Seasonal Variability in Coral Immunity in the Florida Keys, the US Virgin Islands, and Puerto Rico

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This project primarily addresses Program Priority a) Monitoring and assessment of coral reefs or reef resources

Geographic Location of the Project: Florida Keys, USVI, and Puerto Rico

Scope of Project:

This project aimed to 1) establish a baseline for coral immunity in two keystone coral species in three geographic locations, 2) monitor coral immunity levels over time and, 3) monitor microbial dynamics in coral surface mucus, surrounding water mass, and regional sources of invasive microbes (sediment), over time and space. Data gathered is expected to fill a gap in existing data important for the management of numerous coral reef species and their respective habitats.

PROJECT SUMMARY

Work done in 2005-2006 in the Florida Keys addressed the health of *Acropora palmata* in a temporal manner, demonstrating that this coral species has antibiotic activity associated with its surface mucus that is lost during summer months when temperatures increase (Ritchie, 2006). This study also resulted in the discovery that numerous potentially pathogenic bacteria (many implicated in coral bleaching world-wide) are present in reef waters during warming (Ritchie, 2006). These results show a correlation between the loss of coral immune defense and the presence of potentially pathogenic microbes, describing a mechanism for the observations that coral disease (and possibly bleaching) increases during warm trends (Harvell, et al 2002). This was the first study of its kind, and suggested that other healthy coral species may utilize innate immune responses that are lost when temperatures change (Ritchie, 2006). With the aid of this funding, we have additionally developed a computational model for microbial community dynamics in the mucus layer in order to understand how the surface microbial community responds to changes in environmental conditions, and under what circumstances it becomes vulnerable to overgrowth by pathogens (Mao-Jones et al, 2010).

The scleractininan corals *Montastraea faveolata* and *Acropora* palmata are major framework builders in the Caribbean and are important to the ecology of the region. Due to the diminishing numbers of *A. palmata*, and high disease susceptibility of both *A. palmata* and *Montastraea* spp, I aimed to document temporal changes in *both* of these important reef-building corals in the Florida Keys, Puerto Rico and US Virgin Island. Specifically, to monitor 1) temporal and

comparative *A. palmata* and *M. faveolata* innate immune responses via assays measuring antibiotic properties of coral mucus, and 2) microbial associates of coral surface mucus and surrounding water mass, including temporal microbial shifts. This project was designed to answer the following questions: 1) Do the same coral species differ from region to region with respect to antibiotic properties? 2) Do bacterial dynamics in the surrounding water and coral surface vary regionally and seasonally? 3) Are these changes indicative of coral health?

Information generated from this project will contribute to coral reef conservation by providing baseline and seasonal data in both innate immune responses and microbial dynamics between these two species and surrounding water mass. This data will be applicable to the management of coral reefs worldwide and is important for the larger understanding of coral bleaching and disease "footprints" relative to local stressors and seasonal temperature changes. Project results will be disseminated at regional, national, and international meetings, reports to NOAA/NMFS, and will be published in peer-reviewed journals, and disseminated to the public via outreach and education at MML. Partners include USGS/USVI, the University of Puerto Rico, and the Florida Keys and Southeast Regional National Marine Sanctuaries.

OBJECTIVES

1) **Quarterly monitoring of *Acropora palmata* and *Montastraea faveolata* innate immune response via assays measuring antibiotic properties of coral mucus.**
2) **Quarterly monitoring of microbial associates of coral surface mucus and surrounding water mass (including temporal microbial shifts in microbial populations).**
3) **Within-region comparisons of temporal data.**
4) **Between-region comparisons of individual species data.**

