

2019 Undergraduate Research Abstracts

Seasonal changes in male Oyster Toadfish in response to sound

Nora J. Curtis, Alayna G. Mackiewicz, Rosalyn L. Putland, and Allen F. Mensinger

University of Minnesota Duluth, Duluth MN, Marine Biological Laboratory, Woods Hole MA,

During the mating season, male Oyster Toadfish (*Opsanus tau*) establish nesting sites and produce courtship calls called boatwhistles to attract females. The boatwhistles consist of a broadband grant followed by a longer tonal segment and can be generated continuously from mid-May to mid-August. The fundamental frequency of the tonal portion of the call is correlated with water temperature; it is thought that females are attracted to this portion of the call. Females must localize the call to find the nest and deposit the eggs which the male guard throughout development. Males also will produce short grunts to jam the tonal portion of rival males' calls. The toadfish population in Eel Pond was monitored acoustically throughout the mating season using a linear hydrophone array. Throughout the mating season, pure tones and boatwhistle calls were broadcast using an underwater speaker at four different frequencies (150 Hz, 175 Hz, 200 Hz, and 225 Hz) to determine the effect of extraneous sound on the number and timing of toadfish calls. Toadfish were more responsive to lower frequency sounds (150 Hz) early in the season when the water was 19°C. However, as the water temperature increased to 22°C, they shifted their responses to higher frequencies (200 Hz and 225 Hz). The results show that boatwhistle production will increase in response to both pure tone and boatwhistle playback. Additionally, as the fundamental frequency of their own calls increase with rising water temperature, so does their response to higher frequency calls.

Funding by Biological Discovery in Woods Hole at the Marine Biological Laboratory - NSF REU Award # 1659604

Unlocking the role of the symbiotic community in the calcification process of *Astrangia poculata*

Zoe Dellaert^{1,2}, Loretta Roberson²

¹University of Chicago, Chicago, IL, ²Marine Biological Laboratory, Woods Hole, MA.

Calcification is the process by which reef-building corals create their skeletons. This mechanism is still not wholly understood. Symbiotic and aposymbiotic colonies of *Astrangia poculata* were reared in 15°C, 27°C, or ambient conditions. Scanning electron microscopy (SEM) was used to describe how these physiological and environmental conditions impact skeletal structure. Buoyant weight data over time revealed that symbiont state and temperature both significantly affect growth rates. SEM of *A. poculata* skeletons revealed that aposymbiotic colonies appear to have a lower density of calcium carbonate at growing septal spines. Quantitative analysis of roughness of septal spines revealed that aposymbiotic colonies have a rougher surface texture than symbiotic colonies. This roughness trend is strongest in the colonies reared at 27°C, which were also the fastest growing colonies. Subsequently, scanning electron microscopy was used to examine the calicoblastic ectoderm of *A. poculata*. Initial results reveal that calicoblastic cells appear to form a fine mesh across the skeleton. SEM of both the skeleton and tissue revealed a pervasive presence of bioeroders in the skeleton. Light sheet microscopy using the L-SPI Single Plane Illumination system was used to confirm and characterize this community of bioeroders. Finally, we studied skeletons of the tropical corals *Porites astreoides* and *Acropora cervicornis* to understand how *A. poculata* skeletal structure compares to its tropical counterparts. Few studies have examined the skeleton of *A. poculata* or corals in general using SEM. These results unlock new insights into the skeletons of temperate corals and the associated community.

Jeff Metcalf Summer Undergraduate Research Fellowship – The University of Chicago

A New Ligase (& Nuclease): Studying Ligase K in Bdelloid Rotifers

Hannah Burd^{1,2}, Bette Hecox-Lea², Anna Maglio², David Mark Welch²

¹Dartmouth College, Hanover NH; ²Marine Biological Laboratory, Woods Hole MA

Bdelloid rotifers are asexual microinvertebrates found world-wide in aquatic and terrestrial habitats. While simple with a complexity comparable to nematodes, they are resistant to radiation and can survive prolonged periods of desiccation (extreme water loss) by entering a dormant stage called anhydrobiosis during which the rotifer shrivels up and ceases metabolic activity. One gene that may contribute to desiccation recovery is Ligase K (LigK), a gene acquired through horizontal gene transfer with structural similarities to kinetoplastid kDNA ligase. A previous RNASeq study found LigK expression upregulated in desiccation recovery in the bdelloid, *Adineta vaga*. I aimed to characterize the LigK protein from *A. vaga*. *A. vaga* LigK is predicted to localize to the nucleus, and has two zinc-finger domains, which have been previously observed to act cooperatively in other proteins. However, the specific zinc fingers observed in Ligase K have (PAR)-binding zinc finger (PBZ) domains. The PBZ region is associated with endonuclease and exonuclease functions. Thus, Ligase K may also be able to cut ends of DNA that become damaged in the desiccation process, removing blocks to synthesis or ligation. I isolated the gene from *A. vaga*, transformed *E. coli* to express the protein, purified the protein for testing against the most commonly used DNA Ligase, T4 DNA Ligase, and screened for nuclease activity.

Dartmouth College E.E. Just Program

The impacts of temperature changes on the coral *Astrangia poculata*

Celina Ceballos^{1,2}, Mayra Sánchez-García², Loretta Roberson²

¹Eckerd College, St. Petersburg, FL, ²Marine Biological Laboratory, Woods Hole, MA

With fluctuating temperatures due to climate change, corals worldwide are experiencing massive coral bleaching events. Corals lose the symbiotic relationship they have with the dinoflagellate *Symbiodinium* sp. that provide energy, and eventually starve and die. *Astrangia poculata*, a wide ranging coral species, can tolerate large temperature fluctuations unlike their more tropical cousins. They naturally exist in both a symbiotic state with zooxanthellae and aposymbiotic state without zooxanthellae. This project analyzes the physiological attributes of *A. poculata* at warm, cold, and changing temperatures to determine how these corals adapt to temperature fluctuations of the climate which can help research in the conservation of coral species that are suffering from climate change. We measured critical parameters (buoyant weight, photosynthetic yield, and density of symbionts) before and after a temperature change. This experiment showed, despite some exceptions, an overall decrease in the weight, photosynthetic yield, and symbiont density after a temperature change, whether the corals experienced warming or cooling. However, warming the corals seemed to have more of an impact, with a mortality rate of 100% after 29 days. Preliminary confocal microscopy imaging of the corals also allowed us to analyze the degree of stress on the corals by demonstrating fluorescence of reactive oxygen species and chlorophyll A. This project will help future scientists by understanding how *A. poculata* can withstand widely fluctuating temperatures and the how temperature changes might impact corals in general.

