

2013 MBL Undergraduate Research Symposium

Thursday, August 15, 2013

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And

NSF-REU “Biological Discovery in Woods Hole” program

Program Schedule and Abstracts

Organizer:

Robert Paul Malchow
University of Illinois at Chicago

Session Chairs:

Robert Paul Malchow, University of Illinois at Chicago
Allen Mensinger, University of Minnesota-Duluth
Gerardo Morfini, University of Illinois at Chicago
Jianwu Tang, Ecosystems Center, MBL

2013 MBL Undergraduate Research Symposium

August 15, 2013, Lillie Auditorium

8:50 **Opening Remarks, Joshua Hamilton¹ & William Reznikoff².** ¹Chief Academic & Scientific Officer & ²Director of Education, Marine Biological Laboratory, Woods Hole, MA.

Session One – Allen F. Mensinger, University of Minnesota, Duluth – Chair

9:00 **The Effect of Light Intensity on Iridescent Expression in Long-fin Squid (*Doryteuthis pealeii*).** Emily Cardinal¹, Trevor Wardill², Paloma Gonzalez-Bellido², Roger Hanlon². ¹Dept. Biological Sciences, Saint Catherine University, Saint Paul, MN; ²Program in Sensory Physiology and Behavior, Marine Biological Laboratory, Woods Hole MA.

9:15 **How Peripheral Injury Alters Squid Behaviour.** Julia Carroll¹, Robyn Crook², Roger Hanlon³. ¹Williams College, Williamstown, MA; ²Department of Integrative Biology and Pharmacology, University of Texas Health, Houston, TX; ³Program in Sensory Physiology and Behavior, Marine Biological Laboratory, Woods Hole, MA.

9:30 **3D Particle Tracking to Analyze Predator-Prey Interactions in *Mnemiopsis leidyi*.** Roshena MacPherson¹, Sean Colin², Brad Gemmell³ and Jack Costello⁴. ¹University of California - Berkeley, Berkeley CA; ²Roger Williams University, Bristol RI; ³Marine Biological Laboratory, Woods Hole, MA; ⁴Providence College, Providence RI.

9:45 **The Functional Significance of Papillae of the Superficial Neuromasts in *Opsanus tau*.** Halvor N. Adams IV¹, Beth Giuffrida², Allen F. Mensinger^{3,4}. ¹Adelphi University, Garden City, NY; ²Wareham Middle School, MA; ³University of Minnesota, Duluth; ⁴Marine Biological Laboratory, Woods Hole, MA.

10:00 **Synuclein Distribution in Regenerating Lamprey Spinal Cords.** Julia Eisen¹, Stephanie Fogerson², Jennifer Morgan². ¹Barnard College, New York, NY; ²Eugene Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory, Woods Hole, MA.

10:15 **Calcium Increases in Poor Surviving Neurons after Spinal Cord Injury in Lampreys.** Helena Lane¹, Stephanie Fogerson², Jennifer Morgan². ¹Oberlin College, Oberlin, OH; ²Eugene Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory, Woods Hole, MA.

10:30 **SV2 Expression and Synapse Distribution in Regenerating Spinal Cords of the Sea Lamprey, *Petromyzon marinus*. Scott R. Allen¹, Jennifer R. Morgan².**
¹Department of Biology, Bridgewater State University, Bridgewater, MA; ²Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory, Woods Hole, MA.

10:45 **Break**

Session two – Gerardo Morfini, University of Illinois at Chicago, Chair

11:00 **Searching for the Missing Link in the Pathogenic Pathway of Mutant SOD1 in ALS. Stephen Formel¹, Yuyu Song², Arthur L. Horwich², Gerardo Morfini³, Scott Brady³.**
¹Department of Biology, Hunter College, New York, NY; ²Department of Genetics and Howard Hughes Medical Institute, Yale School of Medicine, New Haven, CT; ³Department of Anatomy and Cell Biology, University of Illinois, Chicago, IL.

11:15 **Evaluating the Effect of Chaperones on Mutant SOD1. Izrail Abdurakhmanov¹, Scott T. Brady², Gerardo Morfini², Weiming Ni³, Arthur L. Horwich³, Yuyu Song³.**
¹Department of Biological Sciences, CUNY Hunter College, New York, NY; ²Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, IL; ³Department of Genetics and Howard Hughes Medical Institute, Yale School of Medicine, New Haven, CT.

11:30 **Investigating the Variant Repeats of C9ORF72 in ALS. Jennifer Purks¹, Gerardo Morfini², Yuyu Song³, Scott T. Brady².** ¹Department of Biology, Georgetown University, Washington, D.C.; ²Department of Anatomy and Cell Biology, University of Illinois, Chicago, IL.; ³Department of Genetics and Howard Hughes Medical Institute, Yale School of Medicine, New Haven, CT

11:45 **A pathogenic pathway common to familial ALS forms of different genetic etiology. Nicola Kriefall¹, Saul Penaranda¹, Yuyu Song², Scott Brady³ and Gerardo Morfini³.**
¹Department of Biology, CUNY Hunter College, New York, NY; ²Department of Genetics and Howard Hughes Medical Institute, Yale School of Medicine, New Haven, CT; ³Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, IL.

12:00-1:00 **Lunch**

Session 3 – Jim Tang, Marine Biological Laboratory, Chair

1:00 **From Cell to Satellite: Seasonality of Internal Leaf Structure and Relationship with Leaf Surface Reflectance. Shalanda Grier¹, Xi Yang², Jim Tang².** ¹Hampton University, Hampton, VA; ²Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA.