DESIGN AND RESULTS

Sampling. In November 18 of 2008, samples were taken from each coral species listed at the NW shore of Hawksnest Bay (3 colonies samples of each species) Dennis Bay (3 colonies samples of each species) and Leinster Bay in the US Virgin Islands. Because most *M. faveolata* colonies have died-out in these regions we sampled *M. annularis* in these sites. July 14th, 2009 samples were retaken as described above for November of 2008. Due to hurricane and sampling

logistics, samples have been taken from Puerto Rico for Quarters 1, 2 and 3, the Florida Keys for Quarter 1, 2, 3, 4, and USVI Quarters 1, and 2. Samples were taken during field collection using a non-invasive sampling technique via 5-20 ml needleless syringe samples of the surface mucopolysaccharide layer (SML) of healthy coral (Figure 1a). Carolyn Rogers (USGS) selected sample sites and accompanied and aided the PI during sampling. Samples were taken from a mid-palmate region of the coral frond (5 and 8 cm from the growing edge in *Acropora palmata*) and from the upward facing region of the colony in the boulder coral *Montastraea annularis* or *M. faveolata*. Pictures were taken of colonies before (Figure 1) and after (Figure 2) sampling so monitoring of samples regions can be maintained.

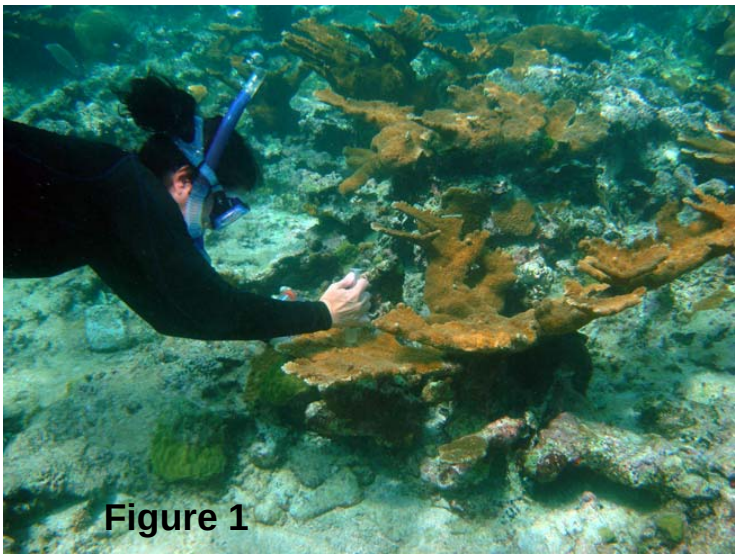
Figure 1

Samples taken during field collection included a non-invasive sampling technique via 5-20 ml needleless syringe samples of the surface mucopolysaccharide layer (SML) of healthy coral. Samples were taken from a mid-palmate region of the coral frond (5 and 8 cm from the growing edge in *Acropora. palmata*) or from the upward facing region of the colony in the boulder coral *Montastraea faveolata.* Samples were maintained at ambient temperature and processed within 2 hours of collection, with the exception of July of 2009 USVI samples that were kept cold and processed within 48 hours of sampling. Baseline water sampling was conducted in each region analyzed for coral mucus microbial composition.