Biological Discovery in Woods Hole at the Marine Biological Laboratory NSF REU Award #1659604

Site-Directed RNA Editing Using TadAs

Rosalind Pan^{1,2}, Juan Felipe Diaz Quiroz², Joshua J.C. Rosenthal²

¹The University of Chicago, ²Marine Biological Laboratory

Site-Directed RNA Editing (SDRE) is a strategy to modify genetic information at the mRNA level. The SDRE system developed by the Rosenthal Lab catalyzes adenosine (A) to inosine (I) conversion using the Deaminase Domain (DD) of ADAR2 linked to λ N peptides. The λ N peptides interact with boxB hairpins located in guide RNAs (gRNA) that direct the DD to the target As. The system can precisely drive A-I conversion in mRNAs, but it has many limitations including sequence context dependency and off-target editing. In this study, we replaced ADAR with TadA, a different adenosine deaminase that catalyzes A-I conversion in *E. coli* tRNAs. This change will allow us to use random mutagenesis in bacteria to select for TadA variants with improved editing efficiency and novel substrate recognition. To investigate whether TadA fused to λ N could edit RNA, we performed in cellula and in vitro editing assays using recombinant enzymes that contained TadA homo or heterodimers. For both assays, we designed an RNA substrate that contained several target As within sequence contexts preferred by TadAs, including a target site mimicking the tRNA anticodon loop (ACL) naturally edited by TadAs. In cellula results suggested that TadA- λ N could edit at the ACL target site in the absence of gRNAs. The in vitro assay recapitulated ACL editing at high efficiency and revealed an additional editing site within a motif that partially overlaps the ACL sequence. This suggested that TadA- λ N activity is dependent on specific neighbouring contexts but does not require the full tRNA ACL sequence. In vitro and in cellula results also showed that TadA- λ N editing may be independent of binding to gRNAs. These preliminary data suggest that using TadA as the enzymatic component for SDRE is promising but it may be necessary to develop new strategies to direct TadA to specific target sites.

Jeff Metcalf Summer Undergraduate Research Fellowship – The University of Chicago

Phosphorylated Tau Activates Signaling Pathways That Inhibit Fast Axonal Transport in Squid Axoplasm

Sarah Aloe^{1,2}, Sarah Morris^{2,3}, Amanda Onoichenco^{1,2}, Gerardo Morfini^{2,3}, Scott T. Brady^{2,3}

¹Hunter College, CUNY, New York; ²Marine Biological Laboratory, Woods Hole, MA; ³University of Illinois at Chicago

Tau tangles, a hallmark of Alzheimer's Disease (AD), are formed when tau, a microtubule associated protein, misfolds and aggregates in neurons. Previous work has shown that aggregated tau inhibits fast axonal transport (FAT), a process essential for maintenance of axons and synapses, via a specific phosphatase activating domain (PAD) at the N-terminus of tau. The PAD, which is not exposed in tau monomers, activates protein phosphatase 1 (PP1), which activates the protein kinase GSK3 β via dephosphorylation. GSK3 β phosphorylates the motor protein kinesin, leading to the release of its vesicle cargoes. Although aggregated tau in AD has no mutations, it is known to be highly phosphorylated. A closer look at tau phosphorylation has identified sites that are phosphorylated in normal and AD brains. Using vesicle motility assays in squid axoplasm, the current study aims to understand potential roles of these sites in regulating the PAD exposure. Three phosphorylation sites located in the proline-rich domain of tau are S199, S202, and T205. We found that concurrent pseudophosphorylation of these sites inhibits anterograde FAT, imitating the effect of tau aggregates. This effect was blocked by a PP1 inhibitor, suggesting the PAD was exposed. Additionally, each of the phosphorylation sites were tested individually. S199E inhibited both anterograde and retrograde FAT, while T205E inhibited only anterograde transport and S202E showed no effect. Further experiments used an antibody, TNT1, which binds specifically to the exposed PAD. TNT1 successfully blocked inhibition of anterograde FAT by T205E tau, but did not prevent inhibition of FAT by S199E tau, suggesting that T205E exposes the PAD but S199E does not. These results show that phosphorylated tau can inhibit FAT and suggests different phosphorylation sites can expose different biologically active domains of tau, a notion consistent with a potential function of tau for regulating FAT in both disease and non-diseased brains.

NINDS, Tau Consortium, Hunter College Institutional Overhead Funds

Training *Octopus bimaculoides*: Isolating Arm and Sucker Movements to Evaluate Sensory Capabilities

Alex Megerle^{1,2}, Lane Kennedy², and Roger Hanlon²

¹Lehigh University, ²Marine Biological Laboratory

Octopus arms are capable of performing a wide range of flexible motions due to their advanced musculature. Four distinct muscle groups (transverse, longitudinal, and two oblique) allow for elongation, shortening, bending, and twisting. Suckers lining each arm allow octopods to both manipulate objects and obtain physical and chemical cues from their environment. To study the sensory capabilities of octopus arms and suckers, we developed a protocol to train *Octopus bimaculoides* to reach through a hole in a plexiglass divider and grasp an object. By associating successful touches with a food reward, in three weeks we were able to achieve a rate of successful touches of ~75%. This classical conditioning represents a key first step in isolating the movements of a single octopus arm and its suckers to experimentally test their range of sensory capabilities.

Partial funding for this project was provided by ONR Grant N00014-19-1-2445 to R. Hanlon.

Funding by Biological Discovery in Woods Hole at the Marine Biological Laboratory - NSF REU Award # 1659604

Differences in Axonal Growth of Dopaminergic Neurons Exhibited in Familial Parkinson's Disease Mouse Model

Olivia Morales^{1,2}, Jack Tang³, Shobana Subramanian³, Rongmin Chen³, Elizabeth Jonas^{2,3}

¹The University of Chicago, ²Marine Biological Laboratory, ³Yale University

Familial Parkinson's Disease (PD) is a neurological disorder which results in tremors, bradykinesia, and stiffness of movement. PD is caused by the degeneration of dopaminergic neurons, which are nerve cells responsible for the production of dopamine. The dysfunction and death of dopaminergic neurons results in a lack of dopamine and disrupts normal motor function. How this degeneration of dopaminergic neurons occurs, however, is still not fully understood; PD can be due to either environmental or genetic conditions. One cause of familial PD is mutation of the gene for the protein DJ-1. Wild type DJ-1 functions to decrease the uncoupling of the mitochondrial inner membrane by binding to the β subunit of the ATP synthase and thus increasing the efficiency of ATP production. It is hypothesized that this increase in efficiency results in an increase in neuronal process outgrowth. Therefore, it is expected that tyrosine hydroxylase (TH +) dopaminergic neurons of model mice lacking DJ-1 will have impaired dopaminergic neuronal outgrowth. Comparing the intensity of TH staining in substantia nigra neuron axonal arbors within brain slices will determine whether this difference exists.

McCarter Metcalf Fellowship

The Proline-Rich Domain Mediates Toxic Effect of Mutant Huntingtin on Fast Axonal Transport

A. Onoichenco^{1,2}, S.L. Morris^{2,3}, S. Aloe^{1,2}, G. Morfini^{2,3}, S.T. Brady^{2,3}

¹Hunter College, CUNY, New York; ²Marine Biological Laboratory, Woods Hole, MA; ³University of Illinois at Chicago, IL

Huntington's disease (HD) is a neurodegenerative disorder caused by the mutant huntingtin (mHtt) protein, which has an aberrantly extended polyglutamine (polyQ) region longer than 36 glutamines. This expansion results in a toxic gain of function in the huntingtin gene, but the underlying mechanisms remain unknown. Although neuronal cell death may occur at the late stages of disease, axonal and synaptic pathology in early stages of the disease suggest the involvement of an axonal mechanism. A well-established pathogenic effect of mHtt includes the inhibition of fast axonal transport (FAT), a cellular process necessary for the maintenance of axonal connections and synaptic function, in both anterograde and retrograde directions. This inhibitory effect has been linked to the activation of cJun amino terminal kinase 3 (JNK3), a mitogen-activated protein kinase (MAPK) preferentially expressed in the brain that is capable of phosphorylating motor proteins. However, the mechanism by which mHtt activates JNK3 is not yet clear. In this study, we demonstrate a key role for the proline-rich domain in the toxicity of mHtt and propose a mechanism in which expanded polyQ exposes the PRD, thus activating a MAPK signaling cascade. Utilizing vesicle motility assays of squid axoplasm allowed us to assess the effects of the PRD and subregions within the PRD on FAT to show that specific portions featuring SH3 binding motifs are responsible for toxicity. Furthermore, we were able to demonstrate the protective effects of antibodies targeting the PRD and of competitive binding of exogenous peptides with SH3 domains. Elucidation of such a mechanism and identification of toxic regions of the mHtt may be critical in the development of novel HD treatments and therapies.