- 1:15 **Climate Imprint on Carbon Isotopic Signatures of Leaf Wax in Arctic Vegetation: Groundtruthing Paleoclimate Proxies.** Tanner Cunningham¹, Maureen Conte², J.C. Weber². ¹Bates College, Lewiston, ME; ²Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA.
- 1:30 **Effects of Nitrogen Fertilization on *Spartina Alterniflora* Morphology.** Caitlin Bauer¹, Thomas Mozdzer¹. ¹Bryn Mawr College, Bryn Mawr, PA.
- 1:45 **The Effects of Saltmarsh Nutrient Enrichment on *Fundulus heteroclitus* Diet and Growth Rates, Plum Island, MA.** Nathan E. Andrews¹, David Behringer², James Nelson³. ¹Department of Marine Biology, University of Rhode Island, Kingston, RI; ²Department of Environmental Sciences, Washington & Jefferson College, Washington, PA; ³TIDE, Marine Biological Laboratory, Woods Hole, MA.
- 2:00 **The Effects of Nutrient Enrichment on *Fundulus heteroclitus* Demographics in Northern Massachusetts Saltmarshes.** David Behringer¹, Nathan E. Andrews², James Nelson³. ¹Department of Environmental Studies, Washington & Jefferson College, Washington, PA; ²Department of Marine Biology, University of Rhode Island, Kingston, RI; ³TIDE, Marine Biological Laboratory, Woods Hole, MA.
- 2:15 **Variation in *Z. marina* Morphology and Sediment Composition with Depth in West Falmouth Harbor, MA.** Mary Gibbs¹, Melanie Hayn^{1,2}, Robert W. Howarth^{1,2}, Roxanne Marino¹. ¹Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, NY; ²Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA.
- 2:30 **Great Egret and Snowy Egret Population Density and Foraging Habits in the Plum Island Sound and Surrounding Marsh Ecosystems.** Wesley Clark¹, Robert Buchsbaum². ¹Eckerd College, St. Petersburg, FL; ²Mass Audubon, Wenham, MA.

2:45 **Break & Symposium Group Photo**

Session 4 – Robert Paul Malchow, University of Illinois at Chicago, Chair

- 3:00 **Scanning Electron Microscopy Analysis of the "Plastisphere": Microbial Succession on Plastic Marine Debris.** Jessica Fields¹, Kiera Saleem², Erik Zettler³, Linda Amaral Zettler^{4,5}. ¹Division of Biology and Medicine, Brown University, Providence, RI; ²Department of Environmental, Earth, and Geospatial Sciences, North Carolina Central University, Durham, NC; ³SEA Semester, Sea Education Association, Woods Hole, MA; ⁴Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory, Woods Hole, MA; ⁵Department of Geological Sciences, Brown University, Providence, RI.

- 3:15 **What You Didn't Want to Know About Your Mouth: Visualizing the Structure of Oral Biofilms.** Braden Tierney¹, Jessica L. Mark Welch², Blair J. Rossetti², and Gary G. Borisy². ¹Duke University, Durham, NC; ²Bay Paul Center, Marine Biological Laboratory, Woods Hole, MA.
- 3:30 **Mechanisms of Nutrient Availability in the Adaptive Plasticity of Anamniote Hatching.** Jason Sloan¹, Tessa Comstock Hulburt², Rebecca T. Thomason³, Jonathan D. Gitlin³. ¹Department of Biology, Hunter College, New York City, NY; ²Department of Biomedical Engineering, Worcester Polytechnic Institute, Worcester, MA; ³Eugene Bell Center for Regenerative Biology & Tissue Engineering, Marine Biological Laboratory, Woods Hole, MA.
- 3:45 **NQO1, NAD(P)H-dependent Cytosolic Oxidoreductase Modulates β -cell Redox Status and Insulin Secretion.** Delaine M. Zayas-Bazán Burgos¹, Joshua P. Gray², Emma Heart³. ¹Department of Natural Sciences, University of Puerto Rico, Cayey, PR; ²Department of Science, US Coast Guard Academy, New London, CT; ³Cellular Dynamics Program, Marine Biological Laboratory, Woods Hole, MA.
- 4:00 **Primary cell culture of teleost vocal motoneurons and an analysis of the extracellular matrix.** Molly J. Kirk¹ and M. Jade Zee¹. ¹Program in Behavioral Neuroscience, Northeastern University, Boston, MA.
- 4:15 **Evaluating the Effects of Mutant FUS in Axonal Transport.** Saul Penaranda¹, Scott T. Brady², Gerardo Morfini³, Reddy Sam³, Daryl A. Bosco⁵, ¹Department of Biological Sciences, CUNY Hunter College, New York, NY.; ²Department of Anatomy & Cell Biology, University of Illinois at Chicago, Chicago, IL.; ³Department of Neurology, University of Massachusetts Medical Center, Worcester, MA.; ⁴Marine Biological Laboratory, Woods Hole, MA.
- 4:30 **Closing remarks, Robert Paul Malchow**¹ ¹University of Illinois at Chicago, Chicago IL.

Evaluating the Effect of Chaperones on Mutant SOD1

Izrail Abdurakhmanov¹, Scott T. Brady², Gerardo Morfini², Weiming Ni^{3,4}, Arthur L. Horwich^{3,4},
Yuyu Song^{3,4}

¹Department of Biological Sciences, CUNY Hunter College, New York, New York;

²Department of Anatomy and Cell Biology, University of Illinois at Chicago, Chicago, Illinois

³Department of Genetics, Yale University, New Haven, CT

⁴Howard Hughes Medical Institute

Amyotrophic Lateral Sclerosis (ALS, Lou Gehrig's disease), is a fatal neurodegenerative disease affecting upper and lower motor neurons. Five to ten percent of ALS cases result from autosomal dominant inheritance of certain disease genes (Familial ALS), whereas the majority of cases have no known familial history and are thus called Sporadic ALS. Mutations in the gene that codes for the enzyme superoxide dismutase 1 (SOD1) have been discovered to cause twenty percent of familial ALS cases. Wild type SOD1 has been proposed to play a role in reducing free oxide radicals in the cytosol and is involved in redox signaling. It has been demonstrated that pathological SOD1 mutations give rise to a gain of toxic function as opposed to loss of dismutase activity. We proposed that mutant SOD1 may cause toxicity by impairing axonal transport, which leads to motor neuron degeneration. Vesicle motility assays in the squid giant axon with the G85R SOD1 mutation showed that there was a dramatic decrease in Fast Axonal Transport (FAT) in the anterograde direction (towards the synapse). In order to rescue transport from the inhibitory effect of the G85R SOD1 mutant, which is misfolded, we looked into chaperone proteins, known for their role in recognizing foreign or misfolded proteins. The molecular chaperones Hsc70 and Hsp110, were found to associate with SOD1 G85R in the mouse spinal chord. When the effect of Hsc70 on mutant SOD1 was tested, a partial rescue in inhibition of anterograde transport was observed. However, when repeated with Hsp110, which has been demonstrated to act as a nucleotide exchange factor for Hsc70, a full rescue was observed. Due to the observed rescuing effect of these chaperones, a new means for inhibiting the toxic effect of misfolded G85R can be utilized.

