rhythms Over Time And Space In Siders Pond

Petra K. Byl[1,2], Emily Reddington[2], Rika Anderson[3], Joseph J. Vallino[2], Julie A. Huber[2]
[1]University of Chicago, Chicago, IL; [2]Marine Biological Laboratory, Woods Hole, MA

Diverse bacterial and archaeal metabolic networks cycle life-essential elements throughout our biosphere and can locally influence the habitability of an ecosystem. However, we have limited knowledge on the dynamics that govern interactions between members of a microbial community in the environment over time and space. Here, we examined the structure and function of the microbial community in Siders Pond— a permanently stratified, meromictic kettle pond in Falmouth, MA— with a focus on the relationship between phototrophs and other members of the microbial community. We sampled 7 casts at 8 chemically and physically distinct depths over a 24-hour diel cycle and analyzed biogeochemical constituents as well as constructed metagenomic and metatranscriptomic libraries. Results show a vertical gradient of phototrophs over depth with mixotrophic and aerobic phototrophic lineages dominating the water column above 6 meters and anaerobic phototrophic lineages dominating the water column at 8 meters. Our metagenomic and geochemical data indicates that dissolved oxygen, microbial biomass, and regulatory circadian rhythm genes (kaiABC, ldpA, and cikA) peaked at 3-4 meters depth, where phototrophic cyanobacteria dominated the water column. In the lower water column, a coupled decrease in hydrogen sulfide and dissolved inorganic carbon during daylight hours provided evidence for a diel metabolic cycle in phototrophic green sulfur bacteria (*Chlorobi*). Based on our preliminary data, we hypothesize that microbial phototrophs express genes on an internal circadian rhythm. We are currently analyzing the metatranscriptome for the expression of regulatory circadian rhythm genes, phototrophic metabolism, and nutrient uptake. Combined with data from the spatial and temporal changes in water column geochemistry, results will be presented in the context of understanding the complex microbiome that drives biogeochemical cycling in Siders Pond.

Funding by NSF grant GEO-1451356

Fight or Flight: analyzing the metrics and behavioral context of rapid neural polyphenism in cephalopod mollusks

Olivia Cattau[1,2] and Roger T. Hanlon[2]
[1]University of Chicago, Chicago, IL; [2]Marine Biological Laboratory, Woods Hole, MA

Cephalopods are renowned for the extreme speed with which they can change a single phenotype for camouflage and communication. This is due to direct neural control of their skin pigmentation as opposed to slower, hormonally controlled camouflage, which is used by many fishes, lizards, and other animals. We addressed two complementary questions: (i) exactly how fast can each species of cephalopod change full body patterns, or components of patterns, each involving millions of skin elements and (ii) what is the behavioral context that influences the speed or type of pattern each type of cephalopod can deploy? We analyzed 336 video sequences from fieldwork over the past decade, primarily focusing on European cuttlefish (*Sepia officinalis),* giant Australian cuttlefish (*Sepia apama*), Caribbean reef squid (*Sepioteuthis sepioidea*), and the Caribbean octopus (*Octopus vulgaris*). We separated the behavioral contexts into three groups: primary defense (unthreatened animal goes into camouflage), secondary defense (deimatic and protean behaviors), and sexual signaling (fighting and courtship). *Sepia officinalis* produced the single fastest component change: (170 milliseconds) but *Sepioteuthis sepioidea* produced the fastest full body patterning deployment (300 milliseconds). Regardless of species, it was during secondary defenses that the fastest neural responses occurred, suggesting that immediate danger of predation was the likely selective force that led to the evolution of this extreme adaptation.

Funding by ONR grant #N00014-10-1-0989, AFOSR grant #A9550–09–0346, MBL/UChicago grant, and Metcalf SURF program (University of Chicago)

FMRP localizes adjacent to mitochondria in synaptic spines of rat hippocampal neurons

Nicole M. Cruz -Reyes[1,4], Nikita Mehta[2,4], Han-A Park[3], Paige Miranda[3], Nelli Mnatsakanyan[3], Elizabeth Jonas[3,4]
[1]University of Puerto Rico, Cayey, PR, [2]University of Chicago, Chicago, IL, [3]Yale University, New Haven, CT, [4]Marine Biological Laboratory, Woods Hole, MA

Fragile X Mental Retardation Protein (FMRP) is a well-studied mRNA binding protein necessary for the repression of protein translation in neurons. In Fragile X syndrome, an X-linked neurological condition (FXS), CGG repeats in DNA shut down transcription of the *Fmr1* gene through a process called methylation. As a consequence, FMRP production either stops or is very low, resulting in abnormally high protein synthesis of a large number of synaptic proteins. FXS causes intellectual disability and autistic behaviors, among other impairments. Although the learning deficit experienced by the patients is not well understood, it may result at least in part from the inability to produce Long-Term Potentiation (LTP) of synaptic transmission in the hippocampus and areas of the neocortex. Previous findings suggest that, in addition to its role as an mRNA binding protein, FMRP may be localized to, or associated with, mitochondria and may act similarly to B-cell lymphoma-extra large (Bcl-xL) to protect neurons from stress by increasing mitochondrial efficiency. This mitochondrial effect may also reduce unnecessary protein translation. Our investigation focuses on finding evidence to support subcellular localization of FMRP. Using immunocytochemistry of isolated rat hippocampal neurons we localized anti-FMRP antibody to sites overlapping with the mitochondrial antibody COX IV. Microscopy was performed with a Zeiss confocal microscope and resulting images were analyzed with ImageJ. We find that FMRP appears to be present in puncta throughout the cell, and that it prominently co-localizes with COX IV mitochondria-labeled sites in areas that appear adjacent to, or part of, dendritic spines. Future work will attempt to localize FMRP to a specific subcellular fraction in biochemical experiments and by electron microcopy.

