2021 Undergraduate Research Abstracts

Quantifying edge design in Sepia officinalis camouflage patterns

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To avoid detection or recognition by visual predators, cuttlefish change their mantle and edge body patterns in relation to the visual background. Our goal is to quantify and compare the edge designs used in mottle and disruptive body patterns. Mottle body patterns have medium-sized light and dark patches with moderate contrast between them. Disruptive body patterns show large light and dark patches of varying shapes and orientations with high contrast between them. We took high-resolution images of 10 cuttlefish on six natural substrates that varied in scale and contrast; these substrates were designed to elicit either a mottle or disruptive body pattern. Using MATLAB to analyze the images, we quantified (1) the texture of the mantle pattern compared to the background and (2) the specific pattern designs of the mantle edges used in mottle versus disruptive body patterns. We hypothesized that the mottle body pattern would have edge designs correlated with the adjacent background to enhance blending. Conversely, we predicted disruptive patterns to have edge designs with little correlation with the background, and that instead the edge designs would have high contrast and pattern irregularities. The expected results will provide insight into how cuttlefish disguise their body outline and orientation by altering segments of their edges with contrast, concavity, or convexity in relation to visual surroundings. This approach can help reveal principles of pattern and edge design used in cuttlefish and many other animals that use mottle and disruptive camouflage.

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Cell Intercalation and Follicular Elongation in Butterfly Eggs

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In Drosophila, follicle cells secrete a molecular corset that restricts elongation along an anterior-posterior axis, producing oblong eggs. Butterfly and moth (Lepidoptera) egg shape ranges from hemispheres to peaked obelisks, yet we do not understand whether the same mechanisms that shape Drosophila oocytes apply. Through robust live imaging techniques in the Painted Lady (Vanessa cardui) and Blue Morpho (Morpho peleides), we observed that follicle cell rotation does not occur in elongated butterfly eggs like it does in Drosophila. In elongated eggs of V. cardui, follicle cells surrounding the developing oocyte undergo intercalation—organizing into pairs of columns with cells of each column pair weaving past each other like a zipper producing a single column, going from the posterior to the anterior pole of the egg. In contrast, the hemispherical eggs of M. peleides do not intercalate and instead their follicle cells are hexagonally close-packed, forming a honeycomb-like lattice that expands during development. To hopefully understand the mechanisms involved in intercalation, we disrupted myosin (light-chain) binding to actin in our ex vivo ovarian culture system with a Rho-Kinase inhibitor (Y-27632), we concluded that the actomyosin cytoskeletal network is necessary for intercalation, since the youngest oocytes are unable to form stacked columns and instead follicle cells pack as in a hemispherical egg. To further investigate elongation, we collected, dissected, and imaged Cabbage White butterflies (Pieris rapae) that produce eggs around 50% longer than V. cardui eggs. Although P. rapae did not culture well in laboratory settings, it was possible to

conclude that the actomyosin network was contributing to the final oocyte shape and that similar principles of growth constriction act to orient growth to one direction on an anterior-posterior axis as are occurring in V. cardui. Sampling of more species will help us clearly understand how particular forces drive elongation and yield an overall greater understanding of which developmental events control the variety of butterfly egg shapes.

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Using CRISPR Cas9 to Create Xenopus Models of Neurodegenerative Diseases (NDD's)

