

2010 MBL Undergraduate Research Symposium Abstracts

Oral and poster presentations

**Complexities with Arsenic Exposure: How Concentration Affects Levels of Gene Expression, Specifically Those Regulated by Hormones and Endocrine Receptors.** *Chelsea Marian Connolly, Biology, Valdosta State University Dr. Fokko Zandbergen (Post Doc), Vansa Chatikavanij (Research Assistant), and Dr. Joshua Hamilton (Mentor), Marine Biological Laboratory*

Arsenic has been known as a toxin for centuries. It is found naturally in the environment and its distribution is widely present at low levels in food and water. Government standards set the allowable levels of As for drinking water at 10ppb (10uM/L). Previous studies have proven that As is an endocrine disruptor; that even at low levels below the standard, transcription of genes controlled by nuclear hormone receptors is affected, and at specific concentrations, an increase in transcription occurs. In Human Embryonic Kidney cells (HEKs), we tested a nuclear hormone receptor to see if HEKs elicit a similar response as shown in previous studies. As well, we imaged the treated HEKs to attempt to visually investigate the transport of the receptor from the cytosol to the nucleus under the conditions where low dose enhancement occurs. Through this method of exploration, we aim to get a better understanding about where and how As creates this enhancement.

**The Comparative Geography of Bacteria and Muco-polysaccharides in Mouse Intestines.** *Kurt M. Isaac-Elder, Dr. Jessica Mark-Welch, Yuko Hasegawa, & Dr. Gary Borisy.*

This study analyzed the relationship of mucus and microbiota in the mouse small and large intestine via three methods; 1. Use Alcian Blue as a general stain for mucus, examine distribution of mucus in sections of mouse gut. 2. Use wheat germ agglutinin (WGA) as a marker for the sugars N-acetylglucosamine and N-acetylneuraminic acid, and compare the distribution of bacteria and WGA in gnotobiotic mice. 3. Compare the relative abundance of *Bacteroides thetaiotaomicron* and *Eubacterium rectale*, in a dual colonized and a 15 taxon colonized mouse, in the large intestine, in areas with and without mucus. In this preliminary study no trends were found in the localization of bacteria relative to WGA staining.

**Testing the H<sup>+</sup> hypothesis of lateral inhibition: Confocal localization of the pH sensitive dye HAF in isolated retinal neurons.** SOPHIA E. BOOTH,<sup>1</sup> JASON JACOBY,<sup>2</sup> and ROBERT PAUL MALCHOW,<sup>2,3</sup>  
<sup>1</sup>*Department of Psychology, University of Colorado at Colorado Springs*<sup>2</sup>*Department of Biological Sciences, University of Illinois at Chicago*<sup>3</sup>*Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago.*

Alterations in extracellular H<sup>+</sup> concentration have been hypothesized to play a key role in mediating feedback inhibition from retinal horizontal cells onto vertebrate photoreceptors. According to the H<sup>+</sup> hypothesis of lateral feedback inhibition, depolarized horizontal cells release H<sup>+</sup>, which bind to calcium channels on photoreceptor synaptic terminals and act to shift the voltage activation curve of the calcium channels to more depolarized levels and reduce the magnitude of calcium influx. This hypothesis predicts that depolarization of isolated horizontal cells should lead to an extracellular acidification adjacent to the extracellular membrane of horizontal cells. Experiments to test this hypothesis have resulted in conflicting findings. Jouhou et al. (2007) claim an extracellular acidification induced by either glutamate or depolarization by potassium of isolated carp and goldfish horizontal cells as measured using the pH-sensitive fluorescent indicator HAF. However, Molina et al. (2004) and Kreitzer et al. (2007) report an extracellular alkalinization induced by glutamate and depolarization by potassium from skate and catfish horizontal cells measured using self-referencing H<sup>+</sup>-selective microelectrodes. In the present study, we have used confocal microscopy to examine the cellular distribution of the pH-sensitive dye HAF in neurons isolated from the retinae of goldfish and catfish. Our data indicate that HAF is not localized exclusively to the external face of the plasma membrane of isolated retinal horizontal and bipolar cells. Rather, HAF staining is detected throughout these cells. Further, HAF staining of catfish cone horizontal cells co-localized in part with stains that are specific for mitochondria. The presence of intracellular HAF makes it likely that this pH-sensitive fluorescent dye will report the intracellular acidification known to occur in horizontal cells when activated by extracellular glutamate (cf. Dixon & Copenhagen, 1993). We suggest that the acidifications reported by Jouhou et al. (2007) using the dye HAF may well represent changes in intracellular acid levels rather than extracellular alterations in H<sup>+</sup> concentration.

