

# **2015 MBL Undergraduate Research Symposium**

**Wednesday-Thursday  
August 19-20, 2015  
Speck Auditorium  
1:00pm to 5:00pm**

## **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**

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**Program Organizers:**

**Allen Mensinger-University of Minnesota Duluth  
Beth Simmons - MBL**

**Special Thanks to**

**Emily Cardinal for program support**

**2015 MBL Undergraduate Research Symposium  
August 19-20, 2015, Speck Auditorium**

**Wednesday, August 19<sup>th</sup>, 2015**

**1:00 Opening Remarks – Steve Zottoli MBL Education Department**

**Session One – Chair: Susie Banks, MBL**

**1:10 Effects of ocean acidification and flow on oxygen and pH conditions of developing squid (*Doryteuthis pealeii*) egg cases. Apryle Panyi<sup>1</sup>, Matthew Long<sup>2</sup>, T. Aran Mooney<sup>3</sup>, Casey Zakroff<sup>3</sup>. <sup>1</sup>*Stockton University, Galloway, NJ*; <sup>2</sup>*Marine Chemistry and Geochemistry Department, Woods Hole Oceanographic Institution, Woods Hole, MA*; <sup>3</sup>*Biology Department, Woods Hole Oceanographic Institution, Woods Hole, MA.***

**1:25 The effects of temperature and environmental noise on oyster toadfish (*Opsanus tau*) mating calls. Jacey C. Van Wert<sup>1,2</sup> and Allen F. Mensinger<sup>2,3</sup>. <sup>1</sup>*Santa Barbara City College, Santa Barbara, CA*, <sup>2</sup>*Marine Biological Laboratory, Woods Hole, MA*, <sup>3</sup>*University of Minnesota Duluth, Duluth, MN.***

**1:40 Surviving in extreme environments: novel adaptations of *Legionella* to the Rio Tinto. Irene Zhang. *University of Chicago.***

**1:55 Global Distributions and Putative Hosts of *Legionella* Bacterial Species. Louise Barias<sup>1</sup>, Linda Amaral-Zettler<sup>2</sup>. *Dartmouth College*<sup>1</sup>, *Josephine Bay Paul Center- Marine Biological Laboratory*<sup>2</sup>.**

**2:10 Molecular Mechanisms of Parkinson's Disease: Investigating Potential Interactions between  $\alpha$ -synuclein and Phosphoinositides at Synapses. Yangtian Yi<sup>1</sup>, Lindsey Soll<sup>2</sup>, Audrey Medeiros<sup>2</sup>, Jennifer R. Morgan<sup>2</sup>. <sup>1</sup>*The University of Chicago, Chicago, IL*; <sup>2</sup>*The Eugene Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory, Woods Hole, MA.***

**2:25 Optimizing the performance of an algae-to-methane coupled bioreactor system through experimental and modeling approaches. Ruby An<sup>1,2</sup>, Joseph J. Vallino<sup>2</sup>, Zoe G. Cardon<sup>2</sup>. <sup>1</sup>*The University of Chicago, Chicago, IL*; and <sup>2</sup>*Marine Biological Laboratory, Woods Hole, MA.***

**2:40 The impact of nitrogen loading in Waquoit Bay, MA on estuarine food webs constructed by stable isotope analysis. Skelton, Sarah E<sup>1</sup>, Ivan Valiela<sup>2</sup>, Javier Lloret<sup>2</sup>, Caroline H Owens<sup>2</sup>, and Elizabeth J Elmstrom<sup>2</sup>. <sup>1</sup>*Department of Ecology and Evolutionary Biology, Brown University, Providence, RI;* <sup>2</sup>*Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA.***

**3:00 Refreshment Break in Speck Lobby**

**Session Two – Chair: Drew Latimer, MBL**

**3:15 Assessing Microbial Coordination over Time and Space in Siders Pond. Byl, Petra K.<sup>1,2</sup>, Leslie Murphy<sup>2</sup>, Julie Huber<sup>2</sup>, Joseph Vallino<sup>2</sup>. <sup>1</sup>*University of Chicago, Chicago, IL;* <sup>2</sup>*Marine Biological Laboratory, Woods Hole, MA.***

**3:30 Role of Copper in the Adaptive Plasticity of Hatching in *Danio rerio*. Melissa Li<sup>1</sup>, Rebecca T. Thomason<sup>2</sup>, Jonathan D. Gitlin<sup>2</sup>. <sup>1</sup>*The Division of Biological Sciences, University of Chicago, Chicago, Illinois;* <sup>2</sup> *Eugene Bell Center for Regenerative Biology & Tissue Engineering, Marine Biological Laboratory, Woods Hole, MA.***

**3:45 A Critical Role for Copper in the Regulation of Energy Homeostasis. Clara Kao<sup>1</sup>, Andrew Latimer<sup>2</sup>, Jonathan Gitlin<sup>2</sup>. <sup>1</sup>*The University of Chicago, Chicago, IL;* <sup>2</sup>*Marine Biological Laboratory, Woods Hole, MA.***

**4:00 Seasonality and inter-annual variation in pteropod flux in the Sargasso Sea. Emily Maness<sup>1,2</sup>, Dr. Maureen H. Conte<sup>2</sup>, JC Weber<sup>2</sup>, Dr. Amy Maas<sup>3</sup>. <sup>1</sup>*University of Tampa, Tampa, FL;* <sup>2</sup>*Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA, and* <sup>3</sup>*Bermuda Institute of Ocean Sciences, St. George's, Bermuda.***

**4:15 Phosphorus Phase Associations in the Northern Sargasso Sea. Leonard Shaw<sup>1</sup>, Maureen Conte<sup>2</sup>, J.C. Weber<sup>2</sup>, Alice Carter<sup>2</sup>. <sup>1</sup>*University of Chicago, Chicago IL;* <sup>2</sup>*Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA.***

**4:30 Effects of changing nitrogen loads on vegetation patterns in Waquoit Bay, 1990-2014. Caroline Owens<sup>1,2</sup>, Ivan Valiela<sup>1</sup>, Javier Lloret<sup>1</sup>, Elizabeth Elmstrom<sup>1</sup>, and Sarah Skelton<sup>1,3</sup>. <sup>1</sup>*Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA,* <sup>2</sup>*University of Chicago, Chicago, IL* <sup>3</sup>*Brown University, Providence, RI.***

**4:45 Hyperspectral imaging of dynamic camouflage in juvenile winter flounder reveals how color- and pattern-matching help avoid detection by shallow-water predators. Weller, Hannah I.<sup>1</sup>, Chuan-Chin Chiao<sup>2,3</sup>, Andrea Rummel<sup>1,2</sup>, and Roger T. Hanlon<sup>2,4</sup>. <sup>1</sup>*University of Chicago, Chicago, IL;* <sup>2</sup>*Marine Biological Laboratory, Woods Hole, MA;* <sup>3</sup>*Department of Life Science, National Tsing Hua University, Hsinchu, Taiwan;* and <sup>4</sup>*Department of Evolutionary Biology, Brown University, Providence, RI.***

**Thursday, August 20<sup>th</sup>, 2015**

**Session Three – Chair: KathiJo Jankowski, MBL**

**1:00 Alkalinity enrichment enhances growth of the larval blue mussel, *Mytilus edulis*. Morgan Bennett-Smith, Dave Bailey, Daniel McCorkle, and Scott Lindell.** *Scientific Aquaculture Program, Marine Biological Laboratory Woods Hole, MA.*

**1:15 Examining chlorophyll fluorescence and spectral signal to understand ecosystem functions and plant physiology in a temperate forest. Jonathan M. Michelsen<sup>1,2</sup>, Jim Tang<sup>2</sup>, and Mary Heskell<sup>2</sup>.** <sup>1</sup>*University of Chicago, Chicago, IL;* <sup>2</sup>*Marine Biological Laboratory, Woods Hole, MA.*

**1:30 Predicting the effects of salt marsh restoration on CO<sub>2</sub> sequestration and CH<sub>4</sub> emissions. Jasmine Prat<sup>1,2</sup>, Faming Wang<sup>2</sup>, Jianwu Tang<sup>2</sup>, Kate Morkeski<sup>2</sup>.** <sup>1</sup>*University of California Santa Barbara, Santa Barbara, CA;* <sup>2</sup>*The Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA.*

