Impacts of predation on the chemical ecology and microbiome during coral restoration
University of Rhode Island
Biological and Environmental Sciences: Evolution and Marine Biology

PROJECT SUMMARY

Overview

Coral reefs are critical to the health of the planet, but reefs around the world are dying due to increasing threats from human activity, especially climate change. The Florida Keys Reef Tract is one of the longest barrier reefs in the world, but has also seen some of the greatest stress from human development. To restore reefs in degraded areas such as the Florida Keys, scientists have developed practical coral restoration methods. When restoring slower growing boulder corals, coral colonies are "microfragmented" in the lab by cutting them into <1cm by 1cm pieces. The fragments are grown on small ceramic plugs for 6-12 months, and then planted out on the reef (Page et al. 2018). While microfragmenting promotes fast coral growth and corals survive well while still in the lab, fragments can die under wild conditions (Forsman et al. 2015). The higher mortality rates seen in the wild often decrease again after one month. Decreased predation from parrotfish and a shift in coral color has also been observed around this time.

We propose that the initial mortality in transplanted corals is due to fish predation, and that the subsequent decrease in mortality is due to changes in the microbiome and development of chemical cues once corals acclimate to wild conditions. We hypothesize that during acclimation in the wild, newly transplanted corals produce chemical cues, likely stemming from associated changes in their microbiomes, resulting in a distasteful signal to predators.

Focusing on the boulder coral species *Orbicella faveolata*, we aim to quantify the effects of chemical cues and microbial composition in the coral holobiont on fish predation during coral restoration.

Intellectual Merit

Despite coral restoration efforts, little is known about the fate of transplanted fragments. This project will provide new insights into the chemical ecology of corals and ultimately how microbial symbionts mediate ecological interactions. Fish predation is a key ecological process shaping modern reefs, and this project will provide evidence on the role of fish predation on transplanted corals as well as generate guidelines to increase restoration yields. Coral reefs support at least 25% of the world's marine biodiversity, act as CO_2 sinks, and are key to the health of our oceans. While the state of our reefs will not change unless we slow down CO_2 emissions, the acclimation capacity of corals to adjust their chemistry and microbiomes is a natural asset to reef restoration that may allow reef survival long enough for reductions in global emissions to occur.

Broader Impacts

Results from this study will be incorporated into a coral restoration workshops using Mote's established communication channels in the Florida Keys. This will advance coral restoration and help to generate strategies for researchers around the world to aid in their conservation efforts. Coral restoration is also a subject that is accessible to people with varying levels of scientific education. Researchers in the field will also have ample opportunities to mentor interns at Mote Marine Lab, as a central piece of their internship program is to work with visiting scientists. At URI, members of this project are on the leadership team for the Society for Women in Marine Science. This organization involves mentoring, networking, professional development, and public outreach. Researchers will develop a coral focused curriculum for local elementary school students as part of SWMS' education initiative. Researchers on this project have extensive previous experience in outdoor education, and are fully prepared to use this to teach children, teens and the general public about marine biology and conservation.

1. Background

Coral reefs support at least 25% of the world's marine biodiversity, act as CO_2 sinks, and are key to the health of our oceans. While the state of our reefs will not change unless we slow down CO_2 emissions, the acclimation capacity of corals to adjust their chemistry and microbiomes to stress is a natural asset to reef restoration that may allow reef survival long enough for reductions in global emissions to occur. As we lose more corals to climate change, ocean acidification, and other stressors, developing methods to restore massive corals quickly have become vitally important. The group under the greatest stress are the scleractinian, or stony corals. Stony corals are a benthic species that grow clonally, building a hard calcium carbonate skeleton as they expand. Over thousands of years, they build entire reefs. However, they grow extremely slowly and may only grow 1mm of new tissue a year. Thus, in the past, coral restoration efforts have focused on faster growing branching corals, such as *Acropora cervicornis*.

Fragmenting corals and planting the pieces back on the reef is a practice that is widespread globally and has proven an effective way to "farm" large amounts of branching coral (Lirman et al. 2010). Coral "gardening" efforts, where corals are grown in fragments either on shore or in a monitored nursery and are then transplanted out to the reef, have achieved high survivorship and higher growth rates (Rinkevich 2014, Horoszowski-Fridman 2017). Recently, researchers at Mote Marine Laboratory discovered that fragmentation techniques could be applied to boulder corals as well (Page et al. 2018, Forsman et al. 2015). In the massive coral species *Orbicella faveolata*, microfragmented corals produced up to 6.5 times more tissue than non-fragmented colonies (Fig. 1, Page et al. 2018). However, heavy predation in the first month of coral outplanting limits their survival (McCarthy 2017). Predation scars are typically not observed after one to three months, and preliminary data suggests predation mortality can be avoided by caging corals during an initial acclimation period (Fig. 3). While microfragmentation has proven effective in the field, there remain few quantitative studies on the various factors influencing long-term coral fragment survival.