**Do cells in the Magnocellular Octaval Nucleus respond to auditory frequencies?** *Solymar Rivera Matos (University of Puerto Rico – Cayey) & Peggys Edds-Walton (MBL).*

The magnocellular nucleus (magno) is one of five octaval nuclei that receive input from the three end-organs of the ear that may be involved in hearing. In most fishes, the saccule is the major source of auditory input. In toadfish, the saccule responds well to frequencies in the sounds produced by male toadfish to attract females to the nest, as well as lower frequencies (50 – 303 Hz). The saccule sends afferents to all five of the octaval nuclei, including magno, but magno does not send projections to higher auditory centers of the brain. Magno has been divided into three subdivisions (M1,2,3) based on cell morphologies and inputs. We asked whether cells in one or more of the divisions of magno respond to auditory frequencies. We conducted extracellular recordings in magno and injected neurobiotin into successful recording sites to confirm location and label local cell types. Sinusoidal particle motion stimuli (50 – 303 Hz) were presented at multiple stimulus levels to assess sensitivity. Our data indicate that cells in M2 and M3 respond to auditory frequencies at biologically relevant levels, with a similar frequency response distribution to cells in the descending octaval nucleus, which is the primary contributor to the ascending auditory circuit in toadfish. We suggest that magno may be involved in spinal-cord mediated, reflex responses to sound; however, many attempts to record in the divisions of magno were negative, indicating that only a subset of cells are auditory.

**Papillae expression in *Sepia officinalis*.** Kelly Harrington<sup>1</sup> Mentors: Justine J. Allen<sup>2,3</sup> and Dr. Roger T. Hanlon<sup>2,1</sup> Bridgewater State University, Bridgewater, Massachusetts<sup>2</sup> Marine Biological Laboratory, Woods Hole, Massachusetts<sup>3</sup> Brown University, Providence, Rhode Island

Coleiid cephalopods are known for their ability to rapidly and adaptively camouflage in a diversity of visual environments. Color change is mediated by chromatophore organs and reflective elements in the skin. Octopus and cuttlefish are also able to change the texture of their skin by adaptively expressing papillae. In *Sepia officinalis*, papillae of different sizes and shapes cover the dorsal and lateral surfaces of the mantle, eyes and arms. Cuttlefish were tested in the presence of foam cones with different degrees of lateral “spikiness” to simulate natural 3D objects. Three sets of papillae, the Major lateral mantle papillae (MLMP), the Major lateral eye papillae (MLEP), and the Posterior mantle tip papilla (PMTP) were measured to test whether these papillae are evoked in response to pronged objects in the visual field. The three foam cones with projections elicited stronger expression of MLMP when compared to the controls. The MLEP were most strongly expressed in the presence of the cone with the fewest prongs. The PMTP were more commonly expressed when cuttlefish were in the presence of the spiky objects. Determining the cues that govern papillae erection can assist in the study of visual perception for quick, adaptive camouflage and may give us clues as to how papillae contribute to camouflage in nature.

**Sphingomyelin inhibits fast axonal transport.** Shavonn Smith (Ursinus College) & Scott Brady (University of Illinois Chicago).

Lysosomal storage diseases (LSDs) are a heterogeneous group of inherited diseases, caused by the malfunction of one of the lysosomal hydrolases, the enzymes responsible for the degradation of specific substrates inside lysosomes, resulting in the accumulation of undigested substrates in the lysosomes. This results in the malfunction of the entire lysosomal system. Although each LSD has a distinct clinical picture, several LSDs are characterized by neurological impairment. Among these, the infantile form of Niemann-Pick disease (NPD) is caused by the deficiency of the sphingomyelin-degrading enzyme acidic sphingomyelinase (ASM). Lack of ASM results in accumulation of the sphingolipid sphingomyelin in the cells of the nervous system and of several peripheral organs, like the heart and the lungs. The loss of ASM is believed to lead to neurodegeneration in NPD patients and this, in turn, leads to the death of the patient within 3 years. Although the role of sphingomyelin in membrane composition and trafficking has been studied, little is known about the link between its accumulation and the neurodegeneration. In this work, we hypothesize that sphingomyelin directly affects axonal transport. Since neurons deeply rely on the transport of molecular constituents along their processes, the interference of sphingomyelin on the transport of vesicles along neurons might explain the severe degeneration observed in NPD patients. By performing vesicle motility assays in isolated squid axoplasm, we show that sphingomyelin inhibits fast axonal transport. Importantly, this effect can be pharmacologically prevented with SB203580, an inhibitor of the MAP Kinase cascade, indicating that sphingomyelin affects axonal transport through the activation of one of these kinases.

