Adaptive Sensory Processing in the Lateral Line of *Leucoraja erinacea*

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Sensory self-stimulation from an animal’s own behavior often produces much stronger signals than important stimuli in its surrounding environment, yet the brain manages to filter out and ignore these self-generated signals. We have previously shown that the principal neurons in the primary electrosensory nucleus of the little skate (*Leucoraja erinacea*) learn to recognize and reject electrosensory signals that are consistently associated with the animal’s own behavior or fictive behavior. This adaptive filter mechanism is mediated by a distinctive anatomical organization that is also characteristic of the cerebellum. The same cerebellar-like organization is also found in the medial nucleus, which processes incoming mechanosensory lateral line information. Because of this similarity in structure, the medial nucleus is hypothesized to contain the same adaptive filter mechanism as a means of filtering out self-generated mechanosensory stimulation. Our goal is to test the presence of the adaptive filter in the lateral line of the skate. We experimentally coupled an external mechanosensory stimulus to each cycle of the skate’s fictive swimming in hopes of observing decreased, and therefore adaptive, responses from the principal neurons in the medial nucleus. Because getting sustained fictive swimming proved difficult, too few experiments have been completed thus far to fully test the hypothesis of the adaptive filter in the lateral line. In addition to testing more cells with a mechanosensory stimulus coupled to fictive swimming, we are also testing cells with the same stimulus coupled to fictive ventilation, which is a continuous and reliable ongoing behavior. Testing the presence of the adaptive filter in the lateral line would help piece together a model of how the cerebellum integrates, processes, and predicts sensory consequences of self-generated behavior.
Spinal regeneration in double transected lampreys

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In the United States alone, over 250,000 people live with spinal cord injury (SCI) and
approximately 12,000 more are afflicted each year. In humans, SCI can lead to irreversible
deficits in sensation and motor control beyond the injury site. There are animal models, however,
that possess mechanisms for spinal cord regeneration after SCI including Petromyzon marinus,
the sea lamprey. This model shows full behavioral recovery 11 weeks after a complete spinal
transection. Although its recovery from SCI has been studied extensively, little is known about
what limits this regenerative ability may have. Therefore, we set out to investigate whether
lampreys transected a second time would recover through behavioral analysis of double
transected animals as well as structural analysis of their spinal cords. Using a combination of
biweekly behavioral scoring and fluorescence microscopy, we found that double transected
animals show behavioral recovery 11 weeks post injury, as well structural recovery of axons and
microtubules across the transection site. In addition, there is evidence that the synaptic density
was not fully restored across the transection site. Despite this deficit, our initial data show that
double transected lampreys recover behaviorally after 11 weeks, suggesting that lampreys
possess pro-regenerative mechanisms that allow them to recover from multiple spinal
transections.

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Recording the dynamic passing cloud display in cuttlefish
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The static and dynamic skin patterns typical of cuttlefishes are largely produced by the expansion and contraction of millions of pigmented chromatophore organs, each operated by multiple innervated muscles. Passing cloud is characterized by synchronized bands of dark expanded chromatophores traveling across the upper mantle. The fundamental question concerns whether muscle-muscle interactions contribute to some of the synchrony; that is, perhaps not all the passing cloud may be controlled via nerves originating in the brain. This infers some peripheral control of patterning. For this study, European common cuttlefish (Sepia officinalis) and flamboyant cuttlefish (Metasepia pfefferi) were used to model passing cloud. Multiple high-resolution videos, ranging from 1x-5x magnification, were taken of the animals performing passing cloud while in modified tanks. Trimmed videos were then sent to modelers who will use diffusion-driven Turing instability to replicate the peripheral emergent behavior of traveling waves by processing the spatial symmetry of chromatophore expansion and contraction. Through the addition of other parameters, including timescale, refractory period, feedback control, boundary conditions and exogenous signals, the team hopes to model passing cloud and identify the level of influence on wave dynamics of the central nervous system versus local peripheral control via muscles in the skin.
Using passive acoustics to determine the effect of abiotic and biotic sound on Oyster Toadfish (*Opsanus tau*) vocalization rates
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Acoustic communication is critical for reproductive success in the Oyster Toadfish, *Opsanus tau*. Passive acoustics allows for non-invasive monitoring of fish vocalizations and previous studies have determined the seasonality of toadfish calls in Eel Pond in Woods Hole, MA using a single hydrophone. Over the last century, human activities have increasingly added artificial sounds to the aquatic environment. The purposes of this study were to determine the number and location of vocalizing toadfish in Eel Pond using a multiple hydrophone array and to monitor how different abiotic and biotic factors impact toadfish calls. Numerous motorized watercraft armored in Eel Pond, including the RV Gemma, which presents a unique opportunity to monitor toadfish vocalizations in response to anthropogenic sound. A four-hydrophone linear array was deployed to record underwater sound. The number of calls, amplitude, time interval and location of fish recorded from the hydrophones were analyzed. Six different vocalizing toadfish were located and monitored over the course of the breeding season. Anthropogenic sound produced by the RV Gemma and heavy rain events depressed vocalization rates in toadfish. Boatwhistle playbacks using a previously recorded toadfish call increased the amplitude and number of toadfish calls during the peak mating season. The multiple hydrophone array allowed for individual toadfish locations to be determined and monitored and provided data that shows toadfish vocalization rates can be influenced by anthropogenic, environmental and conspecific sounds.

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Actin cytoskeleton interactions with Kv3.3 and Hax-1 in MNTB neurons
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Neurons in the Medial Nucleus of the Trapezoid Body (MNTB) are involved in locating the source of sounds as well as processing high frequency auditory stimuli. The Kv3.3 channel, which is highly expressed in these neurons, is responsible for the rapid repolarization of the membrane potential after firing. Kv3.3 is expressed at particularly high levels in the large presynaptic terminals, called Calyces of Held, which provide synaptic input to MNTB neurons. The Kv3.3 channel has been shown to bind to Hax-1, an anti-apoptotic protein that binds to Arp 2/3, which is an actin-recruiting protein complex. The Hax-1/Arp 2/3 complex binds the C-terminus of Kv3.3 and nucleates actin at the plasma membrane. In transfected cells, this creates a channel-associated subcortical cytoskeleton that regulates the activation and inactivation of Kv3.3. It is not yet known, however, whether Hax-1 is localized with Kv3.3 channels in any type of neuron. Using immunofluorescent labeling techniques, as well as phalloidin staining, we imaged actin, Kv3.3 channels, and Hax-1 in the presynaptic Calyces of Held and in postsynaptic MNTB neurons in the rat brain stem. We were able to demonstrate co-localization of Hax-1 with the dense actin cytoskeleton in the presynaptic terminals. This indicates that Hax-1 and actin are closely associated with one another in the MNTB.