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Animal models of human disease are important research tools used to study disease diagnosis, development, and treatment. Xenopus tropicalis is a popular genetic model and superior in comparison to mice in regard to their large brood size and also compared to zebra fish which, despite having large broods and cheap husbandry, have less synteny compared to mammals. Provided these reasons, we chose to utilize Xenopus tropicalis to genetically modify two genes involved in neural development. Low expression of either of these two genes, ntf3 or nrg1, is associated with neurodevelopmental disorders such as schizophrenia, while mutations of ntf3 are associated with neurodegenerative disorders including Alzheimer's. Similarly, loss-of-function mutations of nrg1 have been linked to another neurodegenerative disease, ALS. To generate animal models with which researchers may use to study these diseases, we used CRISPR-Cas9 gene-editing technology to knock-out these two genes by creating indels in their protein-coding regions. The knockout of these genes involved designing and synthesizing single guide RNA (sgRNA) that Cas9 could use to cut the gene at the start of its protein domain, thereby rendering the translated protein functionless. After injecting both sgRNA and Cas9 into Xenopus tropicalis embryos, surviving tadpoles were screened for guide-effectiveness and observed to have phenotypic differences in comparison to embryos injected with a control mixture of water and fluorescent dye lacking sgRNA and Cas9. Tadpoles in which ntf3 was edited showed differences in their swimming behavior. Meanwhile, tadpoles in which nrg1 was mutated exhibited exogastrulation, a process in which nervous system development is halted due to the endoderm shifting from residing within the embryo to outside the embryo. The prevalence of these phenotypes suggest mutations in these genes affects nervous system development in Xenopus tropicalis and have the potential to be exploited to create model organisms for neurodevelopmental and neurodegenerative diseases.

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Effects of potassium nitrate and phthalates in regeneration in cnidarian Nematostella vectensis.

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The cnidarian Nematostella vectensis is a sea anemone adopted as a study organism due to its ability to regenerate within 14 days of amputation. Pollutants such as potassium nitrate and phthalates are found in salt marshes, the anemones home. Potassium nitrate is used in fertilizers commonly used on lawns and agriculture land, the run-off from this ends up in the marshlands and oceans. Phthalates are chemicals found in plastics, utilized as plasticizers to provide flexibility and durability to modern made plastics. Numerous studies have linked phthalates to damages in the reproductive and endocrine system, from unborn babies to fully developed adults. These chemicals seep into bodies of water and the full extent of these on ecosystems in the marshes and oceans and those who utilize them is unknown.

Using Nematostella, we tested the effect of exposure to different concentrations of these pollutants on the animals ability to regenerate the oral region which is composed of it's pharynx and tentacles, both necessary for feeding. Juvenile animals were amputated mid body or below the pharynx and incubated in different concentrations of the chemical. Defects in the tentacles and various phenotypes have been observed in the higher concentration animals. Feeding assessments have been done in order to identify a difference in the behavior and cnidocyte staining to quantify the presence of cnidocytes in the treated and untreated animals.

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Acute Behavioral Responses to Partial Spinal Cord Transections in Lampreys

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Unlike most mammals, including humans, non-mammalian vertebrates are better equipped to recover from spinal cord injuries. For example, sea lampreys (Petromyzon marinus) are able to recover swimming and burrowing behaviors after a complete spinal cord transection by 10-12 weeks post injury. While the long-term neuroplasticity that supports functional recovery after spinal cord transections is somewhat understood, much less is known about the acute, short term neuroplasticity that occurs. This is especially true after partial transections. We therefore set out to determine how partial lesions of the spinal cord acutely impact locomotor function. We performed video imaging (60 fps) on 5 lampreys with medial spinal lesions and 5 lampreys with bilateral lesions at 60 before their respective injuries, and at 2, 24, 48, and 72 hours post injury. The kinematics of the lampreys' swimming behavior were analyzed using custom software written in R (trackter; https://cran.r-project.org/web/packages/trackter/index.html), followed by confirmation of the spinal lesion site using histological methods. Lampreys that underwent bilateral spinal cord lesions typically exhibited lower amplitude sinusoidal swimming motion, difficulty starting and stopping locomotion, and decreased swimming activity compared to their pre-injury status. Lampreys that underwent medial spinal cord legions were less impaired, but still often fatigued quicker and started and stopped swimming at irregular times. When we looked at how the animals' swimming changes post injury, the data suggested that the medially transected animals retained nearly full swimming capabilities throughout recovery. In contrast the bilaterally transected animals surprisingly lost functionality over three days post-injury, which may be due to the fact that some of the transected axons underwent post-injury retraction. These preliminary findings suggest that the neural circuitry that controls locomotion in lampreys is likely located in the lateral tracts of the spinal cord, as opposed to the medial tracts. In the future we hope to analyze our videos with other software packages in order to get a better understanding of how the animals' behavior changed post-injury.

