

Marine Biological Laboratory



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2018 Undergraduate Research Abstracts

Effect of Alzheimer's Disease and Progressive Supranuclear Palsy Tau Aggregates on Fast Axonal Transport

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Tauopathies are a family of neurodegenerative diseases characterized by formation of aggregates of the protein tau, a predominantly neuronal microtubule binding protein, in different regions of the brain. Previous work in the squid axoplasm model showed that, unlike soluble tau, recombinant tau aggregates cause inhibition of anterograde fast axonal transport mediated by the multisubunit motor protein conventional kinesin. This occurs through activation of the PP1 GSK3 β signaling pathway. Once activated by PP1, GSK3 β phosphorylates the kinesin light chain subunits, causing detachment of conventional kinesin from vesicles. PP1 is activated by the phosphatase-activating domain (PAD) at the N-terminal of the tau protein, which is generally not exposed in soluble wild type tau monomers. The current study aims to further understand the toxic species of tau by analyzing tau aggregates isolated from brains of patients with two tauopathies: Alzheimer's disease (AD) and Progressive Supranuclear Palsy (PSP). We hypothesize that aggregates from both diseases will cause inhibition of anterograde axonal transport and that the antibody TNT1, which has specificity for the PAD region, can be used to effectively bind to the domain and block its effect. We found that tau aggregates from both AD and PSP inhibited anterograde axonal transport, but AD tau aggregates additionally inhibited retrograde axonal transport. For aggregates from PSP, the rate of transport was almost fully recovered with TNT1. However, TNT1 only partially rescued the effect of AD aggregates. This suggests that exposure of the PAD is playing a crucial role in the decline of axonal transport triggered by PSP-derived tau aggregates, but additional PAD-independent pathways that negatively impact axonal transport are most likely activated by AD-derived tau aggregates.

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Marine benthic algal response to salt marsh nutrient enrichment

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Anthropogenic addition of nitrogen via runoff in coastal watersheds has been shown to affect both structural and ecological components of salt marsh systems. Benthic microalgae, found in photosynthetic ranges of marsh sediment, is a key primary producer, and may contribute to sediment stabilization. However, there is currently little research focused on how eutrophic conditions stimulate these algae. This project aims to evaluate the response of benthic microalgae to a thirteen-year period of experimental nutrient enrichment, and subsequent response to two years of marsh recovery, or non-enrichment. It is predicted that benthic algal biomass will increase in nutrient enriched marshes during the thirteen-year enrichment period, as nitrogen availability is no longer a limiting factor. However, it is expected that benthic algal biomass in enriched creeks will stabilize early in the recovery period.

Dorr Foundation

Elucidating Interactions Between the Parkinson's disease-associated protein, α -Synuclein, and Dynamin-1

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Familial Parkinson's Disease is associated with aberrant aggregation of a small, synaptic protein called α -synuclein. Under physiological conditions, α -synuclein exists as monomeric, dimeric and possibly tetrameric species. While the native form of α -synuclein is still under debate, it is generally agreed that an imbalance of these molecular species leads to pathology. However, the roles of each molecular species remain unknown. Past studies from our lab indicate that, at elevated concentrations, the acute introduction of both monomeric and dimeric α -synuclein into lamprey axons disrupts clathrin-mediated endocytosis at synapses, leading to loss of synaptic vesicles. While monomeric α -synuclein disrupted the uncoating of clathrin coated vesicles, dimeric α -synuclein induced a phenotype characterized by an increased number of clathrin-coated pits (CCPs). The phenotype resembles the inhibition of dynamin-1, a GTPase critical for fission of CCPs during clathrin-mediated endocytosis. We therefore hypothesized that α -synuclein dimers selectively interact with dynamin-1 or its binding partners. To study this, we looked for physical interactions and colocalisation of the two proteins. Biochemical interactions were examined through GST pulldowns using recombinant proteins. We encountered technical problems with pulldowns using recombinant dynamin-1. Therefore, as an alternative, we tested for interactions between α -synuclein and endophilin, a dynamin binding partner that also participates in vesicle fission. Though Endophilin A1, A2, and A3 pulled down dynamin, none pulled down α -synuclein monomers or dimers. As a second test for interactions, we performed immunofluorescence for α -synuclein and dynamin-1 in lamprey spinal cord sections. Synuclein appeared punctate within axons. In comparison, axonal dynamin staining appeared diffuse with few punctate areas. There was some punctate colocalisation of these two proteins at synapses. Overall, the data reveal that monomeric and dimeric α -synuclein do not interact with endophilin but confirm partial α -synuclein and dynamin-1 colocalisation at synapses. Studying other dynamin-1 binding partners may elucidate how dimeric α -synuclein inhibits vesicle fission.