Funded by an HHMI Undergraduate Science Education grant to Hunter College.

The Functional Significance of Papillae of the Superficial Neuromasts in *Opsanus tau*

Halvor N. Adams IV¹, Beth Giuffrida², Allen F. Mensinger^{3,4}

¹Adelphi University, Garden City, NY; ²Wareham Middle School, MA; ³University of Minnesota, Duluth; ⁴Marine Biological Laboratory, Woods Hole, MA.

The oyster toadfish, *Opsanus tau*, is a benthic fish that inhabits shallow coastal waters along the Eastern United States. The toadfish anterior lateral line consists of canal neuromasts situated in 4 subdermal canals along each side of the head and numerous superficial neuromasts. Each superficial neuromast is surrounded by paired fleshy appendages termed papillae. The hair cells are arrayed perpendicular to the papillae and it has been hypothesized that the papillae restrict water flow through the neuromast causing them to function as canal neuromasts. However, recent neurophysiological studies found evidence of both canal and superficial neuromasts in the anterior lateral line. The current study tested the alternative hypothesis that the papillae evolved to protect the hair cells from sedimentation. Sand or glass beads, ranging in size from 100 nm to 1 mm were added to a one cm diameter circle centered around a single superficial neuromast and the tissue examined with scanning electron microscopy (SEM). The combination of mucous production and/or fish movement displaced the sediment from the neuromasts quickly with 98 % of the foreign objects displaced within four hours. Tissue was fixed within 10 minutes of bead or sand application and SEM examination was unable to detect any sediment within the papillae or on the cupula, although sand and glass beads were prominent on the outside of the papillae and the surrounding epidermis. The result suggests that the papillae act to protect the hair cells from sediment deposition.

This research was funded by NSF DBI 1005378 REU: Biological Discovery in Woods Hole.

SV2 Expression and Synapse Distribution in Regenerating Spinal Cords of the Sea Lamprey, *Petromyzon marinus*

Scott R. Allen¹, Jennifer R. Morgan²

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Sea lampreys, *Petromyzon marinus*, exhibit robust spinal cord regeneration after a complete spinal cord transection. Synapse regeneration has been characterized for the large descending giant RS axons. But there are thousands of other synapse-forming axons present throughout the spinal cord, which are made by intraspinal neurons. Here we characterize these synapses in the spinal cord of uninjured control lampreys and those at 10-12 weeks after spinal cord transection. To do so, we used an antibody against SV2, a marker of synaptic vesicles. We demonstrate that SV2 expression increases in regions less than 1 mm proximal and distal to the lesion site, suggesting increased synapse density. We also demonstrate that SV2 expression decreases within the lesion site, suggesting decreased synapse density. SV2 expression remains at control levels greater than 1 mm proximal and distal to the lesion site. This has implications for the plasticity of the spinal cord and suggests nearby neurons may compensate for the spinal transection by forming additional synapses.

Funding support: Bridgewater State University, NSF DBI 1005378 REU: Biological Discovery in Woods Hole, and the Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory.

The effects of saltmarsh nutrient enrichment on *Fundulus heteroclitus* diet and growth rates, Plum Island, MA

Nathan E. Andrews¹, David Behringer², James Nelson³

¹Department of Marine Biology, University of Rhode Island, Kingston, RI; ²Department of Environmental Sciences, Washington & Jefferson College, Washington, PA; ³TIDE, Marine Biological Laboratory, Woods Hole, MA

Fundulus heteroclitus, the mummichog, is a common omnivorous saltmarsh fish, and plays an integral role in the ecosystem of the Plum Island Ecosystem LTER. Two experimental creeks are enriched with nitrate fertilizer on incoming tides to mimic anthropogenic nutrient pollution, which are monitored by the TIDE program. We collect and examine mummichogs from the two experimental creeks, one long-term fertilized and one short-term fertilized, from which we expect see the effects of the bottom-up stimulation brought on by the moderate eutrophication of the creek. The nutrient enrichment of the saltmarsh creeks causes substantial growth of algae and degradation of the creek walls and *Spartina alterniflora* habitat, which affects the fish diet, available food sources, growth rates, population demographics, and energetics. The experimental creeks and their mummichogs are compared against control creeks with similar geometrics. In order to determine the growth rates and to monitor the cohorts' growth from those hatched this summer and the ones spawning this summer from the past summer(s), length frequency analysis (LFA) was conducted on approximately 1000 mummichogs from the 4 creeks by use of a seine net. The demographics and gut contents were examined by collecting fish on the high marsh while flume netting. Those fish were then dissected and the gut content identified and sorted into 2 tins for each fish, one detritus and one protein, then dried, weighed, and the percent protein was determined. Both the LFA and gut content was examined for the months June and July. These data were analyzed to determine the differences in the experimental creeks from the control creeks pertaining to diet and trophic bottlenecking. We observed differences in diet by size between the experimental and control creeks.

This research was funded by NSF-DEB-0816963, "Collaborative Research: Interacting controls on ecosystem function: nutrient state and omnivory in salt marsh ecosystems".

Effects of Nitrate Fertilization on *Spartina Alterniflora* Morphology

Caitlin Bauer¹, Thomas Mozdzer¹

¹Bryn Mawr College, Bryn Mawr, PA.

Salt marshes are uniquely important ecosystems, which provide a number of ecosystem services including shoreline stabilization and nutrient retention. Along the Atlantic Coast of North America, salt marshes are dominated by the foundation species, *Spartina alterniflora*. There is a great deal of uncertainty to how these ecosystems will respond to anthropogenic nitrate pollution. While many studies have shown increases in ecosystem productivity with nitrate fertilization, research at the TIDE project has shown that the system may cause marsh collapse. In addition, our unpublished data suggests that there has been a shift in genetic identity in fertilized marshes with only ten years of nitrate fertilization. Our objective is to determine if this shift in genotype has resulted in different plant phenotypes, which may destabilize the salt marsh. In order to understand how nitrate pollution can alter phenotypes, we studied plant morphology across the four creeks by measuring specific leaf area, stem density, and shoot specific weight. These data provide greater insight to the above ground biomass of the tall form *Spartina alterniflora* across both salt marshes fertilized with nitrate and control creeks without added nitrate. Preliminary analysis suggests that added nitrate causes a shift in standard leaf area. Our research sheds light on how nutrient-induced genotypic shifts can result in altered resource allocation and resulting changes to ecosystem stability.