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Field study provides insight into arm flexibility: Octopus vulgaris uses multiple arm actions simultaneously to achieve complex behaviors.

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Characterizing the flexibility of octopus arms is of keen interest to researchers in biomechanics, neuroscience and soft robotics. While the morphology and arm deformations of octopus arms have been studied in laboratory experiments, the arm actions used to achieve such flexibility have yet to be studied comprehensively in freely-moving octopuses under natural field conditions. This study aims to quantify arm flexibility in naturally behaving octopuses by visually characterizing the arm actions that constitute a majority of octopus behaviors. Twelve arm actions were defined using field videos of Octopus vulgaris from the western Atlantic and Caribbean regions. More than 3,900 arm action incidences were observed in 26 minutes of field video from six field sites. Remarkably, all eight arms performed all 12 arm actions. Moreover, individual arms performed more than one action at the same time and multiple arms performed multiple actions simultaneously. Anterior arms performed more arm actions than posterior arms, but there were no differences between left and right arm pairs. The diverse arm flexibility of complex behaviors can be analyzed by using discrete arm actions to help understand the dexterity of octopus arms, which can ultimately inform the biomimetics field in establishing soft robotics.

Undergraduate Research Opportunities Center, CSUMB

Antioxidants may reduce endosymbiont expulsion and prevent bleaching in corals

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Corals have a symbiotic relationship with photosynthetic Symbiodineacea, an intracellular micro-algal dinoflagellate group that reside within the gastrodermal tissue of the host coral. The nutrients produced by these endosymbionts provide sustenance to the coral, fulfilling energy needs for growth, reproduction, and nutrient efficiency. ‘Bleaching’ occurs when thermal stress causes a buildup of reactive oxygen species (ROS) in coral tissue, resulting in the expulsion of the endosymbionts and is related to rise in sea temperature. Previous studies on Porites asteroides suggest antioxidants such as L-ascorbic acid and catechin are ROS scavengers and help reduce ROS buildup in coral tissue, thereby reducing the number of expelled endosymbionts. We are testing whether these antioxidants reduce stress in the coral Astrangia poculata when used with a camphor bleaching treatment. A. poculata is a scleractinian coral native to the western Atlantic to the Gulf of Mexico, can function in both aposymbiotic and symbiotic states, and is highly tolerant of temperature changes, making it an ideal model system for this study. The treatments consist of the exposure of corals to antioxidants, camphor to mimic cold stress, and both camphor and antioxidants simultaneously. Photosynthetic efficiency (Fv/Fm), symbiont density, and calcification were measured throughout. While there was not a substantial change in calcification, we found that exposure to antioxidants during bleaching reduced endosymbiont expulsion and helped maintain normal levels of photosynthetic yield.

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Ontogeny of auditory sensitivity and ranges in the little skate (Leucoraja Erinacea)

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Relatively little is known about elasmobranch auditory ranges and how auditory sensitivity changes with age. The little skate (Leucoraja erinacea) has become a model organism partly because it is easily raised from its egg case in the lab. Changes in auditory sensitivity of little skates post-hatching were examined while submerged using auditory evoked potentials (AEP). The most sensitive frequency range for the little skate was determined to be 100 to 300 Hz which is similar to findings in a previous study on adult little skates. Auditory sensitivity was detectable at 200 Hz the first week post-hatching and increased in sensitivity and frequency range during at least the first 4 week post-hatching. During the last stages of development in the egg case, skates beat their tail to aid in respiration. Therefore, to also assess auditory sensitivity, a behavioral assay of stage 32 or 33 skates was developed that examined tail beating rate in response to sound. Approximately 50% of embryos displayed noticeable cessation of tail beating when presented with sound. Behavioral results suggest that the auditory sensitivity of the embryo might be greater than that newly hatched skates which support the claim that behavioral experiments can be more sensitive than AEPs. This research provides groundwork for future studies examining the role hearing plays in skate predator-prey interactions or the impacts of anthropogenic sound.

