SEASONAL CHANGES IN THE PROTECTIVE PROPERTIES OF ELKHORN CORAL SURFACE MUCUS

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Summary

Recent work 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).

Funds appropriated from the POR Grant, 2007, were to continue to document seasonal changes in *A. palmata* mucus with regards to antibiotic effectiveness against potentially invasive microbes. However, funds available were to continue quarterly, rather than monthly, sampling. Goals achieved under this continued funding are as follows:
1) Coral mucus has been collected quarterly from three study corals located on Looe Key Reef, on the Florida Reef Tract. Specifically corals at MB9, MB10, and MB20.

2) Antibiotic activities are being continually monitored.

3) Bacterial libraries have been generated from both water column and coral mucus samples and have been assayed for antibiotic activities monthly. In addition, Vibrio shifts are being monitored and data analysis is underway to visualize monthly shifts over the course of 3 years.

4) DNA has been extracted from both the water column and from coral mucus and is being used for Vibrio specific DGGE analysis. Based on these, and earlier results funded by POR grants, collaborations with USF College of Marine Sciences to develop an autonomous detection device for vibrio presence are being developed.

5) These results are being compiled and compared to results obtained from *Acropora palmata* sampling in the USVI and Puerto Rico. Comparative results are pending data completion and analysis.

This work continues to address seasonal variability in coral mucus and mucus-associated bacteria, and is already adding a new dimension to the database that is largely composed of coral disease etiologies. Specifically, collaborations with USF College of Marine Sciences engineering and microbiology departments are underway to develop an autonomous detection device for Vibrio presence in the reef environment.
Summary of major findings
We looked at antibacterial isolates by season (analysis ongoing, Figure 1), antibiotic activity on the coral surface as well as Vibrios (potentially opportunistic bacteria) in the water column and on the surface of the coral (Figure 2) and mean sea surface temperatures (SST; Figures 1-4). We used Vibrios as a proxy (a model system) for opportunistic pathogens because it is a known pathogen in many marine organisms and implicated in problems in corals. And there are numerous works that describe the growth rates of Vibrios.

Figure 1. Antibacterial Isolates by season. Y-axis = % antibiotic producing bacteria. Degrees Celsius are plotted in the secondary Y-axis. Data represent samples taken from April 2005 to October 2007.
Figure 2. Sea surface temperature effects on coral resistance and pathogen growth. Measurements were shown for seawater and coral mucus from April of 2005 to September of 2005. *Vibrio* ratios were measured as percent growth on TCBS media. Antibiotic activity (AB) in surface mucus was measured as ability to inhibit growth of seawater microbes (fold-inhibition) and was multiplied by 10 to scale.
Figure 3. Sea surface temperature and pathogen growth. Measurements were shown for seawater July of 2006 to October of 2007. Based on growth on differential media, Vibrio ratios are shown in yellow and there seems to be a correlation between decrease in SST and decrease in % of Vibrios present.
Figure 4. Impact of seasonality on pathogen dynamics. These results are consistent with a computational model on coral microbes (done by a group at Cornell) where a hysteresis model for pathogen lag is predicted – pathogen adherence to and overgrowth in mucus results in pathogen persistence even after conditions return to favorable conditions for healthy state microbiota. 

This suggests a lag time in coral recovery and a potential cause of disease, AFTER a warming event. That may also help to explain why corals are more susceptible to disease during AND EVEN AFTER a warming trend. Management applications are obvious as even when corals return to "normal" state, pathogen persistence and overall coral innate immunity are concurrent, making it more imperative to keep other stressors off of a reef system.
Matching Funds Obtained for Comparative Study in USVI and Puerto Rico

Funds were obtained (41K), with help from this POR funding, to do quarterly studies of *Acropora palmata* in Puerto Rico and the US Virgin Islands specifically to compare to the corals of the Florida Keys. These funds were obtained via the **NOAA/NMFS Coral Reef Conservation Grants** and will provide much needed comparisons of both the microbial communities associated with and the antibiotic (innate immunity differences) properties of *A. palmata* within this region of the Caribbean. The scope if this comparative project aims to 1) establish a baseline for coral immunity in *A. palmata* 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. Due to logistical difficulties and extension of this project has been requested and granted.

Education, Outreach, Funding and Publications

This work greatly primed funding for similar projects and publications, as listed below. Invited seminars based specifically on this study have been presented at the 11th International Coral Reef Symposium in Ft. Lauderdale, July 2008, and at the International Society of Microbial Ecology in Cairnes, Australia, in August of 2008. The work supported by POR has led to two Masters theses (Cory Krediet, and Stephanie Halbig) and one PhD Dissertation (C. Krediet) through collaborations with Researchers at the University of Florida, and one PhD Dissertation topic in collaboration with Pam Hallock Muller at USF Marine Sciences. It has also provided is providing research projects to 4 student interns who presented results at the International Coral Reef Symposium in July (Ross Cunning, Nick Jackowski, Jon Onufryk, Carmel Norman). Funding helped to develop a new session at the 11th International Coral Reef Symposium, 2008, on Coral Microbial Interactions that was well attended. I am currently editing the
Proceeding of this meeting. I am currently serving on a committee of 12 (the Acropora Recovery Team) to develop a management plan for this species under the Endangered Species Act. This committee is administered via the NOAA National Marine Fisheries Service. Information gained through this funding has influenced elements of the proposed recovery plan for this species. Papers that are directly or indirectly resulted from this work are listed below:

**Peer-Reviewed Publications**


Krediet, CJ, KB Ritchie, M Cohen, E Lipp, K Sutherland, and M Teplitski (In Press and online) Utilization of mucus from the coral Acropora palmata by environmental and pathogenic isolates of Serratia marcescens. Applied and Environmental Microbiology 75(12): online.


Ritchie, KB, Myers, RL, Thurmond, J (In Prep) Bacteria Associated with Symbiodinium spp. in culture.


Grants:


NOAA/NMFS Coral Reef Conservation Grant. 41K. Seasonal Variability in Coral Immunity in the Florida Keys, the US Virgin Islands, and Puerto Rico.


Munson Foundation. The Role of Microbial Communities in Coral Health and Disease 20K. 2005-2008