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Hippocampal Synaptic Plasticity in Parkinson's-Associated DJ-1 Knock-Out Mice

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Parkinson's Disease (PD) is characterized by the neurodegeneration of dopaminergic neurons. Familial PD has been associated with the mutation of multiple mitochondrial proteins, including alpha-synuclein and DJ-1. These two proteins play a critical role in metabolic function by binding to the beta-subunit of the ATP synthase in the inner mitochondrial membrane which closes a hydrogen leak thereby increasing the efficiency of ATP production. Previous research has shown that the presence of anti-apoptotic mitochondrial proteins similar to DJ-1, specifically Bcl-xL, is crucial for the mechanism of long-term potentiation (LTP), an energy-dependent process that results in a persistent strengthening of the synapse. The purpose of this study was to determine the importance of DJ-1 in the formation of LTP, as it serves a similar role to Bcl-xL in the mitochondria. Using brain slices from either Wild-Type (WT) or DJ-1 knock-out (KO) mice, we recorded the stimulus dependent voltage changes in the CA1 dendrites of the hippocampus as a model for synaptic plasticity. LTP was induced by stimulating the pre-synaptic nerve bundle (Schaffer collateral) at high frequency, which results in a long-term increase of the post-synaptic response compared to baseline responses. Short-term synaptic plasticity was studied using paired-pulse facilitation in which two depolarizing pulses are injected at increasing intervals. DJ-1 KO mice and WT mice showed no significant difference in their response to the induction of LTP, nor in the paired-pulse protocol. These preliminary data suggest that genetic depletion of DJ-1 does not inhibit the mechanisms required for short-term and long-term synaptic plasticity in the hippocampal CA3-CA1 synapse. Further research is required to elucidate the functional differences between the actions of, or localization of, Bcl-xL and DJ-1, and how these discrepancies lead to the varying synaptic phenotypes.

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Generating RFX6 Mutants via CRISPR/Cas9 to Model Mitchell-Riley Syndrome in *Xenopus tropicalis*

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RFX6 is a transcription factor crucial for anterior endoderm and pancreas development with an identical DNA binding domain between humans and *Xenopus*. The RFX6 gene is associated with Mitchell-Riley Syndrome, characterized by neonatal diabetes, intestinal atresia, pancreatic hypoplasia, and gallbladder agenesis. In a previous study, morpholinos have been used to block RFX6 translation in *Xenopus laevis*, causing down-regulation of foregut and pancreas markers, including *foxa2* and *insulin*. Here, we investigate whether a gene knockout via CRISPR/Cas9 will result in similar phenotypic effects in *Xenopus tropicalis* and hypothesize that genomic changes will produce similar phenotypes.

Four single guide RNAs (sgRNAs) were designed to target the RFX6 DNA-binding domain, assessed *in silico*, synthesized, and microinjected with Cas9 enzyme and Texas Red into *Xenopus tropicalis* embryos. Sequencing the genomic DNA of injected embryos has shown that these four sgRNAs were not effective at editing the RFX6 gene. More sgRNAs will be designed and tested *in vivo*. In situ hybridizations have been performed to assess *insulin* expression patterns in Stage 41 Control embryos, and anti-sense DIG-labeled RNA probes are being synthesized for the *foxa2* and *insulin* markers for future in situ hybridizations to compare phenotypes in control and RFX6 knockout embryos.

Once effective sgRNAs are identified, we will inject them into embryos, assess phenotypic effects in founders, raise F0 animals to adulthood, and then cross them to obtain homozygous RFX6 null mutants, which will serve as models to further understand disease progression and pancreas development.

Jeff Metcalf Summer Undergraduate Research Fellowship – The University of Chicago

Linking Landscapes: salt marsh geomorphology structures mummichog diet

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Trophic links can bridge different, otherwise separated, food webs by facilitating the transfer of energy between them. In New England salt marsh ecosystems, mummichog (*Fundulus heteroclitus*) function as a trophic link by gathering energy produced in the high marsh and moving it down into the creek food web. We studied how local geomorphology effects the strength of this trophic link by examining stomach contents of mummichog in creeks that range in their degree of low marsh degradation. We found that creeks with intact low marsh habitat exhibit mummichog diets that consist of a higher percentage of high marsh prey compared to creeks with greater fragmented low marsh. This suggests that landscape can play a large role in the transfer of energy and function of ecosystems.

Does *Hydra* Have Social Behavior?

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Social behavior plays an essential role in survival and reproductive success across the animal kingdom, yet the origins of social behavior are still unknown. We have carried out experiments using the simple animal Hydra, with the goal of determining when during animal evolution social behavior first appeared. Hydra is a member of Cnidaria, a phylum that evolved over 500 million years ago. Because of its simplicity, Hydra provides scientists with a system to study and understand complex concepts in neuroscience on the most basic level. While Hydra's basic behaviors have been known since Abraham Trembley's work in the 18th Century, no research has been conducted to determine if Hydra has social behavior. In this study, we used a variety of approaches to search for evidence that Hydra exhibits social behavior. We recorded time lapse videos of Hydra in Petri dishes under various conditions that we expected to elicit social behavior. These included comparing behavior of Hydra in various sized groups, various combinations of sex, and various combinations of species. We are carrying out analysis of our videos by tracking movements, angles of orientation, spatial distribution, and floating patterns. The results from these data will provide insight into the extent to which Hydra polyps show awareness of each other and group organization which, in turn, will allow us to determine if Hydra has social behavior. This novel research will provide a foundation for additional studies which should ultimately define when social behavior first appeared in animals.