**1:45 Agricultural intensification in the Amazon: tracking fertilizer nitrogen from soy-maize double cropping to streams. Vanessa Cabrera<sup>1</sup>, KathiJo Jankowski<sup>2</sup>, Christopher Neill<sup>2</sup>, Marcia Macedo<sup>3</sup>, Linda Deegan<sup>2</sup>, Paulo Brando<sup>3</sup>, Sebastião Aviz do Nascimento<sup>4</sup>, Sandro Rocha<sup>4</sup>, Ebis Pinheiro do Nascimento<sup>4</sup>, Darlison Nunes da Costa<sup>4</sup> and Michael Coe<sup>3</sup>.** <sup>1</sup>*University of California, Santa Cruz, Santa Cruz, CA;* <sup>2</sup>*The Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA;* <sup>3</sup>*Woods Hole Research Center, Falmouth, MA;* <sup>4</sup>*Instituto de Pesquisa Ambiental da Amazônia, Canarana, Brazil.*

**2:00 An analysis of shrub encroachment into grasslands using historical aerial imagery of Naushon Island. Champlin, Lena and Christopher Neill.** *Marine Biological Laboratory, Woods Hole, MA.*

**2:15 TBD. Luke Hästen O'Brien.**

**2:30 Impact of soil nitrogen levels on species richness in Northeastern grasslands and the role of disturbance in mediating the nitrogen-diversity relationship. Eva Kinnebrew<sup>1</sup>, Christopher Neill<sup>2</sup>.** <sup>1</sup>*University of Chicago, Chicago, IL;* <sup>2</sup>*Marine Biological Laboratory, Woods Hole, MA.*

## **2:45 Refreshment Break in Speck Lobby**

### **Session 4 - Chair Allen Mensinger, UMD**

#### **3:00 Making Knock-out and Knock-in Alleles of the Pancreatic Genes**

**ABCC8 and Insulin-A in *Xenopus* using CRISPR/Cas9. Irene Coffie<sup>1,2</sup>, William Ratzan<sup>1</sup> and Marko Horb<sup>1</sup>. <sup>1</sup> Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory, Woods Hole, MA. <sup>2</sup> Dartmouth College, Hanover, NH.**

#### **3:15 A neurotransmitter map of *Doryteuthis pealeii* and *Sepia officinalis*.**

**Lauren Chacho<sup>1</sup>, Kyle Fisk<sup>2</sup>, Robert Gould<sup>3</sup>, Alan Kuzirian<sup>4</sup>. <sup>1</sup>Eckerd College, <sup>2,3,4</sup>Marine Biological Laboratory Woods Hole.**

#### **3:30 Response of elaborate pupils in *Leucoraja erinacea* to changes in light**

**intensity. Corey Okinaka<sup>1</sup>, Lydia Mathger<sup>2</sup>, and Alan Kuzirian<sup>2</sup>. <sup>1</sup>University of Chicago, Chicago, IL; <sup>2</sup>Marine Biological Laboratory, Woods Hole, MA.**

#### **3:45 Genomic analysis of opsins in the skate (*Leucoraja erinacea*). Vesel,**

**Claudia<sup>1</sup>, Robert Paul Malchow<sup>1</sup>, and David Mark Welch<sup>2</sup>. <sup>1</sup>University of Illinois at Chicago, Chicago IL; <sup>2</sup>Marine Biological Laboratory, Woods Hole MA.**

#### **4:00 Tau-related Deficits in Fast Axonal Transport in Alzheimer's Disease.**

**Alison Klein<sup>1,4</sup>, Benjamin Combs<sup>2,4</sup>, Gerardo Morfini<sup>3,4</sup>, Scott Brady<sup>3,4</sup>. <sup>1</sup>CUNY Hunter College, New York, NY; <sup>2</sup>Michigan State University, Grand Rapids, MI; <sup>3</sup>University of Illinois, Chicago, Chicago, IL; and <sup>4</sup>Marine Biological Laboratory, Woods Hole, MA.**

#### **4:15 Activation of p38 MAP Kinase and Inhibition of Fast Axonal Transport in Familial ALS. Zachary Gershon<sup>1,3</sup>, Gerardo Morfini<sup>2,3</sup>, and Scott T. Brady<sup>2,3</sup>.**

<sup>1</sup>Department of Biological Sciences, CUNY Hunter College, New York, NY. <sup>2</sup>Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, IL. <sup>3</sup>Marine Biological Laboratory, Woods Hole, MA.

#### **4:30 Inhibition of fast axonal transport: Mutant huntingtin as a cause of**

**dying-back neuropathy. Henry Thomsett<sup>1,3</sup>, Minsu Kang<sup>2,3</sup>, Scott T. Brady<sup>2,3</sup>, Gerardo Morfini<sup>2,3</sup>. <sup>1</sup>Department of Chemistry, Skidmore College, Saratoga Springs, NY, USA. <sup>2</sup>Department of Anatomy and Cell Biology, University of Chicago at Illinois, Chicago, IL, USA. <sup>3</sup>Marine Biological Laboratory, Woods Hole, MA, USA.**

#### **4:45 MLK activation by mutant Huntingtin leads to inhibition of fast axonal**

**transport in Huntington's disease. Abdelmesih, Brenda<sup>1,3</sup>, Morfini, Gerardo<sup>2,3</sup>, and Scott Brady<sup>2,3</sup>. <sup>1</sup>Hunter College, New York, NY; <sup>2</sup>University of Illinois at Chicago, Chicago, IL; and <sup>3</sup>Marine Biological Laboratory, Woods Hole, MA.**

#### **5:00 Closing Remarks –**

**BBQ for presenters and mentors immediately following in MBL Quad**

## **MLK activation by mutant Huntingtin leads to inhibition of fast axonal transport in Huntington's disease**

Abdelmesih, Brenda<sup>1,3</sup>, Morfini, Gerardo<sup>2,3</sup>, and Scott Brady<sup>2,3</sup>.

<sup>1</sup>Hunter College, New York, NY; <sup>2</sup>University of Illinois at Chicago, Chicago, IL; and <sup>3</sup>Marine Biological Laboratory, Woods Hole, MA

Huntington's disease (HD) is an inherited neurodegenerative disease that is caused by expansion of a polyglutamine (polyQ) region located near the N-terminus of the Huntingtin (Htt) protein. However, the mechanism by which polyQ expansion leads to the pathogenesis of Huntington's disease is not clearly understood. Mutant Huntingtin (mHtt) with the polyQ expansion has previously been shown to disrupt fast axonal transport (FAT) in various models of HD. Compromised FAT results in synaptic dysfunction, ultimately causing a dying-back neuropathy where the distal portion of the axon degenerates toward the cell body. Previously, we showed that mHtt inhibits FAT by activation of c-Jun N-terminal kinase 3 (JNK3). JNK3 phosphorylates kinesin, a microtubule-based molecular motor protein, resulting in the detachment of kinesin from microtubules. JNK3 is a member of mitogen-activated protein kinases (MAPKs), which are activated by upstream kinases mitogen-activated protein kinase kinases (MAP2Ks), which are activated by mitogen-activated protein kinase kinase kinases (MAP3Ks). Mixed-lineage kinases (MLKs), a subset of MAP3Ks, are regulated by intramolecular interaction between a Src homology 3 (SH3) domain and an SH3-binding motif. Interestingly, Htt features SH3-binding motifs within the proline-rich domain adjacent to the polyQ region, leading us to hypothesize that Htt may directly bind and activate MLKs. Here, we demonstrate that the SH3-binding motifs in Htt are necessary for the FAT inhibition by mHtt using vesicle motility assays in isolated squid axoplasm.

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

## Optimizing the performance of an algae-to-methane coupled bioreactor system through experimental and modeling approaches

Ruby An<sup>1,2</sup>, Joseph J. Vallino<sup>2</sup>, Zoe G. Cardon<sup>2</sup>

<sup>1</sup>The University of Chicago, Chicago, IL; and <sup>2</sup>Marine Biological Laboratory, Woods Hole, MA

The bioproduction of methane is a potential alternative to the environmentally harmful processes associated with obtaining and burning fossil fuels. Algal bioreactors are a promising approach to methane production as they can support high primary productivity while requiring minimal land usage. We set up an exploratory algae-to-methane (A2M) bioreactor system that converts light and CO<sub>2</sub> to methane through a coupled aerobic algal reactor and anaerobic microbial digester. The algal reactor contained a mutant of *Chlamydomonas reinhardtii* (*CHL 75*), which lacks a cell wall to facilitate anaerobic fermentation. The microbial digester was inoculated with anaerobic groundwater obtained from sampling wells near Little Pond. Light levels, gas flow rates, CO<sub>2</sub> input, and buffering capacity were adjusted experimentally to optimize algal growth. Chlorophyll fluorescence measurements were taken daily to approximate algal concentration. Despite the lack of a cell wall, we found the *CHL 75* mutant to be highly resilient to environmental perturbation. Gas data (O<sub>2</sub>, CO<sub>2</sub> and CH<sub>4</sub>), pH, and dissolved oxygen (DO) from both reactors were monitored continuously and used to develop mathematical models of the A2M system. These models were designed using both classical approaches and Maximum Entropy Production (MEP) concepts. Both experimental manipulations and model results indicated that CO<sub>2</sub> availability was the major limiting factor of algal growth. Model results from the algal reactor submodel will be presented along with experimental data of the coupled system to date.