Funding by the NSF-REU grant DBI-1359230

Understanding changes in estuarine food webs under the influence of increased nitrogen loads: An analysis of carbon and nitrogen isotopic signatures

Emily DeFries[1,2], Elizabeth Elmstrom[2], Javier Lloret[2], Lindsay Levine[3], Clara Maynard[3]
Linda Deegan[4], Ivan Valiela[2]. *[1]Purdue University, [2]Marine Biological Laboratory, [3]Brown University, [4]Woods Hole Research Center.*

Stable nitrogen isotope signatures ($\delta^{15}N$) can determine position of a species within a food web and sources of nitrogen transported into receiving coastal waters. We sampled estuarine species from three estuaries with differing levels of watershed urbanization in the Waquoit Bay estuarine system in Cape Cod, MA. The goals of the study were 1) to corroborate if isotopic signatures defined trophic levels within the estuarine food webs, and 2) whether the signatures were related to the degree of urbanization. Increased urbanization increases levels of nitrogen contributing to eutrophication. Since $\delta^{15}N$ from wastewater tends to be heavier than $\delta^{15}N$ from fertilizer and atmospheric deposition [10–20, 2– 8, and (-3)– 3, respectively for N from wastewater, atmospheric deposition, and fertilizer], discharge from more developed watersheds is heavier. While % N differed among producers, omnivores, and predators, there was no effect of N loading rate on these components of food webs. These taxa reached an asymptotic level of N-sufficiency and lacked N storage capacity. In contrast, $\delta^{15}N$ values across trophic levels rose and differed significantly in estuaries subject to higher N loads. These results corroborated the sensitivity of $\delta^{15}N$ as an indicator of N sources and trophic position. The variances in $\delta^{15}N$ values among the estuaries receiving differing N loads emerged mainly among the lower trophic levels. Whereas $\delta^{15}N$ values were similar at the higher predator trophic levels, suggesting that stable isotopes of producers are the better indicators of N sources in estuaries. $\delta^{13}C$ values in producers ranged widely (-25 to -8 ‰), indicating broad differences in carbon fixation metabolism. The range of $\delta^{13}C$ in consumers was smaller, but indicated that a broad base of different producer biomass was consumed. Stable isotopic analysis is a useful tool for sensitively assessing trophic position evaluating differing N sources entering estuarine food webs.

Funded through the Valiela Lab and the National Science Foundation

Inhibition of fast axonal transport in neurons is mediated by mutant huntingtin protein

[1,3]Karen G. Ebenezer, [2,3]Minsu Kang, [2,3]Gerardo Morfini, [2,3]Scott T. Brady
[1]CUNY Hunter College, New York City, NY; [2]University of Illinois at Chicago, Chicago, IL; and [3]Marine Biological Laboratory, Woods Hole, MA.

Huntington's disease (HD) is a fatal adult-onset neurodegenerative disease characterized by an involuntary jerky movement called Huntington's chorea as well as cognitive deficits and mood disorders. HD is caused by a mutation of a trinucleotide repeat in the huntingtin gene which results in an expansion of the polyglutamine region of the huntingtin protein (Htt). There is no known treatment for the disease, and it affects 1 in 10,000 people in the United States. A growing body of evidence indicates that the inhibition of fast axonal transport (FAT) by mutant Htt (mHtt) is a major pathogenic event in HD. Several studies using different model organisms of HD report inhibition of both anterograde and retrograde axonal transport of various molecular cargoes mediated by motor proteins kinesin and cytoplasmic dynein respectively. Using squid axoplasm isolated from squid giant axons, our laboratory demonstrated that mHtt leads to inhibition of FAT via activation of a mitogen activated protein kinase (MAPK) called c-Jun N-terminal kinase 3 (JNK3), which phosphorylates kinesin and inhibits its microtubule-binding activity. JNK3 itself is activated by a series of upstream kinases, as part of a signaling pathway called a MAPK cascade. Our laboratory analyzed changes in the activity of these kinases in the presence of mutant proteins, as well as the resulting phosphorylation in the described signaling pathway.

Funding from NIH (STB), HHMI REU (Hunter College)

Molecular identification of neuron types in the *Xenopus Laevis* vocal circuit

Claire P. Everett[1,3], Darcy B. Kelley[2,3]
[1]Barnard College, New York, NY; [2]Columbia University, New York, NY; [3]Marine Biological Laboratory, Woods Hole, MA.

Patterned behaviors-such as breathing, speaking, and walking- are incorporated seamlessly into everyday life. Diverse neural circuits, endocrine signaling and muscle effectors govern these patterned behaviors. The Kelley lab uses the sexually differentiated vocal behaviors of the South African Clawed frog, *Xenopus laevis*, as a model system to study how male- and female-specific patterns are produced. While some components of vocal circuits have been identified, local hindbrain interneurons and subpopulations of laryngeal motor neurons are poorly understood. Transcription factor expression has been useful in identifying neurons that contribute to locomotion in spinal cord and respiration in the hindbrain. We are using transcription factor and calcium binding protein expression - combined with dye tracing - to identify participants in vocal patterning in *Xenopus*. Calbindin, Isl1, En1 and androgen receptor expression is mapped in late stage tadpole brains using immunohistochemistry (IHC). Specific neurons are identified in the vocal circuit by injecting a fluorescent tracer (rhodaminated dextran) into the vocal motor nucleus and the pattern-generating nucleus and following axon projections and synaptic contacts. Dye-tracing and IHC together will provide clues into how populations of neurons develop and interact to contribute to this endocrine-responsive call circuit.

Acknowledgments: We thank the Marine Biological Laboratories and specifically National Xenopus Resource and the Horb lab for research support. Thank you to the Tow Research Fellowship and the Charles A. Huebschman Prize for supporting this research.