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Studying the Role of RNA Editing in Regenerating *Octopus Bimaculoides*

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RNA editing is a process in which genetic information can be altered at the RNA level. Editing occurs when the enzyme Adenosine Deaminase Acting on RNA changes Adenosine to Inosine. This process is interesting because in coding regions of mRNA, Inosine is read as Guanosine during translation; thus, editing changes the amino acid sequence and possibly protein function. While RNA editing rarely occurs in coding regions in vertebrates, recoding events occur several orders of magnitude higher in cephalopods. In addition to their incredible RNA editing capabilities, cephalopods also have the capacity to regenerate fully functional arms after amputation. Given that cephalopods can both recode proteins through RNA editing and regenerate, our study's goal was to illuminate whether RNA editing may be involved in arm regeneration in *Octopus Bimaculoides*. To do so, we looked for differences in editing patterns (editing sites and percentage of editing) in genes involved in regulating stemness during arm regeneration. 12 juveniles were amputated on their right first arm on day 0 and tissue was collected for RNA extraction to have the basal levels of editing in the genes of interest. To detect changes in editing during regeneration, the regenerating tip of three animals was collected for RNA extraction 1, 3, 11 and 25 day(s) after amputation, as well as the left first arm as a control. In preliminary results, we observed a trend for the transcription factor Sox2 where control arms displayed higher levels of editing than regenerating arms on day one. Interestingly, this trend was reversed at day three, where regenerating arms had higher levels of editing than controls. Though the study of other genes, more animals, and other time points is required to draw conclusion, the results strongly suggest that RNA editing could play an active role in arm regeneration in *Octopus Bimaculoides*.

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Plant community composition in an experimentally eutrophied salt marsh

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Coastal ecosystems are highly influenced by human activities, such as nutrient enrichment (from septic tanks and farms) and altered food webs (from introducing invasive species). The TIDE Project has been using the Plum Island Estuary for the past 15 years as a large scale experimental study to test the capacity of marshes to tolerate these threats. The goal of the TIDE project is to contribute information that may help protect and restore our marshes. Nitrogen was added to two primary tidal creeks where plant response, physical structure, and food webs were monitored to study the effects of eutrophication. Plant production is predicted to increase with nutrient enrichment. We have been monitoring the percent coverage of dominant marsh plant species (ex: *Spartina alterniflora*, *Spartina patens*, and *Distichlis spicata*) along these creeks to see how the plant community responds to nutrient addition. This study provides an overview of historic plant coverage data across 18 transects at two fertilized creeks and two reference creeks.

Dorr Foundation

Maternal age effects on offspring life and healthspan

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Transgenerational effects on aging have been shown in multiple organisms. The life history of F0 generations can have lasting effects on the life and health of future generations, often through epigenetic mechanisms or by germline resource allocation to offspring. Here, we use the rotifer *Brachionus manjavacas* — a microscopic, aquatic invertebrate — as a model system to study maternal age effects on male offspring. Observed phenotypic health differences between male and female offspring may help explain the mechanism of transgenerational aging effects, as the two sexes are meiotically and mitotically derived, respectively. We found male lifespan to be positively correlated with maternal age, the opposite of previous studies of female offspring. Because of the importance of mitochondria in aging and their maternal inheritance, we also examined mtDNA copy number and mitochondrial function in male offspring from young, middle aged, and old mothers. We found increased mtDNA copy number per cell in offspring from older mothers, suggesting there may be a declining ability to remove mitochondria once damage accumulates. We assessed changes in mitochondrial oxidative potential, which is directly related to the functional ability of mitochondria to power the cell, with fluorescent dye and confocal image analysis. Offspring health through maternal aging was assayed through heat stress survival and behavioral phototaxis. Phototaxis declines in male offspring from mothers of advanced age, indicating a behaviorally observable degradation in health. Our results support the hypothesis that maternal age can affect lifespan and healthspan in future generations, and that these effects may differ based on the genetic origin of offspring. By exploring the transgenerational effects of aging, we hope to further elucidate the mechanisms of aging as they apply to all living things.

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Particulate Carbon Cycling in the Sargasso Sea Water Column: Insights from Lipid Biomarkers to Evaluate Seasonality

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The cycling of organic carbon in the ocean is a key process in the global carbon cycle. Despite the fact that over 90% of particulate material in the deep water column is suspended, the particulate organic carbon (POC) pool remains relatively understudied in terms of its sources, chemical composition, and processing by deep water ecosystems. This study evaluates lipid biomarker composition in suspended particles (20-1500m depth) collected at the Oceanic Flux Program site in the northern Sargasso Sea off Bermuda during the four seasons: early winter (January 2018), following the spring bloom (April 2015, previously analyzed), summer (June 2018), and fall, the minimum productivity period (November 2015, previously analyzed). Key lipids biomarkers are used to assess the contributions of phytoplankton, zooplankton and bacterial sources to the POC, diagenetic transformations of suspended POC throughout the epipelagic and mesopelagic layers, and seasonal temporal variability of the biological pump. This study focuses on how lipid composition and abundance (fatty acids, fatty alcohols, and sterols) vary seasonally in the epipelagic and mesopelagic layers. The lipid concentration variability between high (January and April) and low (June and November) productive periods mirror seasonality in primary production. Changes in phytosterol and cholesterol composition during the four seasons reflect shifts in phytoplankton community structure and zooplankton abundance. In the mesopelagic zone, the cholesterol/phytosterol ratios and the percentages of C16 and C18 alcohols, and odd-chain and branched fatty acids, document the contrasting transitions from algal to animal OM sources and bacterial reprocessing of labile OM depending on the season. This study will better characterize lipid profiles in the Sargasso Sea and add on to the larger project of particulate carbon cycling in oligotrophic regions. These findings will also provide supporting evidence of the factors that influence the biological pump, and by extension, ocean ecosystems and the global carbon cycle.