Funding from NSF-OCE-1058747 "Plum Island Ecosystems LTER".

The Effects of Nutrient Enrichment on *Fundulus heteroclitus* Demographics in Northern Massachusetts Saltmarshes

David Behringer¹, Nathan E. Andrews², James Nelson³

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²Department of Marine Biology, University of Rhode Island, Kingston, RI; ³TIDE, Marine Biological Laboratory, Woods Hole, MA.

Eutrophication associated with agriculture and population density in coastal regions is a problem throughout the world. Salt marshes however, were thought to be resistant to nutrient loading due to their ability to absorb and take in excess nutrients. The TIDE project is a long-term experiment, in its tenth year, which studies the effects of nutrient enrichment at the ecosystem level in the Plum Island Sound in northern Massachusetts. One treatment creek has been fertilized for ten years and a short-term treatment that has been fertilized for five years. Nitrate fertilizer is added, increasing nitrate levels to 70 μ molar, to mimic the real world effects of nutrient enrichment. In order to determine the effects of nutrient enrichment on the demographics of salt marsh creek nekton, flume nets were utilized during the spring tides to collect organisms that utilize the high marsh. We collected nekton during June and July flood tides. All specimens were counted, measured and weighed. Length weight ratios were used to produce a growth curve for *Fundulus heteroclitus*. We found that *Fundulus heteroclitus* is by far the most abundant nekton in the creek systems, contributing 45% of the total biomass and 73% of total individuals. The fish in the short-term fertilized creek had a mean length of 47.291 and a mean weight of 1.675. The fish in the control creek had a mean length of 46.479 and a mean weight of 1.702. The fish in the long-term treatment were smaller, with a mean length of 40.707 and a mean weight of 1.209. The short term fertilized creek had the highest length weight ratio, the long term fertilized creek has a slightly lower length weight ratio, and the control creek had the smallest length weight ratio.

This research was funded by NSF-DEB-0816963, "Collaborative Research: Interacting controls on ecosystem function: nutrient state and omnivory in salt marsh ecosystems".

The effect of light intensity on iridescent expression in long-fin squid (*Doryteuthis pealeii*)

Emily Cardinal¹, Trevor Wardill², Paloma Gonzalez-Bellido² and Roger Hanlon²

¹Department of Biology, Saint Catherine University, Saint Paul, MN; ²Sensory Physiology and Behavior Program, Marine Biological Laboratory, Woods Hole, MA.

Squid are coleoid cephalopods known for their rapid changes in skin coloration with both neurally controlled pigmentary coloration, via chromatophores and structural coloration, via iridophores. Structural coloration uses nanoscale platelets that reflect specific peak wavelengths of light through interference. We sought to discover if iridophore expression would match the environmental light intensity as its behavioral role is unknown. The right fin nerve was denervated to neurally deactivate chromatophores ensuring iridophores are clearly visible and squid were placed in darkness for two hours to reduce iridophore expression. Using a BlueMax Sunrise System and neutral density filters, different maximum light intensity levels were reached for each trial. Photographs were taken and analyzed through standard image processing. Results showed no statistically significant differences between the mean light intensity levels of the iridophores before and after each light intensity treatment as expected because intensity was used as a threshold to segment images for iridophore profiles. However, this use of intensity based segmentation revealed the percent area and number of iridophores expressed differs significantly ($p < 0.05$) between the before and after adaptation conditions to each light intensity treatment, except for the number of iridophores for 2.0 kLux and the percent area of iridophores for 0.5 & 2.0 kLux treatments (demonstrating rescue of iridophore expression after exposure to natural light levels). This indicates that squids match their skin iridescence reflectivity to the visual light levels detected by the eye, but variation in the data suggests iridescence may potentially be used for intraspecific signaling or camouflage.

Funding support: NSF DBI 1005378 REU, Biological Discovery in Woods Hole, and ONR grant N00014-10-1-0989.

How Peripheral Injury Alters Squid Behaviour

Julia Carroll¹, Robyn Crook², Roger Hanlon³

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Almost all animals exhibit defensive responses to physical, external stimuli. Activity in nociceptive sensory pathways usually results in reflexive behavioural responses and may or may not result in cognitive changes that alter ongoing behaviour. Many animals, including squid, form schools to increase survival chances. Schooling works more effectively in populations that experience high levels of predation. One major benefit of schooling, increased vigilance with large numbers, offers a potential counter-defense in nature's evolutionary arms race. Although vigilance in school groups serves a great function, animals cannot spend all their time actively searching for predators and wondering if there is a predation threat. This risk of schooling includes the fact that schools require much energy, and animals need to save some of this energy for other survival tasks such as foraging and reproducing. Another risk for schooling is possible predation of animals by each other especially if one animal of the group appears injured or less viable in any way.

I conducted a behavioural experiment to gain insight into how basic mechanisms of injury-induced neuronal plasticity affect the short- and long-term behavioural responses in schooling squid. I tested to see if minor, peripheral injury altered the defensive behavioural responses of squids that form schools, if site-specific sensitization around an injury allowed for squids to "know" where they are injured, and if squids attempt to protect that vulnerable area by concealing it from aggressive conspecifics. My results show that injured squid do have a higher tendency to either avoid schooling or swim along the edges of school groups with their injury turned away from the school. In contrast, control and injured but non-sensitized squids are more likely to stay in the school group and closer towards the middle than injured squids.

Funding support by NSF grants IOS-1145478 and NSF DBI 1005378 REU: Biological Discovery in Woods Hole.

Great Egret and Snowy Egret population density and foraging habits in the Plum Island Sound and surrounding Marsh ecosystems

Wesley Clark¹, Robert Buchsbaum²

¹ Eckerd College, St. Petersburg, FL; ²Mass Audubon, Wenham, MA.