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Effects of Fragile X Syndrome on the conductance of mitochondrial membrane channels
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Fragile X Syndrome (FXS) is the most common form of inherited intellectual disability. In FXS, the $Fmr1$ gene is silenced, leading to pathologically low levels of Fragile X Mental Retardation Protein (FMRP). FMRP is an mRNA-binding protein that inhibits mRNA translation and represses protein synthesis, primarily in dendrites. The absence of FMRP is thought to be responsible for the long and immature dendritic spines that characterize FXS neurons. The Jonas lab has previously discovered that FMRP plays an important role in regulating normal mitochondrial function and hypothesizes that abnormalities due to the absence of FMRP may disturb the regulation of protein synthesis, thus disrupting dendritic spine formation, synaptic plasticity, and learning. Recent experiments provide evidence of a leak channel in the c-subunit of the $F_1F_0$ ATP synthase, which is embedded in the mitochondrial inner membrane. We hypothesize that FMRP regulates this leak channel by binding to the $F_1$ subunit of the ATP synthase and that the absence of FMRP causes increased inner mitochondrial membrane conductance. To test this hypothesis, we performed patch clamp recordings to compare FMRP-knockout mitochondrial conductance to the conductance of control mitochondria. These recordings are currently being analyzed. Future studies will involve adding FMRP to the FMRP-knockout mice in order to determine whether or not the protein closes the leak and lowers membrane conductance.

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Cellular and Molecular Characterization of Head Regeneration in *Lumbriculus variegatus.*
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*Lumbriculus variegatus* is a freshwater annelid that has robust regenerative capabilities. Previous work in our lab has demonstrated that proper head regeneration is a crucial step for downstream mechanisms that mediate functional recovery of behaviors within the original worm fragment. In this study, we describe cellular changes occurring at the head blastema and identify proteins that are changed in their expression within the regenerating head and in segments located just behind the head. Experiments removing thirty-segment fragments from either the anterior or posterior region of the worm, demonstrate that the head always regenerates on the anterior end of the fragment, regardless of which position along the body axis the fragment was removed (n=10). This data suggests that polarity of the injured fragment is maintained. However, when worms are amputated at varying positions along the anterior-posterior axis, asexual fission planes form more readily (n=17; 76.5%) in the anterior 1/3 region in comparison to injury within the posterior 2/3 region. Molecular expression in regenerating head segments was characterized first using antibodies raised against Drosophila wingless protein and antigens isolated from regenerating blastemal tissue in planaria. Of the two antibodies, the antibody raised against blastemal antigens demonstrated the most unique staining patterns in regenerating head and tail tissue with puncta found throughout each region and along the top of the ventral nerve cord. Immunoblots were later performed on regenerating anterior and posterior worm fragments at 24hr, 1wk, and 3wk post-amputation. Immunoblot detection using the anti-blastemal antibody identified the presence of 3 positive protein epitopes: one that is 126-129KDa, one that is 103-109KDa, and another that is 87KDa in size. Differential expression of the antigen was detected in regenerating head and tail tissues. Taken together these data demonstrate cellular and molecular events that are specific to head regeneration.

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Cuttlefish produce a remarkable display called passing cloud, wherein dark waves appear to travel across the mantle, which requires sequential synchronization of the muscle-pulled expansion of thousands of pigmented chromatophores organs. Notably, both denervated cuttlefish and denervated squid produce traveling waves, although less synchronized, when pulled or chemically stimulated. Squid share the same basic chromatophore anatomy with cuttlefish but do not produce any sort of traveling wave display when living. This suggests that traveling waves in cephalopod skin are not controlled solely by the central nervous system, but that peripheral anatomy plays a role in propagating chromatophore actuation in waves. We aim to test this hypothesis with two approaches: computational modeling of cuttlefish passing cloud dynamics and confocal microscopy of de-innervated squid skin. For the first, we filmed high-speed, high-resolution video of free-swimming cuttlefish passing cloud at both high and low magnification, to visualize both individual chromatophores and the dynamics of the entire wave period. The modeling team will test models of these data to see whether muscle-to-muscle interactions can account for some of the observed dynamics, or if complete central control of chromatophores is necessary. For the second approach, we cut one of the ipsilateral large second order nerve bundles in the squid mantle to produce blanching of the now denervated mantle. We observed that the skin is still able to produce the traveling waves previously seen in dead squid. To assess the state of the peripheral anatomy controlling these blanched chromatophores, we use confocal microscopy to examine the tissue and see if the nerves show signs of atrophy; if so, it suggests that muscles alone can aid the synchronized actuation of passing cloud. The results from our two approaches will help determine future study of the mechanisms of traveling waves in cephalopods.

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Location and interactions of Bcl-X_L and actin in the brain stem
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The Medial Nucleus of the Trapezoid Body (MNTB) contains postsynaptic neurons that receive input from very large presynaptic terminals which are called the Calyces of Held. These function within the ascending auditory pathway and are required for the nervous system to be able to detect the location of sounds in space. The large Calyx of Held synapse has been used by many investigators to study synaptic transmission. Our experiments aim to examine the localization of the anti-cell death protein Bcl-X_L in this region of the brain stem. Bcl-X_L is associated with several processes such as the formation of synapses, regulation of synaptic responses, synaptic vesicle recycling and mitochondrial metabolism. The cytoskeletal element, actin, also plays a role in presynaptic vesicle recycling, and previous work has shown that the calyces are marked by heavy actin staining. Although Bcl-X_L mRNA has been reported in the MNTB, it is not known if or where the protein is expressed. Using immunofluorescent labeling, we set out to determine if Bcl-X_L and actin are colocalized, which would suggest that they work together to achieve vesicle resupply to the presynaptic active zones. Our experiments examined whether Bcl-X_L is localized to axons, to the calyx, and/or to the postsynaptic neurons. Our preliminary findings show that Bcl-X_L is found in both the presynaptic and postsynaptic neurons of the MNTB, colocalizing with actin in the axons and possibly in the Calyx of Held. Punctate organization of Bcl-X_L in the axons of brain stem neurons suggests that Bcl-X_L is present within mitochondria. This feature may function to supply energy to active synapses for vesicle recycling and for re-arrangement of the cytoskeleton. We conclude that the function of Bcl-X_L in the Calyx of Held may be necessary to acutely alter structural elements of the synapse for the process of strengthening synaptic transmission.