Oceanic Flux Program

Assessing calcification of *Astrangia* under different temperature and physiological conditions using Scanning Electron Microscopy

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Symbiotic and aposymbiotic colonies of *Astrangia poculata* were reared for four weeks in seawater at 10°C, 20°C, or 27°C. Buoyant weight measurements were used to investigate the effects of temperature and symbiont state on growth rate. Scanning electron microscopy (SEM) was used to describe how these physiological and environmental conditions are reflected in the crystal structure of the coral skeletons. As reflected in both SEM images and buoyant weight data, aposymbiotic colonies in all temperatures and all colonies kept in 10°C showed higher occurrences of bioerosion than other colonies. The buoyant weight data also revealed that symbiont state and temperature both significantly affect growth rates. SEM of corals kept at 27°C revealed that symbiont absence appears to result in a lower density of calcium carbonate at growing septa tips. Furthermore, the crystal structure of growing septa tips appears to be different in colonies kept at different temperatures. This is the first study examining the skeleton of *Astrangia* using SEM, so these results unlock new insights into the skeletons of temperate corals. In today's changing climate, tropical corals face higher frequencies of warmer ocean temperatures, ocean acidification, and coral bleaching events. This study has the possibility to inform further studies regarding the effects of symbiont state and temperature on the calcification of these integral at-risk corals. This study can also provide insight into historical coral health and symbiosis based on the crystal structures of coral skeletons from the past.

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Micron-Scale Spatial Structure of Microbial Communities on the Kelp *Nereocystis luetkeana*

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Macroalgae are photosynthetic organisms that play a key role in global primary productivity and provide habitat for a plethora of marine organisms. Microbes on the algae surface form a close relationship with the algal host, providing secondary metabolites and influencing its life history, morphology, and resistance to fouling and disease. Although metagenomic sequencing can provide information about the types of bacteria living on macroalgal surfaces, these methods cannot show the spatial arrangement of microbial communities. To better understand the interactions between microbial communities and the algal host, we must study their biogeography on the micron scale. To do this, we obtained samples of the kelp species *Nereocystis luetkeana* collected from Tatoosh Island, WA and Squaxin Island, WA. Sequencing data shows that gammaproteobacteria are more abundant on the Tatoosh samples and alphaproteobacteria are more abundant on the Squaxin samples. Our goal was to use Combinatorial Labeling and Spectral Imaging Fluorescence In Situ Hybridization (CLASI-FISH) to observe the composition and micron scale spatial structure of bacterial communities on the kelp samples. We examined samples from the base and tip of the Tatoosh kelp fronds and from the middle of the Squaxin kelp fronds. The Squaxin samples were sparsely colonized by bacteria whose identification by FISH was consistent with the sequencing data. The relatively new tissue at the base of the Tatoosh samples was sparsely colonized by bacteria, while the older tissue near the tip was more densely colonized. Clusters of *Granulosicoccus* bacteria were observed throughout samples from the frond tip. Alphaproteobacteria were often observed around the periphery of these clusters, but also form patches away from *Granulosicoccus*. Bacteroidetes filaments were distributed throughout the community. Close spatial localization of diverse microbes suggests metabolic interactions among members of the kelp microbial community as well as between the microbes and the kelp itself.

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Origins of deep sea fatty acids assessed using compound specific stable isotopes

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The oceans' biological pump is the transfer of material and energy from the surface to the deep ocean via biotic interactions within ecosystems. Critical in both atmospheric and oceanic processes, it is responsible for controlling atmospheric carbon dioxide uptake, the cycling of elements and nutrients, and providing a food source for deep sea organisms. Lipid biomarkers are powerful tools to identify the sources and transformations of carbon in such ecosystems, but questions remain regarding transport and synthesis. We analyzed carbon isotopic signatures in lipids collected from the Sargasso Sea to determine whether polyunsaturated fatty acids (PUFAs) found in the deep ocean are produced by phytoplankton and transported down, or whether there is a significant deep ocean source. We found a rapid increase in $\delta^{13}\text{C}$ from the production zone to 500 m, and a general trend of increasing $\delta^{13}\text{C}$ with depth. This can potentially be explained by isotopic fractionation during degradation, but does not appear to indicate a bacterial source of PUFAs in the deep ocean. While much work remains to be done to determine if these trends continue, and to more precisely identify sources, these results provide a significant basis of understanding the role of deep sea communities in the global carbon cycle.

Jeff Metcalf Summer Undergraduate Research Fellowship – The University of Chicago

Quantifying camouflage: how well do cephalopods resemble the color of the background in the eyes of different predators?

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An animal's effective ability to use camouflage and remain undetected by predators is due largely to coloration. Cephalopods such as *Octopus vulgaris* seem to have effective camouflage against a large range of predators with various combinations of cone receptors. However, this has rarely been studied quantitatively. We aim to quantify animal coloration and compare it to substrates within its immediate environment under the perspectives of various predators with differing cone receptors. In doing so, we answer the questions: "What does a camouflage prey look like solely in the color space of predator vision" and "What quantifiable differences in color are present in cephalopods and their immediate surrounds?" These questions are explored by analyzing RGB images of *Octopus vulgaris* camouflage on different coral reef habitats such as Puerto Rico, Little Cayman, and Bonaire in the Caribbean Sea. A caveat to this, however, is that captured images introduce non-uniform variables to the image quality and its perception. To control for the discrepancies in captured images, ImageJ and the Image Calibration and Analysis Toolbox is used to extract and quantify the coloration data from these images. We determined that camouflaged octopuses resemble their environment in such a way that allows them to remain undetected, in terms of color, in the eyes of monochromatic and dichromatic predators. However, due to insufficient data, it is still relatively inconclusive as to how camouflaged prey look like solely in the color space of predator vision.

