University of Rhode Island
Research and Innovation
(URI) Grant Application

Dear (URI)'s Search Specialist,

We are undergraduate researchers working in the Prada lab at the University of Rhode Island. We are requesting funding to start a new project to study coral symbiont community variation across environments. Our work is titled: "*Acclimatization of the Symbiont Community to Different Light Environments"*. The (URI) undergraduate funding would allow us to expand upon our knowledge of the scientific process and gain valuable research experience, facilitating finding future career opportunities.

Cat started working in the Prada lab in Spring 2022 and Willow started in Winter 2020. Our research project relates to Ph. D. student Taylor Lindsay's project titled *"Morphological, Physiological and Isotopic Acclimatization of Corals to Different Light Environments"*. We would like to begin our own project in conjunction with Taylor's work that focuses on how the symbiont community fluctuates with depth. We plan on completing the lab work in Fall 2022 and data analysis by Spring 2023.

At URI, we have both sought out experiences to expand our knowledge of research and laboratory skills. Our collective experience in research and coursework from three different majors has provided us with the necessary skill sets to conduct high level, interdisciplinary research. We are excited at the prospect of starting our own project studying how symbiont communities fluctuate in corals. The (URI) Grant is essential for funding the materials necessary to start our own work and enhance our skills and open new research opportunities for us in the near future.

Thank you for your time and consideration. Please feel free to contact us with any questions or concerns at willow_dunster@uri.edu and catherine_eno@uri.edu

Sincerely,
Cat Eno and Willow Dunster

Statement of Preparation - Willow:

At URI, I am thrilled to fulfill my lifelong dream as a Marine Biology and Marine Affairs double major and student-athlete. As someone who is driven by curiosity, I find the ocean intriguing because it is so uniquely complex, unexplored, and under critical stress from human interactions. I strive to contribute to marine research in hopes of helping to preserve this incredible ecosystem.

At URI, I have gained practical lab experience as an undergraduate research assistant with Dr. Carlos Prada. I started assisting Ph. D. student Taylor Lindsay in December 2020 with water quality monitoring on the lab's aquarium system as well as coral feeding and cleaning. I continued to work with the Prada lab as a RI NSF EPSCoR summer undergraduate research fellow (SURF) and conducted data analysis for fish environmental DNA samples in the Narragansett Bay. This year, I received a $500.00 BSURA grant to start my own project where I am learning to amplify and sequence symbiont DNA to assess whether symbiont communities are adapted or plastic in two key Caribbean coral species. Variation in the symbiont community plays a critical role in the success of corals in different environments and I am interested in further understanding how and why this variation exists. I have also continued to work in the Prada lab as a research assistant, helping with sample and data management, preparing coral samples for analysis, and helping to mentor other undergraduates in the lab.

My time in the Prada lab has guided me to pursue a career in molecular ecology. As a recipient of a (URI)2 grant, I would be able to continue to expand upon my research skills and develop a better understanding of the scientific process. Additionally, the grant would allow me to create a more robust dataset that seeks to increase our understanding of coral adaptation and acclimatization and guide restoration efforts. I believe I have the skill set, drive, and curiosity to carry out this research project.

Statement of Preparation - Cat:

As a Rhode Islander, the ocean has always fascinated me as it holds a vast ecosystem of known and unknown marine life. Since beginning my double major career at URI as a Marine Biology and Ocean Engineering, I have wanted to do research with the ocean and combine my two areas of study. My experience in marine biology has given me the tools to understand biological and ecological processes and my experience in ocean engineering has given me the tools to better analyze and understand data.

I have recently joined Dr. Carlos Prada's Lab this Spring of 2022. Here, I have had the opportunity to assist Ph. D. student Taylor Lindsay with her dissertation studying the morphological, physiological and isotopic acclimatization of corals to different light environments. I have learned the protocols of how coral is analyzed to look at their different physiological parameters, and worked in the lab to prepare these samples for further analysis. As a recipient of a (URI)2 grant, I would be able to enhance my skills and have a better understanding of the scientific process. Researching marine life, specifically with a concentration into how climate change has affected ecosystems, is exceptionally important to me and this project will allow me to gain experience in this area of study.

Project Description:

Background

Coral reefs are the most diverse ecosystems in the ocean, providing home to about 25% of all marine life[4]. Worldwide coral cover has declined by 15-20% since ~1950[1]. At the core of these ecosystems is the symbiotic relationship between corals and single-celled algae that provides the majority of the energy a coral needs to survive. Studying the relationship between corals and these single cell algae is key to understanding symbiont community structure and how they change in response to environmental variables and allow corals to live in different habitats.