Funding by Anonymous Donor

## Global Distributions and Putative Hosts of *Legionella* Bacterial Species

Louise Barias<sup>1</sup>, Linda Amaral-Zettler<sup>2</sup>  
Dartmouth College<sup>1</sup>, Josephine Bay Paul Center- Marine Biological Laboratory<sup>2</sup>

*Legionella* is a pathogenic bacterium that causes Legionnaire's disease in humans. Genes associated with this genus were detected in large relative abundances in the Rio Tinto, a very acidic and heavy metal-rich river in southwestern Spain. *Legionella* is not known to be acidophilic or even tolerant to acidic environments. This prompts the question: How is *Legionella* surviving in the Rio Tinto? Our hypothesis is that *Legionella* must be protected from the acidic environment of the Rio Tinto by living inside a protistan host or with acidophilic bacteria in a protected biofilm. We tested this hypothesis by constructing a network analysis on sequence data from Rio Tinto samples and looking for highly correlated occurrences of *Legionella* and putative hosts. In our network analysis we found that *Legionella* isolated from the Rio Tinto samples did not correlate strongly with amoebae, but rather with *Euglena mutabilis*, a ciliate and a mixotrophic flagellate. We are further exploring the possibility that *Legionella* may have multiple hosts in the Rio Tinto, and using genetic information from *Legionella* to find clues about the host interactions. In order to characterize global trends of *Legionella* outbreaks (including Spain) and the sources ascribed to these outbreaks, we used information stored in the GIDEON (Global Infectious Disease and Epidemiology Network) database to map the distribution of Legionnaire's disease outbreaks recorded for the past thirty years. We found trends that suggested most outbreaks were sourced in infected hospitals and cooling towers. Spain, along with the United States and United Kingdom, has had the most recorded outbreaks globally in the past thirty years; Spain has had 32 recorded outbreaks, the United Kingdom 23 outbreaks, and the United States 65 outbreaks. The deadliest outbreak of Legionnaire's disease in the United States is the current outbreak in South Bronx, New York.

Funding provided by: The Dartmouth Shifman-Segal Program and the National Science Foundation Research Experience for Undergraduates at the Marine Biological Laboratory in Woods Hole, MA

## **Alkalinity enrichment enhances growth of the larval blue mussel, *Mytilus edulis***

Morgan Bennett-Smith, Dave Bailey, Daniel McCorkle, and Scott Lindell  
Scientific Aquaculture Program, Marine Biological Laboratory  
Woods Hole, MA

The seawater saturation state with respect to calcium carbonate mineral phases ( $\Omega$  aragonite or  $\Omega$  calcite) is an important factor in the shell growth of marine bivalves, including the Pacific oyster, *Crassostrea gigas*, and the Mediterranean mussel, *Mytilus galloprovincialis*. Seawater that is less strongly supersaturated with respect to aragonite leads to slower development during key early-life stages; related chemical changes, such as elevated carbon dioxide partial pressure and lower pH, seem less directly tied to larval growth. While many studies have examined the negative effects of decreased saturation state on calcifying organisms, there has been little work on the effects of increasing alkalinity (and thus saturation state), as could be done in an aquaculture setting. To determine whether or not chemically elevating seawater alkalinity above natural levels could be advantageous to blue mussel (*Mytilus edulis*) aquaculture, larval rearing experiments were conducted starting with newly fertilized embryos, grown for 8 days under 3 increased aragonite saturation states (1.5, 2.5 and 3.5 times that of the control); the alkalinity enrichments were achieved using sodium bicarbonate additions. Shell length was greater with each enriched aragonite saturation state, and was greatest at the highest saturation state ( $\Omega$  7, 3.5 times control). We are currently examining larvae from the different treatment groups with a scanning electron microscope, in order to analyze saturation state effects on shell integrity and structural mutations at early development stages. Our findings so far suggest that sodium bicarbonate enrichment could be a good option in East Coast shellfish hatcheries, particularly in the face of rising ocean acidity.

Funding provided by NSF grant # DBI-1359230

## Assessing Microbial Coordination over Time and Space in Siders Pond

Byl, Petra K.<sup>1,2</sup>, Leslie Murphy<sup>2</sup>, Julie Huber<sup>2</sup>, Joseph Vallino<sup>2</sup>

<sup>1</sup>University of Chicago, Chicago, IL; <sup>2</sup>Marine Biological Laboratory, Woods Hole, MA

Life on Earth is limited by the availability of Carbon, Hydrogen, Oxygen, Nitrogen, Phosphorus, and Sulfur, as well as various trace metals, and most organisms can only utilize discrete forms of these elements. Microbial communities consisting of diverse bacteria and archaea cycle life-limiting elements through Earth's biosphere via their metabolic pathways. Over space and time, these pathways contribute to complex metabolic networks, which mediate biogeochemical cycles and influence the habitability of an ecosystem. In this study, we are examining the organization and coordination of microbial metabolic networks by applying geochemical, molecular, microbial, and modeling approaches to a local Cape Cod ecosystem, Siders Pond in Falmouth, MA. Siders Pond is permanently stratified, meromictic kettle pond, and due to the strong chemical and physical gradients of the water column, we hypothesize that a large diversity of metabolic pathways will be represented. We sampled 7 time points over a 24hr period at 8 chemically and physically distinct depths (0.5m, 2m, 3m, 4m, 6m, 8m, 10m, 12m). The water column was characterized using a Hydrolab Sonde and samples were collected for nutrient, trace metal, and biomass quantification, as well as for metagenomic and metatranscriptomic sequencing. To date, we have quantified biomass, nucleic acids, sulfide, and DIC throughout the water column. Results suggest that dissolved oxygen remains stratified over the diel cycle, although biomass fluctuates. Soon, metagenomic data will be available to define the potential metabolic pathways available, and the metatranscriptome will demonstrate the realized metabolic pathways over time and space. We will then compare the metabolic network that the environmental data suggests to the metabolic network that the thermodynamic model predicts. The ability to predict how microbial metabolic networks organize and function will facilitate our understanding of how microbial metabolic networks will adapt to anthropogenic pressures and therefore alter biogeochemical cycles.

Funding by NSF grant GEO-1451356

## **Agricultural intensification in the Amazon: tracking fertilizer nitrogen from soy-maize double cropping to streams**

Vanessa Cabrera<sup>1</sup>, KathiJo Jankowski<sup>2</sup>, Christopher Neill<sup>2</sup>, Marcia Macedo<sup>3</sup>, Linda Deegan<sup>2</sup>, Paulo Brando<sup>3</sup>, Sebastião Aviz do Nascimento<sup>4</sup>, Sandro Rocha<sup>4</sup>, Ebis Pinheiro do Nascimento<sup>4</sup>, Darlisson Nunes da Costa<sup>4</sup> and Michael Coe<sup>3</sup>

<sup>1</sup>University of California, Santa Cruz, Santa Cruz, CA; <sup>2</sup>The Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA; <sup>3</sup>Woods Hole Research Center, Falmouth, MA;

<sup>4</sup>Instituto de Pesquisa Ambiental da Amazônia, Canarana, Brazil

Globalization and the increasing demand for food create pressure to both expand and intensify agriculture. These changes have potentially large consequences for the transfer of nutrients across the landscape and the functioning of streams. In the Brazilian Amazon, crop agriculture expanded greatly during the last 20 years. More recently, farmers have intensified production on existing cropland by double cropping soybeans followed by maize (corn) during the same growing season. Maize, a novel crop for the region, requires applications of nitrogen (N) fertilizer while soybeans do not. To determine whether this novel land use and associated N addition influenced N concentrations in groundwater below agricultural fields and in adjacent streams, we measured N concentrations. Samples from groundwater wells and streams were taken from small headwater watersheds across three land uses (soy-maize, soy, and tropical forest) in the Upper Xingu Basin, a region of rapid cropland intensification in the southern Amazon. Each watershed contained six groundwater wells arranged in a transect from the stream, through riparian forest, to the cropland field edge on either side of the stream. Total inorganic N concentrations were higher in wells where double cropping with N fertilization occurred. The nutrient concentration in streams, however, did not differ overall among land uses suggesting the riparian zone is critical in removal of N. As the intensification of agriculture continues the ability of the riparian zone to prevent N from traveling to streams is unknown. Their protection is critical to the functioning of tropical watersheds.