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Mummichog Size Distribution in Creeks of a New England Salt Marsh

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TIDE project under James Nelson Ecosystems Lab

Mummichogs, or Fundulus heteroclitus, are small fish in the Killifish family that live primarily in marsh environments. Uniquely accustomed to harsh water fluctuations, Fundulus heteroclitus can be found in intertidal zones from Nova Scotia to Florida (reviewed in Petersen et al. 2010; Able, 2002). This project was conducted with mummichogs within three creeks of Plum Island, Massachusetts. Each creek has previously received different experimental levels of nutrient enrichment leading them to have differences in geomorphology. The three levels of creek enrichment include; not enriched until this year, short term enrichment, and long term enrichment. This has led to three varying levels of breakage in the high and low marsh; very little breakage, moderate breakage, and significant breakage, respectively (Nelson and others 2018). Previously, mummichogs in these varying creeks were studied to see if their stomach content and food web role were impacted by creek geomorphology. These findings showed that fish from the reference creek consumed more high marsh organisms than both other creeks, followed by the short term enrichment creek, and lastly the long term enrichment creek. This was attributed to the gaps between high and low marsh decreasing the mummichogs' likelihood to safely swim to the high marsh and feed. While their food source differed, their caloric value ultimately did not (Nelson and others 2018).

For this project, Fundulus heteroclitus were sampled from each of these creeks and then weighed and measured to see where trends in these aspects may lie. Since the mummichogs in the reference creek have access to more than one energetic pathway, we can hypothesize that size and weight will be greatest in this creek, followed by the short-term enriched creek, and lastly, the long term enriched creek.

TIDE project

Neofunctionalization of Trypanothione Synthetase Amidase in the bdelloid Adineta vaga

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Bdelloid rotifers are a freshwater micro-invertebrate that can survive periods of desiccation, contract their body, and lose 95% of unbound water. As a result of desiccation, the rotifers DNA is broken. However, when they are reintroduced to water, they rehydrate, and transcription occurs, implying that RNA polymerase can still function. Trypanothione is a small antioxidant molecule unique to bdelloids and some protists, such as trypanosomes. Trypanothione may play a role in bdelloid desiccation resistance by preventing oxidative damage. The bdelloid genome contains four divergent pairs(eight copies) of Trypanothione Synthetase Amidase(TSA). TSA catalyzes four reactions to produce and break down trypanothione. We hypothesize that the copies of TSA are not identical in function and behave differently in various conditions. We compared the activity of the four copies of TSA in the bdelloid Adineta vaga with the single copy in Trypanosoma brucei (TryS), in different salts with varying concentrations. Some copies seem to be inactive at all concentrations for the forward reaction. Other copies of TSA respond differently to salt concentrations than TryS. Understanding the role of TSA in rotifers can provide key insights into evolution and function of genes responsible for DNA repair and protection from oxidative damage. In the future, we would like to create knockout rotifers for TSA and evaluate any impacts on their desiccation response and recovery.

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Combined effects of nutrient enrichment and sea level rise on salt marsh vegetation

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Salt marshes serve a variety of important functions. Marshes filter pollutants before they reach the sea, sequester carbon, serve as a storm buffer, and prevent coastal erosion, among other services. Great Sippewisset Marsh is a local salt marsh in Cape Cod, MA that has been utilized as a study site for scientists at the Marine Biological Laboratory for more than fifty years. In an ongoing experiment that started in the 1970s, circular plots (10m-radius) have been fertilized with nitrogen and phosphorous every year to assess plant community response to nutrient enrichment. Nutrient enrichment is a common problem along coastal communities because sewage, wastewater treatment, and agricultural runoff often feed into salt marshes, altering their landscape. Sea level rise has also impacted the area, altering the structure of the plant community due to different species tolerance to salinity and submergence. Vegetation distribution and abundance within the experimental plots has been continuously recorded every year by field surveys, aerial images, and ground truth measurements. In this work we utilized GIS to digitize available maps of the experimental plots to analyze changes in vegetation over time (1976-2020). We compared differences in vegetation assemblages between control plots and plots fertilized with nitrogen (urea) and phosphorus. Our results show that fertilization has caused profound changes to the plant community composition. We have also observed that, over the past few decades, the plant community, in both fertilized and control plots, has shifted towards species with higher tolerance to salinity and increased submergence, in response to the rise in sea level.