One of the biggest environmental changes across reefs is light along depth gradients. Light decreases with depth and directly impacts the photosynthetic ability of the symbiont and therefore the energetic potential of the coral[4]. Symbiont communities fluctuate along depth gradients as the result of the distribution of light[2]. However, it is unclear whether these changes are reversible so that corals have the flexibility to change their symbionts (acclimatize) or are fixed adapted irreversible processes Symbionts are divided into clades each having distinct photosynthetic capability. Clade C is typical of deeper water corals while clades A and B are characteristic of shallow water corals. Additionally, symbiont cell density and chlorophyll can fluctuate in order to optimize light capture. Shallow water corals typically have higher abundance of chromatophores and a lower cell density while deep water corals have symbiotic cells and higher levels of chlorophyl[5]. A higher density of cells increases energy potential but is energetically costly to maintain. Chlorophyll is the primary pigment of photosynthesis and is responsible for absorbing light in the orange to red and violet to blue spectrum[3]. Chlorophyll is an accessory pigment that is found in marine algae and is specifically on the blue-light absorbance spectrum[6]. [CPM1] Shallow water corals will have a higher chlorophyll concentration and a larger symbiont community which allows them to make use of the higher light availability associated with shallow environments. Deep water corals will have a higher chlorophyll concentration as blue light is the main light that penetrates deep water and in turn will have a smaller symbiont community.

Orbicella faveolata and *Orbicella franksi* are massive mounding corals found widely across the depth gradient in the Caribbean. The objective of the project is to test if symbiont communities are fixed (irreversible) or plastic (flexible) within *Orbicella* species by conducting a common garden experiment and analyzing symbiont community structure, cell density, and chlorophyll content. If symbiont structure changes after transplanting then the symbiont community will be considered plastic. If the symbiont structure stays the same after transplantation, then the community will be considered fixed. A fixed community would suggest that *O. faveolata and O. franksi* have adapted their symbiont communities based upon their depth, and therefore their light environment.

The Prada lab has previously documented the emergence of shallow and deep cryptic lineages in *O. faveolata* as seen in Figure 1. We suspect that because the deep and shallow samples are genetically distinct groups, their symbiont structure will also be distinct for each depth.

In coral reef restoration projects, it is common practice to pay little attention to the depth a parent colony comes from, instead of planting propagules to any location on the reef. Our study will help to explain the variation in symbiont clade, chlorophyll, and density across two key Caribbean reef building species and identify whether deep and shallow water corals can adapt to the opposite environment. This can help conservationists to select the corals that are best suited to transplantation, and therefore have the highest chance of surviving and restoring the reef

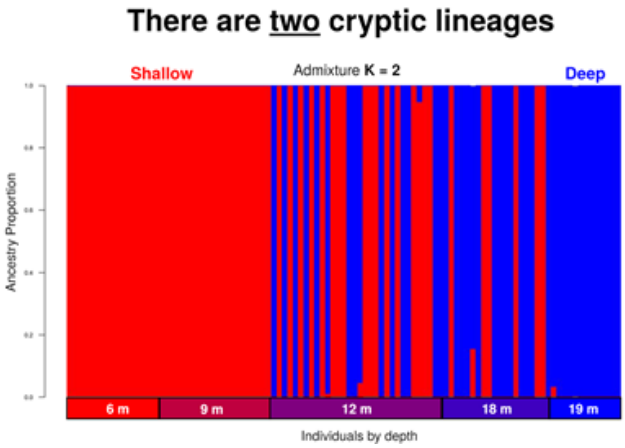

Figure 1. Admixture [2] [3] *plot showing two genetically distinct populations of coral that are highly segregated across depth. It is expected that symbionts will follow a similar pattern (Adapted from Ph. D. student Matias Gómez).*

Aim 1. To acquire the Chlorophyll and Chlorophyll concentrations from the transplanted coral samples to test if the symbionts have adapted or acclimatized to depth via a reciprocal transplant experiment. It is expected that deep water corals will have a higher concentration of Chlorophyll as that is along the blue-light absorbance spectrum which are the wavelengths that travel farthest down the water column. It is expected that shallow water corals will have a higher concentration of Chlorophyll because they are exposed to more sunlight and thus have a larger symbiont community[8].

Aim 2. To test if symbiont cell density is adapted or acclimatized to depth via a reciprocal transplant experiment. A higher cell density is characteristic of corals in deep water environments to optimize light capture. We expect to see an increase in symbiont cell density in corals transplanted from shallow to deep and the inverse for corals transplanted from deep to shallow.

