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**A Rapid Response to Cold Water Stress on Corals - Monitoring coral health and immunity
across habitat gradients in the Lower Florida Keys**

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Abstract:

The cold water event of January of 2010 resulted in mortalities in the mid-channel and nearshore corals of the Florida Keys National Marine Sanctuary. Coral species affected included members of the *Montastraea* spp. complex and the threatened staghorn coral, *Acropora cervicornis*. As the last cold-water mortality event on record was in 1977, this cold water event offered an opportunity to study the types of microbial community changes that may occur, and persist, following this type of stress event. In this study we aimed to observe coral immunity (as measured by microbial profiling) in nearshore, mid-channel, and outer reefs of the Florida Keys Mid to Lower Keys Region. Results indicate that bacterial communities associated with apparently healthy coral tissue are similar to those in other years, reflecting the temporal variations usually observed (ie higher incidences of vibrios in warmer periods) with little indication that the remaining healthy coral microbial communities, or coral immunity associated with each, were compromised after this event. Of interest were our observations that coral associated bacteria from these libraries lose antibiotic activity when temperatures are increased *in vitro*. Results were similar when compiled by month (data not shown). These observations are of great significance in the case that beneficial coral bacteria contribute to the innate immunity of the coral host by producing antibiotics *in situ*. This specific *in vitro* study has never been performed with corals and proves to be an important contribution to understanding how coral bacterial communities may affect coral health.

Submitted to:

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Scope of Work:

- 1) Assess *Montastraea faveolata* colonies in nearshore, mid-channel and outer reefs for coral immunity, microbial profiles and the presence of beneficial microbes
- 2) Assess water column microbes in nearshore, mid-channel and outer reef environment for presence/absence of disease causing microbes
- 3) Assess recovery over time of partially, non affected and affected *M. faveolata* in nearshore, mid-channel and outer reef environments.
- 4) Comparison of conditions to conditions established during a warming event (Ritchie, 2006) using *Acropora palmata* in an outer reef (Looe Key) environment

Methods:

Sampling. Coral mucus from unaffected coral tissue was sampled via standard needless syringe methods (Ritchie, 2006) removing 5-30 mls of coral mucus. Five *M. faveolata* colonies were sampled per site across habitat gradients in the lower Florida Keys (FIGURE 1; TABLE 1): American Shoals), Looe Key Reef Sanctuary Preservation Area (20 foot depth, MB #10), Munson Reef mid-channel habitat (18 foot depth) and a nearshore site (18 foot depth). Three *A. palmata* colonies were sampled only at Looe Key Reef (coordinates as above for *M. faveolata*) as this is the only habitat sampled that harbors this coral species. Note that Looe Key Reef is Ritchie's established study site for the past 6 years and provides a baseline of data for a seasonal comparison of microbes and coral immunity during multiple types of events. Water was sampled at each site by filling 1 liter bottles approximately 1 meter away from corals prior to mucus sampling. All samples were kept at ambient temperature until processed at Mote's Tropical Research Laboratory. All processing took place within 4 hours of collection. Water samples were filtered onto 0.2 um filters and stored frozen for future analysis (pending). **NOTE that no live *M. faveolata* tissue was found at the nearshore site after February of 2010.** Since this time we have continued to monitor water from this site and have made observations of phase shifts from hard corals to *Oculina* spp. and various gorgonian corals (personal observation). We additionally observed that various corals, such as *Siderastrea siderea*, and some individuals of *Colpophilia natans* fared well during this die-out. However we aborted efforts to sample *M. faveolata*. DNA was extracted from each coral colony. Mucus was pooled from *M. faveolata* (only) from each site for purification of culturable bacteria, as described below. Remaining coral mucus from individual coral colonies from each site and date was frozen at -80°C for antibiotic assays.