Great and snowy egrets are common aquatic birds within Plum Island Sound and adjacent estuaries. These birds commute daily to the Sound and adjacent sections of the Great Marsh from offshore nesting islands in order to feed. As part of the Plum Island Sound Long Term Ecological Research Project, we carried out weekly surveys at forty-seven points in order to evaluate how these two species use the different habitats within the estuary. The sites were chosen to include a variety of habitat types: tidal creeks, pannes, open water, and vegetated marsh. We recorded the number of birds at each point, their habitat, and whether they were feeding. Occasional repeated counts from the same site were carried out to determine short-term variability in counts and egret activity and the effect of tides. Typically, around 50 egrets of each species were recorded during these surveys, which represented about 25 percent of the egrets in the region based on nest surveys. Our results showed that egrets are opportunistic feeders. The most consistent feeding hot spots were shallow edges of larger tidal inlets. Their use of salt pannes and the vegetated marsh surface at high tide was variable, probably in response to variations in the amount of their prey in those habitats. However, the data indicates a trend towards the time in pannes being used mostly for foraging whereas time in the marsh is split equally between feeding and other behaviors. The research provides a baseline of current wading bird use of Plum Island Sound within the framework of anticipated change in marsh habitats due to sea level rise as well as a way in which to inform marsh reconstruction and rehabilitation.

Funding: WC funded by the NSF REU program through the Plum Island Ecosystem LTER project.

Synuclein Distribution in Regenerating Lamprey Spinal Cords

Julia Eisen¹, Stephanie Fogerson², Jennifer Morgan²

¹Barnard College, New York, NY; ²Eugene Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory, Woods Hole, MA.

Spinal cord injury causes permanent damage to neurons, leading to impairments of movements and sensations. It is therefore imperative to understand ways to improve axon regeneration and spinal cord repair. The sea lamprey, *Petromyzon marinus*, is a beneficial experimental vertebrate due to its ability to fully regenerate its spinal cord in just 11 weeks post injury and because of the presence of large, identified neurons. We recently found that after spinal cord injury, synuclein – a protein linked to Parkinson’s Disease – accumulates specifically in non-regenerating neurons in the brain. Based on these data, further experiments were pursued in order to study how spinal cord injury affects the levels of synuclein within the spinal cord itself. A pan-synuclein antibody raised against human α -synuclein was tested on control lamprey spinal cords and found to strongly stain neurites, most likely axons. In transected spinal cords (11 weeks post injury), immunofluorescence analysis revealed that synuclein expression decreased in locations both proximal and distal to the lesion site. Thus, unlike the brain’s response to injury in which synuclein appears to aggregate, in the spinal cord synuclein may not form such aggregates but expression is in fact decreased. Preliminary evidence shows that knockdown of synuclein using a morpholino further decreased synuclein expression in the spinal cord. Further experiments will be performed to study axonal and synaptic structures within the spinal cord in the presence and absence of synuclein knockdown. These data have implications for the mechanisms that underlie successful regeneration of the vertebrate central nervous system.

Funding support from the Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory.

Climate Imprint on Carbon Isotopic Signatures of Leaf Wax in Arctic Vegetation: Groundtruthing Paleoclimate Proxies

Tanner Cunningham¹, Maureen Conte², J.C. Weber²

¹Bates College, Lewiston, ME; ²Ecosystems Center, Marine Biological Laboratory, Woods Hole, MA.

This study aims to groundtruth paleo-environmental studies by examining modern day relationships between leaf waxes in plants and sediments and the effects of climate. Waxes are ablated from the leaf surface and distributed throughout the environment. Highly resistant to degradation and recording the molecular and isotopic signature of the vegetation, waxes are an excellent proxy for the plant community and climatic change. Dominant species of tundra vegetation (grasses, sedges and shrubs) were collected in 2003 & 2004 at Imnavait Creek, Alaska. In 2004, species were collected along a latitudinal transect. Waxes were extracted and isolated into component classes (n-alkanes, n-alcohols, and fatty acids). Alcohols were dominant, with unique distributions observed between genera. Wax of the sedge *Carex bigelowii* was predominantly C₂₈ fatty alcohol, while the evergreen *Cassiope tetragona* was primarily shorter chain C₂₂ & C₂₄. Molecular distributions within a given species were consistent along the transect. Waxes in surface sediments from Toolik and Fog2 Lakes had similar distributions to one another and were a mosaic reflecting the surrounding vegetation, making them excellent paleo-proxies capturing changes in tundra vegetation. Bulk carbon and compound-specific carbon within the wax classes covaried similarly between plant species. All tundra species had a typical C₃ isotopic signature, however differences were observed between species. *Salix pulchra* had alcohols that were up to 2‰ heavier, which could have implications in a warmer arctic climate where shrub abundance increases. No significant differences in the isotopic signature were observed along the transect; however, the signal was significantly heavier at Imnavait Creek for all of the species collected, possibly due to drier conditions. Heavier δC¹³ signatures were observed in 2004 compared with 2003 in all the Imnavait Creek species, likely the result of decreased precipitation and accompanying moisture stress causing the stomata to constrict, limiting carbon fractionation.

Funding Support: The Arthur Vining Davis Foundation.

Scanning Electron Microscopy Analysis of the "Plastisphere": Microbial Succession on Plastic Marine Debris

Jessica Fields¹, Kiera Saleem², Erik R. Zettler³, Linda A. Amaral-Zettler^{4,5}

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As global demands for plastic have increased to about 245 million tonnes over the past decades, plastics have become the most abundant type of marine debris and have been found in all five of the world's oceans. Most Plastic Marine Debris (PMD) consists of small pieces of polyethylene (PE), polypropylene (PP), or polystyrene (PS). These small pieces, known as microplastics, have dimensions smaller than 5mm. Although PMD's tendencies to both concentrate persistent organic pollutants and be ingested by marine animals have been well documented, studies of the microbial communities developing on the microplastic debris are only just beginning. Here, we sought to examine the effects of three parameters on the development of microbial biofilms on PMD: (1) The type of substrate on which the biofilm forms; (2) The type of marine environment in which the PMD exists, and; (3) The amount of time the debris has been immersed in a marine environment. We used Scanning Electron Microscopy (SEM) to examine samples of PE, PP, PS, as well as glass beads (GL) from parallel, year-long incubation experiments off the coast of Grenada and Woods Hole, MA. We systematically analyzed samples through the second week of each experiment, counting and cataloguing the types of microorganisms that initiate biofilm formation on the samples, as well as measuring the area each organism occupied on the plastic. This type of survey is key in the process of better understanding the microbial communities present on PMD, a necessary first step in assessing their broader environmental and health impacts.