Aim 3. To test if symbiont communities can acclimate to different depths via a reciprocal transplant experiment. Genomic data for symbiont communities, known as ITS2 will be amplified and sequenced to determine symbiont clade for each genotype and treatment. It is expected that corals moved to environments will be dominated by Clade C, while corals in the shallow habitat will be dominated by Clades A and B.

With this grant, we will investigate the effects of adaptation vs acclimatization of the symbiont community. Our work will help provide insight into how the symbiont community changes over a depth gradient.

Experimental Design

In summer 2021, a reciprocal transplant was carried out in Media Luna, Puerto Rico on the species *Orbicella faveolata* and *Orbicella franksi*. 40 genotypes of each species were collected from deep (16.8m) environments. *O. faveolata* was also collected from shallow (4.6m) environments. Replicate cages of the genotypes were transplanted to the foreign environment following the format in figure 1. Fragments were allowed to acclimatize for two months. A small section of each sample was preserved in DNA/RNA shield for genetic analysis and the remainder was snap-frozen in liquid nitrogen. The objective is to analyze the variation of the chlorophyll and cell density concentrations of *Orbicella faveolata* and *Orbicella franksi* across a depth gradient. The size and structure of the community directly impacts the photosynthetic capability of the coral. We will quantify the variation and understand whether it results from adaptation or acclimatization.

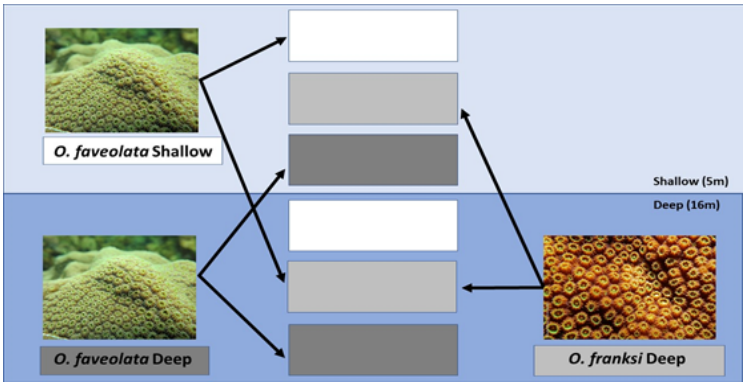

Figure 2. A common garden experiment [CPM4] *was conducted between deep and shallow environments in Puerto Rico with 40 genotypes of Orbicella faveolata were collected from shallow and deep and were reciprocally transplanted in cages. Orbicella franksi was only collected from deep and was translated to shallow. All three sets of genotypes also had control cages deployed at the original depth.*

Procedures

Cat will be responsible for quantifying chlorophyll content, using a Synergy HTX Multi-Mode Microplate Reader. The reader records wavelengths at 630, 663, and 750 nm which are used to determine the type of chlorophyll. Equation's 1 and 2 are used to determine the chlorophyll a and chlorophyll concentration.

$$Chlorophyll\ a = 11.43E_{663} - 0.64E_{630} \quad (1)$$

$$Chlorophyll\ c_2 = 27.09E_{630} - 3.63E_{663} \quad (2)$$

Willow will quantify cell density. The symbiont pellets produced from a tissue homogenate will be resuspended then mixed and loaded onto the haemocytometer slide. Cells will be counted using the grid pattern on the haemocytometer. We expect to see an increase in symbiont cell density in corals transplanted from shallow to deep and the inverse for corals transplanted from deep to shallow.

Willow will also continue to characterize the symbiont clade. DNA extractions will be amplified using ITS2 primers for the region around 300 base pairs that can be used to identify symbiont Clades. Symportal, a novel analytical framework will be used to organize sequencing data. If corals change their dominant symbiont clade in their non native environment then the symbiont community will be considered plastic (flexible). If the symbiont community does not change it will be considered fixed (irreversible).

The above data will be combined to analyze how the coral is regulating its symbiont community in response to variations in light availability.

Original Project Ideas

Though our project is a subset of Taylor's dissertation research, it was our idea to specifically look at the symbiont community. We chose to pursue cell density, symbiont clade, and chlorophyll content, as these factors can inform how the coral is regulating its symbiont community in response to variations in light availability. Taylor's work is focused on the whole holobiont, while ours is focused solely on the symbiont community.

Value of Project

The value of this project is that it will provide insights into how corals adapt across a depth gradient. This is key as corals are dying across the world and it is believed that deep water corals can help repopulate shallow water corals. Our work would suggest whether this is true or, if instead, corals are specialized to the depths they occur, and thus matching donor and transplanting sites would enhance yield during coral restoration, an expensive activity now massively in progress across the world.

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