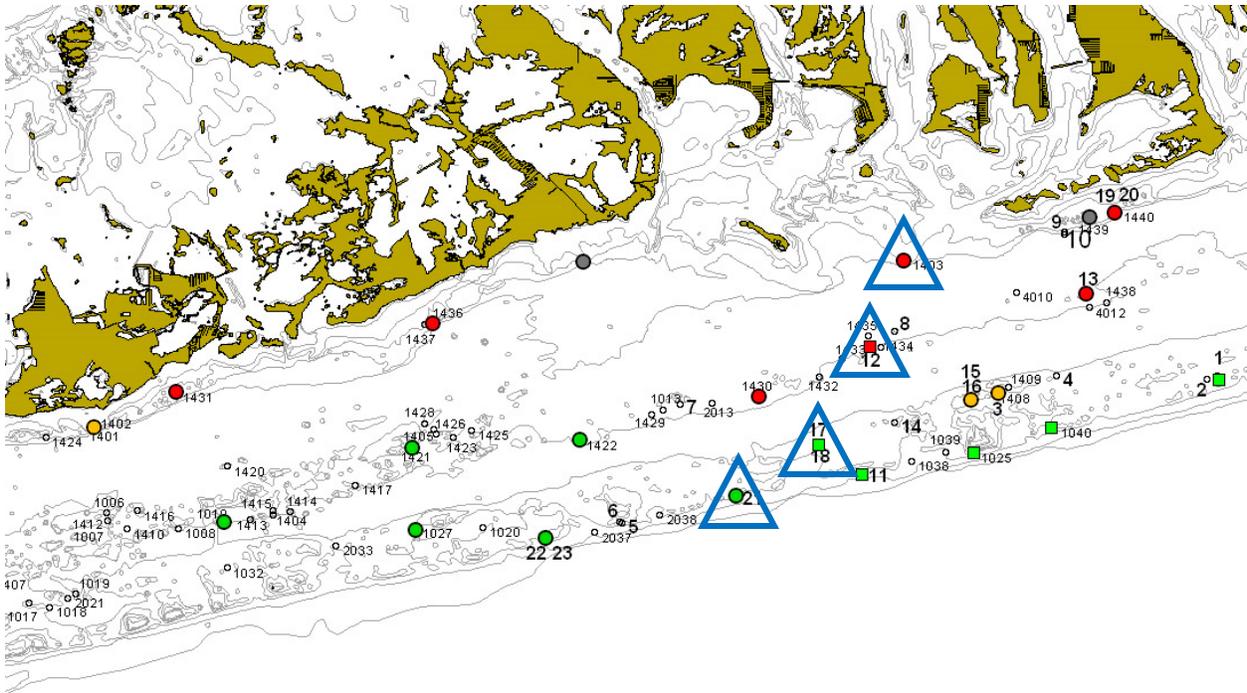


Figure 1 - Preliminary overview of surveyed sites in the Lower Florida Keys. Circles indicate FRRP surveys (E Bartels, NOAA/Stimulus funding), squares indicate observations made prior to the initiation of the FRRP-DRM response (E Bartels/MML). General impressions are color-coded to indicate overall mortality (green = healthy, yellow = some impacts but limited, red = significant recent mortality, gray = not enough coral in survey area or visibility too poor to properly assess). Site surveys monthly are indicated by blue triangles and range from offshore to nearshore sites: American Shoals, Looe Key Reef, Munson Reefs and an unnamed nearshore site.

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 includes a panel of marine invertebrate pathogens and human pathogens, as follows: *S. marcescens* (White Pox pathogen), *Vibrio shiloi*, *V. coralliilyticus*, *V. splendidus*, *V. complanada*, Methicillin-Resistant *Staphylococcus aureus* (MRSA, ATCC 43300), Methicillin-Sensitive *S. aureus* (MSSA, ATCC 29213), Vancomycin-Resistant *Enterococcus* (VRE), *Bacillus subtilis* (ATCC 6633 Km resistant), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* O157. Environmental libraries were grown on Marine Agar (Difco) medium 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 overlaid 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 scored as mm distance from the outside of clearing zone to the outer edge of the tested colony.

Bacterial sensitivity to temperature change. In addition to our specific aims, we also used cultured libraries for a test of coral bacterial sensitivity to temperature change and how this change affects antibiotic activity of the associated bacterial. To do this we simply incubated bacterial libraries at 25°C, 30°C, or 35°C. prior to antibiotic testing (as described above).