Funding by NSF

## **A neurotransmitter map of *Doryteuthis pealeii* and *Sepia officinalis***

Lauren Chacho<sup>1</sup>, Kyle Fisk<sup>2</sup>, Robert Gould<sup>3</sup>, Alan Kuzirian<sup>4</sup>  
<sup>1</sup>Eckerd College, <sup>2,3,4</sup>Marine Biological Laboratory Woods Hole

Cephalopods are organisms that possess unique anatomical features, specifically their rapid skin color change regulated by radial muscles and their jet propulsion controlled by the stellate ganglia. However, little is known regarding the distribution and organization of neurotransmitters affecting these structures. Skin and stellate ganglion tissues were dissected and preserved from juvenile squid, *Doryteuthis pealei* and adult cuttlefish *Sepia officinalis*. Immunohistochemical labeling of sections was utilized to analyze what neurotransmitters were associated with the chromatophore nerves and neurons of the stellate ganglia. Neurotransmitters investigated included serotonin (5-HT), FMRFamide (FMRF), gamma-amino butyric acid (GABA), and L-glutamate. Multi-labeled immuno-reacted samples were examined using laser scanning confocal microscopy. Of the various transmitters investigated, the most successful antibody labeling occurred in the stellate ganglia with 5-HT and FMRF. In *D. pealei* and *S. officinalis*, FMRF was concentrated centrally in the stellate ganglia. In specific nerves coursing the length of the radial chromatophore muscles, 5-HT and FMRF were predominantly present. 5-HT and L-glutamate staining occurred prominently in the cell bodies of the stellate ganglia. GABA staining was seen in small numbers of cells located on the periphery of the stellate ganglia. These findings have resulted in a neurotransmitter map of the stellate ganglia of both *D. pealei* and *S. officinalis*, that has previously been unavailable, and a newer understanding of the organization and functioning of the cephalopod nervous system. This research is still continuing in order to understand the function of the neurotransmitters in the stellate ganglion and how this affects cephalopod behavior.

This research was supported by NSF grant # DBI-1359230 “REU Site: Biological Discovery in Woods Hole”

## **An analysis of shrub encroachment into grasslands using historical aerial imagery of Naushon Island**

Champlin, Lena and Christopher Neill  
Marine Biological Laboratory, Woods Hole, MA

The overgrowth of biodiverse grasslands by shrublands on Naushon Island is representative of the recent decline of grasslands throughout the Northeast US. Reintroduction of grazing livestock may be one method to maintain grassland and reduce shrub invasion. I used historic aerial images to calculate changes in shrub spreading rate during periods before and after introduction of cattle to a 59-ha pasture on Naushon Island. Three aerial images from 1994, 2005, and 2014 were manually “heads-up” digitized in ArcGIS with polygons for shrubs, grass, and trees. This time range included 10 years before cattle were introduced and 10 years after introduction. Data from vegetation monitoring points were used for ground-truthing. Results indicate that shrub cover was the greatest in 2014 and shrub patches were the most connected. Based on total area, the rate of shrub expansion increased slightly from 2005 to 2014 when cows were present suggesting that this intensity of grazing pressure on this time scale did not decrease shrub spread. Other landscape metrics indicated that the number of shrub patches, particularly many small patches, was greatest in 2005 but that patch density was reduced in 2014, potentially because grazing reduces the number of new shrub patches established. Next steps will include digitizing older images to relate shrub expansion to the removal of sheep that were present in high density in the early and mid 1900s.

## **Making Knock-out and Knock-in Alleles of the Pancreatic Genes ABCC8 and Insulin-A in *Xenopus* using CRISPR/Cas9**

Irene Cofie <sup>1,2</sup>, William Ratzan <sup>1</sup> and Marko Horb <sup>1</sup>

<sup>1</sup> Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory, Woods Hole, MA. <sup>2</sup> Dartmouth College, Hanover, NH

The emerging CRISPR/Cas9 technology has been shown to efficiently induce targeted modifications in the genomes of a variety of eukaryotic model organisms. Cas9 is an RNA-guided endonuclease that creates targeted double-stranded breaks (DSBs) in chromosomal DNA, which often results in 'knock-out' alleles due to the error-prone, predominant mechanisms of DNA repair. Additionally, it is possible to create 'knock-in' alleles by providing a DNA template for the cell to repair the induced DSB through homologous recombination, leading to precise genome modifications. Our lab is employing CRISPR/Cas9 to investigate the role of two pancreatic genes, ABCC8 and Insulin-A, using the model organisms *Xenopus tropicalis* and *Xenopus laevis*. ABCC8 encodes a subunit of a potassium ATP channel known as SUR1. Patients with mutations in ABCC8 are at increased risk for diabetic illnesses, such as congenital hyperinsulinism. Our first aim is to generate knock-out mutations in the ABCC8 gene of *Xenopus tropicalis* and analyze the resulting frogs for phenotypic beta-cell dysfunction. Our second aim is to generate a *Xenopus laevis* line harboring a fluorescent protein knock-in allele, IRES-GFP, of the Insulin-A gene. Results from the Insulin-A GFP transgenic frogs and ABCC8 knock-out mutants will be useful for analyzing the function of the genes, for possible applications in understanding human diabetic diseases.

This research was supported by NSF grant DBI-1359230 "REU Site: Biological Discovery in Woods Hole"

## Activation of p38 MAP Kinase and Inhibition of Fast Axonal Transport in Familial ALS

Zachary Gershon<sup>1,3</sup>, Gerardo Morfini<sup>2,3</sup>, and Scott T. Brady<sup>2,3</sup>

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Amyotrophic lateral sclerosis (ALS), a fatal adult-onset neurodegenerative disease (AOND), affects the function and survival of both upper and lower motor neurons. Although no single cause has been elucidated for all ALS cases, several genetic mutations have been identified that produce familial forms of ALS (FALS), which account for approximately 10% of all ALS cases. Similar clinical presentations of sporadic ALS (SALS) and FALS suggest that they may share pathogenic mechanisms. Mutations in the enzyme superoxide dismutase 1 (SOD1) are one of the most common and well-defined causes of FALS. It was previously demonstrated that multiple mutations in SOD1 (G93A, G85R, etc.) and an aberrantly oxidized wild type SOD1 protein share conformational epitopes and inhibit fast axonal transport (FAT), a hallmark of many AONDS including ALS. The effect is mediated through phosphorylation of the kinesin motor protein by the p38 mitogen-activated protein kinase (MAPK) pathway. Disruption of FAT will lead to deficits in synaptic transmission, and eventually a dying-back pattern of degeneration in which the distal end of the axon deteriorates towards the cell body, ultimately culminating in cell death. Another common mutation linked to FALS is a hexanucleotide expansion in the non-coding region of the C9ORF72 gene. This region undergoes repeat associated non-ATG (RAN) translation in both directions and produces five variant gene products consisting of glycine-proline (GP), glycine-alanine (GA), glycine-arginine (GR), proline-alanine (PA), or proline-arginine (PR) repeats. Previously, only GP, GA, and GR were tested for their effect on FAT and only GP was found to be toxic. Here we co-perfused the isolated axoplasm from the squid giant axon with a selective inhibitor for p38 $\alpha$  and G85R SOD1 as well as PA and PR independently to examine their respective effects on FAT.

Funded by an HHMI Undergraduate Science Education grant to Hunter College

## A Critical Role for Copper in the Regulation of Energy Homeostasis

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Confronted with limited nutrient availability, living organisms demonstrate evolutionarily conserved responses, slowing or arresting metabolic processes to permit adaptation and survival. One such essential nutrient is the metal ion copper, which functions as a cofactor for enzymes involved in many vital cellular processes including energy production, oxygen transport, metabolism, and signal transduction. Tight homeostatic regulation of copper acquisition, distribution, and use is necessary to ensure normal function. Copper enters eukaryotic cells at the plasma membrane via the high-affinity copper transporter Ctr1. In a mouse Ctr1 knockout model, embryo development fails at early stages *in utero*. To gain a better understanding of how Ctr1 functions in a vertebrate animal we made a zebrafish Ctr1 knockout. *ctr1* mutant embryos lack melanin, a phenotype observed in copper deficient embryos. However, the most striking phenotype we observe is that development is markedly delayed in *ctr1* mutant larvae compared to siblings. As copper is required for the assembly of cytochrome c oxidase, the regulatory key for energy availability, we hypothesized that this developmental delay is due to decreased mitochondrial energy production. In support of this we observe a decreased amount of the cytochrome c oxidase subunit Cox1 in *ctr1* mutants, leading us to analyze ATP production and markers of mitochondrial abundance and health. Understanding how zebrafish adapt to perturbations in copper regulation may provide new insights into the role of these pathways in vertebrate development and energy homeostasis.