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Analysis of *Doryteuthis pealeii* Squid Locomotion Using a Novel Bio-logging Tag: Effects of Attachment, Water Flow, and Noise

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Squid are important invertebrates in marine ecosystems as prey to many organisms in higher trophic levels and as predators in food webs. Thus it is in situ observations of their behaviors in response to stimuli in their natural environment. These stimuli can include human activity, such as pile driving pulses generated from offshore construction sites. In this study, we describe behavioral responses of small-bodied squid, *Doryteuthis pealeii*, with iTag attachment (a soft-bodied invertebrate eco-sensor tag), flow, and pile driving pulses. The iTag (invertebrate Tag) is a novel biologging device containing 3D accelerometer, gyroscope, light, and temperature sensors. iTag remained attached to squid for 15 minute flow conditions and 15 minute pile driving pulsing conditions. Using video analysis, locomotion behaviors (resting, finning, flapping, jetting) were identified. Control periods with no stimuli (iTag, flow, and pile driving pulses) showed that all squid (iTagged males, control males, control females) exhibited finning locomotion most frequently, with a significant increase in resting behavior in iTagged males between control periods before iTag attachment and after iTag attachment (F= 7.19, P< 0.001). No significant differences were in locomotion were found in flow and pile driving conditions for any squid. These data indicate that the current model of this eco-sensor (iTag) has an impact on squid locomotion, but with minor modifications, can be used to quantify behavior of small-bodied squid species.
Pancreas beta cell development in Xenopus
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Malfunction of the pancreas is linked to many diseases including pancreatitis, diabetes, and pancreatic cancer. Recent research has focused on developing techniques to create insulin producing beta cells \textit{in vitro} through direct reprogramming of stem cells or differentiated cells for beta cell replacement therapy. In order to efficiently promote mature beta cell fates, it is necessary to define the beta cell gene regulatory network in high resolution. Through controlled expression of Neurogenin3 (Ngn3), a bHLH transcription factor, we can preferentially promote beta and delta cell fates in Xenopus endoderm over other endocrine cell fates. To further define the beta cell lineage, we used RNA-seq on endoderm at hourly intervals after Ngn3 activation to measure changes in RNA transcripts. Two genes that showed increased expression within the first 6 hours were Delta-like 4 (dll4), a putative notch ligand, and POU class 4 homeobox 3 (pou4f3), a homeodomain transcription factor. This expression was similar to that seen for known Ngn3 target genes. Neither gene, however, has been shown to play a role in endocrine development. Here, we use CRISPR-Cas9 and morpholinos to examine the function of dll4 and pou4f3 in pancreas development.

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Eutrophication of Waquoit Bay estuaries: Effects of land-derived nitrogen loading on the assemblages macroalgal taxa
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Increased nitrogen (N) loading due to human development and activity on watersheds affects the abundance of macroalgae in receiving estuaries. Though total macroalgal biomass increases as N load increases, species diversity found in macroalgal assemblages may differ in response to external N loads, affecting consumption at the food chain level. To test this hypothesis, we collected macroalgal samples from three Waquoit Bay estuaries that are subject to different land-derived N loads: Childs River (high N load), Quashnet River (intermediate N load), and Sage Lot Pond (low N load).

Dissolved inorganic nitrogen (DIN) concentrations in the water column increased as N load increased, and total macroalgal biomass responded to DIN. There were no clear seasonal trends in the responses of different macroalgal species. Different taxa responded differently to changes in nitrogen supply. Biomass of taxa such as Cladophora vagabunda did not significantly respond to increased N loads. Other taxa, such as Ulva lactuca and Gracilaria tikvahiae significantly increased in biomass in response to increased nitrogen. The biomass response to larger N loads in Childs River resulted in an assemblage dominated by G. tikvahiae and U. lactuca, replacing the assemblage that was prevalent under low N supply, where C. vagabunda and G. tikvahiae shared dominance. The trend in biomass increases resulting from different N loads was not paralleled by N uptake in the taxa. Differing resilience to nitrogen between taxa may account for the difference between N uptake and biomass responses.

The finding that total macroalgal biomass responds positively to N loads corroborates many earlier reports. Our results show that the percent biomass of various taxa differs within the macroalgal assemblage. These differences may be meaningful at the food chain level, because the different macroalgal species alter ambient nutrient regimes, decomposition rates, grazing potential, and palatability of biomass for consumers.

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Responses of inorganic nitrogen concentrations and producers in Cape Cod estuarine systems subject to differing rates of nitrogen loading
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Land-derived nitrogen loads to estuarine systems can have a significant effect on water quality as nitrogen is a principal element regulating biological activity. To examine the effect of nitrogen on phytoplankton and macrophytes as well as assess the fate of nitrogen as it moves through estuarine system to the ocean, we sampled nitrate and ammonium concentrations, salinity, chlorophyll concentration, and macroalgal biomass in five estuaries in Cape Cod subject to different nitrogen loads — Childs River, Quashnet River, and Sage Lot Pond in Waquoit Bay as well as Wareham River and Buttermilk Bay in Buzzards Bay. Nitrate concentrations decreased as salinity increased, suggesting that nitrate in the water column was mainly land-derived. Additional evidence of a terrestrial source of nitrate is provided by the similarity in groundwater concentrations of nitrate entering estuaries and the predicted concentration found in nitrate versus salinity curves. Nitrate concentrations decreased with increased salinity at a faster rate than would be the case of passive mixing of nitrate-high freshwater and nitrate-poor seawater. These estuaries have high nitrate retention capacity, and therefore help maintain marine water quality, lowering discharge of nitrate into near-shore ocean water. Ammonium concentrations were not correlated to salinity and may depend largely on in-estuary processes. Within estuaries, chlorophyll concentration and macroalgal biomass increased with an increased nitrogen load. Phytoplankton and macrophyte abundance within estuaries are therefore affected significantly by larger nitrogen loads, but that high retention of nitrogen within estuaries actively protects water quality in the near-shore ocean waters that receive nitrogen from the estuaries.