Hunter College Institutional Overhead Funds, Tau Consortium, NINDS

Microplastics everywhere: The history of microplastic pollution in Cape Cod salt marsh sediments

Nicole Vandal^{1,3}, Ruby Rorty², Kelsey Chenoweth³, Rut Pedrosa-Pàmies³, Ivan Valiela³, Javier Lloret³

¹Amherst College, Amherst, MA; ²University of Chicago, Chicago, IL; ³Marine Biological Laboratory, Woods Hole, MA

Since the 1950s, plastic production and use has increased worldwide. Large plastic debris breaks down into microplastics (particles < 5mm) that accumulate everywhere, including salt marsh sediments. The relatively minor bioturbation and hydrological disturbances observed in salt marshes, make salt marsh sediments a natural relatively stable time-sensitive repository of microplastics. Microplastic abundance in salt marsh sediment cores was measured to determine the historical time course of the accumulation of microplastic particles across recent decades. Three \approx 24 cm-deep sediment cores were collected in salt marshes of two estuaries within the Waquoit Bay estuarine system: Childs River, an estuary with a relatively urbanized watershed, and Timm's Pond, with a forested watershed. These cores represented more than eight decades of sediment accumulation in those sites. Then the cores were brought to the lab and sliced into 2 cm segments, these segments were then sieved. Finally, microplastics were extracted and analyzed after floatation and oxidation of organic matter in the sample. The abundance of microplastic pollution was quite variable at any depth but has increased 9-fold since the early 1960s. In addition, Microplastics were far more abundant in Childs River than in Timm's Pond sediments. In recent decades the increase in global plastic usage and the degree of urban development in Cape Cod is reflected in the accumulation of microplastics in urbanized areas and beyond.

Biological Discovery in Woods Hole at the Marine Biological Laboratory - NSF REU Award # 1659604

Woods Hole Sea Grant

Protein palmitoylation via a specific palmitoyl transferase facilitates Golgi dispersal observed with nicotine exposure

William Ramos^{1,3}; Okunola Jeyifous^{2,3}; Anitha Govind²; William N. Green^{2,3}

¹The College of the University of Chicago; ²The Department of Neurobiology at the University of Chicago; ³Marine Biological Laboratory

Our lab has observed dispersal occurring in nicotine treated neurons and human embryonic kidney cells (HEK) expressing α 4 β 2-type nicotinic acetylcholine receptors (α 4 β 2Rs). α 4 β 2Rs bind nicotine with high affinity and initiate the addictive process with nicotine binding. Typical GA morphology is observed as a set of membrane stacks in the soma of neurons. During nicotine-induced dispersal we find that the stacks disperse into mobile membranes throughout dendrites and axons. Preliminary data from our lab has implicated the palmitoyl transferase, DHHC2, in the downstream events after nicotine binding to α 4 β 2Rs causing GA dispersal. The identification of DHHC2 as being involved in Golgi dispersal is also consistent with additional evidence from our lab that the posttranslational modification, palmitoylation, is part of the signaling that leads to GA dispersal. To further test whether DHHC is involved, we overexpressed DHHC2 in HEK cells and imaged for changes in GA morphology. Fluorescently tagged sialyltransferase 3 (eGFP-ST3) and DHHC2 (myc-DHHC2) were assayed on the Zeiss spinning disk confocal system with or without the expression of tagged α 4 β 2Rs (HA- α 4 β 2Rs). Preliminary results suggest that DHHC2 overexpression does facilitate GA dispersal and that the GA dispersal induced by DHHC expression occurs independent of α 4 β 2R expression.

Jeff Metcalf Summer Undergraduate Research Fellowship – The University of Chicago

Function of reverse transcriptase-related (rvt) genes in metal stress response

Samuel Maffa^{1,2}, Fernando Rodriguez², Irina Yushenova², Irina Arkhipova^{1,2}

¹Brown University, ²Marine Biological Laboratory

Reverse transcriptase-related (rvt) genes comprise a remarkable class of reverse transcriptases found across several domains of life, including fungi, plants, invertebrates, protists, and even certain bacteria. Among other notable properties, rvts contain highly conserved coiled-coil N-terminal domains demonstrated in bacterial rvt to confer the ability to multimerize and a C-terminal domain purportedly responsible for protein priming. Prior experimentation has shown significant transcriptional stimulation of various rvts in response to transition metal ions, particularly to Ni²⁺ in the ascomycete fungus *Neurospora crassa* and Fe²⁺ in the filamentous bacterium *Herpetosiphon aurantiacus*, suggesting that rvts may play a role in the metal stress response. Here we investigate Ncrvt in the model fungus *N. crassa* and express Harvt from *H. aurantiacus* introduced into the heterologous host *Escherichia coli* by transformation to explore the potential function of rvts in mediating the metal stress response. Rvt activity in *N. crassa* is monitored using the transformant T147 strain with the GFP reporter regulated by the Ncrvt promoter. Mutants of T147 obtained through random somatic mutagenesis under UV radiation are grown in different nickel concentrations and examined for GFP induction and cellular localization. *E. coli* strains expressing recombinant plasmids with functional mutations in key domains of HaRVT are grown across concentration gradients of various transition metals to investigate their growth and survivability. The distinct induction patterns observed in *N. crassa* implicates Ncrvt in different pathways of homeostatic control, while experimentation with *E. coli* suggests that Harvt may provide an advantage in iron-rich environments. Although the mechanism of rvt activity in relation to transition metals is still uncertain, our observations made with fungal and bacterial versions of rvt are consistent with their role in the transition metal stress response.

Brown-MBL Rosenthal LINK Award program

Creating Rfx6 Knock-Out Mutants of *Xenopus*

Corbin Hanna^{1,2,3}, Marcin Wlizla^{2,3}, and Marko Horb^{2,3}

¹Northwest Missouri State University, ²Marine Biological Laboratory, ³National *Xenopus* Resource

Xenopus are an excellent model system for human disease due to their external development, their large spawning and embryo size, their relatively rapid development, fate mapping, and their accurate genome sequencing. Mutations in the rfx6 gene can cause neonatal diabetes and digestive system defects. Diabetes is a chronic disease caused by a disruption in the pancreas, where β -cells cannot properly produce insulin. To better understand what role rfx6 plays in early endoderm development, we are producing mutant *Xenopus laevis* frogs using genome editing. To create mutants, we used CRISPR-Cas9 (Clustered Regularly-Interspaced Short Palindromic Repeats). Cas9 is an enzyme that can cut the two strands of DNA at a specific target point. An RNA guide binds to the pre-designed sequence and directs Cas9 to the correct area on the genome. We examined the exon-intron structure of rfx6, and identified potential sgRNA target sites. Rfx6 has 19 total exons, with our specific sites in exon one and three. These sites are close to the start of the gene and thus likely to completely disrupt the protein function. We microinjected the sgRNA along with Cas9 protein into *Xenopus* embryos at the one/two cell stage to induce site-specific double strand breaks (DSBs) in genomic DNA. Inducing indels at a target site, CRISPR-Cas9 causes a frame-shift mutation. Endogenous DSB repair mechanisms are error prone and commonly introduce insertions and deletions (indels) that result in frame-shift mutations which disrupt protein structure and function. These F0 embryos would have mosaic mutations, which vary between cells. Following injection, embryos are grown and 24-48 hours later we collect genomic DNA from several embryos to ascertain whether mutations were induced. We PCR amplify the targeted region from genomic DNA and send the PCR product for sequencing to interpret whether Cas9 cut the target sequence.