Funding: NIH DK R37 44464 (JDG), Jeff Metcalf Internship Program

## **Impact of soil nitrogen levels on species richness in Northeastern grasslands and the role of disturbance in mediating the nitrogen-diversity relationship**

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In the past century, soil nitrogen levels in Northeastern grasslands have risen significantly above pre-industrial levels due to N deposition from intensive agriculture and fossil fuel combustion. This is problematic because nitrogen can accumulate in the soil and impact plant communities by favoring faster-growing species, which outcompete native grasses and lower species diversity. In this study, we examined the effects of increased nitrogen on Naushon Island, the largest of the Elizabeth Islands off Cape Cod. In the past fifty years, woody shrubs such as catbrier, huckleberry, and bayberry have been threatening and overtaking the coastal grasslands of Naushon Island. To better understand the relationship between shrub spreading and soil nitrogen levels, we collected soil samples from 208 sites on Naushon Island and analyzed them for both ammonium and nitrate. We found that shrub plots, especially those containing catbrier, contained soil with significantly higher nitrogen levels. We also found that total species richness correlates with levels of nitrate in the soils, which supports our original hypothesis. Our study took place in the context of a larger project at the Marine Biological Laboratory that aims to assess the efficacy of cattle grazing and mowing as management techniques. We therefore used the experimental treatments of this project to test whether cattle grazing and mowing impact the relationship between nitrogen and species richness. We found that enclosure plots, where cattle were contained for one week every summer, had drastically lower nitrogen levels compared to surrounding plots. With these findings we hope to help create better coastal grassland management plans.

John Metcalf Program and the John and Judith McCarter Family

## **Tau-related Deficits in Fast Axonal Transport in Alzheimer's Disease**

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Alzheimer's disease is an adult onset neurodegenerative disease (AOND) affecting 1 in 9 Americans above the age of 65. One hallmark of this disease shared with some other AONDS are aggregates of hyperphosphorylated tau, which led to classification of these diseases as tauopathies. Pathogenic forms of tau have been shown to inhibit anterograde fast axonal transport (FAT) by activating axonal protein phosphatase 1 (PP1) and glycogen synthase kinase 3 (GSK3). When FAT is disturbed, synaptic function is also compromised. Recently, it was shown amino acids 2-18 of tau, which comprise the phosphatase-activating domain (PAD), are necessary and sufficient for the activation of PP1. In normal nontoxic forms of tau, the N-terminal PAD is sequestered through its interaction with the C-terminus in what is known as the paperclip conformation. Hyperphosphorylation and aggregation are two likely mechanisms for disrupting the normal conformation of tau, exposing PAD, and disrupting FAT in disease. There are six naturally occurring tau isoforms, that can be classified into two subtypes, those with four microtubule-binding repeat regions (MTBRs), or 4R isoforms, and those with three MTBRs (3R). In a normal brain, the ratio of 3R to 4R isoforms is 1:1. However, many tauopathies are characterized by altered isoform splicing or preferential aggregation of 3R or 4R isoforms which may alter PAD exposure. In order to further investigate the effects tau isoforms have on FAT, we conducted motility assays in axoplasm from squid giant axons. Our data suggests that aggregated forms of both 4R and 3R tau cause reductions in overall anterograde FAT. Additionally, we found that insoluble protein from an AD brain inhibits both anterograde and retrograde FAT compared to an age-matched control brain. These results provide further evidence that inhibition of FAT by pathogenic forms of tau is a toxic mechanism associated with AD and other tauopathies.

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## **Role of Copper in the Adaptive Plasticity of Hatching in *Danio rerio***

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Phenotypic plasticity is an important contributor to fitness in a variable environment. The timing of hatching serves as a critical factor for survival, and the adaptive developmental plasticity of this behavior is well defined in many species. Work in our laboratory has revealed the essential role of copper and the cuproenzyme peptidyl-glycine alpha-amidating monooxygenase in the hatching of teleost embryos. The availability of copper is rate-limiting to the activity of this oxygenase that is specifically expressed in the floor plate of the developing neural tube and functions to activate secreted neuropeptides. To determine the mechanism of this process, we examined the activity of cathepsin L, a serine protease synthesized and secreted from the hatching gland, in embryos treated with a specific copper chelator or antisense oligonucleotides known to abrogate the activity of peptidyl-glycine alpha-amidating monooxygenase. Cathepsin L functions in an essential enzymatic cleavage of the chorion matrix, weakening the structure and allowing embryos to hatch upon gaining locomotive ability. Cathepsin L was readily detected in the developing hatching gland by in-situ hybridization and immunohistochemical staining, and no differences in the abundance of cathepsin L specific mRNA or protein were detected between wild-type and treated embryos. In contrast to these findings, cathepsin L activity was markedly reduced in the perivitelline fluid from chelator or antisense oligonucleotide treated embryos at developmental stages corresponding to maximum hatching gland function. These findings support the concept that peptidyl-glycine alpha-amidating monooxygenase functions to activate a peptide essential for cathepsin L secretion from the hatching gland. Taken together, these data reveal an evolutionarily conserved neuroendocrine mechanism determining hatching rate and timing. The dependence of this mechanism on the availability of copper suggests a specific role for nutrient availability in the adaptive plasticity of hatching in zebrafish embryos.

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## How dynamic 3D skin texture is controlled visually by cuttlefish

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Cuttlefish, in a form of rapid adaptive camouflage, can quickly change both the color and texture of their skin. Many of the visual cues that control skin color have been worked out; however, little is known about the visual cues that control the expression of papillae, the small structures that determine the skin's texture. Because field images show cuttlefish maximally expressing papillae when masquerading as algae, we examined whether the silhouette of objects with algae-like branching would elicit strong papillae expression in *Sepia officinalis* in laboratory experiments. We placed *S. officinalis* in an arena with printed silhouettes of objects with and without branching, and we then quantified the papillae expression in each case. Our preliminary data, despite showing correlation between the level of branching in the image and skin pattern, showed no correlation between the level of branching in the image and papillae expression as measured by our metrics. We also found that individual cuttlefish showed consistency in papillae expression as measured by our metrics across all trials, regardless of image presented.

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## Seasonality and inter-annual variation in pteropod flux in the Sargasso Sea

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Pteropods are marine microzooplankton and an important component of oceanic ecosystems. Pteropods synthesize ornate shells of aragonite carbonate, and their shells are a major fraction of the oceanic carbonate flux. Because aragonite is an easily dissolvable mineral, pteropods are at risk due to decreasing ocean pH. I studied the deep ocean pteropod shell flux at the Oceanic Flux Program (OFP) time-series site in the northern Sargasso Sea to determine pteropod species composition, seasonality, inter-annual variability, and sensitivity to non-seasonal environmental forcing. My study spanned a ten year period (July 2004- October 2014) at a two-week sampling resolution. Three species (*Limacina inflata*, *Styliola subula*, and *Creseis conica*) comprised approximately 75% of the total pteropod shell flux. Most pteropod species showed strong seasonality, which remained consistent over our ten year study period. However the timing of the seasonal maximum varied among species. Many species showed significant inter-annual differences in the amplitude of the seasonal cycle. In particular some species- but not all- showed a large response to non-seasonal environmental forcing (e.g. passage of productive mesoscale eddies and water mass changes). These results, the first decadal high-resolution time-series of pteropod shell flux, indicate that pteropods are keenly sensitive to changes in the oceanic environment including episodic environmental influences.