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Understanding Particle Cycling in the Deep Sargasso Sea through the Use of Lipid Biomarkers
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Organic material in the oceanic water column consists of dissolved, suspended, and sinking material. Primary productivity in the sunlit surface waters drives the flux of organic material that eventually reaches the sea floor. The flux of this material is a major food source for ecosystems below the surface and plays an important role in many biogeochemical processes such as nutrient cycling and atmospheric carbon dioxide uptake. Molecular composition can provide insights on the sources of organic matter and processes controlling its degradation and remineralization in the water column and sediments.

I studied the organic composition of suspended and sinking particles, and at the sediment/water interface at the Oceanic Flux Program site located in the northern Sargasso Sea in 4500m of water. Suspended particles were collected by in situ pumps (30m-4400m), sinking particles by sediment traps (500m, 1500m, 3200m), and surface sediments (upper 0-0.5cm) by a Van Veen sampler.

This study used biomarkers (fatty acids, fatty alcohols, sterols, and hopanoids) to understand the coupling between suspended and sinking particles in the deep ocean and the fate of organic material after delivery to the sea floor. Suspended and sinking particles from the deepest part of the water column collected in April, October, and November, and surface sediments collected in June and August, were compared to determine seasonal patterns. Even in the deepest ocean (>3200m), lipid composition of particles reflects seasonal productivity in surface waters. The June surface sediments (post spring bloom) contain fresher material characteristic of bloom detritus, while the August samples show more evidence of degraded material with greater bacterial and animal contributions.
The effect of marsh zonation on amphipod distribution and predation
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The salt marsh amphipod \textit{Orchestia grillus} is an important component of salt marsh food webs as both facilitators of dead \textit{Spartina} spp. decomposition and likely prey items for birds, fish, and crabs. However, the identity of major \textit{O. grillus} predators, relative predation rates, and the influence of habitat type and \textit{O. grillus} density on predation rates all remains unknown. We designed surveys and tethering experiments to investigate (1) How \textit{O. grillus} abundance varies throughout the marsh, (2) variability in \textit{O. grillus} consumption rates and (3) the identity of key \textit{O. grillus} predators. To determine how amphipod distribution changed with marsh vegetation type, we conducted a survey of amphipod abundance at six habitat types in a gradient from the creek edge to the upland border at two different sites at the Plum Island Estuary (PIE). To determine how predation rates vary by habitat, we deployed 60 tethers in 4 habitat types along three transects per marsh (i.e. 5 tethers per plot for four plots and three transects). We also conducted bird surveys and deployed GoPros during each tether to determine predator identity. Surveys show that amphipods are most common in the upland border/high marsh boundary zone as well as the low marsh to high marsh boundary zone. The highest predation rate on amphipods occurred in the upland border/high marsh boundary zone. Data regarding predator identity has yet to be analyzed. These results indicate the critical role of amphipods in driving the behavior of salt marsh predators and the role of marsh vegetation and tidal inundation influencing the density of this semi-terrestrial marsh invertebrate.

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Carbon cycling in temperate salt marsh ponds
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Salt marshes provide important ecosystem services, one of which is global carbon sequestration. Permanently inundated ponds are prominent facets in the landscape, composing up 60\% of the total marsh area, but they are rarely considered in biogeochemical assessments. Here, we studied metabolism in two salt marsh ponds within the Plum Island Ecosystems LTER. Oxygen and pH measurements were continuously recording using YSI sensors. Surface water samples and sediment cores were taken on selected days. Surface water and porewater samples were analyzed for dissolved inorganic carbon (DIC). The ponds became hypoxic or anoxic at night, which indicates the importance of anaerobic decomposition. During these times and during periods of high photosynthesis, changes in oxygen concentrations were lower than those in DIC concentrations. Thus, we estimated net ecosystem production, gross primary production and respiration based only on changes in carbon fluxes. One pond was net autotrophic and the other net heterotrophic. Differences between the two ponds could be explained by the absence/presence of \textit{Ruppia maritima}. Overall, marsh ponds are heterogeneous in their carbon cycling role in the surrounding landscape.
Effects of tidal restriction on greenhouse gas emissions in New England coastal wetlands
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Coastal wetlands have been the focus of substantial research interest because of their high rate of carbon sequestration per unit area. While this makes them useful for greenhouse gas (GHG) emission offset projects, coastal wetlands are often subjected to a number of anthropogenic factors, such as tidal impoundment and colonization by invasive species, that modulate their effectiveness in sequestering carbon. Past research has produced models showing that restoring tidal flows to impounded wetlands reduces methane emissions. In this study, we collected empirical evidence to better inform this claim, by measuring both carbon dioxide (CO\textsubscript{2}) and methane (CH\textsubscript{4}) fluxes. The GHG fluxes were compared between undisturbed tidal salt marshes and tidally-restricted marshes, between March and August 2017, at various plant species-defined sites. There were two undisturbed tidal salt marsh sites: high marsh (\textit{Spartina patens}) and low marsh (\textit{Spartina alterniflora}); and three tidally-restricted sites: invasive (\textit{Phragmites australis}), native (\textit{Typha latifolia}), and unvegetated zones. The CO\textsubscript{2} fluxes across the sites were used to calculate and compare primary productivity of the various sites. The net CO\textsubscript{2} flux into biomass was compared with CH\textsubscript{4} emissions to determine the overall contribution of the sites to global warming effects. As a result of the unequal contribution to global warming by CH\textsubscript{4} and CO\textsubscript{2}, the proportion of CO\textsubscript{2} sequestration with CH\textsubscript{4} emission by coastal marshes is a crucial consideration in designing policies that are concerned with offsetting GHG emissions.