Effects of Flow and Hypoxia on Developing Squid (Doryteuthis pealeii) Egg Capsules

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Squid are often considered a keystone group of animals in the marine environment; they are predator or prey to a wide range of marine organisms. Larval animals form the foundation of populations, and larval recruitment can be effected by environmental conditions, but these effects are not well described for squid. They naturally exhibit low oxygen concentrations in their egg capsules. Each egg capsule contains around 200 developing embryos all respiring and requiring oxygen to support their rapid growth. The objective of the study was to test if hypoxia, such as found in oxygen minimum zones and some benthic environments, has effects on the development of egg capsules and if water flow can alleviate or exacerbate these effects. It was expected that the oxygen consumption, examined through boundary layer micro-optode profiling, would change in relation to development and flow rate.

The egg capsules were raised in one of two levels of oxygen, 100% saturation and 50 % saturation to examine hypoxia effects in micro-flumes with 3 different flow rates, to examine flow effects. Capsules were then placed inside the raceway of one of 3 micro-flumes each with a different flow rate: 10 cm/s (high), 1 cm/s (low) or 0 cm/s (control). 3-4 capsules were profiled on Day 1, 6 and 10. Separate eggs were placed in cups with mesh and placed in the flume for hatching counts. Flow rate had the highest effect on the 1 cm/s flume Oxygen respiration increased over time with the ambient trial exhibiting higher metabolic rates than the hypoxic trial. Hypoxia also impaired development; the eggs showed mutations and did not hatch after 14 days (typical ambient hatching time). It seems that while squid develop their own oxygen minimum zones inside the egg capsule, they are quite sensitive to decreased environmental oxygen.

Funded by Biological Discovery in Woods Hole REU Program, and WHOI Biology Department- Mooney Lab and the NSF Ocean Acidification Program.

Investigation of choanocyte-like structures on the ovary epithelium in the bat star *Patiria miniata*

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Choanocytes in porifera have a flagella surrounded by a collar of actin filopodia used for feeding. In echinoderms, Norrevang and Wingstrand (1970) observed a similiar structure on the surface of coelomic epithelia, except that the collar consisted of radial lamellae. Additional observations were made using ovary epithelia from the bat star *P. miniata*. By scanning electron microscopy, there is one flagellar structure per cell, usually with 13 lamellae surrounding the base. The tissue was stained with fluorescent phalloidin, and actin labeling was present within the entire radial lamellae. By brightfield light microscopy, the flagella were observed to be highly motile. Further experimentation may be done to elucidate the biological function of the flagella structure, and examine its presence and distribution on other organs such as testis.

Acknowledgements: Sid Tamm, Louie Kerr, Kasia Hammar, The Central Microscopy Facility

Measurement of extracellular changes in acidity mediated by radial glial cells (Möller cells) of the vertebrate retina.

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Voltage-gated calcium channels are essential regulators of synaptic transmission and are extremely sensitive to small changes in extracellular pH. In studies conducted in the vertebrate retina and the greater central nervous system, small alterations in extracellular pH have been shown to impact neural communication, presumably due in part to the sensitivity of these calcium channels to changes in pH. In the retina, the radial glial cells (Müller cells) play important roles in the regulation of ions and neurotransmitters in the extracellular milieu. Using highly sensitive, H^+-selective self-referencing microelectrodes, it has previously been shown that stimulation of tiger salamander (*Ambystoma tigrinum*) Müller cells by extracellular ATP, a molecule commonly released at synapses, causes a large extracellular acidification near the apical head of enzymatically isolated Müller cells. Using this technique, we observed that extracellular ATP also induces a significant extracellular acidification at the level of the basal end foot of the Muller cell. Furthermore, in the African clawed frog, *Xenopus laevis*, application of extracellular ATP also induced a pronounced acidification from enzymatically isolated Müller cells. We are currently working to generate a transgenic line of *X. laevis* which will express the pH-sensitive fluorophore calipHlourin in retinal photoreceptors. This sensor links pHlourin to a subunit of voltage-gated calcium channels and its expression is driven by a photoreceptor-specific promoter (TaCP). Using this sensor, we hope to examine extracellular acidifications mediated by glial cells within the outer plexiform layer of the retina, and to determine whether such alterations alter synaptic transmission.

This work was supported by NSF grants 0924372 & 1359230, NIH P40 OD010997, & support from the Marine Biological Laboratory. We thank Richard H. Kramer of the University of California at Berkeley for the generous gift of the calipHluorin construct originally expressed in zebrafish.

Mutant huntingtin-mediated inhibition of Fast Axonal Transport involves activation of Mixed Lineage Kinases.

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Huntington's Disease (HD) is a neurodegenerative disease caused by an autosomal dominant mutation in the huntingtin gene (Htt) resulting in an expansion of the polyglutamine (PolyQ) region in Exon1. Inhibition of fast axonal transport (FAT), a cellular process critical for the maintenance of axonal connectivity, has been indicated to be a major pathogenic event in the disease. Inhibition of transport occurs through a kinase cascade resulting in activation of c-Jun N-terminal kinase 3 (JNK3), a mitogen-activated protein kinase (MAPK). Once activated JNK3 phosphorylates the heavy-chain on the motor protein kinesin, causing it to dissociate from microtubules, inhibiting transport. JNK3 is activated by a mitogen-activated protein kinase kinase (MAP2Ks), which is in turn activated by a mitogen-activated protein kinase kinase kinase (MAP3Ks). Despite this knowledge, mechanisms linking mHtt to JNK3 activation remained unknown. Our lab has recently shown that mHtt activates upstream of JNK3 by selectively activating mixed ligase kinases (MLK), a subset of MAP3Ks. MLKs are regulated by intramolecular interactions between Src homology 3 (SH3) domain and an SH3-binding motif. Htt features an SH3 binding motif adjacent to the PolyQ region within a proline-rich domain. Here we show the SH3-binding domain in mHtt is necessary for inhibition of transport by mHtt using vesicle motility assays in isolated squid axoplasm. Findings from this work suggest that inhibition of MLKs may represent a novel therapeutic strategy to prevent loss of neuronal connectivity in HD.