**Plasma membrane electron transport in the insulin secreting pancreatic  $\beta$ -cell line INS-1 832/13**  
*Timothy Eisen<sup>1</sup>, Joshua P. Gray<sup>2</sup>, Emma Heart<sup>3</sup>* <sup>1</sup>*Department of Chemistry, Brown University, Providence, RI;* <sup>2</sup>*Department of Science, US Coast Guard Academy, New London, CT;* <sup>3</sup>*Cellular Dynamics Program, Marine Biological Laboratory, Woods Hole, MA*

Plasma membrane electron transport (PMET) is a ubiquitous system that oxidizes NAD(P)H and passes electrons to extracellular targets. Here we demonstrate the presence of robust glucose-dependent PMET activity in the insulin secreting pancreatic  $\beta$ -cell line INS-1 832/13, as assessed by the reduction of the plasma membrane impermeable dyes WST-1 and ferricyanide. PMET activity was several fold higher at concentrations of glucose that stimulated insulin secretion (10 mM) when compared to basal (non-stimulatory concentrations (2 mM)). The mitochondrial inhibitors rotenone, antimycin A and potassium cyanide elevated basal PMET activity. Both basal as well as glucose-stimulated PMET activity were greatly enhanced by the application of aminooxyacetate (AOA), an inhibitor of the malate-aspartate shuttle, suggesting that PMET plays an important role in reoxidation of NADH in  $\beta$ -cells and is enhanced in response to mitochondrial dysfunction.

Dicoumarol, an inhibitor of the PMET cytosolic enzyme NAD(P)H:quinone oxidoreductase (NQO1), abolished glucose-dependent PMET activity. In parallel, over-expression of NQO1 elevated PMET activity, arguing for the crucial role of NQO1 in the PMET pathway of INS-1 832/13 cells. Glucose-stimulated PMET activity was inhibited by the application of the flavoprotein inhibitor diphenylene iodonium (DPI) and various antioxidants, but enhanced by the application of the redox cyler menadione, suggesting that the generation of reactive oxygen intermediates (ROI) is an important function of the PMET pathway.

**Validating *Hermisenda* as an Alzheimer's model.** *Cassandra Childs<sup>1</sup>, George Bell<sup>2</sup>, and Alan Kuzirian<sup>2</sup>* <sup>1</sup>*Wittenberg University, Springfield, OH 45501;* <sup>2</sup>*Marine Biological Laboratory at Woods Hole, MA 02543.*

Amyloid precursor protein (APP), through its proteolysis, forms beta amyloid, which increases susceptibility in humans to Alzheimer's Disease (AD). Beta amyloid is also the primary component of plaques found on the brains of Alzheimer's patients. *Hermisenda crassicornis* has been well studied as a model organism for associative learning and also experimental pharmacology linked to AD. To increase the validity and use of *Hermisenda* as a model organism for AD, it would be beneficial to determine not only the presence of APP but also an age index for the animal. Gonad indices using weight and surface area indices were obtained and regression lines calculated. Brains from trained and non-trained animals were sectioned and labeled with rabbit polyclonal primary antibody for APP.  $R^2$  values for gonad indices of pre-ovipositional animals were larger than post-ovipositional animals (categorized by number of egg masses laid). Gonad indices were consistent between weight and surface area methods, and are reliable predictors of age for *Hermisenda*. Immunopositive staining of brain sections indicated the presence of APP in the axonal hillock region and extending through the axons in neurons in all major ganglia. APP was present in both trained and non-trained animals. Age differences in learning acquisition and memory retention can now be documented using gonad indices, and further research linked to Alzheimer's Disease and the role of amyloid precursor protein may be done using *Hermisenda* as a biomedical model organism.