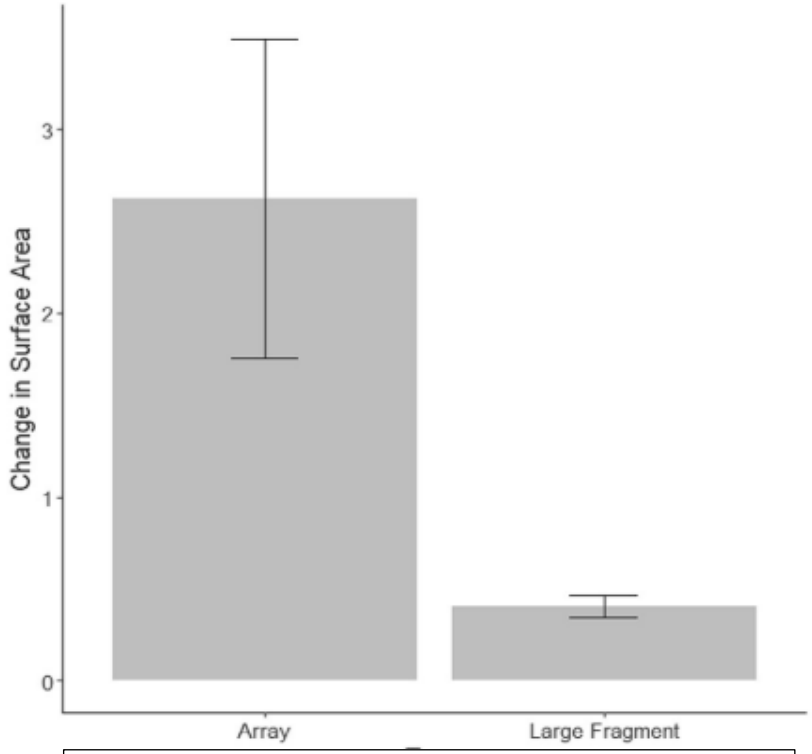

Figure 1: Average change in surface area, per initial cm2, of microfragment arrays and larger fragments of *Orbicella faveolata* 167 days after outplanting within a nearshore site. Error bars represent standard error of the mean. (from Page et al. 2018)

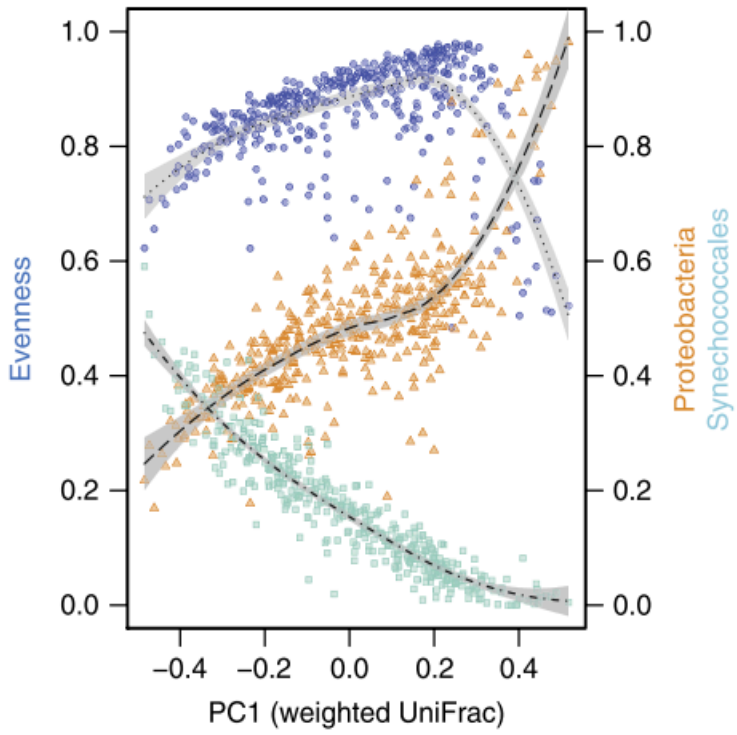

Figure 2: Displacement of Synechococcales by varied Proteobacteria structured differences between stressed coral microbiomes. Points plot the 1st PC axis against the relative abundance of Synechococcales, Proteobacteria, and overall microbial community evenness. Lines show local regression (Loess regression, span=0.75), with grey bars shading extending to twice the s.e. of the regression. (from Zaneveld et al. 2016)