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Coral Bleaching Research as a Tool for Effective Science Communication
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Coral reefs make up the most biodiverse ecosystems on earth, while only accounting for 0.2% of earth’s surface. These complex living assemblages are estimated to contain nearly 9 million species, with about 60,000 or so known and documented. For many countries, the ecosystem services coral reefs provide (fisheries, tourism, etc.) drive economies. Additionally, reefs provide protection for coastal cities from storm surges, and potentially damaging waves. As earth’s ocean temperatures rise as a result of climate change, the corals that form reefs undergo bleaching, a stress response in which the photosynthetic algal cells (zooxanthellae) living within the corals are expelled. When corals are unable to regain their zooxanthellae for an extended period, they become susceptible to disease and competition for space by macroalgae, and eventually die. Among the general public, and even in the science community immediately outside of coral research, the importance of corals and the significance of mass bleaching events are not fully understood. “What is coral bleaching?” and “Are bleached corals dead?” are among questions that highlight the confusion and need for greater outreach on this complex issue. Furthermore, while the general public has begun to accept climate change, misconceptions still exist in the understanding that climate change has been exacerbated by human impacts. To address these issues, we explored the use of a research project on the effects of thermal stress on the Caribbean coral *Porites astreoides* as a tool for effective science communication. Methodology included identifying the audience for the greatest impact, identifying different technologies and approaches to study coral bleaching, identifying and using strategies such as social media platforms to communicate science, and effectively communicating results. As ocean temperatures continue to rise, the number and intensity of threats to coral reefs increase as well. Clear, effective communication of this time-sensitive issue is crucial, and social media applications may play a large role in educating the masses.

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**Improved Site-directed RNA editing by using ADARs from diverse organisms**

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RNA editing could potentially become a powerful tool that would benefit medicine and scientific research. The natural process of RNA editing is catalyzed by adenosine deaminases acting on RNA (ADARs), a class of enzymes that convert adenosine nucleosides into inosines, which are read as guanosines during translation. Our laboratory is interested in developing methods of guiding ADARs to adenosines of our choosing. Certain genetic diseases, such as Cystic Fibrosis, are caused by point mutations that create premature termination codons. In some cases, site-directed RNA editing through the redirection of ADAR function could repair these mutations. The Rosenthal laboratory developed a simple method of site-directed RNA editing. It can be used to edit a specific target by linking the catalytic domain of ADAR to an antisense oligonucleotide for guidance. The antisense oligo and the catalytic domain of human ADAR2 are linked using the λN peptide and the boxB RNA hairpin that it binds. The basic system can edit some adenosines very efficiently, and others less so, due to the intrinsic selectivity of human ADAR2. For example, ~70% of mRNAs carrying a UAG premature termination codon can be corrected in human cells. However, A’s with other neighbors are edited far less efficiently. Our current research tests whether ADAR catalytic domains from other organisms can edit A’s in specific neighboring contexts better than the human enzyme. To accomplish this, we linked ADAR deaminase domains from different organisms to four λN peptides and transfected HEK293T cells with this construct, an RNA guide and a fluorescent protein with a premature termination codon. Thus far we have shown that deaminase domains from two other organisms can edit a UAG premature termination codon at levels similar to the human enzyme. We are currently working on testing deaminase domains from other organisms on adenosines in less favorable contexts.

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An ancestral role for RNA editing in innate immunity
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The ADAR family of RNA-editing enzymes is largely conserved in all metazoans, suggesting it has an essential regulatory function. However, the precise nature of this function remains unclear. ADARs deaminate adenosines to inosines within the cell. Since cellular machinery reads inosines as guanosines during translation, ADARs have the ability to alter mRNA coding sequences. This powerful recoding ability has been a topic of great intrigue; however, in vertebrates, ADARs rarely alter codons. Instead, most editing takes place in non-coding regions. Recently, editing by the ADAR1 protein has been implicated in innate immunity in mice. In order to determine whether this function is evolutionary conserved, we looked at the expression of ADARs in a relevant invertebrate system: the squid. Unlike vertebrates, squid use their ADARs to recode extensively, particularly in the nervous system. They also possess homologs to the vertebrate ADAR1 and ADAR2 proteins. Using Western Blotting and qPCR, we examine the expression levels of squid ADAR1 and ADAR2 in the white body, an immune organ, is comparable to that of nervous tissue, suggesting that ADAR could also play a role in controlling immunity in squid.

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Modeling aging: the eyesight of *Brachionus manjavacas*

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In aging studies, rotifers are a useful model organism due to their short two-week lifespan and the ease with which they can be mass-cultured. Rotifers also have longevity-related genes in common with humans that *D. melanogaster* and *C. elegans* have lost. These facts make rotifer studies an efficient and relevant tool for screening aging-related treatments. Like humans, *B. manjavacas* rotifers experience a deterioration of eyesight with age. The purpose of my experiment was to test if Trolox, a vitamin E analog, could partially rescue this effect. Rotifer eyesight is basic and easy to assess—they exhibit positive phototaxis. Four treatments were tested—a control containing no Trolox, a 25μM Trolox solution, a 50μM Trolox solution, and a 100μM Trolox solution. Age-synchronized rotifers were cultured in each of these treatments and assessed every 2–3 days for phototactic behavior. Although the phototaxis rates were consistently slightly higher in the 25μM and 100μM treatments than in the control treatment, this effect was not statistically significant at any timepoint. In addition to this work on Trolox, I will discuss my ongoing project—using RNA interference to knockdown expression of the PAX6 gene in rotifers. PAX6 is involved in eyespot development, so knockdown should result in a reduced or absent eyespot and a decline in phototaxis.