Funding by Biological Discovery in Woods Hole at the Marine Biological Laboratory - NSF REU Award # 1659604

Examining symbiont selection and polyp connectivity in *Astrangia poculata*

Juliette M. Thibodeau^{1,2}, Mayra A. Sánchez-García², Loretta M. Roberson²

¹University of Chicago, Chicago, IL, ²Marine Biological Laboratory, Woods Hole, MA

Given the recent acceleration in global coral reef decline, there is a growing interest in understanding resilient corals. Increasing ocean temperatures cause a breakdown of the symbiotic relationship between corals and the dinoflagellate *Symbiodinium*, leading to expulsion and eventually colony mortality due to lack of nutrition normally provided by symbionts. *Astrangia poculata* is distinctive in that it can survive in both symbiotic and aposymbiotic states, unlike most tropical corals that have an obligate relationship with *Symbiodinium*. It is therefore an exceptional model system to understand this symbiosis. Our study focuses on the reintroduction of *Symbiodinium* following a bleaching event and the connectivity between polyps in a colony. In this study, we reintroduced symbionts in a single polyp of naturally and chemically bleached colonies of *A. poculata* using two different clades of cultured *Symbiodinium*, as well as tissue from a symbiotic *A. poculata* colony. Image analysis was used to evaluate symbiont density following reintroduction. We found that successful reintroduction of symbionts was independent of how the coral was bleached and the clade of symbiont used, showing that tissue reinfection is the most effective method. This study demonstrates that reintroduction of symbionts is possible in a laboratory setting for *A. poculata*. We also found that there was a lack of polyp connectivity in grown *A. poculata* colonies as well as connectivity with surrounding polyps. This suggests that *A. poculata* polyps lose connectivity after development, unlike those in tropical corals like *Acropora* and *Porites*, species that maintain connectivity as developed polyps. These results offer insight into the symbiosis and colonial interaction of *A. poculata* and how they relate to wholly tropical corals, providing a deeper understanding of resilient corals in the face of climate change.

Jeff Metcalf Summer Undergraduate Research Fellowship – The University of Chicago

Eutrophication and Sea Level Rise in Great Sippewissett Marsh: Differential Effects on Marsh Platform Elevation

Claire Bekker^{1,2}, Samantha Valentine^{1,2}, Ivan Valiela², Javier Lloret²

¹Brown University, ²Marine Biological Laboratory

Salt marshes around the world are experiencing the combined effects of increased eutrophication and sea level rise. Accelerating sea level rise poses a significant threat to these coastal ecosystems through increased erosion and submergence. Previous work suggests that coastal eutrophication acts synergistically with rising sea levels to reduce geomorphic stability and drive salt marsh loss. We used experimental plots in Great Sippewissett Marsh that have been chronically enriched with different doses of nitrogen, and, in addition, have been exposed to a 6-fold increase in sea level rise since 1992. Using these plots, we assessed the combined effects of nitrogen enrichment and rising sea levels on marsh elevation within the plots. The median elevation in plots with the highest fertilization rates (7.56 g N m⁻² wk⁻¹) was 65.3 ± 10.5 (median absolute deviation) cm—higher than the median elevation in plots exposed to low fertilization (0.85 g N m⁻² wk⁻¹) [57.0 ± 3.8 cm] or control plots [44.2 ± 7.6 cm]. As nitrogen doses rose, maximum elevation inside the plots increased significantly, while minimum elevation did not differ significantly across treatments. Thus, median elevation increased with higher nitrogen doses because fertilization increased the height of high marsh platforms. However, fertilization had no significant effect on low marsh elevation. These results indicate that higher doses of nitrogen can foster increased accretion rates, reduce submergence, and consequently, counter the effects of sea level on salt marshes.

Brown University LINK Award Program

Assessing the suitability of *Sepioloidea lineolata* as a genetically tractable model

Claudia Rodríguez López^{1,2}, Namrata Ahuja², and Joshua C. Rosenthal²

¹Williams College, ²Marine Biological Laboratory

The use of genome editing reagents for the development of new genetically tractable models has received worldwide attention for its potential to offer novel insight into broad areas of biology. In cephalopods, it could uncover the molecular innovations that drive complex behavior. Accordingly, the MBL's cephalopod program is investing significant resources towards creating a genetically tractable cephalopod model. Recently, a targeted knock-out of the tryptophan dioxygenase (TDO) gene was achieved in *Euprymna scolopes* and *Doryteuthis pealeii* using the CRISPR-Cas9 system. TDO catalyzes the first committed step in ommochrome biosynthesis, which pigments chromatophores and the retina of cephalopods. Currently, the MBL cultures 6 cephalopod species through their life cycle. Of these, *Sepioloidea lineolata* is promising for model development because it is relatively easy to culture and withstands high stocking densities. The goal of this study is to assess the suitability of *Sepioloidea lineolata* for generating gene knockouts. Given the lack of literature on the embryonic development of this species, the first steps required exploring how to inject embryos and keep them alive in culture. As a guide, we turned to ongoing experiments using *Euprymna scolopes*. Early stage embryos were collected and removed from their protective gelatinous coat, injected with Cas9 and two CRISPR guide RNAs targeting the TDO gene, and then cultured in filtered seawater with antibiotics. Overall, the survival rate of embryos was low, with few living past two weeks. Several factors could conceivably contribute to the low survival rate, some of which include microinjection technique, quality of broodstock, and culture conditions post-injection. Despite the low survivorship of injected embryos, some are viable and developing. A phenotypic assessment of the surviving embryos injected with CRISPR-Cas9 is required to further gauge the success of these experiments. This work provides a basis for further studies on gene knockouts in this species.

Funding by Biological Discovery in Woods Hole at the Marine Biological Laboratory - NSF REU Award # 1659604

Identifying Markers of Tergal and Precoxal Tissues in *Parhyale hawaiiensis*

Eric Chen^{1,2}, Heather Bruce², Nipam Patel²

¹The University of Chicago, ²Marine Biological Laboratory

The origin of the insect wing as an evolutionary novelty is a longstanding problem in arthropod biology. One school of thought suggests that the wing was modified from a dorsal lobe (e.g. gill or plate) on the leg of ancestral crustaceans, while another theory suggests that the wing is a novel projection from the body wall that is unique to insects and is not present in the crustacean ancestor. Recent work from the Patel lab supports the hypothesis that the insect wing is homologous to a lobe on the leg of the pan-crustacean ancestor. In this model, the most proximal leg segment has vestigialized, becoming flatter and broader, and now forms much of the lateral body wall of insects. This caused the lobe on that leg segment to migrate dorsally, to a position consistent with wings in modern insects. According to this model, the lateral aspect of the dorsal shell (the tergum) actually consists of the most proximal leg segment (precoxa) as well as true body wall tissue. While morphological data supports the model that insects incorporated the proximal leg segment into their body wall, genetic evidence for this model is lacking. In this study, I follow a candidate gene approach to identify markers that will distinguish true body wall from the precoxa in a crustacean model, the amphipod *Parhyale hawaiiensis*. To this end, I am using fluorescent in situ hybridization (FISH) to study a panel of genes known to pattern the *Drosophila notum* and characterize their expression domains in *Parhyale* embryos. I expect to see a clear boundary between medial expression of dorsal genes and the lateral expression of proximal leg genes during embryonic limb development.