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Mercury Absorption in Aboveground Biomass on Plum Island Estuary, Plum Island Ecosystems Long Term Ecological Restoration Site, Rowley, MA

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Plum Island Estuary is a location for nutrient and contaminant cycling via biogeochemical processes in coastal New England. Mercury is a contaminant which has effects on the environment and wildlife as a neurotoxin via biomagnification. The importance of the Mercury research that we conducted is to understand its cycles through the environment because Mercury is transferred to organisms. Our results will help us understand differences in vegetation species' Mercury assimilation in the high marsh ecosystem. We primarily studied measurements of Mercury absorption in aboveground vegetation throughout the summer growing season. Samples were collected from the Parker River National Wildlife Refuge in Rowley, Massachusetts during June and July. The studied species were Spartina alterniflora and Spartina patens at 8 sites; 4 dominant sites for each. We hypothesized that S. alterniflora would have a higher Mercury concentration because its leaf has a higher surface area, which would allow for more Mercury to be absorbed. To test our hypothesis, we collected speciated sub samples by separating the live vegetation of S. alterniflora and S. patens. We washed the samples with MQ water and dried them in an oven. We used a Direct Mercury Analyzer (DMA) to measure the Mercury concentrations in the sub samples. Our results showed that S. patens had a higher Mercury concentration within its aboveground biomass. This could be because S. patens dominant sites are more dense with vegetation than S. alterniflora dominant sites. The June to July growth progression showed that Mercury remained at constant concentrations. Studying the two species separately allowed us to see the differences in Mercury levels in two vegetation species. Salt Marsh vegetation at PIE LTER receives inputs of Mercury from both the ocean and the atmosphere, so further research may allow us to see where exact sources of Mercury come from.

REU through NSF

RVT function in bacterium, Herpetosiphon aurantiacus, related to survival in iron-rich environments

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Reverse transcription, a process in which DNA is built from an RNA template, is a specialized function found in nature mainly in selfish genetic elements like viruses and transposons. Reverse-transcriptase related (rvt) genes utilize reverse transcription and have been evolutionarily conserved within almost every major taxonomic group, although patchily, with shared ancestry between both prokaryotes and eukaryotes. While the cellular function of these rvt genes is unknown, rvt genes from rotifers, fungi, and bacteria have been found to impact survival in various transition metals, responding by increased expression. However, the domain responsible for improving host survivability is unknown. Initially, the N-terminal coiled-coil domain of the rvt genes allowing for the multimerization of RVT proteins was hypothesized to involved in the interaction with these metal ions. The RVT protein found in Herpetosiphon aurantiacus (HaRVT), a filamentous gliding bacterium, is expected to interact with Fe2+ based on previous gene expression assays. Specific domains of HaRVT were mutagenized to target the domains potentially involved in the response to iron. Escherichia coli Rosetta 2(DE3) strain was transformed with mutant harvt genes cloned into the pET-45b expression vector. Transformed E. coli with induced RVT protein expression were grown on LB-agar plates with increasing concentrations of iron chloride (II) ranging from 0 mM to 2.5 mM. In addition, Herpetosiphon aurantiacus was grown on skim milk agar plates, supplemented with different concentrations of iron chloride, and imaged every day to measure the area of growth. We

found that the presence of recombinant HaRVT improves growth of E. coli in the presence of iron and allows Herpetosiphon aurantiacus to grow in Fe2+ concentrations up to 1.0 mM. Additionally, the reverse transcriptase domain seems to be more important for HaRVT's response to iron ions than the coiled-coil domain.

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