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## **The effects of Bcl-x<sub>L</sub> on readying the synapse for synaptic plasticity (Ready for LTP or “rLTP”)**

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Bcl-x<sub>L</sub> is an anti-apoptotic protein which also regulates metabolism and other aspects of synaptic function. When over-expressed, Bcl-x<sub>L</sub> enhances spontaneous synaptic transmission, evoked transmission and synaptic vesicle recycling. In addition, it increases the size and number of synapses, increases mitochondrial targeting to synaptic sites and translocates to mitochondria during intense synaptic or neuronal stimulation, increasing the efficiency of ATP production by mitochondria. We now suggest that these important changes that Bcl-x<sub>L</sub> brings about within the synapse may mean that Bcl-x<sub>L</sub> acts as a coordinator molecule for the readying of the synapse for the process of long-term potentiation (LTP). LTP occurs in response to a bout of high-frequency electrical stimulation that induces an immediate and long lasting enhancement of synaptic transmission. LTP in the hippocampus has been strongly correlated with memory formation. We suggest, therefore, that if Bcl-x<sub>L</sub> is inhibited, the synapse will not be ready to make changes during the LTP stimulus, and LTP will not occur, accompanied by a loss of memory formation in the animal, synaptic depression and eventual synaptic loss. We have now shown that one crucial event in the readiness for LTP is enlargement of the size of the readily releasable pool of synaptic vesicles. After analysis of electron microscopy (EM) images of resting hippocampal synapses from three to four week old rat brain slices, docked vesicles at the edge of the active zone that are ready to be released during synaptic transmission are significantly decreased in number in ABT-737-exposed slices. Two possibilities may cause this finding: Bcl-x<sub>L</sub> inhibition could decrease recovery of exocytosed vesicles, or the docked vesicles may have been removed in preparation for proteolytic destruction.

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## **Examining chlorophyll fluorescence and spectral signal to understand ecosystem functions and plant physiology in a temperate forest**

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Understanding ecosystem functions at a regional and global scale becomes increasingly important with the advent of rapid changes associated with global warming. Automated near- and remote-sensing at various scales can fill a critical gap by allowing increased temporal sampling. We used a multi-method approach to measure leaf-, canopy-, and regional-scale chlorophyll fluorescence at Harvard Forest, a temperate, deciduous forest in Petersham, MA, USA. The aim was to link physiological processes across scales and gain a more refined representation of phenological changes in ecosystem productivity.

Remote-sensing of fluorescence provides an opportunity to detect real-time rates of gross primary productivity; however, there is a need to validate canopy and satellite measurements with direct leaf-level observations. Accurate leaf-scale measurements provide a basis for interpreting global satellite data. Detectable chlorophyll fluorescence is emitted during photosynthesis, as a result of leaf chlorophyll pigments absorbing photons. Since chlorophyll fluorescence is a direct consequence of activity in photosystem II, its probing confers the physiological meaning of these signals. Data from the GOME-2 satellite were compared to ground-based fluorescence retrieval, in order to determine the efficacy of airborne retrieval methods for studying photosynthesis and gross primary production.

To complement these emissions, spectral signals of the canopy area were collected using a standard color camera (RGB), as well a camera that images and quantifies the Normalized Difference Vegetation Index (NDVI). These signals, which provide estimates of plant productivity, were compared and found to correlate with each other, and with measurements of chlorophyll fluorescence.

Significant positive correlation was found between the various scales—thus, the detection of chlorophyll fluorescence and spectral signals provides us with powerful tools to monitor and understand various ecosystems and their functions. Proper utilization of these methods enables better projections, improving our ability to predict and respond to the inevitable future changes afflicting ecosystems and climates.

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## Synaptotagmin expression in lamprey reticulospinal neurons after spinal cord injury

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The sea lamprey has the ability to regenerate its spinal cord ten to twelve weeks after complete spinal cord transection. Although lampreys can fully recover, a subpopulation of descending reticulospinal neurons is destined for degeneration and eventually apoptosis after injury, while another subset survives and regenerates. A goal of this project is to determine molecular changes that happen in the regenerating neurons in contrast to the degenerating neurons. Two proteins we particularly focused on were  $\alpha$ -synuclein and synaptotagmin-I.  $\alpha$ -Synuclein is a protein involved in synaptic vesicle trafficking, and its accumulation is known to be detrimental to neurons in spinal cord injury and neurodegenerative diseases such as Parkinson's and Alzheimer's disease. Synaptotagmin-I is a protein that acts as a  $\text{Ca}^{2+}$  sensor for synaptic vesicle exocytosis. Our lab previously showed synuclein accumulates in the poorly surviving neurons following injury and is linked to subsequent neuronal death. The specific goal of my experiments was to test whether other synaptic vesicle proteins accumulate after injury along with synuclein in dying neurons. To address this, I performed immunofluorescence staining on the brains of spinal transected and control lampreys to observe the expression of another vesicle-associated protein, synaptotagmin-I, compared to synuclein. Currently, my results indicate synaptotagmin-I did not accumulate at all with  $\alpha$ -synuclein, suggesting that post-injury synuclein accumulation is specific and is not due to a general impairment of axonal transport of synaptic vesicles. Further studies will be done to determine if other synaptic vesicle proteins accumulate in the neurons of transected lamprey brains to corroborate the notion that synuclein accumulation is specific.

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**Grassland restoration and management on Naushon Island. Luke Hästen O'Brien, Christopher Neill<sup>2</sup>. <sup>1</sup>Boston College, <sup>2</sup>Marine Biological Laboratory, Woods Hole, MA.**

The Island of Naushon, situated northwest of Martha's Vineyard, has undergone a significant transformation in vegetation cover in recent years past. Much of what was once grassland has seen a drastic turnover to expansive shrub thickets, which threaten the biodiversity and ecological stability of this remote isle. The Naushon Grasslands project aims to determine the efficacy of mowing and cattle grazing as methods to control or deter the expansion of said shrub thickets on the island. While nearly all of the islands' fauna are native, the expanding thickets have manifested overwhelmingly homogenous and low-diversity regions that are comprised of one or two species and often rendering these areas impassible. The primary focus of the project is centered on the shrub *Gaylussacia baccata* [Huckleberry] and a liana species, *Smilax rotundifolia*, commonly referred to as Catbrier. The preservation of the island's grasslands is vital to the well being of the local ecosystem and the fauna that inhabit and utilize it. The Naushon Grasslands project aims to conserve the rich biodiversity of threatened sand-plain grasslands. The method through which the project attempts to investigate how this conservation may be accomplished is facilitated by four experiments: vegetation monitoring, cattle enclosures, mowing intensity, and exclosures. It is through these experiments that the various methods and intensities of mowing and grazing may be systematically analyzed, so as to better understand the processes at play. Through vegetation monitoring, it is better understood how untreated plots continue to change with time. In the cattle enclosure experiments, three differing concentrations of cattle facilitate different grazing stresses. The mowing intensity experiment implements three intensities of mowing. The enclosures implement all possible combinations of mowing and grazing to further the understanding of this complex conservation study.

### **Response of elaborate pupils in *Leucoraja erinacea* to changes in light intensity**

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The eyes of many animals, vertebrates and invertebrates, have pupils that restrict in response to light. Various pupil shapes are known, such as circular, slit-shaped and pinhole pupils, and the optical functions of these relatively simple pupils are well understood. Another group of animal pupils exists that are referred to as “elaborate” pupils because of their complex shapes, frills and protuberances. The underlying reasons for this design have been poorly investigated. Some elasmobranch fishes, such as the little skate (*Leucoraja erinacea*), have an elaborate pupil, which consists of a distinctive crescent shaped aperture with multiple dorsally arranged pupillary finger-like protuberances. So far, there has only been circumstantial evidence that the pupil of *L. erinacea* changes shape in response to changes in light intensity, and the extent of any pupillary transformation was unknown. In an effort to understand the optical functions of the elaborate pupil of *L. erinacea*, this project set out to test whether the pupil of *L. erinacea* responds to different light intensities by changing the shape of its aperture. To this end, we measured pupillary area after adapting animals to 8 different light intensities, ranging from (~0.0001lx to ~700lx). Eight skates were used, and we found that all animals decreased their pupil area in response to increasing light intensity. The underlying mechanism of this change is the movement of the dorsal pupil part that bears the finger-like protuberances. The pupil changes shape from a near-circular shape (0.0001lx) to an aperture consisting of multiple triangular pupillary apertures arranged in a crescent (700lx). The data obtained by this project will aid in elucidating the optical functions of the elaborate pupils in *L. erinacea*. These will be discussed.