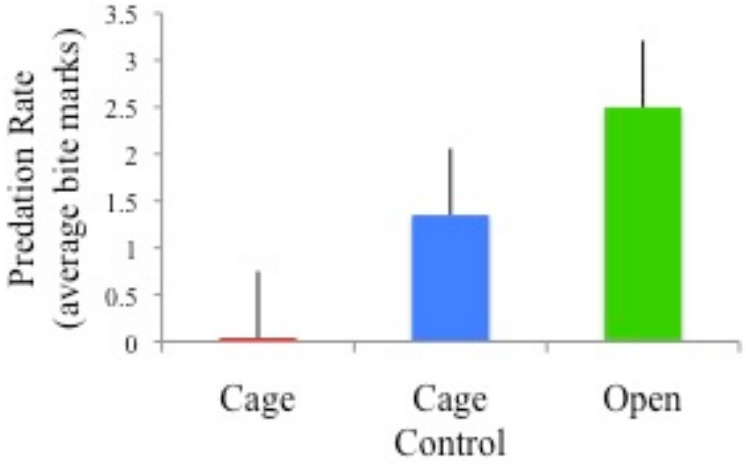

Figure 3: Mean predation rates in bites per coral fragment after 13 days of exposure under reef conditions. Note caging protects corals against predators. Error bars show standard errors. (data from McCarthy 2018)

In recent years, research has begun to focus on the coral microbiome. Corals themselves are part of a diverse holobiont, including bacteria and photosynthetic dinoflagellates (*Symbiodinium spp*.) (Bourne et al. 2016). The Global Coral Microbiome Project seeks to describe corals' diverse microbial communities. The coral microbiome can be affected by stress, including predation, which can have long-term effects on survivorship (Fig. 2). Transplanted corals show a shift in their microbial communities, which can leave them more susceptible to pathogens (Casey et al. 2015). Thermal stress to the coral holobiont can also put the microbiome under greater risk from disease (Bourne 2016).

Predation, especially from parrotfish, is increasingly acknowledged as a major source of mortality for many stony corals (Rotjan and Lewis 2005). Other boulder coral species, such as *Porites astreoides,* do not re-grow tissue over previous parrotfish bites (Rotjan and Lewis 2005). Parrotfish predation has been found to have a significant impact on the microbiomes of transplanted *Acropora cervicornis* fragments, which may lead to increased mortality (Shaver et al. 2017). Coral nurseries, either on shore or in the ocean, traditionally protect corals from predation, which helps corals achieve high growth rates before transplant (van Oppen et al. 2015). And although the changes in the coral microbiome as a result of climate stress have been thoroughly examined, few studies focus on the microbiome response to predation. Previous research on octocorals has found that they

produce secondary metabolites, which have been hypothesized to be an anti-predation adaptation (Sammarco 1996, Puglisi 2002). Six of seven species studied were found to have chemicals unpalatable to fishes located in the sclerites at the tips of their colonies (Puglisi 2002). No studies have attempted to identify specific changes as they relate to restored boulder corals responding to predation stress.

2. Specific Aims

Aim 1: Quantify the acclimation period (number of weeks) over which corals become less vulnerable to fish predators. A decline in predation over time has been observed in the field, but this decline has not been monitored quantitatively for specific coral species (McCarthy 2017). Predation rates in acclimated and un-acclimated colonies that have been planted in the field will be measured over time to both determine over what time period predation decreases, and to determine if acclimated colonies see significantly less predation.

Aim 2: Identify the chemical compounds that differ among: 1) lab raised, 2) newly transplanted (un-acclimated) and 3) acclimated fragments. Chemical profiles for all three groups throughout the transplant process, both before and after fish predation decreases, will be used to identify the biochemical cues by which corals deter fish. They may also provide a chemical framework to assist the analysis of their microbiome compositions. We will use bioinformatics to compare chemical profiles to existing databases to identify key fragments (Quinn et al. 2016).

Aim 3: Measure changes in microbial diversity from: 1) lab raised, 2) recently transplanted (un-acclimated) and 3) acclimated fragments. The microbial community in the coral holobiont can change in response to stress. Identifying changes in the microbiome during restoration will allow for identification of the microbial symbionts that could underlay the production of chemical cues that deter fish predators. We will characterize changes through metabarcoding using the hypervariable V4 region of the 16S rRNA gene (Zaneveld et al. 2016, Shaver 2017).