Jeff Metcalf Summer Undergraduate Research Fellowship – The University of Chicago

Eutrophication and Sea Level Rise in Great Sippewissett Marsh: Effects on Vegetation Cover and Species Dominance

Samantha Valentine^{1,2}, Claire Bekker^{1,2}, Ivan Valiela², Javier Lloret²

¹Brown University, ²Marine Biological Laboratory

Salt marshes across the world are threatened by eutrophication and rising sea levels. Experimental plots in Great Sippewissett Marsh have been chronically enriched with different doses of nitrogen, the limiting nutrient in salt marshes, from 1970 through today. Sea level is now rising at 6x the rate of before 1990. The experimental plots offer the opportunity to study the synergistic effects of eutrophication and sea level rise on salt marshes. Elevation data shows that the elevation of the salt marsh platform increased with increasing N dose. On the other hand, accelerated sea level rise may favor expansion of low marsh species that are adapted to greater submergence. Field measurements showed that cover of low marsh species such as *Spartina alterniflora* decreased by 42% in the highest fertilized plots compared to control. Species that grow beyond the tidal range, such as *Suaeda maritima* and *Iva frutescens* increased from control to high fertilization by 47% and 3% respectively. *Spartina patens* decreased by 11% from control to the highest fertilized plots, as this species was outcompeted by more opportunistic species such as *Distichlis spicata*. Additionally, three different forms of *Spartina alterniflora* also showed differential spatial cover. The tall form of *S. alterniflora*, that prefers areas of highest nutrient concentrations, increased by 6% from the control to the high fertilized plots. This result can also be owed to the N enrichment. These changes in species cover were facilitated by the increases in elevation of the salt marsh platform in the highest fertilized plots. The increase in elevation of the salt marsh platform resulting from the N enrichment, and the related knowledge about the vegetation changes, suggests that carefully designed N enrichments may be a possible means of slowing marsh submergence by sea level rise, while maintaining marsh-specific vegetation.

Brown-MBL LINK program

Role of the transcription factor Sall4 in axolotl tail regeneration

Hannah Geller^{1,2}, Marcela Arenas Gomez², Karen Echeverri²

¹Yale University, New Haven, CT, USA; ²Eugene Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory, Woods Hole, MA, USA

The salamander *Ambystoma mexicanum*, known as the axolotl, has the remarkable ability to regenerate its appendages - including its spinal cord. Following tail amputation, radial glial cells proliferate and create a tubular outgrowth that later differentiates into the neurons and glial cells of the new spinal cord. Soon after wounding, the gene Sal-like 4 (Sall4) is upregulated. Sall4 is a transcription factor known for its role in repressing differentiation as well as maintaining pluripotency and self-renewal in embryonic stem cells. Outside embryonic stem cells, Sall4 is upregulated in regenerating skin cells and has been shown to play a role in collagen regulation during wound healing in the axolotl. The specific role of Sall4 during spinal cord regeneration is unknown. To determine where Sall4 is localized during spinal cord regeneration, Sall4 antibody stains were performed on cross sections of the regenerating axolotl tail at 3, 7, and 10 days post injury (dpi). CRISPR-Cas9 was used to perform a targeted knockout of Sall4 in the spinal cord, and then a whole mount β -III tubulin stain was carried out on Sall4 knockout animals and control tails to visualize axon regeneration. Compared to the uninjured tail, Sall4 expression was greater in 7dpi tails, where it was expressed in the epithelium, spinal cord, and blastema cells. Sall4 knockout led to incomplete regeneration, demonstrating that Sall4 plays a role in tail regeneration. Further studies are necessary to more precisely quantify Sall4 expression at various time points during regeneration and to identify exactly which cells upregulated Sall4 after injury and what the downstream targets of Sall4 are in specific cells. Understanding the role of Sall4 during tail regeneration would bring us one step closer to comprehending the complex process of regeneration in the axolotl.

Funding by Biological Discovery in Woods Hole at the Marine Biological Laboratory - NSF REU Award #1659604

Regulation of mitochondrial ion channel activity in Fragile X brain mitochondria by ATP synthase leak modulation

Jingxuan Song^{1,2}, Pawel Licznarski³, Elizabeth A. Jonas^{2,3}

¹The University of Chicago, ²Marine Biological Laboratory, ³Yale University

Fragile X syndrome is caused by mutation in the X chromosome at the *fmr1* gene, which controls *fmrp* protein expression. This protein binds to synaptic protein mRNA and inhibits their translation. In wild type (WT) brain cells, *fmrp* falls off the mRNA after sufficient stimulation, which allows translation to occur. It's known that the F1 domain of the ATP synthase is regulated by *fmrp* in such a manner, and this regulation contributes to a significant change in F1 domain/FO domain ratio after stimulation. Previous research of Dr. Jonas's lab shows that the FO domain can form a leak channel on the inner membrane of mitochondria, and they have found that increasing F1/FO ratio helps close the leak and enhances the efficiency of ATP production. However, *fmrp* is absent in Fragile X (FX) brain cells due to mutation at the *fmr1* gene. Without *fmrp* regulation, mRNAs are continuously being translated, and this disrupts the desired F1/FO ratio during development. It's possible that pharmacological intervention could ameliorate the leak and normalize rates of protein synthesis. We propose that Dextramipexole (dex) is a promising drug for improving brain development in Fragile X syndrome due to previous evidence that it can bind to the F1 domain and promote closure of the FO domain channel in WT mitochondria. Nevertheless, its effects on leak channel activity in the Fragile X mitochondria remain unknown. Therefore, this project explores the effect of Dex on decreasing channel activity on FX brain mitochondria. Membrane channel activity is recorded before and after dex is added to the bath in both WT and FX brain mitochondria using patch clamp technique. The result confirms that dex is able to down-regulates channel activity in WT and FX mitochondria. These findings are encouraging in that dex may show therapeutic potential in Fragile X syndrome.

Jeff Metcalf Summer Undergraduate Research Fellowship – The University of Chicago

NS112706

Top-down control of terrestrial salt marsh invertebrates by the mummichog (*Fundulus heteroclitus*) in a New England salt marsh

Katrina Fedors, Olivia Floyd, Justin Lesser, James Nelson

Nelson Lab, TIDE Project

In theory, predators may exert top-down control on prey populations through trophic interactions. In New England salt marshes, creek bank geomorphology limits access of the mummichog (*Fundulus heteroclitus*) to the terrestrial high marsh zone, where they prey upon terrestrial invertebrates. Here, we tested whether altered mummichog access to the high marsh affects top-down control by assessing the high marsh invertebrate community at creeks with different bank geomorphology both before and after the period during which mummichogs have the greatest access to the high marsh. We found no difference in terrestrial invertebrate abundance before and after the spring high tide, suggesting that mummichogs exert little top down control over invertebrate populations in this system.