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### **Effects of changing nitrogen loads on vegetation patterns in Waquoit Bay, 1990-2014**

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Excess nitrogen in coastal estuarine systems leads to eutrophication, a major environmental concern that can cause the replacement of native eelgrass beds by macroalgal blooms among other harmful effects. Nitrogen loads to estuaries depend both on contributions from anthropogenic land uses within the watershed, such as fertilizers and wastewater, and on atmospheric deposition to the surface area of the watershed or the bay itself. In this study we sought to understand whether changes in land use and in atmospheric deposition of nitrogen that took place between 1990 and 2014 altered N loads to the Waquoit Bay estuarine system in Massachusetts, and what effect these changes have had on macroalgal and eelgrass communities in the various estuaries. We analyzed detailed GIS layers to determine changes in watershed land covers across the decades. The resulting data, input into a well-validated nitrogen loading model, show that over the study period N contributions to the estuaries from local anthropogenic sources increased significantly. These increases have recently been offset by the significant reduction in atmospheric N deposition recorded across the Northeast since 2000. We are in the process of collecting vegetation samples to determine whether these shifts in external N loads have had measurable effects on the biomass of macroalgae, eelgrass, and phytoplankton present in each estuary. After assembling a time series of producer biomasses throughout the growing season (May-October), we will compare our data with those collected during the 1990s and early 2000s to assess the impacts of changing nitrogen inputs on the estuarine system as a whole.

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**Effects of ocean acidification and flow on oxygen and pH conditions of developing squid  
(*Doryteuthis pealeii*) egg cases**

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Little is known about the natural oxygen and pH conditions within the egg cases of key marine organisms. Such information is critical to address concerns regarding potential impacts of ocean acidification on these taxa and changes to the environment in which critical life stages develop. This study investigated the oxygen and pH environment adjacent to and within the egg cases of squid, *Doryteuthis pealeii*, under ambient and elevated CO<sub>2</sub> (400 and 2200 ppm), and across differing water flow rates (0, 1, and 10 cm/s). Under both CO<sub>2</sub> treatments, oxygen and pH in the egg case centers dropped dramatically across development. In the ambient CO<sub>2</sub> trial, oxygen concentrations reached a minimum of 4.351 μmol/L, and pH reached a minimum of 7.36. In the elevated CO<sub>2</sub> trial, oxygen concentrations reached a minimum of 9.910 μmol/L, and pH reached a minimum of 6.79. Oxygen concentrations in the egg case centers were highest in the egg cases exposed to 10 cm/s flow in both CO<sub>2</sub> trials, across all age classes measured. All tested egg cases successfully hatched, demonstrating that developing *D. pealeii* embryos have a strong tolerance for low oxygen and pH, but there were more unsuccessful embryos counted in the 0 and 1 cm/s flow conditions. When considering the impacts of ocean acidification, water flow may play a key role in mitigating developmental stress to egg case bound embryos by increasing available oxygen.

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## **Predicting the effects of salt marsh restoration on CO<sub>2</sub> sequestration and CH<sub>4</sub> emissions**

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Salt marshes have the ability to sequester carbon dioxide (CO<sub>2</sub>) at a rate approximately ten times that of forests, including tropical rainforests (McLeod et al., 2011). The potential for salt marshes to reduce greenhouse gas emissions has motivated efforts to restore and conserve these systems. One such conservation effort is the Herring River Tidal Restoration Project, an initiative to restore tidal flow to Herring River (Wellfleet, MA) after a century of tidal restriction. The goal of this study was to better understand how CO<sub>2</sub> flux and methane (CH<sub>4</sub>) emissions in this once uniform system have changed over time, and how restoration may affect these parameters in the future. Upstream of the dike on Herring River both freshwater marsh and deciduous forest have developed in the place of what was historically salt marsh. Gas emissions were measured in June and July 2015 in these two habitats as well as in a natural salt marsh. Results indicate that the salt marsh had smaller gross primary production (GPP) values than the freshwater marsh (16.99 vs. 28.98  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), but had similar net primary production (NPP) values as the freshwater marsh (8.11 vs. 8.15  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ) due to lower ecosystem respiration in salt marsh. The forest was found to have CH<sub>4</sub> emissions near zero (-0.001  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ), and the freshwater marsh had CH<sub>4</sub> emissions 5.8 times higher than the salt marsh. From these findings we predict that the restoration of tidal flow and concomitant conversion of freshwater marsh to salt marsh will result in overall higher CO<sub>2</sub> sequestration and lower CH<sub>4</sub> emissions.

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### **Phosphorus Phase Associations in the Northern Sargasso Sea**

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Phosphorus is a crucial micronutrient for marine organisms, used in important biological functions such as metabolism and photosynthesis. Particulate phosphorus that is not recycled in the euphotic zone sinks into the deep ocean where it is removed from phytoplankton production. The chemical form or “phase” of phosphorus in sinking particles can determine whether phosphorus in the water column dissolves and eventually upwells to fuel subsequent phytoplankton production or sinks to the seafloor to be buried in ocean sediments. My research is focused on how phosphorus is partitioned among different organic and inorganic phases in the deep ocean flux. I examined deep ocean particles collected by the Oceanic Flux Program sediment traps in the oligotrophic or “nutrient poor” Sargasso Sea. These particles were collected at 500m, 1500m, and 3200m depths over a seasonal cycle, from November 2008 (minimum production) through March 2009 (end of spring bloom). Phosphorus in the samples were partitioned into seven fractions. The first fraction was loosely-sorbed phosphorus released from particles into overlying trap cup brine during sample collection. A sequential extraction method (SEDEX) was then used to separate six “operationally-defined” particulate phases: exchangeable phosphorus, iron bound phosphorus, authigenic apatite and carbonate bound phosphorus, detrital apatite, opal bound phosphorus, and residual organic phosphorus.

We found that the loosely-sorbed phosphorus released into the brine was the dominant reservoir of phosphorus at 500m (77%) and dropped significantly with depth. Particulate phosphorus was the largest reservoir at 1500m (64%) and 3200m (83%). Among particulate phases, authigenic apatite and carbonate bound phosphorus was the largest reservoir at 500m (36%) whereas iron bound phosphorus was the largest reservoir at 1500m (44%) and 3200m (44%). Fluxes of most particulate phases showed similar seasonality, with highest flux during the spring bloom. As certain authigenic minerals and iron oxides have been identified as oceanic phosphorus sinks, this research provides new insights into deep ocean phosphorus geochemistry in the particle flux.

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### **The impact of nitrogen loading in Waquoit Bay, MA on estuarine food webs constructed by stable isotope analysis**

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Waquoit Bay, Massachusetts is a small, tidal bay on the south shore of Cape Cod in Massachusetts, which encompasses several spatially distinct estuarine ecosystems. These estuaries have each been subjected to different degrees of human development. Because of this, the amount of nitrogen loading differs among estuaries in the system, as does the relative contribution of nitrogen from atmospheric deposition and wastewater to the total nitrogen load within each estuary. To test whether different land-derived nitrogen loads created effects that propagated up estuarine food webs, we measured stable nitrogen and carbon values of the taxa in the food web. Three estuaries were examined: Sage Lot Pond (low level of development), Childs River (high level of development), and Quashnet River (intermediate level of development). Over eight weeks, we collected marine flora and fauna using an Ekman dredge from nine stations within each estuary in June to August 2015. Additionally, over a three-day period, we used a seine net to collect larger, mobile animals present in these communities. Collected plants and animals matter were dried and ground, and then underwent stable isotope analysis. Preliminary results indicate that  $\delta^{15}\text{N}$  stable isotope values are heavier for animals living in Childs River, while these values are lowest for animals living in Sage Lot Pond. This implies that degree of land development cascades up the structure of food webs within the estuaries of Waquoit Bay. While faunal stable isotope signatures differed substantially between estuaries, the faunal %N values did not. These differences suggest additional influences from changing sources (atmospheric deposition versus wastewater input) may be possible.

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### **Inhibition of fast axonal transport: Mutant huntingtin as a cause of dying-back neuropathy**

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Huntington's disease (HD) is a devastating neurological disorder characterized by dying-back degeneration of projection neurons within the striatum and cerebral cortex. HD is caused by mutations in the gene encoding Huntingtin (Htt) protein that result in expansion of a polyglutamine (polyQ) tract near the amino-terminus of the protein. Consistent with the axonal degeneration phenotype characteristic of HD, mutant forms of Htt (mHtt) have been shown to inhibit fast axonal transport (FAT), a cellular process critical for appropriate maintenance of the axonal compartment. Moreover, this toxic effect of mHtt involved abnormal activation of the neuronal-specific JNK isoform, JNK3. Despite this knowledge, molecular components linking mHtt to JNK3 activation remained elusive.

Like other members of the MAPK pathway, JNK3 is activated by upstream kinases MAPK kinases (MKKs), which in turn are activated by MAPK kinase kinases (MKKKs). This information prompted us to perform experiments aimed to identify specific MKK and MKKK components mediating the toxic effect of mHtt on FAT.

Results from vesicle motility assays and biochemical experiments using the isolated squid axoplasm this summer indicate that mHtt selectively activates mixed lineage kinases (MLKs) and MKK7 kinases in an axon-autonomous manner. Together, our data identifies a novel kinase pathway mediating inhibitory effects of mHtt on FAT, and provides novel therapeutic targets to correct axonal degeneration in HD.