Total microbial community profiling of associated bacteria. Total DNA from **each coral colony** was extracted by centrifugation followed by a bead-beating, humic acid removal system (Soil-DNA extraction kit, MoBIO). Samples are archived frozen at -80°C for bacterial profiling. Adequate funding was unavailable to process these samples for thorough bacterial profiling.

TABLE 1: Sampling Sites (Sampled Monthly from February 2010 through January 2011)

Site #	Site Name	Habitat Type	Coordinates	Depth (ft)	Coral Species Sampled
1	American Shoals	offshore	24°31.322 N x 81°30.304 W	60	<i>M. faveolata</i> (x5)
2	Looe Key Reef	outer reef	24°32.671 N x 081°24.485 W	20	<i>M. faveolata</i> (x5) <i>A. palmata</i> (x3)
3	Munson Reef	mid channel	24°35.766 N x 81°22.313 W	18	<i>M. faveolata</i> (x5)
4	Staghorn Site #1	nearshore	24°37.284 N x 81°21.776 W	18	No live <i>M. faveolata</i> tissue found at this site after 2/2010

Results

We sampled coral mucus from unaffected coral tissues at the sites indicated in sites indicated in Figure 1 monthly from February of 2010 through January of 2011. **To date, we have archived and assayed 1,824 bacterial isolates, total, for this study.** Rapid assays for the presence/absence of *Vibrio* species were used to assess the general health of tissues sampled over our gradient. Samples results, pooled from each site, indicate that *Vibrio* levels are consistent with other temporal studies (Ritchie, 2008, Ritchie, 2006) with higher percentages of *Vibrio* spp observed on the coral surface in warmer months (**Figure 2**). Antibiotic testing of pooled coral mucus showed similar trends as previously found (Ritchie 2008) but appeared within a relatively “normal” threshold as compared to a heating event (Ritchie 2006) and as correlated with apparently healthy coral tissue sampled (**Figure 3**).

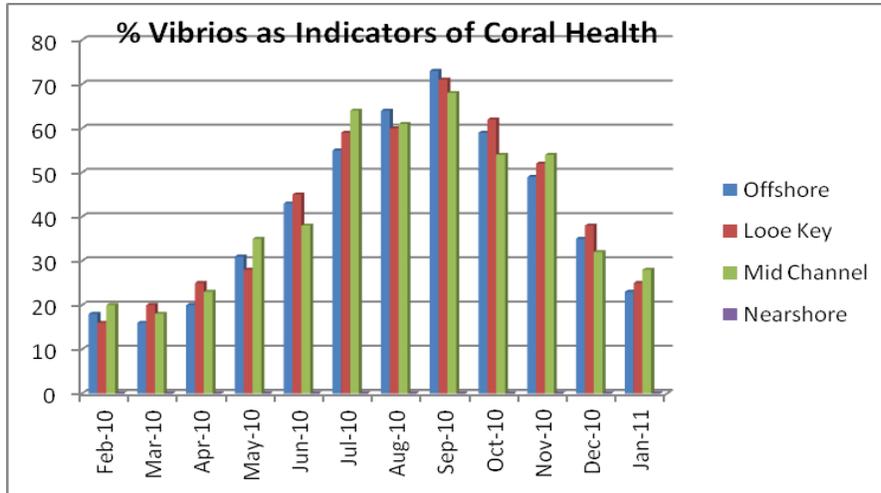
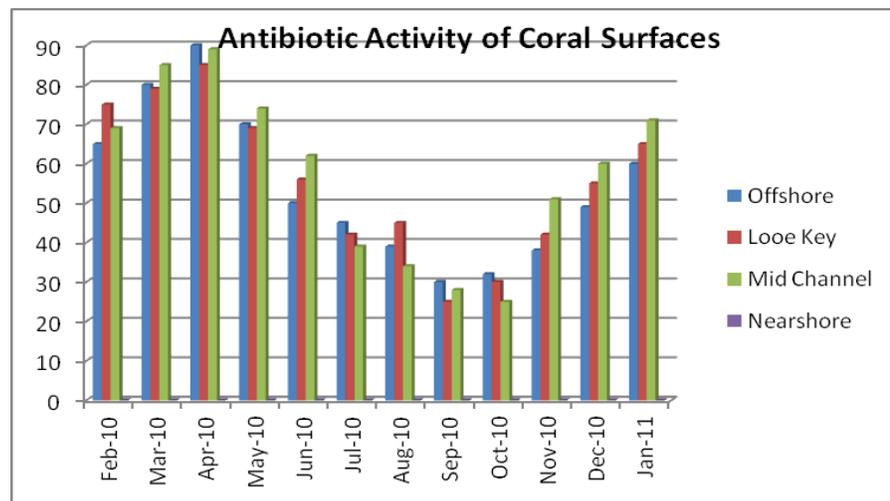