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Optimizing the expression of soluble recombinant protein by a mutant reverse transcriptase gene expressed in *Escherichia coli*

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Reverse transcriptases (RTs) are a unique set of enzymes unusual in that they can synthesize DNA using an RNA template, the opposite direction of customary transcription. A recently discovered class of single-copy, RT-related genes named *rvt* differs from other RTs which are usually associated with mobile elements. A large loop domain that has an unknown function and isn’t present in any known RTs separates the highly conserved motifs 1 and 2 from the rest of the *rvt* gene. This study aimed to aid the determination of the function of this large loop domain by recovering soluble proteins expressed by an *rvt* mutant (IAY1) whose loop domain had been deleted. Ideally, further studies will purify the recombinant proteins expressed by IAY1 and compare their activity to that of the full length *rvt* gene, thereby determining the function of the missing loop domain. To obtain sufficient amounts of soluble recombinant protein to be used in protein purification, protein expression in E. coli had to be optimized. Over the course of this experiment, our laboratory compared many different culturing parameters — various concentrations of IPTG, times and temperatures at which the cultures were allowed to grow, and the inclusion of additives shown to improve solubility — to see what conditions led to an optimal expression of soluble protein. Similarly, different host strains of E. coli were compared.

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Capture of extrachromosomal circular DNA in bdelloid rotifers via Mu transposition cloning pathway
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Bdelloid rotifers, tiny freshwater metazoans, pose an evolutionary conundrum in their genomic makeup coupled with their utilization of asexual reproduction. Genome analysis of the bdelloid rotifer Adineta vaga reveals that transposable elements (TEs) compose only 3% of the genome, contrary to an unchecked TE expansion or total absence expected in asexual organisms. TE content, while held to low copy counts, contains great diversity, related to rotifers’ receptiveness to horizontal gene transfer with 8% of genes likely of non-metazoan origin. Large, complex TEs called Terminons have been recently identified in A. vaga, which act as retroelements that facilitate DNA transposition through insertion of mobile RNA copies via reverse transcriptase. Terminons, along with other TEs, have been found to encode rolling-circle replication initiator proteins (Rep), which generate extrachromosomal, circular intermediates while facilitating transposition within the host genome. As a result, circular rotifer DNA provides insight into transposition events, as they are a direct byproduct of said events. We attempt to identify these circular intermediates through Mu transposition-based cloning, which delivers an origin of replication and a selectable marker to circular DNA. This is achieved through the insertion of the marker-carrying transposon into circular Donor DNA through the interaction of MuA transposase enzyme and the transposon’s Mu sites. The dynamics of this reaction were initially explored with plasmid pUC19 as control, and later with isolated A. vaga DNA. Reaction set up was optimized with different methods deployed for preparing enriched circular DNA template extractions and isolating Mu transposon. Specific cloning pathway included restriction enzyme liberation of Mu transposon, reaction with prepared A. vaga donor DNA, E. coli transformation and colony selection, insertion confirmation by plasmid digest, and eventual sequencing.

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Identifying extrachromosomal intermediates of transposition in bdelloid rotifers
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Transposable elements (TEs) are mobile segments of DNA common in eukaryotic genomes. A class I TE, or retroelement, transposes by creating an RNA copy of itself that is subsequently transcribed back into DNA by an element-encoded reverse transcriptase and inserted into new locations within the host genome. Here, we focus on newly described retroelements, termed Terminons, in the genome of bdelloid rotifers, microscopic freshwater invertebrates known for their ability to withstand desiccation, susceptibility to horizontal gene transfer, and mode of asexual reproduction. Terminons are large and complex, often exceeding 40 kb in length and encoding numerous ORFs. Of particular interest are ORFs encoding rolling-circle replication initiator (Rep) proteins that harbor a striking similarity to geminivirus Rep proteins. In geminiviruses, pathogenic plant viruses with circular single-stranded (ss) DNA, the Rep protein facilitates replication of the viral genome through rolling-circle replication. Rep proteins, while not intrinsic to Terminons, are thought to form extrachromosomal intermediates of transposition in rotifers. In this study, cesium chloride density-gradient centrifugation was used to search for said intermediates either in the form of extrachromosomal circular DNA or nucleoprotein particles. Centrifugation was performed with samples of DNA from three species of bdelloid rotifers: namely Adineta vaga, Adineta sp. 11-2 natural isolate, and Adineta ricciae.

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Species composition of microbial structures in dental plaque
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It is increasingly becoming understood that the properties of microbes depend on their neighbors and their local environment. The recently described “hedgehog” structure found in dental plaque is a prime example of this. \textit{Corynebacterium} provides a core structure with radiating filaments, which cocci surround in a “corncob” formation. The various taxa surround the filaments in a specific pattern due to their micron-scale environment, with \textit{Streptococcus} cocci binding to the outermost edge of the hedgehog, while anaerobic taxa including \textit{Fusobacterium} and \textit{Leptotrichia} bind more proximally.\textsuperscript{3} This microbial consortium is understood at the genus level, but the species identity of these microbes is pertinent to further research, as individual species within a taxon can have diverging morphologies and physiologies. The purpose of this project is to determine the species of \textit{Streptococcus} and \textit{Corynebacterium} involved in corncob structures of dental plaque. We are using fluorescence \textit{in situ} hybridization to visualize and differentiate bacterial species. We have developed probes targeting individual species of \textit{Corynebacterium} and \textit{Streptococcus} and tested these probes on pure cultures to ensure specific hybridization of relevant species. We are applying these probes to dental plaque samples to determine the species involved in corncob structures. Preliminary results indicate that \textit{Streptococcus mitis} is present in corncob structures. These results stand in contrast to literature reports of another species, \textit{S. cristatus}, in these structures.

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Microbial Structures of the Human Tongue
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Microbial communities play a crucial role in the human body, especially when it comes to oral health. The structural organization of bacteria has recently been identified in dental plaque and other parts of the mouth like the cheek and tongue. There are five major types of bacteria on the tongue: Rothia, Streptococcus, Veillonella, Actinomyces, and Neisseria. With the current understanding of the organizational structure of two of the main components of the oral microbiota, Rothia mucilaginosa and Streptococcus mitis, and determine if there are different strains of the same species colonizing the same individual. In order to get all strains from a single individual, a tongue sample will be collected from an anonymous donor, diluted, and streaked to purity. After streaking the bacteria to purity, I will use Fluorescence in situ Hybridization (FISH) to detect the bacteria we are looking for using specific probes. Using PCR targeted to 16S rRNA, I will identify different strains of R. mucilaginosa and S. mitis and select candidates for genome sequencing. I will then compare those genomes to a metagenome from dental plaque to identify whether different species colonize plaque and tongue. This will help us better understand which Streptococcus are present on the tongue vs. in plaque and whether each species is represented by a single colony or different types within each species within each person.