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## **The effects of temperature and environmental noise on oyster toadfish (*Opsanus tau*) mating calls**

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Many animals use acoustic signaling as a form of intraspecific communication. The oyster toadfish, *Opsanus tau*, possess sexually dimorphic sonic muscles surrounding the swim bladder to produce vocalizations, making them an ideal model organism to study bioacoustics. During the breeding season (May - August), male toadfish use boatwhistles to attract females to nests. Although it was known the fundamental frequency of toadfish calls relate to water temperature, the onset of calling, seasonal duration and the effects of environmental noise were unknown in the Cape Cod area. An *in situ* hydrophone monitored several toadfish in Eel Pond, Woods Hole, Massachusetts, from mid May through late August, 2015. Ambient water temperature ranged from 13 - 24°C and male toadfish began producing boatwhistles when temperature reached approximately 15°C in late May. The temperature increase resulted in higher frequencies (110 - 220 Hz) of the tonal portion of the boatwhistle, which is proposed as the primary acoustic attractant. Also, toadfish produced fewer boatwhistles during times of increased ambient noise, which include anthropogenic and meteorological sources. Since acoustic communication is closely associated to ambient water conditions, these results demonstrate the environmental effects on toadfish calling and provide evidence that anthropogenic impacts may alter fish acoustic behavior and breeding success.

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### **Genomic analysis of opsins in the skate (*Leucoraja erinacea*)**

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The retina of the skate is believed to possess a single class of rod photoreceptors expressing a single visual pigment, rhodopsin. This simplified retinal structure has made the skate an attractive experimental model for examining functional questions related to visual processing. While the visual pigment expressed in rod photoreceptors of this species has been cloned, a number of questions regarding its genomic structure remain, and little information regarding the presence or absence of other opsin-related proteins in the skate is available. To address these questions, we analyzed genomic data available through the Little Skate Genome Project to more closely examine the intron-exon structure of skate rhodopsin and to look for other related opsins. Using BLAST to examine the Little Skate Genomic Contigs Build 2 and Transcriptomic Contigs Build 2 data bases, we assembled multiple contigs into a single scaffold to identify and annotate the complete rhodopsin gene, including up- and downstream regions and the location and identity of the first intron – an intron observed in the rhodopsin of other species but not detected previously in the skate. We identified other opsin-like sequences in the assembly and constructed a phylogenetic tree to identify these as pinopsins, melanopsins, and “vertebrate ancient” (VA) opsins. We have not yet found evidence of cone opsins, although our search strategy revealed cone opsins in a shark genome assembly of similar quality to the skate assembly. We are currently examining the accelerated evolution of the skate VA opsin for evidence of positive selection for divergent function. Such information should help to elucidate how the relatively unique structure and function of the skate retina was shaped and developed during the course of its evolution.

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### **Hyperspectral imaging of dynamic camouflage in juvenile winter flounder reveals how color- and pattern-matching help avoid detection by shallow-water predators**

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Flatfish (order Pleuronectiformes) are well known for their cryptic camouflage abilities; many species can rapidly adjust skin colors and patterns to disguise themselves against a variety of natural substrates. Winter flounder, *Pseudopleuronectes americanus*, spend much of the first year of life in shallow water, where they exhibit dynamic camouflage to reduce their risk of predation from seabirds and fishes. Quantifying the efficacy of winter flounder camouflage in eyes of their potential predators is challenging, since typical imaging systems produce RGB images particular to human vision; birds are tetrachromatic and different teleost fishes exhibit a wide variety of dichromatic, trichromatic, and tetrachromatic visual systems not directly comparable with human vision. By using a unique hyperspectral imaging (HSI) camera, which records full-spectrum radiance data spanning from 360 nm to 660 nm (16 total channels), we visualized both color and pattern matching through the eyes of hypothetical predators. Juvenile winter flounder (SL < 6 cm) were tested on local samples of sand and gravel, two visually dissimilar but common shallow-water substrates. Fish were placed in outdoor tanks of either sand or gravel and photographed using the HSI under natural sunlight. The images were assessed for both chromatic and achromatic camouflage. Isoluminance images simulating the color vision of a tetrachromatic avian predator reveal excellent color matching in juvenile flounder on both the medium-contrast gravel and the low-contrast sand. Luminance images processed through the Laplacian of Gaussian edge detector, an algorithm thought to closely model vertebrate edge detection mechanisms, indicate that the flounder are also extremely difficult to be detected using contrast sensitivity. These findings (i) suggest that the dynamic camouflage exhibited by juvenile winter flounder is very effective in evading detection from highly color-competent predators, and (ii) indicate that HSI technology has a high potential for providing valuable insights into studies of biological coloration.

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### **Molecular Mechanisms of Parkinson's Disease: Investigating Potential Interactions between $\alpha$ -synuclein and Phosphoinositides at Synapses**

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Parkinson's disease (PD) is an age-related movement disorder. One pathological hallmark of PD is an atypical aggregation of  $\alpha$ -synuclein in neurons, including their synapses. However, how excess  $\alpha$ -synuclein affects synapses, and the underlying mechanisms, remain unclear. Our lab recently showed that excess  $\alpha$ -synuclein produces severe endocytic phenotypes at stimulated synapses, resulting in a loss of synaptic vesicles and an expansion of the plasma membrane. However, the molecular mechanisms by which excess  $\alpha$ -synuclein inhibits vesicle recycling are unknown. To address this, we aim to determine what are the synaptic binding partners for  $\alpha$ -synuclein. Phosphoinositides (PIs) are good candidates because they are involved in vesicle trafficking, including endocytosis at synapses, and preliminary *in vitro* data indicate that  $\alpha$ -synuclein binds to several PIs. My project is to further investigate whether  $\alpha$ -synuclein may interact with PIs *in vivo* by examining their localization patterns. To determine which PIs are expressed at synapses, neurons were stained using antibodies against PI(4)P, PI(4,5)P<sub>2</sub>, and a synaptic marker SNAP25. In my preliminary studies, I found that PI(4)P colocalizes at synapses with SNAP25 and is also expressed in the cell body and neurites. PI(4,5)P<sub>2</sub>, however, is mostly expressed in the cell body in a punctate pattern and less abundant than PI(4)P. The expression level of PI(4,5)P<sub>2</sub> increases over time in cultured neurons. In contrast,  $\alpha$ -synuclein staining shows that  $\alpha$ -synuclein is expressed in the cell body and neurites as well as at synapses where PI(4,5)P<sub>2</sub> is not found. Taken together, these data suggest that  $\alpha$ -synuclein is more likely to interact with PI(4)P at synapses than with PI(4,5)P<sub>2</sub>. Further experiments will be performed to study whether the overexpression of  $\alpha$ -synuclein changes the amount and distribution of PIs. These results have implications for the mechanisms underlying the synaptic vesicle trafficking defects associated with excess  $\alpha$ -synuclein in models of PD.

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## **Surviving in extreme environments: novel adaptations of *Legionella* to the Rio Tinto**

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*Legionella* is a genus of gram-negative bacteria, some species of which can infect humans opportunistically to cause Legionnaire's disease. Ubiquitous in a range of natural and manmade freshwater environments, *Legionella* is considered intolerant to heavy metals. However, a high abundance of *Legionella*-associated genes in a recent metagenomic survey of biodiversity in the acidic and heavy metal extreme Rio Tinto (RT) in southwest Spain challenges this assumption. One hypothesis for how *Legionella* might persist in this environment is that it exists as an obligate symbiont inside eukaryotic hosts, possibly acidotolerant amoebae, representing novel adaptations in lifestyle that may be reflected in the genomes of RT *Legionella*. We are using existing metagenomics data, archived samples, and laboratory culture-based techniques to explore the microbial and eukaryotic diversity in the Rio Tinto. We ran a network analysis using bacterial and eukaryotic amplicon data from the RT and found co-occurrences between *Legionella* and several eukaryotic protists. We are now carrying out comparative genomics on *Legionella* genomic data derived from RT metagenomes and published complete genomes of cultured strains of *Legionella* species to investigate their evolutionary relationships and examine the possibility of Lateral Gene Transfer from potential hosts to bacteria. In addition, using 43 newly sequenced *Legionella* genomes, we developed a genus-specific *Legionella* probe for FISH that will allow visualization of the spatial distribution of *Legionella* within RT microcosms and enable us to determine whether several pre-existing amoeba cultures, including species from the RT, can take up *Legionella* and serve as hosts.

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