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Role of *Foxl2* in *Xenopus tropicalis* Female Sex-Determination

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Xenopus, a genus of aquatic frogs with an evolutionary history similar to humans, provides a great model system for studying human disease. There are currently 20 species within this genus, though the most frequently studied are the allotetraploid, *Xenopus laevis*, and the diploid, *Xenopus tropicalis*. While research in each species has advantages, there are significant genomic differences between the two. For example, the sex chromosome of *X. laevis* is 2L, while in *X. tropicalis*, it is chromosome 7. This leads to the need for further investigation to distinguish the similarities and differences in sex differentiation between these species. *Xenopus* sex determination is not well understood at a molecular level, with most previous research being conducted in *Xenopus laevis*. There are two main genes involved in sex determination in this species: DMW and DMRT1. In genetically male tadpoles (ZZ), DMW is not present, which allows DMRT1 to trigger the male sex-determining cascade. Genetically female tadpoles (ZW), have and express the gene DMW, which inhibits the expression of DMRT1. This triggers the female sex-determining cascade and acts as an anti-testes gene. As part of the female sex-determining cascade, DMW also upregulates *Foxl2* expression, which consequently upregulates *Cyp19* expression. The ovary-forming gene DMW is not found within the *X. tropicalis* genome, leading to the question: what is the major sex determining gene in *X. tropicalis*? *Foxl2* and its role in sex determination in *X. laevis* and other species suggests that it may play a role in *X. tropicalis* female sex determination as well. The goal of this ongoing research is to knock-out *Foxl2* and determine if this results in sex reversal from female to male in *X. tropicalis*. If so, *Foxl2* could potentially be a major sex-determining gene for this species.

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

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As the only class of reverse transcriptases (RTs) common to multiple domains of life, *rvts* (reverse transcriptase-related genes) have been found in organisms as diverse and evolutionarily disparate as fungi, plants, invertebrates, and certain bacteria. Previous characterization of *rvt* in bacteria determined that, unlike most RTs, the protein could only be coerced into polymerizing very short products in vitro, questioning its ability to yield long cDNA copies. *Rvts* are exceptional among other RTs as well for their highly-conserved N and C-terminal domains. Transition metal ions, and Ni²⁺ in particular, have been demonstrated to induce transcription of *rvt* in the filamentous fungus *Neurospora crassa*, leading to the hypothesis that *rvts* may mediate metal ion stress. Here, we use the model fungus *N. crassa* and the bdelloid rotifer *Adineta vaga* to investigate the potential role of *rvt* in the transition metal stress response. *N. crassa* strains, including deletion and enzymatically-inactive strains, are surveyed for linear, vegetative, and aerial growth over a nickel concentration gradient. The functionality of urease, one of the few enzymes which utilizes nickel across all domains of life, is quantified as well. In the rotifer *A. vaga*, metal stress is determined via movement assays. The results are then compared with transcriptional data obtained from qPCR focusing on *rvt* and a known nickel transporter. While the specific functionality of this enigmatic class of genes remains unclear, the inductive capacities of nickel and the altered growth and behavioral patterns observed support the implication of *rvts* in the transition metal stress response.

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Potential net nitrogen mineralization in Plum Island salt marsh soils

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Woods Hole Research Center

Coastal vegetated wetlands like salt marshes have the ability to sequester and store large stocks of carbon in their inundated soils, making them incredibly valuable ecosystems. One major threat that these coastal ecosystems and their long-term carbon stores face is eutrophication: receiving nutrients in excess to the point that the geomorphology and trophic webs are characteristically altered. One ecosystem process that affects primary marsh function as well as global carbon stores is decomposition. General controls of decomposition rates include both carbon and nitrogen availability, and the ratios that these nutrients exist in their organic and inorganic forms. Thus, an important process to measure is nitrogen mineralization. The TIDE project is a 15-year study that examined the effects of controlled eutrophication on ecosystem processes in a northern Massachusetts salt marsh. Now in a period of recovery from nutrient loading, the rate at which ecosystem processes can also recover is unknown. The goal of this study was to determine how long-term nutrient loading affected rates of potential net nitrogen mineralization in soils. Rates of mineralization (nitrification and ammonification) were compared across three dominant plant communities as well as during both spring and neap tidal cycles. These data give us basic insight to soil nutrient cycling within the marsh, and hints on how quickly these processes may return to their original rates and functions.

Hydrogel enables injection of squid embryos for CRISPR-Cas9 genome editing and other genetic tools in cephalopods