Funding support: Brown-Marine Biological Laboratory LINK Partnership Award and a National Science Foundation Grant to E.R.Z. (OCE-1155379), and L.A.A-Z. (OCE-1155571).

Searching for the Missing Link in the Pathogenic Pathway of Mutant SOD1 in ALS

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Mutations in Superoxide Dismutase (SOD1) cause about 2% of cases of Amyotrophic Lateral Sclerosis (ALS). Like many other neurodegenerative diseases, ALS has a “dying back” neuropathy, indicating an impairment of axonal transport (AT). A well studied example is the misfolded SOD1 protein, G85R, which abnormally activates a Mitogen Activated Protein Kinase (MAPK) cascade ending in kinesin phosphorylation via p38 MAPK. Vesicle motility assays in squid giant axons, allowed us to explore this pathway further, attempting to find the putative link between SOD1 and the MAPK cascade. Using a Domain of Versatile Docking peptide (DVD) as a competitive inhibitor, we narrowed down the MKK to MKK3/MKK6. Significantly, the ASK1 inhibitor, NQDI, prevented the toxic effects of G85R on AT. Combined, this suggests the MKKK is Apoptosis signal-regulating kinase (ASK1). Accordingly, we began to explore the link between G85R and ASK1 by examining the effects of known upstream ASK1 regulators, Casein Kinase 1 (CK1), Akt, and Camodulin Kinase II (CAMKII), on axonal transport. Finding this missing link will aid in understanding the pathogenesis of ALS and shed light on potential therapeutic targets.

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Variation in *Z. marina* Morphology and Sediment Composition with Depth in West Falmouth Harbor, MA

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Zostera marina is a species of marine seagrass that contributes to nutrient cycling, sediment stabilization, primary production, and habitat in coastal ecosystems. Environmental factors, such as depth and light availability, have been shown to influence the morphology and distribution of *Z. marina* in deeper coastal ecosystems. We assessed *Z. marina* morphometry and sediment composition along a depth gradient ranging from 0.7 m to 2.5 m in West Falmouth Harbor, MA. As depth increased, plant density generally decreased. There was no relationship between areal plant biomass or areal blade area and depth. However, we observed an increase in individual plant biomass, average blade area per plant, and average maximum blade height with increasing depth. These results suggest that *Z. marina* resource distribution was depth-dependent at the study site. Light profiles obtained at the study site suggest that light was not a limiting factor concerning *Z. marina* growth. *Z. marina* morphometry measurements suggest that the plants adapted their growth strategy to maximize light intake to compensate for shading in deeper water. Sediment composition analyses revealed that bulk density tended to decrease and organic matter content tended to increase with depth. Increased *Z. marina* blade height and area may play a role in buffering the effects of water flow velocity, allowing for the stabilization of finer sediments and organic matter at increased depths. Our results show that trends in *Z. marina* morphology and sediment characteristics observed in systems with greater depth gradients may also be present in systems with lesser depth gradients. A better understanding of the variation in *Z. marina* morphometry and sediment composition will allow for an assessment of seagrass community heterogeneity in West Falmouth Harbor, which can be utilized to improve the scaling of *Z. marina* and sediment biogeochemical processes.

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From Cell to Satellite: Seasonality of internal leaf structure and relationship with leaf surface reflectance

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Leaf internal structure is one of the key components related to plant photosynthesis. The measurements of leaf reflectance in near infrared (NIR) provided a non-destructive way to estimate leaf internal structure. However, a robust relationship between leaf internal structure and leaf NIR reflectance throughout the season was not established. Understanding this relationship can help infer vegetation on a larger scale using near-surface cameras and space-borne satellites. Reflectance is the ratio measure of incoming light that hits a surface and the amount that reflects back to the atmosphere. In this study we measured the internal structure of the surface area of mesophyll cells per leaf area (Ames/A) of tree leaves and near-infrared NIR reflectance measurements throughout the growing season of year 2012. A spectroradiometer and integrating sphere for light sources were used to take reflectance measurements of sun-lit and shaded red oak (*Quercus rubra*) leaves spanning from May 2012-October 2012 in Harvard Forest, MA. Ames/A for each sample was calculated by analyzing images of leaf cross-sections under a light microscope and software ImageJ. Both Ames/A and NIR showed 'arched' patterns throughout the season. Ames/A and NIR are both significantly higher in sun-lit leaves than in shaded leaves ($p < 0.05$). There was a robust linear relationship between Ames/A and NIR ($r^2 = 0.5$). More data needs to be collected to see if this relationship is consistent throughout the season and across different species.

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Primary cell culture of teleost vocal motoneurons and an analysis of the extracellular matrix

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Vocalizations are hormone-mediated, context-dependent behaviors, which are controlled by distinct vocal control nuclei (VCN) within the vertebrate brain. Because of their relative simplicity, the vocal nuclei located in the hindbrain-spinal region of the toadfish, *Opsanus tau*, represent a robust model of a VCN. The output of this system, the vocal motor neurons (VMNs), are capable of producing remarkably high frequency vocalizations of up to 700 Hz. Obtaining electrophysiological recordings *in vivo* or in semi-intact preparations has proven difficult due to a pervasive extracellular matrix which limits direct access to the VMNs. We characterized the composition of the VMN extracellular matrix using antibodies against common structural components of perineuronal nets including chondroitin sulfate (CS-56; 1:1000), which were previously characterized in the song nucleus HVC of zebra finches. Substantial labeling of chondroitin sulfate within the VMN suggests the presence of a perineuronal net-like structure surrounding these cells.

Prior neurochemical studies showed extensive GABA-like input to the VMN and surrounding regions. We further examined the calcium-binding protein parvalbumin (PARV-19; 1:1000), which is characteristic of fast-spiking neurons, especially inhibitory interneurons. Small parvalbumin-positive cells were found on the dorsal surface of the spinal cord and to a lesser degree, just ventral to the VMN.