Funding from NIH (STB), HHMI REU (Hunter College)

De novo detection and annotation of transposable elements in metazoan genomes

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Transposable elements (TEs) are mobile segments of DNA able to replicate itself within an organism's genome. Existing in virtually all metazoan genomes, TEs are often used as markers to track genetic diversity on a species-level scale based on their composition, frequency, and divergence in individual genomes. Their impact on genome function and evolution can vary from negative to neutral to positive, depending on a combination of different factors. In this study, a *de novo* approach was used to identify TEs in the rotifer *Adineta vaga*, and the arthropods *Cataglyphis hispanica*, *Darwinula stevensoni*, and *Megaphragma amalphitanum* genome sequences, using a comprehensive software package that integrates various TE detection methods. This *de novo* pipeline detects repeated DNA segments within a genome, builds consensus sequences on each family of repeats, and classifies these repeats based on previously-generated TE libraries. Using these consensus TE sequences, it is then possible to measure each TE's divergence from their putative ancestral sequences. Aggregating the results for all TEs within individual genomes, we are given a broader view on the composition and divergence of each genome measured by proliferation of individual TE families over time in the context of a given genome. In doing so, we are able to make phylogenetic comparisons between species and between populations within individual species, furthering insight on the genetic origins of biological diversity. Moreover, the annotation pipeline provides the necessary prerequisites to analyze the genetic and epigenetic environment of TE families, yielding clues to their biological impact.

Funding provided by the Rosenthal Brown-Marine Biological Laboratory Partnership LINK Award. We thank the Unité de Recherche Génomique at the Institut National de la Recherche Agronomique in France for providing the REPET software package used to detect TEs and annotate the genomes.

Spatial-Temporal Responses of Estuarine Phytoplankton to Changes in Nitrogen Loads from Watersheds

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Nitrogen is the well-known limiting nutrient for primary production by phytoplankton in coastal waters. It is important in determining phytoplankton chlorophyll concentrations in many coastal regions, including the Waquoit Bay estuarine system in Cape Cod, MA. Work during the 1990s on Waquoit Bay estuaries reported that estuaries whose watersheds discharged larger DIN loads supported larger mean annual concentrations of chlorophyll *a*. The effects of different N loads also affected seasonal patterns of chlorophyll concentrations, with peaks in late summer. Since the 1990s, urban development on watersheds has continued, with increased wastewater inputs to the estuaries. The goal of our project was to test whether the mean annual and seasonal chlorophyll concentrations during 2016 continued to reflect the 1990 effect of nitrogen loads discharged from watersheds into receiving estuaries. For this experiment, we sampled nutrient and chlorophyll concentrations in three estuaries of the Waquoit Bay estuarine system from May through August 2016. We sampled estuaries subjected to different nitrogen loads from their watersheds (483, 303, and 20 kg N ha^{-1} yr^{-1} for Childs River, Quashnet River, and Sage Lot Pond, respectively). NO_3 concentrations were determined using standard colorimetric assays in a Lachat Autoanalyzer. NH_4 concentrations were determined using a Cary UV-Visible Spectrophotometer. Chlorophyll *a* concentrations were determined by spectrophotometry using a filter fluorometer. The results showed that DIN (the sum of NH_4 and NO_3, with NO_3 being dominant) concentrations increased in estuaries subject to larger watershed-derived N loads. Chlorophyll *a* concentrations were low in spring and increased throughout the season. DIN concentrations were high in spring and diminished later in the season—presumably being taken up by producers. The results are consistent with the 1990 pattern of N loading and phytoplankton production. Greater N loads lead to greater phytoplankton production.

Funding by Brown University LINK Award Fellowship and the Woods Hole Sea Grant. A special thank you to Charles and Phyllis Rosenthal for financing the fellowship. I would like to thank Alison Maksym for her help with administrative and financing tasks. I would also like to thank Tynan Bowyer for his help in the field.

The effects of amyloid beta (Aβ) on mitochondrial function in hippocampal neurons

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Alzheimer's Disease (AD) is a neurological disorder characterized by cognitive and motor deterioration that affects memory, attention, speech, and behavior. One of the factors underlying the progression of AD is the accumulation of amyloid beta (Aβ) protein plaques that may block synaptic transmission. It has been previously suggested that mitochondrial dysfunction may play a role in the onset or progression of AD. We now demonstrate that Aβ contributes to cellular dysfunction by affecting both mitochondrial membrane potential and calcium retention capacity -- two measures of mitochondrial function and efficiency. After isolating and purifying mitochondria through density-gradient centrifugation, we found that Aβ-treated mitochondria exhibited a reduction in calcium uptake ability in comparison to control mitochondria. Using confocal microscopy of the mitochondrial membrane potential indicator, TMRE, we visualized and quantified a significant difference in mitochondrial membrane potential between control and Aβ-treated hippocampal neurons. In addition to drastically reducing the membrane potential, Aβ peptide also affected cell morphology – the neurons and mitochondria were irregular in shape, size, frequency, and distribution. We predict that these findings are the result of an Aβ-induced inner mitochondrial membrane leak, and hypothesize that Aβ's interaction with B-cell lymphoma extra-large (Bcl-xL), a specialized Bcl-2 family protein, may cause the opening of the mitochondrial permeability transition pore (mPTP), resulting in decreased mitochondrial competency. Bcl-xL's binding to the beta subunit of the F1F0-ATP synthase reduces the inner membrane ion leak, thereby increasing the flow of ions through the H+ translocator of ATP synthase to make ATP and enhancing the efficiency of energy metabolism in the mitochondria. Since Bcl-xL, among other regulatory proteins, is vital for preventing the neurotoxicity that leads to AD, our findings suggest that Aβ may disrupt regulation of the gating of the inner membrane leak channel within ATP synthase, leading to functional decline of the mitochondria.