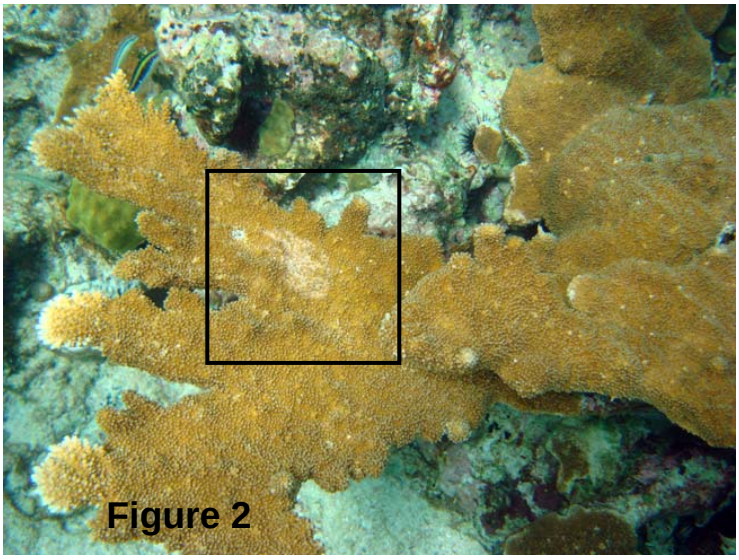
Figure 2

Innate immune response measures via antibiotic assays of coral mucus. This assay was developed to test the potential antibiotic properties associated with the coral mucus against environmentally relevant sources of invasive microbes. This was carried out by mimicking the coral surface microlayer on a growth media plate and plating out dilutions of various environmental sources. Coral mucus was plated onto Glycerol Artificial Seawater Media (GASWA) media, dried, and UV irradiated to inhibit growth of associated microorganisms. All control (GASWA) plates are UV irradiated prior to plating. Environmental sources of invasive microbes were serially diluted and plated onto tester and control plates, grown at 24°C. Colony forming units (CFUs) per milliliter are estimated for each set of experiments. Environmental sources to be tested include water samples from the region surrounding the corals collected as well as standard tester strains (*Serratia marcescens*, *Vibrio corallyliticaus*, and *Vibrio shilonii*,). This assay is currently being standardized via mucus viscosity and polysaccharide dyes to ensure consistency between samples and experiments. Results to date show insignificant levels of innate immunity between *A. palmata* mucus between regions and seasons (See **Table 1**, attached).

Antibiotic production by culturable coral associates. It has been found that the percentage of antibiotic producing bacteria is higher in healthy corals, suggesting that healthy bacteria harbor beneficial surface bacteria (Ritchie, 2006: Myers et al, in review). In light of this finding, mucus associated bacteria were used in a primary screen to test for the production of anti-bacterial compounds. Microorganisms exhibiting a unique colony or cellular morphology (as compared to other colonies on a single plate) were sub-cultured to purification and 96-well plate libraries generated and archived at 80°C for antibiotic screening and species identification. This primary screen included a panel of marine invertebrate pathogens and human pathogens, as follows: *S. marcescens* (White Pox pathogen), *Aurantimonas coralicida* (White Plague pathogen), *Pseudoalteromonas haloplanktus*, spp. *tetraodonas* (sea urchin pathogen), Methicillin-Resistant *Staphylococcus aureus* (MRSA, ATCC 43300), Methicillin-Sensitive *S.* au*reus* (MSSA, ATCC 29213), Vancomycin-Resistant *Enterococcus* (VRE), *Bacillus subtillis* (ATCC 6633 Km resistant), *Salmonella typhimurium* (ATCC 6994), *Enterococcus faecalis* (ATCC 29212), *Shigella* and *Escherichia coli* O157. Environmental libraries were grown on marine agar for two days at 24°C followed by UV irradiation to inhibit cross contamination during overlays. Over-night cultures of tester species are grown in Luria Broth (LB), Tryptic

Soy Broth (TSB) or Glycerol Artificial Sea water media (GASW), as appropriate. Libraries were overlayed with 0.8% soft agar medium containing the tester bacterial species of interest. Antibiotic presence is assayed as a zone of growth inhibition (clearing zone) around the colony that produces an active antibiotic. Antibiotic spectra of library isolates were used to further dereplicate cultured marine isolates and zones of inhibition was scored as mm distance from the outside of clearing zone to the outer edge of the tested colony. 85-100 bacteria were isolated from pooled triplicate samples and assayes as described above. **Figures 3 and 4** illustrate that higher percentages of antibiotic producing bacteria were cultured in November 2008 and May 2009 as compared to July 2009, in both *Montastraea* spp and *A. palmata*, which is consistent with previous results (Ritchie 2006). Routine monitoring will need to be performed to determin the lag effect predicted from the computational model suggesting that even when conditions return to normal, there is a lag period where corals do not recover to previous levels if innate immunity and beneficial microbes (Mao-Jones et al, 2010).