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Cephalopods, are unique invertebrates due to their large brains – with more than 500 million neurons. Studying their neural circuits has been a prime target for many neurobiologists. Despite extensive documentation of cephalopod neural processes, there has yet to be a targeted attempt to build genome editing tools to study circuits in their brains. This study focuses on developing the tools required for a thorough interrogation of neural circuits in the squid species *Doriteuthis pealeii* via CRISPR-Cas9 knockout of Pax-6 and Rhodopsin genes which govern the visual pathway. For this we have developed two different methods of in vitro injection into squid embryos – one which is a variant on the Giraldez method and the other which is a novel technique of embedding embryos in hydrogel, and is an exciting possibility to be applied to pygmy squid *Idiosepius notoides*, where traditional injection methods have proven unsuccessful. For the first injection protocol, we built hydrogel holders (20% w/v) with an aperture such that embryos just fit inside and injected (a) Di-I and dextrin dyes to fluorescently label the embryos to establish a proof of concept for the injection and (b) CRISPR-Cas9 complex for Pax6/Rhodopsin knockout. All injected cells were cultured in agarose and seawater as they developed to the hatchling stage. The cells labelled with Di-I showed the expected fluorescence and developed normally, while the CRISPR injected embryos were phenotyped and genotyped eventually after full development. For the alternate novel hydrogel embedding injection method, we overcame a major limitation of traditional injections because we no longer have to snip and damage the chorion, which makes for an easier and scalable injection technique. Breaking the barrier of genome editing in cephalopods will open up exciting possibilities about investigating the response of neural circuits to varied novel responses, like artificially inducing colored skin pigmentation via optogenetics on the visual pathway.

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Effects of Actin Polymerization Inhibition on Vesicular Exocytosis and Recovery

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Evidence suggests that actin polymerization may be necessary for re-supply of synaptic vesicles after strong stimulation of the presynaptic terminal that depletes these vesicles. Re-supply of vesicles may occur through re-endocytosis of neurotransmitters or the movement of vesicles from farther up the neuron to the synapse. Latrunculin B (LatB), a potent actin polymerization inhibitor that prevents polymerization of f-actin and sequesters g-actin, had previously been studied to play a role in hippocampal synapses, but had yet to be studied in Squid stellate ganglion synapse. Electrophysiological experiments were used to determine the effects of latrunculin B on release of neurotransmitter and resupply of synaptic vesicles as assayed by the slope of excitatory post synaptic potentials (EPSPs). The depolarization slope of the EPSPs was determined to be decreased in LatB-exposed synapses and recovery of the EPSP was delayed after strong stimulation (tetanus) Rundown of the EPSPs during a tetanus was also determined to occur earlier in synapses treated with LatB. Our data suggest that there could be a relationship between actin dynamics and synaptic vesicle re-supply during and after a prolonged tetanus in *Loligo pealeii* (longfin inshore squid) presynapse. The mechanism by which this occurs has yet to be elucidated.

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Cold shocks induce asymbiotic colonies in *A. poculata*, an emerging coral model

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The coral *Astrangia poculata* is capable of living in a facultative symbiosis with the photosynthetic algal dinoflagellates, *Breviolum psygmophilum*, allowing the coral to survive in a completely asymbiotic—or bleached—state in times of stress, unlike most tropical corals. This experiment aims to test whether *A. poculata* can be bleached and then re-infected with *B. psygmophilum*, thus developing a model system for the study of corals symbioses, growth, effects of stress, and the overall ecology between *A. poculata* and *B. psygmophilum*. In this 4-week experiment, the sea anemone *Exaptasia pallida* and *A. poculata* were induced to bleach by cold shocks with menthol (20%) and cold-temperature (4°C). By week 2, symbiont density within *A. poculata* decreased by 84.4% in menthol and 74.8% in cold-temperatures, whereas *E. pallida* decreased by 98.8% and 91.8%, respectively. By week 4, symbiont density decreased by ~99% for all treatments. Photosynthetic efficiency measurements decreased in conjunction with the loss of symbiont density for both species in both shock treatments. There was not complete eradication of all algal symbionts, however, which calls for a modified shock procedure for *A. poculata*. In an asymbiotic state that can be maintained for an extended time, *A. poculata* could be used to study both reinfection of *B. psygmophilum* or more stress-resistant clades of symbionts, as well as the role of the symbiont in the growth and survivorship of the coral itself.

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Testing Age-Dependent Effects on Episodic-Like Memory in Cuttlefish

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Episodic memory involves the recollection of personal experienced events, based on what happened, where, and when. This type of long-term memory is a complex capacity that was once thought to be unique to humans and has age-dependent effects (i.e. it does not develop in children until the age of 4-5 and also declines with age). Studies have now demonstrated that some mammals and birds are also capable of recollecting this type of memory, termed episodic-like memory (ELM). More recently, episodic-like memory has been shown in cuttlefish, a remarkable finding given that cuttlefish diverged from vertebrates over 600 million years ago. Here, we investigate whether cuttlefish exhibit age-dependent effects on ELM. We tested sub-adult (10 month old) and adult (22 month old) cuttlefish (n = 6 for each group) for what-where-when components of a specific foraging event. We found that sub-adults are capable of recalling what they ate, where they ate it, and how long ago. Adults are exhibiting similar abilities but are still in the final phase of testing. These results highlight that unlike many vertebrate species, ELM in cuttlefish does not apparently decline with age. These findings serve as a fundamental baseline of complex learning in species with distinctly different evolutionary histories from vertebrates. This baseline provides a pathway for evolutionary and biomedical researchers interested in unconventional models to investigate memory on a comparative basis.