**Morphological Examination of Toadfish Cirri.** *Beth Giuffrida (Wareham Middle School), Katherine Sipper (Northern Michigan University), Samantha Lindemann, and Allen F. Mensinger (University of Minnesota, Duluth).*

The oyster toadfish, *Opsanus tau*, has been used in a wide variety of research studies. Numerous fleshy appendages, or cirri, project from the head of the fish. However, the functional significance of the cirri remains unknown. Morphological and behavioral studies were initiated to determine the functional significance of the appendages. Cirri were obtained from adult toadfish, fixed, sectioned and stained with haematoxylin/eosin. Morphological investigations showed that mandibular cirri have high density of sensory cells which strongly resemble extraoral taste buds found in other species of fish, while extraocular cirri have few if any sensory cells. The high density of taste buds is consistent with the mandibular cirri's proximity to the oral cavity and we propose the cirri function in gustatory analysis.

**Small molecule Bcl-xL inhibitor prevents long term potentiation: Hippocampal slice electrophysiology.** Allan M. Augillard<sup>1</sup>, Kambiz N. Alavian<sup>2</sup>, Hongmei Li<sup>2</sup>, Jason Shepherd<sup>3</sup>, Elizabeth A. Jonas<sup>2</sup> <sup>1</sup>Xavier University Louisiana, LA <sup>2</sup>Intrnl. Med. Endocrinology Yale University School of Medicine, New Haven, CT <sup>3</sup>Picower Institute for Learning and Memory, MIT, Cambridge, MA

Recent studies have emphasized the importance of mitochondria in synaptic function. Increased expression of the mitochondrial protein Bcl-xL increases mitochondrial localization to synapses and improves mitochondrial metabolism, correlated with the enlargement of presynaptic vesicle clusters, an increase in postsynaptic density proteins, and an increase in the frequency and amplitude of spontaneous synaptic transmission. Elevated spontaneous transmission could be caused by increased plasma membrane targeting of GluR1 subunits in the Bcl-xL expressing cells and this was found to be the case by immunohistochemistry and quantitative fluorescence measurements. In contrast, inhibition of Bcl-xL expression or function decreases synapse formation. One form of plasticity that has been well-studied in the hippocampus is the phenomenon of long term potentiation (LTP) of postsynaptic responses in the CA1 region. To study the role of Bcl-xL in the expression of LTP, we applied the specific chemical inhibitor ABT-737 to rat hippocampal slices before and after high frequency (theta burst) stimulation while recording field postsynaptic potentials (fPSPs). While vehicle-treated control slices showed sustained LTP, slices from the same rat brain that were treated with the Bcl-xL inhibitor failed to manifest LTP. We suggest that Bcl-xL is required for LTP, and that, after theta burst stimulation, a long term metabolic change occurs to synaptic mitochondria.

**Keywords:** Bcl-xL, LTP, Hippocampus, ABT-737, Response

Abbreviations used: Bcl-xL, B-cell lymphoma-extra large; LTP, Long Term Potentiation; fPSP, field postsynaptic potentials; GluR1, glutamate receptor type 1

Poster (only) Presentations

**Primary productivity changes of *Enteromorpha intestinalis* (gutweed) in nutrient enriched salt marsh creeks.** Sam Safran, Austin Ritter & Sallie Sheldon. (Middlebury College).

*Enteromorpha intestinalis* (gutweed) is the primary species of macroalga found within the creeks of salt marsh ecosystems. It is an opportunistic green alga and a known bloom-forming species. We altered whole salt marsh creeks by addition of nutrients (N and P; 15-fold increase over ambient conditions) directly to the flooding tide and sought to determine the effects of this enrichment on *E. intestinalis* productivity. Algae from fertilized and reference creek-walls were collected and their gross primary productivity (GPP), net primary productivity (NPP), and respiration measured using the light and dark bottle dissolved oxygen method.

While significantly higher levels of macroalgal GPP were found in one fertilized creek ( $n = 5$ ,  $p = 0.004$ ) these results become only marginally significant when corrected for differences in phytoplankton productivity ( $p = 0.159$ ). Similarly, while marginally significant increases in phytoplankton-corrected *E. intestinalis* GPP were found in a second fertilized creek ( $n = 5$ ,  $p = 0.115$ ), it is possible that this is simply an artifact of increased microbial respiration in the fertilized-creek ecosystem. We suspect that with additional replication and slight adjustments of methodology as the summer ends, we will find more substantial evidence of increased macro-algal productivity under enriched conditions.