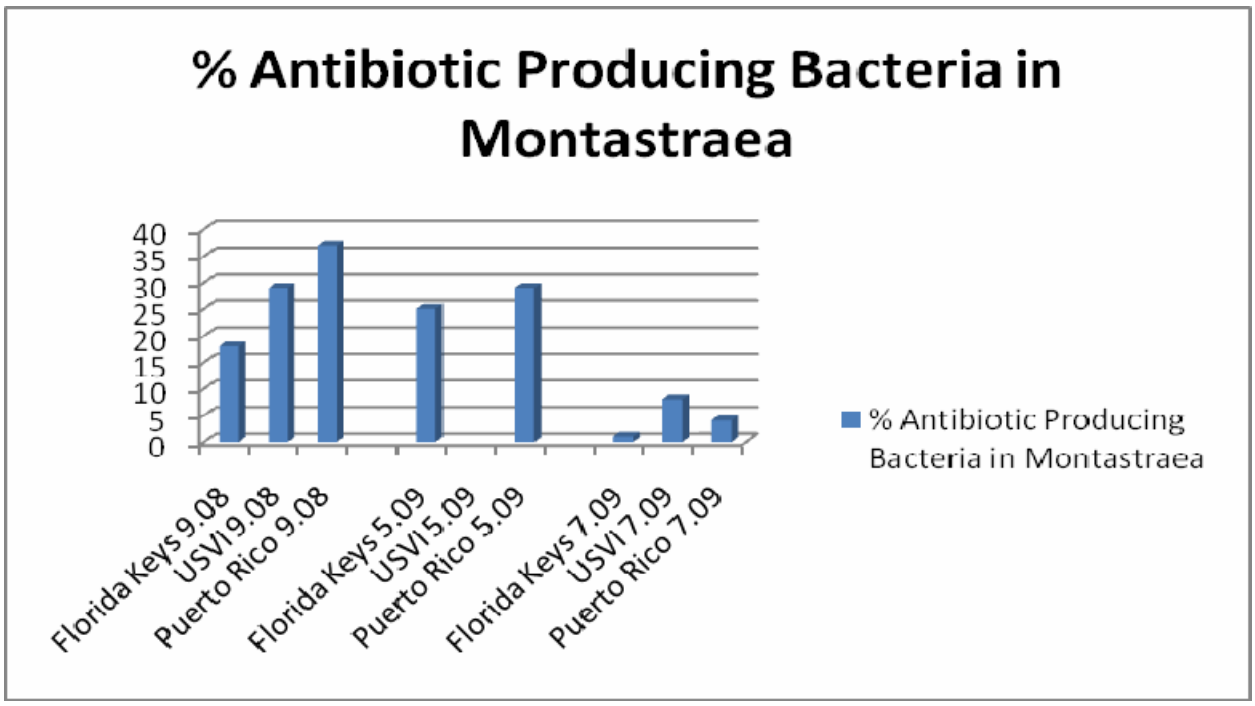

Figure 3 - relative percentage of antibiotic producing bacteria cultures seasonally in *Montastraea* spp. Note that samples were not gathered of USVI in May of 2009.