To further facilitate electrophysiological analysis of VMNs, we also developed an *in vitro* cell culture preparation. Fluorescently-labeled VMNs were dissected from longitudinal slices of the hindbrain-spinal cord, digested using a cold trypsinase enzyme cocktail and seeded onto laminin-containing substrata. VMNs were found to adhere within one week and showed little signs of extracellular matrix that had once been present. VMNs adhere almost selectively in the presence of estrogen-supplemented media suggesting the important role that hormones play in the survival of these neurons.

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A pathogenic pathway common to familial ALS forms of different genetic etiology

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Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease affecting function and survival of motor neurons. Most ALS cases are sporadic with no identified genetic defect, but 5-10% result from mutations in unrelated genes causing familial forms of ALS (FALS). Genes associated with FALS encode proteins of diverse structure and function, including superoxide dismutase 1 (SOD1; reduction of superoxide radicals and redox signaling), FUS, TDP-43, and others. In addition, most FALS cases result from an intronic repeat expansion in the *C9orf72* gene, which results in the production of GA, GR, and GP-rich peptides in affected patients. Loss of axonal connectivity and deficits in axonal transport (AT) represent pathogenic events common to all FALS forms, suggesting possible overlap of pathogenic pathways triggered by mutant proteins. Accordingly, perfusion of FALS-related SOD1 variants and a *C9orf72*-related peptide in squid axons similarly promotes reductions in anterograde, kinesin-dependent AT. However, mechanisms by which these unrelated proteins inhibit AT remained unknown. Here, we present biochemical evidence suggesting activation of a common pathogenic pathway triggered by mutant SOD1 variants and a *C9orf72*-related GP-rich peptide. This pathway involves abnormal activation of the p38 MAPK pathway within axons, in a manner independent of changes in gene transcription, protein aggregate formation, or deficits in ATP production. Immunoblots of squid axoplasms perfused with either mutant SOD1 or with a specific *C9orf72*-related peptide similarly showed increased activation of P38 and its upstream kinase MKK3/6, compared to control axons. Providing a basis for the inhibition of AT triggered by GP-rich peptide, our prior work showed that active P38 phosphorylates kinesin heavy chains, inhibiting conventional kinesin. Taken together, results from our studies indicate that, as observed with FALS-related SOD1 proteins, GP-rich peptides resulting from abnormal *C9orf72* translation inhibit AT through a mechanism involving abnormal activation of axonal P38.

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Calcium Increases in Poor Surviving Neurons after Spinal Cord Injury in Lampreys

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Spinal cord injury in humans is both permanent and costly, making it imperative to understand the factors that promote or prevent regeneration after injury. In the early vertebrate the sea lamprey, *Petromyzon marinus*, robust functional recovery of swimming behavior occurs within 10-12 weeks after a complete spinal cord transection. Despite the recovery of swimming behavior, some injured neurons survive and regenerate their axons, while others die. We therefore want to identify the molecular mechanisms that govern the fate of good surviving and poor surviving neurons. One potential mechanism may involve calcium because calcium plays vital roles in cell growth, gene expression, and cell death via apoptosis. To test this, we use Giant Reticulospinal Neurons (RS), which are located in stereotypical positions in the brain and can be easily visualized under a light microscope making them good candidates to study post-injury effects. To test whether misregulation of calcium could be a difference between good and poor surviving RS neurons, Oregon Green BAPTA, a calcium indicator, was used to stain the brains of uninjured control lampreys and those at 11-14 weeks after spinal cord injury. These same brains were then Nissl stained to identify healthy versus dying neurons. A healthy cell retains Nissl stain while unhealthy cells lose Nissl stain. Of the cells that lost Nissl stain, 74% were also brightly stained with Oregon Green BAPTA indicating that calcium is accumulating in poor surviving cells. Thus, calcium levels increase in poor surviving neurons after injury, suggesting that these cells are dying by apoptosis. These studies have implications for creating therapeutic treatments to enhance spinal cord regeneration.

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3D particle tracking to analyze predator-prey encounters in *Mnemiopsis leidyi*

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Mnemiopsis leidyi is a species of lobate ctenophore that has recently expanded its habitat from the North and South American Atlantic Coast to more distant areas including the Black and Mediterranean Seas. This has piqued interest in the identification of environmental factors that can encourage or discourage its invasion of a region. We examined how different swimming speeds, which may be induced by turbulence in the water, affect the organism's prey capture efficiency. Because of the bilateral symmetry of *Mnemiopsis*, 2D imaging is not a reliable technique; unlike an organism with radial symmetry, the fluid flow in one plane will likely be very different from that in another plane. Therefore we developed two techniques – digital in-line holography and video with multiple orthogonal views using mirrors – to be able to reliably track particles and small organisms in and around *Mnemiopsis*. Once the techniques were developed, we used the mirror method to analyze the variation of *Mnemiopsis*' prey capture efficiency with swimming speed by monitoring encounters between *Mnemiopsis* and *Artemia*, small brine shrimp that are typical prey for the ctenophore. It was found that capture efficiency did not significantly decrease with higher swimming speeds. Particle Image Velocimetry of the fluid flow inside *Mnemiopsis* at different swimming speeds might elucidate the reason for this.

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EVALUATING THE EFFECTS OF MUTANT FUS IN AXONAL TRANSPORT

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Amyotrophic Lateral Sclerosis (ALS) is a disease of motor neurons in the brain and spinal cord that control voluntary muscle movement. Approximately 5-10% of ALS cases are familial (FALS), transmitted as a dominant trait with variable age of onset. One of the recently discovered genes with mutations that cause FALS is FUS (*Fused in Sarcoma*), which encodes a nuclear polypeptide. In healthy individuals, wild-type FUS (WT-FUS) is found in the nucleus and is involved in DNA repair, RNA splicing, and transcription. In contrast, mutant forms of FUS associated with FALS are enriched in the cytoplasm. In the cytoplasm, mutant FUS forms are thought to contribute to FALS by a mechanism involving abnormal aggregation and recruitment of other proteins. However, a molecular basis underlying mutant FUS-induced neuronal toxicity remains unknown. Here, we investigated the effects of a various mutant FUS polypeptides on fast axonal transport (FAT) using vesicle motility assays in isolated squid axoplasm. The effects of different mutations were evaluated in both Anterograde and Retrograde directions using differential interference contrast microscopy. Remarkably, it was found that all FALS-related FUS proteins tested, but not WT-FUS, inhibit both directions of FAT. Significantly, a pharmacological inhibitor specific for the protein kinase p38 prevented the toxic effect of mutant FUS on FAT. Taken together, our data suggests that mutant forms of FUS associated with FALS inhibit FAT through a mechanism involving abnormal activation of axonal p38. Based on this results, p38 might represent a novel therapeutic target for FUS-related forms of FALS.