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Measuring Resolution of the Orientation-Independent Differential Interference Contrast Microscope
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In my presentation I describe a method of using a Siemens star to measure the resolution of an optical microscope. I focus on measuring the orientation-independent differential interference contrast (OI-DIC) microscope developed by M. Shribak. In particular, I examined how this microscope compares to related microscopy techniques, as well as optimized the specific OI-DIC parameters to obtain the clearest image possible. OI-DIC and its pre- and post-image processing techniques were compared to standard DIC, phase contrast, and phase DIC. OI-DIC parameters of interest include wavelength of light, numerical aperture of the condenser lens, and the DIC prism model. The algorithm and GUI for measurement and processing was developed in Python, which processes and analyzes the lateral modulation response to spatial frequency and the axial modulation response for three-dimensional image stacks of any Siemens star test structure. The results for each variable are discussed in detail in the presentation. The information acquired from these measurements confirms some of the resolution improvements of OI-DIC microscopy relative to other techniques, and helps guide users in the selection of OI-DIC parameters for getting the best image possible. These results also indicate specific improvements that can be made to the OI-DIC microscope used in this study.

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Effects of Fragile X Syndrome on the conductance of mitochondrial membrane channels
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Fragile X Syndrome (FXS) is the most common form of inherited intellectual disability. In FXS, the \textit{Fmr1} gene is silenced, leading to pathologically low levels of Fragile X Mental Retardation Protein (FMRP). FMRP is an mRNA-binding protein that inhibits mRNA translation and represses protein synthesis, primarily in dendrites. The absence of FMRP is thought to be responsible for the long and immature dendritic spines that characterize FXS neurons. The Jonas lab has previously discovered that FMRP plays an important role in regulating normal mitochondrial function and hypothesizes that abnormalities due to the absence of FMRP may disturb the regulation of protein synthesis, thus disrupting dendritic spine formation, synaptic plasticity, and learning. Recent experiments provide evidence of a leak channel in the c-subunit of the F.F.\textsubscript{1}F.O\textsubscript{1} ATP synthase, which is embedded in the mitochondrial inner membrane. We hypothesize that FMRP regulates this leak channel by binding to the F.\textsubscript{1} subunit of the ATP synthase and that the absence of FMRP causes increased inner mitochondrial membrane conductance. To test this hypothesis, we performed patch clamp recordings to compare FMRP-knockout mitochondrial conductance to the conductance of control mitochondria. These recordings are currently being analyzed. Future studies will involve adding FMRP to the FMRP-knockout mice in order to determine whether or not the protein closes the leak and lowers membrane conductance.

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Fast axonal transport dysfunction in neurons is mediated by mutant tau protein via activation of phosphotransferases

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Alzheimer’s disease (AD) is a chronic neurodegenerative disease characterized by dementia, memory loss, problems with language and mood disorders. Aggregates of the microtubule-associated protein tau, which results in neuronal degeneration through inhibition of kinesin-based anterograde fast axonal transport (FAT), are hallmarks of AD as well as other tauopathies. However, the molecular mechanisms that link the effect of pathogenic forms of tau to the cause of anterograde FAT inhibition are poorly understood. Using squid axoplasm isolated from squid giant axons, our laboratory has shown that it is through the activation of axonal protein phosphatase 1 (PP1), subsequent dephosphorylation of glycogen synthase kinase 3 (GSK3), and then the phosphorylation of kinesin, that allows aggregated, microtubule-associated tau proteins to inhibit anterograde FAT in AD. In addition, a phosphatase-activating domain (PAD) at the N-terminus of tau, has been shown to be fundamental in directly activating PP1. Increased exposure of PAD in pathogenic forms of tau results in misregulation of axonal phosphotransferases, which greatly affects the activity downstream and provides a molecular explanation as to how anterograde FAT is inhibited in AD.

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Inhibition of fast axonal transport in neurons is facilitated by a tau-mediated molecular mechanism involving exposure of the PAD domain

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Alzheimer’s disease (AD) is a neurodegenerative disease characterized by dementia and loss of memory. Recent studies indicate that the exposure of a phosphatase-activating domain (PAD) in wild type tau protein is a key step in AD pathology and that this exposure results in inhibition of fast axonal transport in neurons. Since fast axonal transport (FAT) is a critical cellular process that maintains axonal connectivity, inhibition of FAT is a major pathogenic event in AD. The regular conformation of the protein tau, a microtubule-associated protein, is folded over like a paperclip. Aggregates of tau and disease-associated mutations in tau alter this paperclip-like conformation to reveal a pathological domain. This pathogenic effect seems to require amino acids 2-18 of the n-terminal end of tau, known as PAD. Using vesicle motility assays in the isolated squid axoplasm preparation to conduct quantitative evaluations of FAT through video microscopy, our lab has shown that aggregates of all tau isoforms appear to selectively inhibit anterograde FAT. We also show that disease mutations in the tau gene that alter the paperclip conformation of tau and increase PAD exposure. In all of these cases, the inhibition of anterograde transport appears to occur via activation of the PPI-GSK3 cascade and requires an exposed PAD to inhibit FAT. However, recent studies on specific phosphorylation variants of tau suggest that additional pathogenic mechanisms may exist, because perfusion of specific pseudophosphorylated tau constructs appears to also activate a MAP kinase based pathway. The role of pathological tau in altering kinase activity may provide new insights in the underlying mechanisms for Alzheimer’s disease and other diseases with tau pathology.