NSF

Driving fish wild? The effects of pile driving on black sea bass feeding behavior

Marie Renahan^{1,3}, Jenni Stanley²

¹Indiana University, ²Woods Hole Oceanographic Institute, ³Marine Biological Laboratory

Expanding offshore construction and wind energy infrastructure increases the importance of understanding the effects of anthropogenic sounds on fishes. Pile driving is a technique used to build foundational support for buildings. It produces a low frequency, impulsive sound commonly exceeding 200 dB re 1 μ Pa at 1 m underwater. The sound, very different from the natural underwater soundscape, has been attributed with causing barotrauma and death in fish swimming in close proximity. Black sea bass is a commercially, recreationally and ecologically significant species along the eastern coast of the United States. Little is known about their auditory abilities, but research suggests their hearing frequency range directly overlaps with pile driving sounds, being most sensitive to frequencies of greatest energy. The effects of chronic exposure to pile driving on the feeding behavior of black sea bass were investigated. A fish was placed within the experimental tank for approximately 15 hours to acclimate it to its surroundings. Food was released and was immediately followed by the start of each exposure period. The fish was exposed to three 15-minute pile driving recordings (average SPL 0-peak 174 dB re 1 μ Pa, taken from field recordings at 500 m from source), punctuated by 15 minutes of ambient sound (or continuous ambient sound if a control trial). Preliminary analyses show that fish exposed to pile driving were more likely not to feed or have a significantly increased time to feed. The exposed fish exhibited freezing and aggressive behaviors and sought out shelter, giving further evidence of the behavioral change due to pile driving exposure. Further research will increase the sample size and will investigate effects of pile driving on spawning, migration, or large-scale population size in the field (rather than controlled setting) to better identify the importance of mitigation strategies to minimize pile driving sound.

Bureau of Ocean Energy Management Environmental Studies Program through Interagency Agreement Number M17PG00029

Funding by Biological Discovery in Woods Hole at the Marine Biological Laboratory - NSF REU Award # 1659604

Effect of phthalates on tentacle regeneration in sea anemone, *Nematostella vectensis*

Advait Ganapathy^{1,2}, Karen Echeverri²

¹University of Chicago, Chicago, IL, ²Eugene Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory, Woods Hole, MA

Nematostella vectensis is a species of sea anemone that has recently been adopted as a model organism for tissue regeneration studies due to its ability to rapidly regenerate amputation tentacles within a week. Phthalates are a group of compounds that are derivatives of phthalic acid and are used as plasticizers to make plastic products more elastic. Despite their known effects on the human reproductive system and nervous, they are widely used in everyday products from erasers to PVC pipes and could potentially leach into water bodies. This could potentially harm a variety of aquatic organisms. In this project I tested the effects of different concentrations of phthalates on the ability of *Nematostella* to regenerate. Two different phthalates were used in the experiments – dioctyl phthalate and bis – ethyl, hexyl- phthalate - with the former being used in printing inks and the latter being used in PVC pipes. Young adult animals were amputated at the middle of the pharynx and were kept at the various concentrations of phthalates. A variety of phenotypes were observed after a week of regeneration, with the most prominent being the outward curling of tentacles. Finally, used real time PCR the impact of the presence of phthalates on gene expression was studied.

Jeff Metcalf Summer Undergraduate Research Fellowship – The University of Chicago

The impacts of changes in marsh geomorphology on the diversity of high marsh biota consumed by Mummichog (*Fundulus heteroclitus*)

Olivia T. Floyd, Justin S. Lesser, Katrina L. Fedors, James A. Nelson

Marine Biological Laboratory

Mummichog (*Fundulus heteroclitus*) serve a fundamental ecological role as transporters of terrestrial productivity from the high marsh to aquatic zones in New England salt marsh ecosystems. However, changes in the geomorphological structure of the low marsh zone brought on by anthropogenic inputs can hinder fish access to the high marsh platform. In order to understand how the diversity of terrestrial organisms consumed by these fish may be affected by reduced access to the high marsh, we collected mummichog using the high marsh at creeks with differing levels of low marsh degradation and examined their gut contents in order to measure the diversity of the species of organisms consumed. Our data indicates high species diversity at both locations, but no overall difference in diversity between creeks. This could suggest that while geomorphological changes in marsh structure may limit mummichog access to high marsh food sources, that the variety of species consumed by mummichog is not altered.

NSF REU Program

Functional recovery of burrowing behavior in sea lampreys after spinal cord injury

Nicole A. McCarthy^{1,2}, Hilary R. Katz², Jennifer R. Morgan²

¹Neuroscience Department, Carthage College, Kenosha, WI, ²The Eugene Bell Center for Regenerative Biology and Tissue Engineering, Marine Biological Laboratory, Woods Hole, MA

Sea lampreys can regenerate their spinal cord axons in 10-12 weeks after a complete transection and consequently are able to achieve functional recovery of swimming. While a great deal is known about the functional recovery of swimming, further investigation of other locomotor behaviors is needed to assess the full potential of lampreys' ability to functionally recover. Larval sea lampreys are generally found burrowed in sand. Unlike swimming, not much is known about lampreys' ability to recover burrowing following spinal cord injury. In this study, we evaluated the lamprey's ability to burrow at multiple post-injury time points during spinal cord regeneration, spanning from 2 to 11 weeks post-injury (WPI). Burrowing behavior has two components. The initial component resembles swimming with fast undulations, while the final component involves large body flexions that pull the tail under the sand. Lampreys at various WPI were video recorded at 60 frames per second to assess burrowing behavior and subsequently compared to control (uninjured) animals. The duration of the initial component did not differ between control and spinal-transected animals across the entire recovery period (Control: 1.82 1.24 sec, 2-4 WPI: 2.25 2.16 sec, 5-8 WPI: 2.08 0.871 sec, 9-11 WPI: 2.06 2.26 sec; Kruskal-Wallis $p = 0.3366$). In contrast, at 2-4 WPI most animals were unable to complete burrowing, leaving a portion of the tail exposed. By 9-11 WPI, most animals finished the final component but took significantly longer than controls (Control: 21.1 16.0 sec, 2-4 WPI: 128 70.5 sec, 5-8 WPI: 129 106 sec, 9-11 WPI: 94.9 81.8 sec; Kruskal-Wallis $p = .000373$). These data indicate that, similar to swimming behavior, lampreys are able to recover burrowing behavior after spinal cord injury, though moderate deficits persist.

Biological Discovery in Woods Hole at the Marine Biological Laboratory NSF REU Award #1659604

MBL/Bell Center

Microplastics everywhere: Watershed urbanization affects microplastic abundance in salt marsh sediment

Ruby Rorty^{1,3}, Nicole Vandal², Kelsey Chenoweth³, Rut Pedrosa-Pamies³, Javier Lloret³, Ivan Valiela³

¹The University of Chicago, ²Amherst College, ³The Marine Biological Laboratory

As worldwide plastic production and use increases, the accumulation of plastic debris in coastal ecosystems has become a major environmental concern. In particular, microplastics (plastic particles <5mm) are believed to be ubiquitous in the world's oceans. The abundance and sources of microplastics in coastal environments such as Cape Cod salt marshes have been barely studied. Here, we analyze surface sediment from six different estuaries in Waquoit Bay, representing a wide range of watershed urbanization, in order to determine (1) what relationship exists between human activity and the abundance of different types of microplastics, and (2) what these abundances suggest about sources and transport mechanisms for the different types of microplastics. Our results show that microplastic fragments are more abundant in more urbanized sites. Contrastingly, plastic microfibers are ubiquitous in the sampled sites, regardless of the degree of urbanization. These findings suggest that the larger and heavier microplastic fragments are less efficiently transported in estuaries, and tend to accumulate in proximity to land sources. Microfibers, in contrast, are less dense and have smaller dimensions. Transportation via currents or through air brings them to all sites, including areas located far from human activity. These results can inform both future research on microplastic abundance in salt marshes and pollution management.