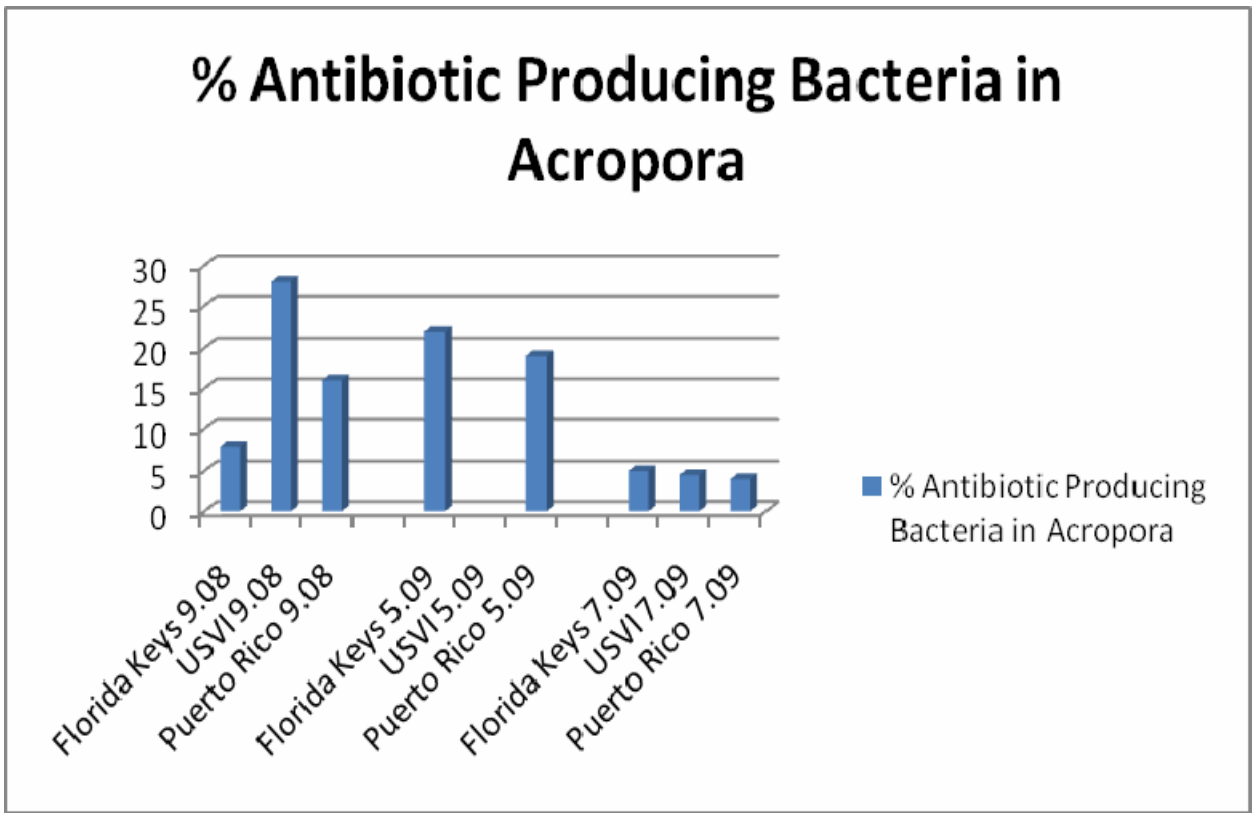

Figure 4 - relative percentage of antibiotic producing bacteria cultures seasonally in *Acropora palmate*. Note that samples were not gathered of USVI in May of 2009.

Total microbial community profiling via T-RFLP analysis of associated bacteria. Total DNA from each sample of coral mucus will be extracted by centrifugation followed by a bead-beating, humic acid removal system (Soil-DNA extraction kit, MoBIO). This system has proven effective in purification of total environmental DNA from organisms that resist standard extraction methods (algae and Gram-positive bacteria) and is valuable for the removal of compounds that inhibit subsequent genetic manipulation (Janus *et al*., 2005). Samples were archived frozen at –80˚C for molecular work. *Total microbial community profiling via TRFLP analysis.* Total DNA from each sample of coral mucus was extracted by centrifugation followed by a bead-beating, humic acid removal system (Soil-DNA extraction kit, MoBIO). This system has proven effective in purification of total environmental DNA from organisms that resist standard extraction methods (algae and Gram-positive bacteria) and is valuable for the removal of compounds that inhibit subsequent genetic manipulation (Janus et al., 2005). Samples are archived frozen at 80 degrees C for TRFLP work. Total DNA is used in a polymerase chain

reaction (PCR) with 16S rDNA TRFLP primers in order to selectively amplify the 16S rRNA genes used for genetic identification of total bacteria present (Moss et al., 2006). Members of the Ritchie lab are routinely using this technique for bacterial profiling of other marine systems (unpublished).

Figure 5 shows an example of TRFLP analysis of *M. faveolata* from the Florida Keys compared to TRFLP profiles from Puerto Rico. Peak differences, bacterial community structures, and similarity indices are being compiled and data analyzed via multidimensional scaling, among other statistical measures. We anticipate that data will determine which bacterial groups are similar between regions and will reflect seasonality in normal communities while corals are NOT diseased.