**Response of primary producers in salt marsh ponds to nutrient enrichment: part of a whole-ecosystem experiment** Austin Ritter<sup>1</sup>, Sam Safran<sup>1</sup>, Sallie Sheldon<sup>1</sup>, Kate Morkeski<sup>2</sup> <sup>1</sup>Middlebury College, <sup>2</sup>MBL

We examined the effects of chronic eutrophication on primary production in salt marsh ponds as part of a larger, long term, whole-ecosystem manipulation in which nutrients were added to flooding water of two tidal creeks throughout the growing season. Nitrate and phosphate were elevated to concentrations approximately 15x ambient throughout the growing season and 30x ambient for 3 d during this experiment. We measured percent cover, gross primary productivity (GPP), and rate of colonization in order to examine the response of algae and the vascular aquatic plant, *Rupia maritima*, to nutrient additions. Preliminary results from two nutrient enriched ponds and two reference (unfertilized) ponds are presented here. We found that the nutrient enriched ponds had larger algal mats, more epiphytic algae per gram of *R. maritima*, higher GPP per gram of *R. maritima* (without removing epiphytes), and faster rates of algal colonization than reference ponds. Further, we found that spring tides, which repeatedly flooded the ponds over a 7 d period, stimulated the rate of algal colonization in nutrient enriched ponds, but not in reference ponds. We did not observe higher percent cover of *R. maritima* in nutrient enrichment ponds, possibly due to competition with algae for light. These results indicate that chronic nutrient loading to salt marshes stimulates algal growth and production in salt marsh ponds and that vascular plants in salt marsh ponds may be directly affected to a lesser degree.

Oral Presentations

**Morphological and electrophysiological development of peripheral glial cells in the olfactory nerve of the moth *Manduca sexta*.** Koussa MA, Hayashi JH, Tolbert LP, Oland LA (University of Arizona)

Two main classes of glial cells invest the olfactory pathway of the moth *Manduca sexta*: (1) central glia associated with the olfactory (antennal) lobe neuropil and with an axon sorting region in the most proximal part of the olfactory nerve, and (2) peripheral glia that migrate from the sensory epithelium into the nerve and enwrap bundles of olfactory receptor axons in the adult antennal nerve. The current work focuses on the peripheral glial cells. Developmentally, these glial cells migrate into the nerve following the olfactory receptor axons and during the period in which the axons become electrically active. We have begun to examine their development by recording the whole-cell currents of developing peripheral glial cells using protocols previously developed by Lohr et al. (2001, 2002) for recording from central glial cells. Whole-cell recordings were made from peripheral glial cells *in situ* from holding potentials of -50, -70 and -90 mV. Perfusion solutions with combinations of K<sup>+</sup>-free, Ca<sup>2+</sup>-free, Ba<sup>2+</sup>, Cd<sup>2+</sup>, 4-aminopyridine, and tetraethylammonium added to the pipette solution, revealed outward K<sup>+</sup> currents and a slow inward Ca<sup>2+</sup> current. The presence of this Ca<sup>2+</sup> current is consistent with previous experiments *in situ* and *in vivo* (Hartl et al. (2007) that showed that peripheral glial cells at these stages responded to activation of the receptor axons with changes in the calcium concentration. The current profiles recorded at different stages during the period of axon ingrowth were generally similar although we detected considerable variance that may be dependent on whether the recorded glial cell was dividing, migrating, or stationary. The extensive dye coupling at early stages of axon ingrowth decreased dramatically by the end of this period. [Lohr C et al. (2001) *Glia* 36:309-20; Lohr et al. (2002) *J Neurobiol* 52:85-98; Hartl et al. (2007) *Eur J NSci* 25:945-56]

**Short and Long Term Effects of Nitrate Enrichment Yield Different Effects on Greenhouse Gas Production in Salt Marshes.** Kelsey Fisher, Serena Moseman<sup>1</sup>, Kevin Kroeger, Sandy Baldwin, Wally Brooks, and Adrian Green<sup>2</sup> <sup>1</sup>Boston College, Chestnut Hill, MA <sup>2</sup>USGS Coastal Marine Science Center, Woods Hole, MA