Funding by the University of Chicago Jeff Metcalf SURF scholarship

Identifying autotrophic microbes at deep-sea hydrothermal vents using RNA-SIP and RT-qPCR

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Deep-sea hydrothermal vents are windows into the microbial community present within the rocky subseafloor of oceanic crust. Subseafloor microbial communities thrive by harnessing chemical energy from the environment and converting this energy into biomass. In this study, we used RNA stable isotope probing (RNA-SIP) to identify and compare the active autotrophic microbes at Axial Seamount, an active submarine volcano located off the coast of Oregon, USA. Fluid samples were collected in 2013 and 2015 from Marker 33, a low temperature diffuse fluid vent. Samples were incubated with both ^{12}C- and ^{13}C-labeled bicarbonate at 30°C, 55°C and 80°C for 36 hours in 2013 and at 55°C for 12 and 18 hours in 2015. RNA was then extracted and mixed into a cesium salt gradient that was centrifuged for 64 hours to allow for the separation of the labeled RNA and then fractionated by density. We applied a new reverse transcription quantitative PCR (RT-qPCR) assay to determine the abundance of bacterial and archaeal 16S rRNA in each density fraction for comparison to total RNA concentrations. RT-qPCR results showed there was a density separation between the ^{12}C and ^{13}C, confirming uptake of the labeled bicarbonate and the presence of an active autotrophic community at each temperature. Interestingly, in the 2015 experiment, there were two peaks present in the ^{13}C experiment, suggesting a separation of possibly slower and faster growing microbes able to take up the labeled carbon. To understand the taxonomic composition of bacteria and archaea across the density gradient and the two peaks, we performed 16S rRNA sequencing on the Illumina MiSeq platform. RT-qPCR and sequencing results will be presented in the context of identifying the active autotrophs living beneath the seafloor at Axial Seamount.

Funding by the NSF REU award#1359230

Exploring the light environment for larval culturing of MBL squid

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Squid eyes and human eyes evolved independently and have similar structures, but work under different light environments. Cephalopods, like squid and octopuses, are very intelligent organisms with alien-like body structures that may inspire future applications. Gene manipulation of embryos may be possible with the right genetic tools but squid culturing remains a barrier for reasons that remain unclear. One possibility is the light environment in the lab is different from the ocean. Squid culturing is being tested using buckets designed with different ranges of light intensities, polarizing film, and other materials to make an ocean-like habitat. Light dimmers and LED lights wrapped around a bucket create different kinds of light environments that can now be tested. In the future behavioral assays will be used to identify favorable light conditions based on hatchling swim patterns. Plankton will then be tested to identify hatchling's preferred diet. Ultimately, we hope to culture squid larvae in the future to apply new applications.

Funding:Bridgewater State University Acknowledgements: Karen Crawford and Antonio Giraldez.

Morphological characterization of chromatophore granules in the squid *Doryteuthis pealeii* and implications for biophotonics

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A defining morphological characteristic of coleoid cephalopods (octopuses, squids, and cuttlefishes) is skin embedded with an array of chromatophores—dynamic, colored neuromuscular organs. These organs confer the ability to camouflage from and signal to other animals. At the center of each chromatophore is an expandable pigment cell containing an elastic sacculus of colored granules. We present a method for the isolation and imaging of individual chromatophore pigment cells and granules. Each color class of chromatophore contains distinct assemblages of granules with characteristic size and shape: brown granules are large, smooth, and ellipsoidal; reds are mid-sized, convoluted, and ring-shaped; yellows are small and fine-grained. The distinct morphology of each granule color class may imply a structural coloration mechanism that contributes to the color of a pigment granule.

This work was funded by AFOSR grant A9550–09–0346 and NSF-REU grant DBI-1359230. We would like to thank Karen Crawford, Kyle Ford, Richard Gates, Kasia Hammar, Louis Kerr, James Kutlowski, Alan Kuzirian, and Lydia Mäthger.

Comparing Scanning Electron Microscopy and Micro-CT Imaging Techniques for the Mechanosensory Lateral Line

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The lateral line system in fish consists of mechanosensory neuromasts, which detect changes in pressure and vibrations through the water. In this study, scanning electron microscopy (SEM) and micro-CT scanning were conducted to demonstrate how different techniques can be used to visualize and quantify neuromasts along the anterior lateral line (ALL) of Bighead Carp (*Hypophthalmichthys nobilis*), Silver Carp (*H. Molitrix*) and Oyster Toadfish (*Opsanus tau)*. SEM was conducted on Bighead and Silver Carp to map the spatial distribution of neuromasts along the ALL and compare the abundance of superficial neuromasts between the two morphotypes. This technique proved useful in obtaining fine resolution images of hair cells within the neuromasts however was limited to surface analysis only. Micro-CT scanning is a nondestructive 3-D x-ray imaging technique that provides an alternative method to analyze small scale features with less preparation than SEM. Micro-CT scanning was compared using two different micro-CT scanners of varying resolutions which obtained images at pixel sizes of 35.3 and 12.9 μm. Scans were then used to create a 3-D model of the anterior portion of the Oyster Toadfish which included the lateral line. This approach allowed for detailed 3-D imaging, but was unable to visualize hair cells with the same detail as SEM. Each technique proved useful at mapping neuromasts, but with structural and detail limitations. The use of these two techniques together allows for hair cell identification and three dimensional reconstruction of neuromasts and the lateral line.