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Characterization of Neoblast population during regeneration in *Lumbriculus Variegatus*

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Abstract: *Lumbriculus variegatus* is a freshwater oligochaete and it is one of a few organisms that is capable of regenerating lost body segments when there has been an injury to the organism. In my current study, I propose to investigate the expression of signaling proteins during Lumbriculid regeneration. We are most interested in characterizing proteins which are important contributors to stem cell development. Using an antibody raised against proteins found in wound blastema (1D9-E11), we detected 2 protein bands that measure 214 kDa and 137 kDa on immunoblots. These 2 bands are present in both anterior and posterior regenerating and non-regenerating fragments. Therefore, it is predicted that these proteins may already be present in segmental tissue prior to an injury event and are increased in expression during regeneration. Immunohistochemical analysis demonstrates 1D9-E11 immunoreactive puncta are present within the neuropile of the ventral nerve cord and within the body cavity more closely associated with the nervous system. Total worm lysates will be further purified using immunoaffinity columns with 1D9 antibody attached to sephadex beads. 1D9 column purified proteins will be then sent for mass spectrometry analysis using the MALDI-TOF method. This work will finally determine the protein identity of the 1D9 immunoreactive protein epitopes which are involved in regeneration in Lumbriculus. These results will allow us to further characterize the stem cell populations that are utilized during lumbriculid regeneration. Finally, co-localization of the 1D9 positive cells with bromo-deoxy-uridine (BrDU) will provide further evidence that 1D9-E11 is positively identifying a subpopulation of stem cells in Lumbriculus. Overall,

understanding the mechanisms of regeneration utilized by *Lumbriculus* will further our understanding of these complex processes and perhaps the knowledge gained will be applicable in higher order organisms.

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Vitamin C transporter gene regulation in early developmental stages of *Porites astreoides*

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The mechanisms by which corals form their extracellular skeletal matrix remains poorly understood, despite their importance for both the coral's structure and the transport of necessary molecules. Potentially due to its role as a cofactor in collagen formation, a Vitamin C Transporter gene (SLC23A) had previously been found to be highly upregulated in recently settled larvae—the stage at which the coral's inorganic matrix begins to form. We sought to analyze the regulation of SLC23A in the prevalent and hardy Caribbean coral *Porites astreoides* during the swimming larval stage and the settled larval stage, with no skeletal formation occurring during the former and the skeletal formation commencing in the latter. Considering increasing global oceanic temperatures, we utilized *Porites astreoides* to further uncover how the gene regulation changes throughout the larval life stages of the coral and determine possible effects of elevated thermal stress on the regulation of SLC23A. Using reverse-transcriptase quantitative PCR, a significant upregulation was found between 1, 3, and 10 days post-settlement as well as between 0 and 10 post-spawning. A significant decrease in expression was also found for day 10 settled larvae under heat-stressed conditions. This suggests that Vitamin C is an essential molecule utilized in the formation of the coral's skeletal matrix in the early stages after settlement, and increasing temperatures may hinder its transport and diminish skeletal growth.

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Effects of Increasing pCO₂ and Temperature on Swimming Behavior of *D. Pealii* Paralarvae

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Increasing temperatures and atmospheric CO₂ levels have decreased ocean pH and have varying impacts on the physiology and behavior of marine organisms, particularly on larval and planktonic forms of ecologically and economically important species, such as the inshore longfin squid, *Doryteuthis pealeii*. This squid is an ecologically key species within the coastal marine food web, and it is an important commercial fishery species. This project aims to determine the effect of rising CO₂ levels and temperature on the swimming ability of *D. pealeii* paralarvae. Squid eggs were raised in CO₂ levels of 400 and 2200 ppm, reflecting ambient and predicted levels in 2300 respectively. After they hatched, we took advantage of the squids' attraction to light and recorded individuals in a tank with alternating lights on each side to encourage them to swim 'laps' across the tank. The temperature in the tanks ranged between 20°C and 25°C. Swimming behaviors were categorized by the shape of each lap, as either linear, curvy, irregular, or showing no response to the light. Preliminary data suggests that higher pCO₂ levels slightly, but significantly, increase irregular and no response laps. Results also suggest a significant decrease in linear laps and increase in curvy laps with rising temperature. The paralarvae's swimming behavior appears to be more impacted by the increased temperature than the pCO₂. Although these paralarvae are relatively resilient to rising CO₂ levels, rising ocean temperatures could impact their swimming ability and, by extension, their ability to avoid predators and hunt for food.

Macrophyte stable isotope signatures as indicators of nitrogen loads and sources in three estuaries of Waquoit Bay

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Nitrogen loading from atmospheric deposition, wastewater, and fertilizer to estuaries can affect the productivity and status of macrophytes in estuarine systems. Childs River, Quashnet River, and Sage Lot Pond are three estuaries of Waquoit Bay in Cape Cod that receive different land-derived nitrogen loads. The differences in N loads across these estuaries derive from differences in land use in each watershed. Because each source of nitrogen has a distinct N stable isotope ratio, the $\delta^{15}\text{N}$ signatures of estuarine macrophytes can identify different sources of land-derived nitrogen inputs to each estuary. Within the present study, we analyzed $\delta^{15}\text{N}$ signatures of seven macrophyte species in three estuaries from 1993 to 2017 to assess decadal scale trends in macrophyte isotopic signatures. No significant seasonal or interannual changes in $\delta^{15}\text{N}$ signatures of macrophytes were found across different years. For most macrophyte species, $\delta^{15}\text{N}$ signatures increased with increasing N loads and wastewater was found to be the greatest source of nitrogen contributing to $\delta^{15}\text{N}$ signatures. Therefore, these results demonstrate that macrophyte $\delta^{15}\text{N}$ values may indicate wastewater contributions to N loads. The relationship between $\delta^{15}\text{N}$ macrophyte signatures and land-derived nitrogen loads implies a link between anthropogenic activities on land and responses by estuarine communities.