Though coastal wetlands are thought to be sinks for carbon, the effects of fertilization on the greenhouse gas source or sink nature of the wetlands are unclear. An increase in denitrification, the process that produces nitrous oxide, is common in response to increased fertilization and nutrient loading. Short term nitrate additions in small plots yielded significantly greater production of nitrous oxide over that of control plots in a *Spartina patens* marsh at Plum Island in Rowley, MA ( $t_3=-2.57$ ,  $p=.04$ ). To test the effects of persistent, whole-creek scale nutrient loading on a *Spartina patens* salt marsh ecosystem, we examined the differences in carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), and nitrous oxide (N<sub>2</sub>O) fluxes between two creeks that are a part of the TIDE project, Sweeney Creek and West Creek in Plum Island, MA, using the static chamber technique. Sweeney Creek has been fertilized with nitrate since 2004, with West Creek serving as the control. Fluxes were measured in mid July 2010 prior to, during, and one day following an experimental doubling of fertilization levels in Sweeney Creek. Surprisingly, the N<sub>2</sub>O fluxes did not differ between creeks; however, average CO<sub>2</sub> fluxes in Sweeney were double those in West ( $t_7=4.08$ ,  $p=.005$ ). No differences were found in CH<sub>4</sub> fluxes. As a next step to further examine the effects of different types of anthropogenic modification on salt marsh activity, we are examining the effects of land use change and restoration on greenhouse gas fluxes as well as denitrification potential. This analysis is currently in progress, and preliminary results will be presented.

**Effects of Biodiversity on Detritus Processing in Salt Marsh Ecosystems.** Ashley Mui<sup>1</sup>, Meghan Short<sup>2</sup>, David S. Johnson<sup>3</sup>, and Linda A. Deegan<sup>3</sup>

<sup>1</sup>University of North Carolina at Chapel Hill, <sup>2</sup>Brown University, <sup>3</sup>Marine Biological Laboratory.

Given the scarcity of grazers in salt marshes, nearly all vegetation is fated to become detritus. Detritus represents the basis of the food web in salt marsh ecosystems, making its decomposition a vital factor in marsh ecosystem functioning. Detritivores, therefore, play a prominent ecological role in salt marshes. How large their impact is may depend on the palatability and nutritional content of the plant matter on which they feed. We studied the effect of detritivore biodiversity on the processing of the most abundant marsh grass, *Spartina patens*, which was harvested from both a fertilized marsh and a reference marsh. Petri dishes containing 5g dry weight of either nitrate enriched or non enriched *Spartina* detritus acted as mesocosms. Biodiversity was manipulated within these mesocosms by creating monocultures, bicultures, and tricultures of three major marsh detritivores: the coffee bean snail *Melampus bidentatus*, the amphipod *Orchestia grillus*, and the isopod *Philoscia vittata*. At the end of four weeks, the amount of litter processing in each mesocosm was examined by measuring communitation, *Spartina* mass loss, and change in microbial respiration. Communitation was quantified by the mass of small grass particles formed from the original 4-cm strands placed into each dish. We expect that litter processing will be greatest in the most diverse mesocosms, those containing all three detritivores, and that enriched litter will experience more processing than reference litter because of its increased nutritional content.

**Nutrient and Detritivore Effects on Decomposition of *Spartina Patens* litter in a Salt Marsh Ecosystem** Meghan Short<sup>1</sup>, Ashley Mui<sup>2</sup>, David S. Johnson<sup>3</sup>, Linda A. Deegan<sup>3</sup>

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Salt marshes are highly productive ecosystems with relatively few consumers of live plant material, making decomposition of plant detritus a crucial process in recycling nutrients. *Spartina patens* decomposition is especially important because of the abundance of *S. patens*—in our study site, *S. patens* covers approximately 80% of the overall marsh area. This study examines the effect of two interacting factors, nutrient availability and abundance of a major detritivore, *Melampus bidentatus*, on *Spartina patens* litter decomposition. Nitrate addition, as well as higher abundance of *M. bidentatus*, was expected to stimulate microbial activity and accelerate litter processing, with possible interactions between these effects. Litter bags containing 15 g dried *S. patens* detritus and various levels of *M. bidentatus* snails were deployed for 5 and 10 week intervals at creeks with reference and elevated nitrate levels. Litter processing was subsequently evaluated based on change in mass, microbial respiration, communitation, and nitrogen content. Preliminary data suggest that nutrient availability and detritivore control are both important factors in *S. patens*

**An Examination of Creek Bank Deterioration in Fertilized Plum Island Sound Salt Marsh Creeks**

Ariella Cohen, Erik Yando, Chris Haight & Scott Warren Connecticut College.