Funding by National Science Foundation Grants IOS-1354745 and DBI-1359230 (AFM) and the University of Wisconsin Superior McNair Scholars Program

Long term responses to high N loading in West Falmouth Harbor: the elusive role of eelgrass meadows in determining water quality.

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Since the Green Revolution of the 1970's, aquatic ecosystems have seen a marked increase in nitrogen nutrient loading due to runoff from over-fertilized agricultural lands and from sewage pollution from increasingly dense urban centers clustered on the coasts. High N nutrient loads have deleterious effects on aquatic systems; such as cultural eutrophication, hypoxia, and loss of eel grasses. West Falmouth Harbor, located in Massachusetts, has received high N loads over the past several decades due to contaminated groundwater flows from a sewage treatment plant. Although there have been upgrades to the sewage treatment plant, high N groundwater is reaching the harbor as groundwater travel times are longer than 10 years. Between 2005 and 2016 there have been substantial changes to the health and spread of the eelgrass meadows within the harbor—specifically, the loss of the eel grass meadows in the inner harbor along with a contraction and decline of eel grasses in the outer and middle harbor. This gives a unique opportunity to study how the loss of eelgrass in the inner harbor can affect the water quality of the outer harbor. Here, we compare sediment and water quality using biogeochemical and biometric assays in the waters of West Falmouth Harbor to data previously collected in 2008. We measured sediment respiration, gross primary productivity (GPP), nitrate efflux, and ammonium efflux from sediment cores in the inner harbor. Additionally, we examined eelgrass meadows using biomass assays to determine the size and health of eelgrass meadows in the middle and outer harbor. Further study to illuminate the role of eelgrasses determining water quality—are needed to see how the ecosystem of West Falmouth Harbor adapts to future levels of N loading in coming years.

Funding provided by the National Science Foundation and the MBL REU Program.

Using Lipid Biomarkers to Understand Deep Ocean Organic Particle Flux

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The oceanic water column hosts a sinking flux of organic matter that consists of the nutrients and detrital products of marine ecosystems. The molecular composition of this organic particle flux can be used to illuminate deep ocean ecological processes. This study examined lipid biomarkers in the deep ocean sinking particle flux and suspended particles collected at the Oceanic Flux Program (OFP) time-series site in the northern Sargasso Sea, an oligotrophic region typical of mid ocean gyres. Deep ocean sinking particles were collected by the OFP sediment traps at 500m, 1500m, and 3200m depths in April 2015 (spring bloom) and November 2015 (minimal production). The lipid biomarker composition of the sinking particle flux was compared to that of the suspended particle pool, which was collected by in-situ pumps at depths ranging from 80m (the phytoplankton production maximum) to 4400m (100 m above the ocean bottom).

This presentation will focus on three main classes of lipid biomarkers: sterols, fatty acids, and hopanoids. These biomarkers can be used to determine the sources of the organic matter in deep ocean particles as well as provide insights on the ecological processes that lead to remineralization and the release of bioavailable nutrients. The organic composition of sinking particles in the spring shows greater enrichment of biomarkers derived from fresh phytoplankton production than that in the fall, which shows greater enrichment in animal-derived materials. Hopanoids and other biomarkers indicative of bacterially-derived materials decreased from spring to fall and increased in relative abundance with depth. Biomarker composition in suspended particles reveals a conversion from fresh phytoplankton-derived organic detritus in the upper ocean to a more bacterially-enriched material in the deep ocean. The findings of this study show the power of lipid biomarkers in providing novel insights on organic particle flux and ocean biogeochemical cycling.

Funding Support: National Science Foundation Chemical Oceanography grant

Gene conversion as a possible mechanism behind the asexual thriving of bdelloid rotifers

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For many organisms, including humans, sexual reproduction has been seen as crucial for the survival of old species and propagation of new species through evolution. Meiosis allows for the reshuffling of allele copies and mutations so that nature can select for or against certain phenotypes. However, bdelloid rotifers have developed a mechanism for propagating beneficial mutations and masking deleterious ones, all while reproducing explicitly through parthenogenesis. It is also known that rotifers are capable of anhydrobiosis in desiccation periods and that they have a phenomenal ability to repair DNA double-strand breaks (DSBs). Therefore, it is believed that rotifers may have adapted an ability to repair DSBs accumulated during desiccation through a process called gene conversion. We were able to look for areas in the genome that may have undergone gene conversion by mapping the cDNA from desiccation lines and a hydrated (control) line back to a reference genome. By comparing levels of expression of gene regions in the desiccation lines to their hydrated counterparts, we have found which regions were likely overwritten by a gene conversion. Primers were then designed for the genes of interest and used in two further tests to detect gene conversions. The first test was to see if possibly desiccated regions would be replicated with primers designed for the coding sequence of the reference genome. The second test was to sequence the replicated regions and compare the SNPs between alleles to see where a gene was likely overwritten. Results from the PCR show amplification of almost all genes of interest, with no significant difference between allelic or ohnologous pairs, as expected. We are currently sequencing regions to check for loss of heterozygosity.

Funding by the NIH R21 AG046899 to D.M.W., and the E. E. Just Fellow and Schifman Scholarships.