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Investigating the Variant Repeats of *C9ORF72* in ALS

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Hexanucleotide expansion (GGGGCC) in the *C9ORF72* gene is a prevalent mutation linked to both Familial Amyotrophic lateral sclerosis (ALS) and Frontotemporal Dementia (FTD), yet the mechanism of toxicity is poorly understood. Previous studies demonstrated Repeat Associated Non-ATG (RAN) translation of the expanded portion in the *C9ORF72* gene produces three variants of repeated copolymers: (glycine-proline)_n, (glycine-alanine)_n and (glycine-arginine)_n which were proposed to be associated with ALS pathology. One common feature of ALS is degeneration of motor neurons and inhibition of Fast Axonal Transport (FAT). Using the squid giant axon preparation we have observed inhibition of anterograde FAT. Therefore, to fully understand the toxicity of the hexanucleotide expansion, (GP)₈, (GA)₈ and (GR)₈ peptides were synthesized and individually perfused in the isolated giant axons of the squid model. The toxic variant of ALS would be expected to inhibit anterograde FAT. Of the three variants, (GP)₈ significantly slowed anterograde transport while the (GR)₈ and (GA)₈ peptides had no significant effect on transport. This suggests that the presence of the GP repeats may be connected to the pathology of *C9ORF72*-associated familial ALS. Furthermore, co-perfusion of an ASK1 inhibitor or a P38 inhibitor rescued the FAT defect caused by (GP)₈ alone. These inhibitors of various levels of the MAP kinase cascade, which further impair kinesin motor function and anterograde transport, suggest a molecular mechanism of GP-induced-toxicity.

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Mechanisms of Nutrient Availability in the Adaptive Plasticity of Anamniote Hatching

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The timing of hatching in anamniotes is a critical developmental event that is adaptively plastic in response to numerous environmental conditions. To elucidate the role of nutrient availability in this process, we took advantage of the well-defined phenotypes in zebrafish with genetic and nutritional differences in the metabolism of copper, a trace element essential for development. Treatment of developing embryos with the membrane permeable copper chelator neocuproine resulted in a dose-dependent decrease in the rate and percent of hatching at 96 hours post fertilization. This effect was abrogated by the addition of copper, replicated utilizing other copper chelating compounds and occurred at concentrations of drug without other alterations in copper homeostasis. Furthermore, this effect of neocuproine on the rate and percent of hatching was markedly increased in copper transport deficient mutant embryos when compared to wild-type siblings. A developmental window was identified between 16 and 32 hours post fertilization when copper is essential for hatching, suggesting that a specific copper-dependent enzymatic pathway is involved in the process. Peptidylglycine α -amidating monooxygenase is an evolutionarily conserved cuproenzyme essential for the active modification of several peptides required in neuroendocrine physiology. Consistent with a direct role for this cuproenzyme in the copper-dependent effect on hatching, antisense abrogation of peptidylglycine α -amidating monooxygenase activity resulted in a similar reduction in the rate and percent of hatching at 96 hours post fertilization. Importantly, this effect was further increased in a dose of neocuproine that alone was without impact on hatching. Expression of peptidylglycine α -amidating monooxygenase is confined to the ventral floor plate during this period and in all cases the development and morphology of the hatching gland is normal. Taken together, these studies reveal a critical role for copper availability in the adaptive plasticity of hatching and define a unique neuroendocrine mechanism mediating this process.

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What You Didn't Want to Know About Your Mouth: Visualizing the Structure of Oral Biofilms.

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Interactions between bacteria of the human oral microbiome can alter microbes' properties, such as their ability to survive in aerobic environments or invade host epithelial cells. Many of the recent findings concerning microbial community composition have come from sequencing, yet this method does not provide information on organisms' spatial relationships. We present a technique of biofilm visualization that allows direct analysis of the location of individual cells. To do this, we collected dental plaque samples from three donors and embedded them separately in plastic resin. Using 4 distinct probe sets consisting of 8 to 9 taxa specific probes which targeted different groups of bacteria, we hybridized adjacent sections of plaque using Fluorescence In Situ Hybridization (FISH) to determine which bacterial taxa were present. To provide greater confidence in identification of cells we used nested probe sets that contained general as well as highly specific probes. This meant that if certain bacteria were present, they would be labeled with two to three fluorophores. Fluorescence spectral images were acquired with a Zeiss LSM 780 confocal microscope and then processed using a linear unmixing algorithm to differentiate the signals from the different fluorophores. Using the data acquired, we were able to identify many of the bacteria in the plaque sections. One characteristic structure consisted of at least six distinct genera. We designed and employed a new probe set which targeted these in order to analyze the spatial organization of the cells in the biofilm. This and similar experiments are revealing clearly coordinated communities and previously unknown micron-scale taxon-taxon interactions.

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NQO1, NAD(P)H-dependent Cytosolic Oxidoreductase Modulates β -cell Redox Status and Insulin Secretion

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Redox status, defined as a ratio between the reduced-to-oxidized forms of redox couples (such as NADH-to-NAD⁺ and NADPH-to-NADP⁺), plays an important role in the overall health as well as in the glucose-stimulated insulin secretion (GSIS) from pancreatic β -cells. Here we have investigated the role of cytosolic NAD(P)H-dependent oxidoreductase NQO1 on the β -cell redox status and quinone-dependent production of reactive oxygen intermediates (ROI). In both clonal insulin secreting β -cells (INS-1 832/13) and isolated rodent islets, NQO1 over-expression blunted quinone-dependent ROI production, while NQO1 knockout islets had enhanced ROI formation. Furthermore, NQO1 has been found to decrease NAD(P)H-to-NAD(P)⁺ ratio, consistent with the NQO1-dependent utilization of NAD(P)H for the intrinsic Plasma Membrane Electron Transport activity in β -cells. Together, these data show that NQO1 plays an important role in maintaining proper redox status and maintains insulin secretion in the face of oxidative stress.

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