3. Research Approach

Study Location: To allow large sample sizes in the predation assays, we will perform the caging experiments in the Florida Keys in collaboration with Mote Marine Laboratory's International Center for Coral Reef Research and Restoration (IC2R3). Mote has been replanting corals for over 20 years, and has the experience and resources to manipulate thousands of fragments. We will use existing study sites with years of photographic data cataloguing coral survivorship and growth. Mote maintains extensive on-shore facilities ideal for growing and monitoring coral microfragments. As our team includes a former Mote employee, we are very familiar with both the field and the on-shore facilities.

Experimental Design – Aim 1: To identify the acclimation period at which corals start deterring fish predators (Aim 1), we will transplant *O. faveolata* fragments in cages at different times, starting with corals at eight weeks, then six weeks, then four weeks and then two weeks. Cages will then be removed from the corals. and fragments will be randomly mixed with lab-raised (un-acclimated) corals. Each outplant population will be a mix of un-acclimated corals and acclimated corals with different times of acclimation. Each mix population will have 500 fragments (100 per treatment), and will be replicated across four reef sites. Predation rates will be quantified in un-acclimated and 2-, 4-, 6- and 8-week acclimated fragments bi-weekly for three months. Predation rates will be determined from the presence of distinctive parrotfish bite scars on coral skeletons (Rotjan and Lewis 2005). Another mix population of 500 fragments will be maintained on shore in order to assess the effects of predation without transplanting. These corals will remain in raceways for the duration of the study, and a percentage comparable to field observations will be randomly selected to be subjected to artificial predation.

Experimental Design – Aim 2: To determine changes in the chemical and microbiome composition (Aims 2 and 3), we will punch 1cm wide disks from 10 fragments each from: 1) lab-raised (un-acclimated), 2) recently transplanted, and 3) acclimated with different weeks of acclimation. Samples will be preserved for analysis in 10 ml of LC-MS/MS grade 70% methanol and 30% water (Quinn et al. 2016). Chemical composition will be characterized using mass spectrometry. Protein concentrations will be determined using a modified Bradford protein assay (Puglisi 2002). To call the molecular features of each metabalome researchers will use the Bruker Daltonic Find Molecular Features algorithm (Quinn et al. 2016). We will compare the chemical footprint of each sample bioinformatically to known databases and identify key molecular segments (Quinn et al. 2016, Welsh 2017). Matrices can be imported into R-Studio for further statistical analysis.

Experimental Design – Aim 3: To examine the coral microbiome, we will use syringes to sample the coral mucus. Sampling the mucus will allow us to repeatedly sample the same individuals over time without excessively harming the corals during this stage of the analysis (Zaneveld et al. 2015). Changes in the microbial community will be described via metabarcoding using the hypervariable V4 region of the 16S rRNA gene (Zaneveld et al. 2016). Samples will be used to generate 16S amplicon libraries. After amplification, triplicate reactions will be pooled for analysis. The QUIIME software pipeline will be used for analysis of microbial community diversity. Functional profiles of each microbial samples will be predicted using the PICRUSt tool. Specific functions will be determined using GeneOntology terms and KEGG profiles. This project builds upon collaboration between the Prada lab at URI and Dr. Jesse Zaneveld from the University of Washington, and the Global Coral Microbiome Project.

4. Intellectual Merit

The proposed research will advance our understanding of the coral microbiome under stress. *Orbicella faveolata* is a foundational coral species on Caribbean reefs, so understanding these dynamics will have strong implications for future conservations efforts. The results from this study also fit into the larger aims of the Global Coral Microbiome Project, especially in describing the microbiology of all reef building corals and examining how the coral microbiome can help explain resistance or overall vulnerability of different coral species to stress. Previously there has been little research into the coral microbiome during restoration of boulder corals. This project will help improve the efficacy of coral restoration efforts.

5. Broader Impacts

At the University of Rhode Island: This project will involve extensive training for one graduate student, as well as at least two undergraduate students at URI. The graduate student will also incorporate their research into various training and mentoring opportunities with URI's chapter of the Society for Women in Marine Science. SWMS provides an opportunity for graduate students to mentor undergraduates who are interested in marine science, and helps them learn about research. The program involves monthly meetings, application advice, and collaboration on established outreach programs to local primary and secondary schools.

At Mote IC2R3: Our goal is to build a curriculum for coral restoration workshops using Mote's established communication channels in the Florida Keys. This curriculum can be used to advance coral restoration and generating strategies for researchers around the world to aid in their conservation efforts. Mote has already started hosting workshops at IC2R3, and the results of this project will make the curriculum more effective. This project will also provide ample opportunities to mentor interns at Mote Marine Lab, who will assist with field methods and data collection during field work at the facility.

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