The effects of nutrient enrichment in salt marshes have recently become an area of heightened interest. Salt marshes are traditionally believed to be nearly limitless sinks for nutrients, thus providing a buffer for estuarine waters. The TIDE project (Trophic Cascades and Interacting Control Processes in a Detritus-based Aquatic Ecosystem) attempts to test one hypothesis implied by this idea: that nutrient enrichment does not have a negative impact on salt marshes. TIDE has been artificially eutrofying two salt marsh creeks (50 – 70  $\mu$ M NO<sub>3</sub>, ca. 10X background) since 2004. Intertidal creek banks, dominated by *Spartina alterniflora*, appear to be deteriorating structurally in fertilized creeks relative to the control creeks. The objectives of our work were to quantify the observed deterioration. In fertilized creeks there was a significantly greater quantity of creek bank cracks and bare mud in comparison to the controls. We conclude that fertilization has potentially contributed to the deterioration of the intertidal creek bank in fertilized sites at a much faster and extensive rate than the control creeks.

**An examination of below ground biomass in fertilized *Spartina alterniflora* communities:  
Determining a mechanism for creek bank deterioration.** Erik Yando, Ariella Cohen, Erik Yando  
Chris Haight, & Scott Warren Connecticut College

The TIDE project (Trophic Cascades and Interacting Control Processes in a Detritus-based Aquatic Ecosystem) attempts to investigate the effects of nutrient enrichment on salt marshes in order to better understand the traditionally believed buffering capabilities of these marshes. Since 2004, TIDE has been artificially fertilizing two salt marsh creeks in Plum Island Sound, Massachusetts (50 – 70  $\mu\text{M}$   $\text{NO}_3$ , ca. 10X background). One of the general aims of the project is to test whether eutrophication of a salt marsh ecosystem affects its ecosystem services. The objective of this study was to test the hypothesis that fertilization weakens peat structure. Twenty peat cores were analyzed from fertilized and control creeks. Factors compared were live and dead belowground biomass, bulk density and water content. Live biomass was significantly greater in control sites. There were no differences in dead biomass. Peat water content was significantly greater in cores from fertilized creeks and bulk density was greater in the cores from control creeks. We conclude that fertilization may be contributing to the decreased peat structural integrity by decreasing live roots and rhizomes and increasing water content.

**MICROBIAL DIVERSITY AT THE VIRGINIA COAST RESERVE LONG TERM  
ECOLOGICAL RESEARCH SITE:** Katelyn Giangregorio & Linda Amaral-Zettler Laboratory, Bay  
Paul Center

Microbes inhabit all parts of the Earth's biosphere and are critical in supporting our ecosystem through nutrient cycling and climate regulation. Because of their rapid growth cycles and environmental sensitivity, marine microbes can be ideal early biological indicators of large-scale ecosystem changes such as sea level rise and global temperature fluctuation. Microorganisms are some of the least understood forms of life on our planet and the study of their diversity and dynamics provides insight into the complexities of species interactions and the relationship between community-level changes and ecosystem dynamics. The MIRADA (Microbial Inventory Research Across Diverse Aquatic LTERs) project is a biodiversity survey study in which baseline diversity and relative abundance of bacterial, archaeal and eukaryal taxa were measured from 13 distinct fresh water and marine LTER sites. As one part of this survey, the Virginia Coast Reserve site is a coastal barrier ecosystem that spans a transect from an inland marsh stream to an oceanic inlet. Eight replicate water samples were taken from four key stations across winter and summer months, and the resulting small subunit ribosomal RNA was analyzed using 454 pyrosequencing. This recently developed high-throughput sequencing technique has opened new frontiers in examining microbes in marine environments and has given us the ability to deeply study microbial communities and the potential to discover novel species. Statistical techniques to examine microbial abundance data is lacking in the field of microbial ecology due in part to the specialized nature of the gene sequence data and subsequent analyses. Recently developed specialized statistical software provided the necessary tools for estimating species richness, the number of microbial species present in a sample. Other mathematical visualization techniques show the varying structure and distribution of microbial species in different physical and chemical environments. Our data analysis thus far has indicated that within the Virginia Coast Reserve there are temporal and spatial trends including seasonal fluxes of organisms and a diversity of species at differing geographical locations.