Woods Hole Oceanographic Institute Sea Grant and the Jeff Metcalf Summer Undergraduate Research Fellowship – The University of Chicago

Assessment of particle cycling processes in the deep ocean using the carbon isotopic composition of fatty acid biomarkers

Rebecca Hopkins^{1,2}, JC Weber², Maureen H. Conte^{2,3}

¹College of Wooster, ²Ecosystems Center, Marine Biological Laboratory, ³Bermuda Institute of Ocean Sciences

Fatty acid (FA) molecular and carbon isotopic composition ($\delta^{13}\text{C}$) is useful in assessment of sources, recycling, and degradation (“remineralization”) of organic material in the environment. My research used FA $\delta^{13}\text{C}$ in particles within the water column of the Sargasso Sea off Bermuda to evaluate carbon sources and remineralization processes, from the sunlit surface zone of photosynthesis to the deep seafloor at 4500 m depth. I extracted and isolated the FAs, and used special SPE columns to separate the individual FAs by degree of unsaturation. The $\delta^{13}\text{C}$ of the individual FAs was quantified using a gas chromatograph- isotope ratio mass spectrometer (GC-irMS). I focused on a seasonal comparison of $\delta^{13}\text{C}$ profiles of polyunsaturated fatty acids (PUFAs) sourced from surface water phytoplankton production and possibly piezophilic (high pressure) deep ocean bacteria production, even-chained saturated fatty acids (SFA) which have many sources, and bacteria FAs (e.g. odd/ branched). In April (post spring bloom), water column $\delta^{13}\text{C}$ profiles of both PUFAs and SFAs show ^{13}C depletion in surface waters (< 100m), a large ^{13}C enrichment (~5‰) from the base of the photic zone to ~500m depth, and then small variability in deeper waters. This pattern is indicative of primary production of FAs by photosynthesis in the photic zone, and shows that significant carbon isotopic fractionation is associated with remineralization of fresh, sinking organic materials in the midwater column. November $\delta^{13}\text{C}$ profiles were enriched by ~3‰ relative to April and showed a smaller $\delta^{13}\text{C}$ gradient between the surface photic zone and 500 m, which is consistent with a greater contribution of recycled carbon (more isotopic fractionation) during low productivity seasons. My study provides the first look at $\delta^{13}\text{C}$ water column profiles of key FA biomarkers and provides conclusive data showing that significant isotopic fractionation occurs during organic carbon remineralization in the oceanic water column.

National Science Foundation, Chemical Oceanography Division Grant OCE 1536644

Regeneration of Negative Phototactic Response in *Lumbriculus variegatus*, an aquatic annelid

Marina Vargas and Veronica G. Martinez-Acosta

Dept. of Biology, University of the Incarnate Word, San Antonio, TX; Marine Biological Laboratory, Woods Hole, MA

Lumbriculus variegatus, an aquatic annelid, is capable of complete regeneration from a few body segments (Martinez and Zoran, 2009). In this study we present an investigation of photoreception and its regenerative properties in *Lumbriculus*. *Lumbriculus* is thought to possess photoreceptors within its posterior-most segments, as rapid tail withdrawals are evoked when a shadow is cast over the water column. Initial studies describe that *Lumbriculus* possesses large photosomal photoreceptors that lie directly below the epithelial layer (Drewes and Fournier 1989). A simple phototactic assay was designed to characterize responses from regenerating worm fragments isolated from the posterior-most segments of the worm. Preliminary data suggest that there is significant difference between the amount of time it takes for anterior (Ant) and posterior (Post) regenerating fragments to move away from a light source (Control (C) vs Ant $p=0.42$, $df=5$; C vs Post $p=0.047$, $df=5$). Immunohistochemical analysis of Anti-Futsch (Developmental Hybridoma Bank) and Anti-G- α Subunit q/11/14 (Santa Cruz Biotechnology), previously described as markers for photoreceptors in other worm species, were utilized to elucidate structural arrangement and composition of putative photoreceptors within Lumbricolid tail segments. Candidate sensory receptor cells were labeled by 22c10, including cells that are found adjacent to serotonergic axonal connections that project to cell bodies. G- α protein is more broadly labeling and is found within small epithelial cells along and throughout the cuticle as well as co-labeling within neuronal cell bodies extending from the ventral nerve cord. Using transmission electron microscopy, we will elucidate more clearly the ultrastructural arrangement of the 22c10 positive cells as well as other candidate photoreceptor cells found within the posterior-most segments. Overall, understanding the mechanisms of photoreception utilized by *Lumbriculus* will further our understanding of these complex processes and perhaps the knowledge gained will be applicable in higher order organisms.

Biological Discovery in Woods Hole at the Marine Biological Laboratory NSF REU Award #1659604

Anaerobic Salt Marsh Microbial Consortia Remain Stable Over Time When Exposed to High Nitrate Concentrations

Rohan S. Kremer Guha^{1,2}, Ashley Bulseco-McKim², Joseph Vineis³, Jennifer Bowen³, Joseph Vallino²

¹The University of Chicago, ²Marine Biological Laboratory, ³Northeastern University

It has been observed that microbial communities respond dynamically to environmental perturbations with respect to metabolism, taxonomy and abundance. While competition is certainly prevalent in microbial communities, we also know that some bacterial communities work cooperatively together to effectively utilize resources in what is called division of labor. However, little is known about the stability of these cooperating sub-communities and how they respond to perturbations. We hypothesized that consortia, representing sub-populations of the whole community, would remain stable over time if isolated from other communities. For our experiment, we collected bacterial samples from Plum Island in Newburyport, MA, using 30 traps sunk 24 cm into the sediment. The resulting consortium was used to inoculate three replicate flasks that had feed mixtures consisting of marine broth with 1000 μ M nitrate. A sterile flask served as control. Purging nitrogen gas through the flasks kept them anaerobic, and 300 mL samples were extracted every three days for analyses followed by the addition of 300 mL of sterile medium to maintain constant culture volume. The consortia were measured over the course of two weeks for metabolic output (through CO₂ production), nitrate concentration (via chemiluminescent analyzer), cell numbers (DAPI counts), and taxonomy (16S gene identification). Preliminary results suggest that the consortia are indeed stable over time.