Brown University LINK Award

Nitrogen load regime change: changes in atmospheric deposition drive trajectory of nitrogen loads in Buzzards Bay estuaries

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Land-derived nitrogen (N) supply determines primary production, water quality, and eutrophication to estuarine systems. To assess the contributions of atmospheric deposition, fertilizer, and waste water nitrogen loads to estuaries, and to determine the fate and effects of nitrogen as it moves through estuarine systems, we examined the changing nitrogen loads and water quality of 23 of the estuaries in Buzzards Bay, MA. Nitrate, chlorophyll, and salinity concentrations were measured from 1992 to 2016 in each estuary and N loads were determined from 1985 to 2015. In Buzzards Bay estuaries, 83% of nitrogen loads increased until 2000 and 78% of nitrogen loads decreased after 2000. This regime shift was primarily caused by decreased atmospheric deposition of nitrogen to Cape Cod from 1998 forward. The responses to this shift included decreased nitrate concentrations from 2002 - 2016 and a slight increase in chlorophyll concentrations from 1992 to 2016. Both nitrate and chlorophyll concentrations were lowest in the saltiest reaches of these estuaries. The effect of changes in N loads on nitrate concentrations were most evidenced in the low salinity reaches of estuaries, and were not significant in higher salinity waters within estuaries. This indicated that nitrogen inputs were from the watershed, and that water quality in Buzzards Bay have not changed across decades.

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Potassium Channel Organizes Actin Structure of the Presynaptic Terminal

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Spinocerebellar ataxia type 13 (SCA13) is a neurodegenerative disease that primarily affects the cerebellum and auditory brainstem nuclei, is associated with motor symptoms, and impairs sound localization. SCA13 is autosomal dominant and caused by mutations in *KCNC3*, the gene that encodes a voltage-gated potassium channel subunit called Kv3.3. Because of their ability to repolarize action potentials rapidly, Kv3.3 channels are highly expressed in regions of the auditory system that require cells to fire at high rates. G592R, a mutation associated with late-onset SCA13, is a substitution mutation that occurs in a region of the C-terminal of the Kv3.3 channel containing a chain of prolines. This one amino acid change results in a functioning potassium channel but disrupts its interaction with actin. Previous studies of wild-type Kv3.3, Kv3.3 knock-out (KO), and G592R knock-in (KI) mice have shown that Kv3.3 influences certain components of endocytosis and found that these channels interact with Arp2/3 to prompt actin polymerization. We have tested the hypothesis that Kv3.3 channels regulate actin structure in the medial nucleus of the trapezoid body (MNTB), a region with a particularly large presynaptic terminal called the Calyx of Held. Using fluorescent-tagged phalloidin, which specifically binds to filamentous actin, we stained slices of mouse brain containing the MNTB to observe differences in actin organization among Calyces of Held from wild-type, Kv3.3 KO, and G592R KI mice. Through super-resolution microscopy, it was found that the actin structure of the synapse is disrupted in Kv3.3 KO and G592R KI mice, indicating that the Kv3.3 channel organizes the structure of actin at the presynaptic terminal.

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Stimulation Changes Actin Architecture in the Presynaptic Terminal

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The readily releasable pool of docked synaptic vesicles is depleted when neurons fire; therefore the pool must be renewed after stimulation. Actin is known to be key in the endocytosis of fused vesicles, but changes in actin architecture after stimulation have not previously been investigated using super resolution microscopy. We hypothesize that the actin architecture of neurons must change after stimulation to allow for the docking of new or recycled vesicles and for the endocytosis of fused vesicles. This study was performed in brain slices from young rats containing medial and lateral nuclei of the trapezoid body (MNTB), auditory neurons that fire at high frequencies and are therefore good models for studying recovery from stimulation. Experimental slices were stimulated while controls were not. Both conditions were imaged for the synaptic vesicle marker synaptophysin and for actin using confocal microscopy. This allowed us to see the localization of pre-synaptic vesicles and actin. In non-stimulated slices, actin fingers wrap around clusters of synaptic vesicles. In our stimulated slices, more colocalization of synaptophysin and actin is present as this structural confinement of vesicles by actin is lost. These preliminary data suggest that actin is important in renewal of the readily releasable pool

of neurotransmitter-containing vesicles, but further study is needed to explain how the change in actin dynamics is regulated.

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Photoreceptor characterization as evidence for retinal specialization and visual adaptation in an elasmobranch

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The pure rod distribution in the retina, and the frilled shape of the pupil of the little skate, *Leucoraja erinacea*, are among the most unique characteristics of the visual system of this benthic, elasmobranch fish. Much of this animal's specific optical anatomy remains unexplored, however, and studying these characteristics provides insight into the visual demands and adaptive specialization of this animal. In this project, we aim to provide the first detailed examination of a particular part of this anatomy, the photoreceptor. Two skate retinas were examined: (1) Using light microscopy techniques, one retina was cut into 38 specimens, for which orientation and location were carefully documented. Semi-thin sections (~65 µm thickness) were cut, stained with Toluidine blue and imaged so we could record photoreceptor length, width and angle with respect to the retinal surface. (2) The other retina was whole-mounted, imaged and the number of photoreceptors was counted for 205 sites in order to calculate the density distribution. We found that photoreceptor morphology and distribution varied across the retina, with a distinct horizontal visual streak pattern of increased photoreceptor density which indicates differences in sampling ability.

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