The mechanism of pupillary movement in the little skate *Leucoraja erinacea*

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Pupils are optical apertures that control the amount of light reaching the retina by dilation (opening) and constriction (closing). Distinct pupil shapes are known in the eyes of vertebrates and invertebrates, the most common types being circular, slit shaped, and pinhole. The functions of these "conventional" pupils are well understood; yet there are some animals with elaborately-shaped pupils, such as the skate, whose functions are so far unstudied. The nocturnal, bottom-dwelling skate *Leucoraja erinacea* has an elaborate closed pupil in bright light that dilates to almost circular in response to low light. We examined (1) the rate of pupil shape change, and (2) the mechanism behind this transformation. In a behavioral experiment, 10 skates were exposed to two light intensities: low light (0.05 lx) and bright light (560 lx). The pupils took longer than 20 mins to dilate from elaborate to near-circular, and constrict from circular to elaborate. When a dark-adapted skate was exposed to bright light, there was an apparent "lifting" of the finger-like frills along the dorsal pupil rim. This behavior ceased when the pupil began to constrict. Using histological techniques, we investigated the anatomy of the skate pupil. We found two zones: the dorsal "folding zone" and a flatter "frill zone." In the folding zone, we found muscle fibers whose orientation suggests they provide the active mechanism for pupil constriction and dilation. In the frill zone, we found an organized epithelium, collagen fibers, and blood vessels. Our findings suggest that the slow rate of pupil dilation may correlate with the slow light accommodation of the retinal photoreceptors (rods only). These structures potentially suffice to maintain the elaborate shape when the frills are lifted and the pupil subsequently constricted (and dilated), as bulky musculature appears kept to a minimum toward the ventral end of the frills.

Funding by the National Science Foundation Research Experience for Undergraduates Program. We thank John E. Dowling, Alan M. Kuzirian, Danielle R. Gregoire, and Louie Kerr for their comments and assistance with this work.

Species composition of microbial structures in dental plaque

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It is increasingly becoming understood that the properties of microbes depend on their neighbors and their local environment. The recently described "hedgehog" structure found in dental plaque is a prime example of this. *Corynebacterium* provides a core structure with radiating filaments, which cocci surround in a "corncob" formation. The various taxa surround the filaments in a specific pattern due to their micron-scale environment, with *Streptococcus* cocci binding to the outermost edge of the hedgehog, while anaerobic taxa including *Fusobacterium* and *Leptotrichia* bind more proximally. [1] This microbial consortium is understood at the genus level, but the species identity of these microbes is pertinent to further research, as individual species within a taxon can have diverging morphologies and physiologies. The purpose of this project is to determine the species of *Streptococcus* and *Corynebacterium* involved in corncob structures of dental plaque. We are using fluorescence *in situ* hybridization to visualize and differentiate bacterial species. We have developed probes targeting individual species of *Corynebacterium* and *Streptococcus* and tested these probes on pure cultures to ensure specific hybridization of relevant species. We are applying these probes to dental plaque samples to determine the species involved in corncob structures. Preliminary results indicate that *Streptococcus mitis* is present in corncob structures. This results stands in contrast to literature reports of another species, *S. cristatus*, in these structures.

Funding by the Jeff Metcalf Program and the National Institute of Health-National Institute of Dental and Craniofacial Research

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Microfluidic Droplets as Substrates for Custom Photolithographic Patterning

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Microfluidic flow devices and positive photolithography are used to create micro-sized structures in polyethylene glycol (PEG) polymers, a biologically inert medium suitable for many scientific and medical uses. The shapes of microfluidic objects, notably droplets, are limited by the internal properties of fluids, whose cohesion encourages simple shapes with radial symmetry. Photolithographic techniques are prized for the intricate patterns they can imprint upon flat films of substrate. Little research has been done at the intersection of these two methods. Combined, they could be capable of fabricating three-dimensional microparticles with previously unattainable structure. To investigate whether advanced photolithographic technologies might be capable of patterning this level of detail in microfluidic objects, samples of polymerisable PEG droplets, treated with a photoinitator, were subjected to UV light through a custom photomask, designed with the digital Polygon 400 system. The designed particles were recovered and analysed upon isolation, and revealed faithful recreation of the design. The particles fabricated with the method described here may be applicable to the analysis of microtubule aster growth, whose study often benefits from the engineering of tortuous micro-sized environments.

Jeff Metcalf Fund

In vitro studies of *Penelope*-like retroelements in the genome of the bdelloid rotifer *Adineta Vaga*

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Penelope-like elements (PLEs) represent a class of unusual retroelements that, despite their widespread presence in eukaryotic genomes, propagate through a poorly understood transposition mechanism. PLEs encode a reverse transcriptase (RT) domain that is highly divergent from that of other retroelements, and have introns within inserted sequences – a counter-intuitive feature for RNA-propagated mobile elements. Further complicating the structure-function relations underlying PLE behavior, it has recently been reported that the pseudo-long terminal repeat (pLTR) regions flanking some PLE insertions contain previously undiscovered variants of Hammerhead ribozymes (HHRs) – a type of small self-cleaving RNA that could play a role in processing PLE transposons during replication. In the context of the "RNA world" view that postulates genetic and catalytic functions for RNA prior to the emergence of DNA- and protein-based life forms, these RT and HHR motifs may provide valuable insight into the history of transposons and the diversity of RNA-based mobile elements. The current work aims to improve biochemical understanding of PLE domains and of these elements' transposition mechanism, focusing in particular on an apparently active family of PLEs identified in the genome of a bdelloid rotifer, *Adineta vaga*. These PLEs are characterized by a continuous open reading frame (ORF) containing RT and endonuclease (EN) domains, flanked by two HHR motif-containing pLTR regions. *In silico* analysis suggests that part of the pLTR may interact with the EN domain. Expression of this recombinant ORF has been moderately successful and suggests that its EN domain is active within the expression host. Separately, the pLTR motif associated with this family has been incorporated into a vector for *in vitro* transcription. Our experiments are aimed at characterizing the self-cleaving activity of the HHRs in these pLTR regions and their interaction with RT, and examining the recognition and cleavage preferences of the EN domain on the pLTR substrate.