Jeff Metcalf Summer Undergraduate Research Fellowship – The University of Chicago

Optimizing a protocol for the electroporation of *Symbiodinium* for delivery of fluorescent markers

Darya R. Khodakhah¹ ; Eldad Gutner-Hoch² ; Joshua Rosenthal² ; Loretta M. Roberson²

¹Middlebury College, Middlebury, VT 05753 USA, ²Marine Biological Laboratory, Woods Hole, MA 02543 USA

Many Cnidarians live in a mutualistic, symbiotic relationship with photosynthetic algae in order to meet their energetic needs. Climate change, however, is creating environmental conditions (such as low ocean pH and high water temperatures) that can cause the breakdown of this symbiotic relationship and result in the loss of the symbionts within the host, a phenomenon called bleaching. This process is a worldwide concern for reef-building corals, where the loss of coral reefs and the ecosystems that they support are proceeding at alarming rates. In most symbiotic cnidarians, bleaching is fatal. However, some species can swap their symbionts to new, more temperature-tolerant varieties, but how this process works is not well understood. Specifically, it is not known whether the symbiotic algae is acquired from the environment, or recolonizes from pre-existing small concentrations within the host tissue. A major limitation in studying this process is that the symbionts can only be distinguished by genetic testing after recolonizations because they lack visible markers. Thus, developing a means to identify different symbionts using fluorescent tags would enable bleaching to be studied in real-time, using microscopic techniques. As a first step to achieving this goal, methods need to be developed to deliver reagents within the symbionts. We chose electroporation. Using a fluorescent green dextran, this project experimented with different electroporation parameters (i.e., buffer type, cell wall removal, and various electroporation settings) in order to fine-tune a set of parameters for the electroporation of *Symbiodinium*.

The Merlin Foundation

Treatment of FMRP KO Rodents with Dexpramipexole

Lucas Brandao^{1,2}, Harshvardhan Rolyan³, Elizabeth A. Jonas^{2,3}

¹Clark University, ²Marine Biological Laboratory, ³Yale University

Fragile X Syndrome is a sex-linked genetic disorder and it is the leading monogenetic cause of autism. Inherited in a dominant fashion, Fragile X Syndrome is caused by CGG repeats at the site of the Fragile X Mental Retardation Protein (FMRP) gene on the X-chromosome, leading to absence of expression of FMRP. FMRP is a translational inhibitor involved in neuronal maturation and synaptic plasticity. It is also an indirect mediator of cellular metabolism. Knocking out FMRP in neurons causes the Fragile X phenotype, which is characterized by excessive levels of protein synthesis, insufficient synaptic plasticity, and morphologically immature synapses. FMRP regulates nervous system development by controlling the rate of translation for a wide variety of mRNAs associated with neuronal maturation. FMRP acts as a translational repressor in response to different kinds of synaptic stimulation. FMRP binds to the mRNA that codes for the Beta-subunit of ATP synthase and inhibits its translation. In response to stimulation, FMRP unbinds and allows for the translation of the Beta-subunit mRNA, which then modifies the metabolism so that it may meet the new energy demands of the developing neurons. In Fragile X Syndrome, this maturation process is disrupted, and upregulated protein synthesis of the c-subunit of ATP synthase is responsible for a metabolic inefficiency of FMRP KO neurons. The inefficiency comes from an inner membrane proton leak at the c-subunit of ATP synthase that disrupts the mitochondrial proton gradient, and therefore, ATP synthesis. By using Dexpramipexole (DEX), we hope to normalize the metabolism of FMRP KO neurons and rescue them. Analysis of the morphology of the dendritic spines of both WT and FMRP KO neurons will indicate the efficacy of DEX as a drug treatment for the FMRP KO phenotype.

Funding by Biological Discovery in Woods Hole at the Marine Biological Laboratory - NSF REU Award # 1659604

The influence of Hurricane Igor on the deep ocean carbon flux

Annagh Devitt^{1,2}; Rut Pedrosa-Pàmies²; Maureen H. Conte^{2,3}

¹University of Chicago, Chicago, IL, ²The Ecosystem Center, Marine Biological Laboratory, Woods Hole, MA, ³Bermuda Institute of Ocean Science, St. Georges GE01, Bermuda

Hurricanes exert large physical forces on the ocean, such as intense mixing and cooling of surface waters and upwelling of nutrient-rich waters which may trigger episodic phytoplankton blooms. While the upper-ocean response to hurricanes is relatively well-established, hurricanes' impact on the deep ocean carbon cycle remains barely known. I focused on Hurricane Igor to research the impact of episodic events on particle flux to the deep ocean. In September 2010, Hurricane Igor tracked through the NW Atlantic Ocean passing 64 km WNW of Bermuda. This study evaluates the organic composition of sinking particles collected before, during and after Igor's passage at the Oceanic Flux Program site offshore Bermuda. Lipid biomarkers were analyzed to characterize the relative contributions of fresh surface-derived phytodetritus, zooplankton, and bacterial biomass to the particulate organic carbon as it was transported through the mesopelagic and bathypelagic zones. Results indicate that the large total mass flux exported to the deep ocean during Igor's passage was mainly composed of carbonates resuspended from Bermuda's platform by internal waves, with a relatively small contribution from particulate organic carbon. However, the organic carbon exported to 1500 m and 3200 m was rich in labile and fresh phytoplankton-derived (e.g. fatty acids 20:5 ω 3 and 22:6 ω 3, and phytosterols), zooplankton-derived (e.g. 18:1 ω 9 and 20:1 fatty acids), and microbial-derived (e.g. 18:1 ω 7 and hopanoids) lipid compounds. This indicates that Igor enhanced the transfer of surface and platform-derived organic carbon and stimulated heterotrophic activity in the deep ocean. Our results demonstrate that hurricanes can enhance oceanic carbon export and impact deep ocean ecosystems. With an increasing number of extreme weather events due to climate change, these results indicate that such events, like hurricanes, can have an impact on global carbon cycling.

Jeff Metcalf Summer Undergraduate Research Fellowship – The University of Chicago

Three's Company: Linking Plant Cover, Tidal Inundation, and Community Structure in the Plum Island Estuary in the Face of Sea Level Rise

Abigail M. Kwiat, Michael S. Roy, and Jarrett E. K. Byrnes

University of Connecticut and University of Massachusetts Boston

The Plum Island Estuary (PIE) is the largest continuous saltmarsh in the northeastern United States. It is an extremely important habitat that supports a wide variety of marine and terrestrial organisms. Increased sea level rise (SLR) due to climate change could significantly alter plant species composition (i.e., percent cover of *Spartina alterniflora* versus *S. patens*) in this vital habitat through time. Previous studies indicate that both marine and terrestrial animals occupy different vegetation zones in PIE. Therefore, it is essential to determine connections among tidal inundation, percent cover of marsh plants, and species diversity to understand how the marsh community may shift with increased SLR. We asked the following questions related to the structure and species composition of this system: 1) What is the abundance and species composition of organisms within different zones of vegetation at high and low tide; and 2) does tidal inundation affect species diversity in each zone? To answer these questions, we utilized numerous sampling methodologies to analyze species diversity and PIE community structure. At two sites, we deployed a series of four transects with 5 evenly spaced plots per transect (n = 20 plots/site). In each plot, we deployed a novel shallow pitfall trap to capture marine and terrestrial invertebrates at low tide, breeder traps to collect marine species at high tide, and tide sticks to determine inundation levels per plot. Finally, we conducted quadrat surveys for each plot to determine percent cover of marsh vegetation. Data from our study offers compelling evidence that species that prefer low marsh environments, with higher levels of inundation, will thrive as sea levels continue to increase, due to increased levels of salt tolerance. Our results importantly provide insight into dynamics within this critical habitat as ecosystems are altered by climate change worldwide.

MBL PIE LTER REU program through the NSF