Figure 2 - Sea surface temperature effects on coral *Vibrio* presence. Measurements were shown for pooled coral mucus from three sites (note that nearshore sites did not have live *M. faveolata* tissue) from February 2010 to January 2011. *Vibrio* ratios were measured as percent growth on TCBS media.

Figure 3 - Antibiotic activity (AB) in surface mucus was measured from pooled corals/site and as ability to inhibit growth of seawater microbes (fold-inhibition). Figures were multiplied by 10 to scale.



Beneficial Bacterial Response to Temperature Increase

In addition to our specific aims, we also used cultured libraries for a test of coral bacterial sensitivity to temperature change and how this change affects antibiotic activity of the associated bacterial. To do this we simply incubated bacterial libraries at 25°C, 30°C, or 35°C. prior to antibiotic testing (as described above). Of 1,824 bacterial isolates total derived from this study, most beneficial antibiotic producers lost antibiotic production capabilities when temperatures increased (**Figure 4**). Results were similar when compiled by month (data not shown). This observation is of great significance in the case that these beneficial bacteria contribute to the innate immunity of the coral host by producing antibiotics *in situ*.

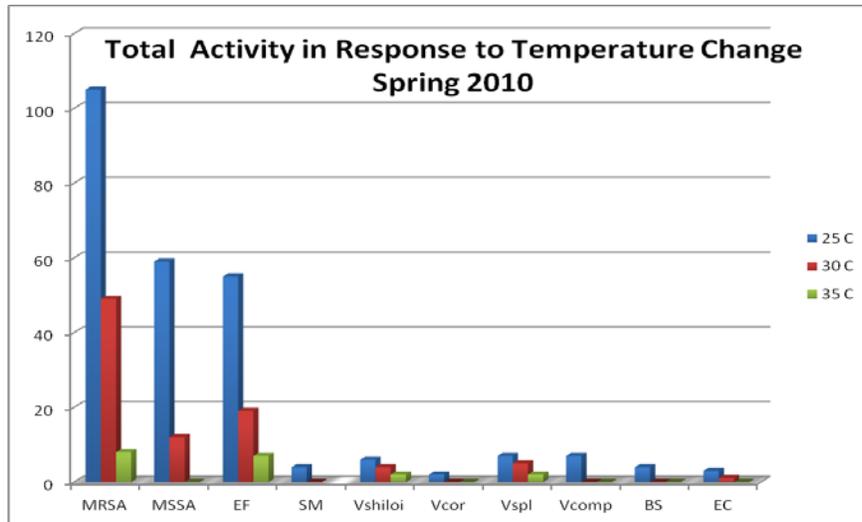


Figure 4 – Antibiotic producing bacteria lose activity at increasing temperatures. 1,824 bacterial isolates total derived from this study were tested against various bacterial pathogen tester strains (indicated on the X-axis; see methods). Isolates were grown at various temperatures (25°C, 30°C, 35°C) in order to determine temperature effect on antibiotic production. Of

interest is the observation that a temperature increase significantly reduces antibiotic production capabilities in most beneficial bacterial. Results were similar when compiled by month (data not shown).

Acknowledgements

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References

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