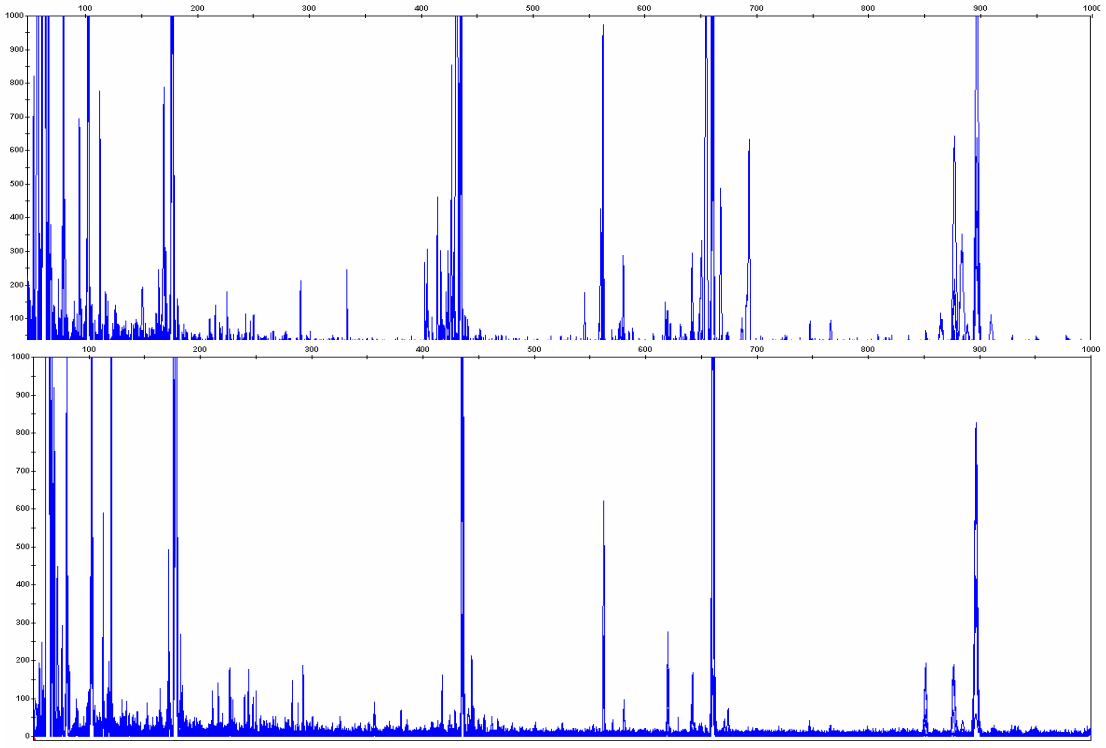

Figure 5. Example of T-RFLP patterns of 16S rDNAs from the mucopolysaccharide layer of Montastraea faveolata from Puerto Rico and the Florida Keys digested with *Rsa*I. 16S rDNAs were amplified with universal primers U9f labeled with FAM dye and U1509R. (A) TRFLP profile of *Montastraea faveolata* from Puerto Rico (B) TRFLP profile of *M. faveolata* from the Florida Keys.

Ongoing data analysis. Data analysis for T-RFLP will be ongoing after this final progress report. Proper statistical analysis (Principle component analysis and others) are currently being best determined for this data set. Comprehensive data from this support and will be supplied after final report date, as will relevant publications resulting from this funding.

SUMMARY OF RELEVANT DATA. To date, innate immunity measures show that differences are not significant between regions and seasons assayed. However, intricate data imbedded in t-RFLP analysis community fingerprinting could ultimately yield subtle differences that were not initially determined. However, beneficial bacterial shifts do appear significant within regions on a seasonal basis. Finally, DNA fingerprinting and statistica analysis between regions, seasons and coral species is ongoing.

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