This work was supported by NSF MCB-1121334 to I.A. and by the Jeff Metcalf Summer Undergraduate Research Fellowship of the University of Chicago to V.S. We additionally wish to thank Fernando Rodriguez for providing invaluable advice in the laboratory.

Isolated squid axoplasm: An experimental system for the study of axon-autonomous events relevant to human neurodegenerative diseases.

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Membrane bound organelles are transported and distributed to their final destination within axons by fast axonal transport (FAT), a cellular process involving the activity of molecular motor proteins that is critical for appropriate maintenance of the axonal compartment. Genetic and experimental evidence indicates that deficits in FAT are adequate to trigger dying-back degeneration of neurons, which is characterized by deficits in synaptic function and axonal connectivity that long precede neuronal cell death. Interestingly, major human neurodegenerative disorders such as Huntington's disease (HD), Alzheimer's disease, and Parkinson's disease all feature dying back degeneration of neurons, therefore the development of methods for the study of axon-specific molecular events is imperative. However, experimental systems that facilitate an evaluation of molecular events within axons remain scarce. Using the isolated squid axoplasm preparation, I will describe biochemical and microscopy-based methods that allow for the evaluation of *axon-autonomous* effects of neuropathogenic proteins on FAT, and describe experiments revealing a novel pathogenic mechanism for HD.

Funding by CHDI Foundation.

The effects of different visual background cues on dynamic expression of cuttlefish 3D skin papillae for camouflage

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Cuttlefish are marine organisms capable of dynamically camouflaging to their surroundings by rapidly altering components of their skin including 3D texture. The dynamic texturing of the skin is accomplished by hundreds of projections known as papillae. Prior research has suggested that visual cues are the primary moderator of papillae expression in *Sepia officinalis*, yet the specific background cues that elicit either extension or retraction are unknown. Over the course of four days, three groups of five cuttlefish were placed in an arena that contained six textured rocks with moderate-to-high contrast, and six uniformly bright white smooth objects. For the analysis of 129 cuttlefish images, eight sets of papillae were evaluated for four grades of expression (0 for no expression, 3 for maximum expression). Cuttlefish next to textured rocks showed significantly greater papillae expression than those next to smooth white objects. These results indicate that background objects with small-scale 3-dimensionality of moderate-to-high contrast elements are a key stimulus for expression of skin papillae. Moreover, the smooth rocks elicited an active retraction of skin papillae. These data provide evidence for at least one specific background stimulus for papillae expression, but undoubtedly there are other stimuli in natural backgrounds that elicit papillae to enhance camouflage for cuttlefish.

We are grateful for funding from AFOSR grant #A9550-09-0346 and the NSF REU grant.

Tracking biofilm growth of early Plastisphere colonizers

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Plastic marine debris (PMD) presents a serious and worsening global pollution problem. PMD permeates even the most distant marine habitats, including deep-sea canyons and polar sea ice layers (Schlining, K, et al., 2013; Obbard, R.W., et al., 2014). Everywhere PMD travels, it comes into contact with microbial life. The surface of PMD provides an exploitable niche for a community dominated by microbes called "the Plastisphere." (Zettler, Mincer and Amaral-Zettler, 2013). While some impacts of PMD on marine animals as consequences of ingestion and entanglement are well-documented, a great number of PMD's ecological impacts remain quite unknown. Understanding the ecology and physiology of the Plastisphere will likely be crucial for our understanding of these so-far-unknown impacts. For example, the behavior of the Plastisphere community may facilitate the transport of invasive algae or bacteria and affect the bioavailability of persistent organic pollutants that adsorb to PMD's hydrophobic surface. Most of the Plastisphere work done so far has been on field samples, which could not be accurately aged. As a result, we are not sure at what rates Plastisphere biofilms form, or how Plastisphere communities change over time, particularly during early community assembly that occurs in the first hours and days of exposure. Our experiments employed lab-grown Plastisphere communities to study biofilm growth rates and interactions between selected microbes on different resins. We found that a non-axenic pennate diatom biofilm formed on four plastic resins at rates greater than one doubling per day, and we found significant differences in growth on different resins. We also found that certain species of Plastisphere bacteria appear to facilitate or inhibit each other's growth in a biofilm.

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Intravital imaging of hepatocyte response to tunicamycin induced ER-stress in zebrafish

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Fatty liver disease (FLD) is the most common liver disease in the Western world. The Unfolded Protein Response (UPR) is activated upon endoplasmic reticulum (ER) stress, and UPR activation has been shown to be alternatively protective or causative for FLD. We sought to determine whether structural changes in the ER was predictive of FLD outcome in zebrafish exposed to the disease causing stressor, tunicamycin. Utilizing confocal microscopy for intravital imaging of 5 days post fertilization (dpf) zebrafish larvae expressing a fluorescent transgene targeted to the ER, we analyzed ER structural changes in 166 fish over 12-48 hours of exposure to tunicamycin, and correlated this with lipid accumulation in hepatocytes. The ER in control hepatocytes is a classical reticular pattern but large, circular and highly motile structures were observed in hepatocytes of most fish after 18 hours of tunicamycin treatment and persisted through 48 hours of exposure. We sought to identify the nature of these structures and found that they labeled with Lysotracker, which stains acidic compartments. Lysotracker labeled small vesicular structures in hepatocytes from control larvae, but the Lysotracker positive structures increased in size and number after 12-16 hours of tunicamycin. This suggests that ER-stress in hepatocytes leads to increased autophagy and lysosome mediated degradation of ER stress, as a mechanism to clear unfolded proteins from the ER.

Funding by New York University Abu Dhabi (NYUAD).

Thank you to the Chu Lab at the Icahn School of Medicine at Mount Sinai, NY, NY, and the support of the imaging team at the Marine Biological Laboratory, Woods Hole, MA.