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Assessing the use of Tg(fabp10a:EGFP-hPLIN2) as a live lipid marker in zebrafish hepatocytes to identify fatty liver disease
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Fatty liver disease (FLD) is the most common hepatic pathology worldwide and is characterized by an accumulation of lipid droplets in the hepatocytes. In FLD, Endoplasmic Reticulum (ER) stress activates the Unfolded Protein Response (UPR) which can protect against or induce FLD. Zebrafish is an ideal system to study the disease due to their transparency, rapid development and ease of gene manipulation. Traditionally, FLD is identified using lipophilic dyes. However, the line Tg(fabp10a:EGFP-hPLIN2) expresses a fluorescent green marker in the hepatocytes on the protein Perilipin-2 (hPLIN2). PLIN2 is a cytoplasmic lipid droplet protein that may have role in preventing the UPR to counter lipid accumulation. Live imaging can be used to screen EGFP-hPLIN2 larvae for FLD, whereas other methods of lipid marking require fixed larvae. The larvae which are screened positive are considered to have an adaptive UPR whereas those larvae who do not exhibit FLD have a stressed UPR. In this way, FLD is more a sign of homeostatic ability rather than a pathological condition. Through confocal microscopy of fish at 4, 5, and 6 days post fertilization (dpf) we found that the line Tg(fabp10a:EGFP-hPLIN2) was inferior to another liver marker Nile Red which is a lipophilic dye that need only be added to the dish of larvae 1 hour before imaging. We found variability in the expression of the fluorescent marker among 10 clutches of 175 EGFP-hPLIN larvae at 4, 5, or 6 and therefore determined the necessity for pre-screening the larvae at 4 dpf for GFP fluorescence in the liver using a fluorescent stereoscope. Moreover, we found that overexpression of hPLIN2 causes change in ER shape, a traditional indication of ER stress. This suggests that hPLIN2 has a complicated relationship with FLD and therefore lacks as an indicator compared to the dye Nile Red.

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Divergent fates of UHRF1 deficient embryonic zebrafish hepatocytes
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Uncontrolled cell proliferation is a hallmark of cancer. Uhrf1 is a master regulator of DNA methylation, and its deficiency leads to a global hypomethylation. As methylation is responsible for silencing gene transcription, Uhrf1 deficient cells are prime models for investigating cell cycle anomalies. As previous research indicated a significant amount of S-phase arrest in uhrf1 mutant hepatocytes, and less hepatic outgrowth is seen the mutants, we sought to investigate the cellular fate of these S-phase arrested cells. Do they simply undergo division slower or undergo apoptosis, and what part of the replication mechanism is responsible for the outcome? A variety of immunofluorescence methods were utilized, including a TUNEL apoptosis assay on cryosectioned larvae, and a 20 minute EdU pulse to visualize differential patterns based on the stage of the S-phase cells are in. TUNEL assay results showed a significantly increased amount of apoptosis in uhrf1 mutants at 4 days post fertilization (dpf). The EdU experiment indicated more cells in general and especially in the late S-phase in the mutants when compared to the wild types. As a long-term BrdU pulse experiment run previously showed higher rates of BrdU retention in uhrf1 mutants and possibly indicated a slower progression through the S-phase in these mutants, this experiment showed that a slower progression was not the case. This short EdU pulse experiment suggests that uhrf1 mutant hepatocytes do not undergo a slower progression through the S-phase of the cell cycle, and is a preliminary step in combining EdU with PCNA or BrdU to capture a longer time-lapse visualization of S-phase progression in zebrafish.

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A survey of marine fungi species and morphological diversity in Woods Hole
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The kingdom of fungi is estimated to include approximately 1.5 million species. Comparatively there are roughly 5,500 known species of mammals and 350,000 known beetles. Counts of fungal species focus primarily on fungi associated with terrestrial environment, overlooking fresh and saltwater environments. Only about 0.6\% of known fungi are found in marine environments, which is surprising given that more than 70\% of the earth’s surface is ocean. In terrestrial environments, fungi are known to be a vital part of the ecosystem, contributing to the decomposition of organic matter and cycling of nutrients. Much less is known about marine fungi and the role they play in their environments. Our goal was to conduct a survey of species of culturable marine fungi, identify them by ribosomal RNA sequence, and analyze their growth behavior and morphology. We collected samples of marine water from several marine environments around Woods Hole with a specific focus on Buzzard’s Bay and Vineyard Sound. Water quality data on temperature, salinity, and pH were also collected at the time of sampling. Water samples were plated on agar plates with antibiotics to select for fungal isolates. Plating was done at three steps in the procedure: unfiltered seawater, concentrated seawater, and matter scraped off the filter. We documented colony growth and found great diversity in colony shape, color, and growth rate. We then documented morphology using differential interference contrast (DIC) microscopy, both still images and time-lapse sequences. Ribosomal RNA sequencing was used to identify species. Species of particular interest included several types of black yeast with varying morphologies. These organisms can grow in extreme environments, and exhibit interesting combinations of budding and hyphae-like growth patterns.

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Why are cephalopod eggs so big? Testing the functional limits of jet propulsion in pygmy squid
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Cephalopod eggs are exceptionally large compared to other molluscs. Large eggs produce large hatchlings. The increased egg size in cephalopods may be due to functional constraints faced by hatchlings using fins and jet propulsion to swim in a low Reynold’s number environment, versus using cilia in other mollusc larvae, such as veligers. At hatching, squid have larger funnels relative to body size than when they are older, which suggests that jet propulsion may be limited by seawater viscosity at small sizes. To test the influence of viscosity on jet propulsion and fins at the size limit of cephalopods, the “world’s smallest” swimming cephalopod, hatchlings of the pygmy squid *Idiosepius paradoxus*, was used. Polyvinylpyrrolidone (PVP) can be added to increase viscosity of seawater, and preliminary results found that jet propulsion broke at a concentration of 2.16%. A larger tank system that minimizes edge effects will decrease variation in the Reynold’s number environment experienced by hatchlings. However, an intermediate system that enabled large-scale collection of data was necessary. X-y plane, and z-axis movements were analyzed to show that at a viscosity of more than 9.0 centipoises, squid are unable to swim. Further analysis will allow us to predict jet propulsion efficiencies for cephalopod sizes smaller than those found in nature, and for sizes matching those of small ciliated larvae. Future studies may include analyzing viscosity in fin and jet propulsion systems of other marine animals, particle imaging velocimetry, and analysis of neural activity during swimming.

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